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Fish Community Diversity and Spatiotemporal Dynamics in the Downstream of the Fujiang River Based on Environmental DNA

Jiaming Zhang [1,2,†], Yifang Chen [1,†], Xinxin Zhou [1,2], Jiaxin Huang [1,2], Xiaohan Dong [1,2], Shuli Zhu [3,*] and Yanjun Shen [1,2,*]

[1] Laboratory of Water Ecological Health and Environmental Safety, School of life Sciences, Chongqing Normal University, Chongqing 401331, China; taiho2333@gmail.com (J.Z.); yifangchen94@outlook.com (Y.C.); zhouxinixin726@163.com (X.Z.); 19971464829@163.com (J.H.); 18325131947@163.com (X.D.)
[2] Chongqing Key Laboratory of Conservation and Utilization of Freshwater Fishes, Chongqing 401331, China
[3] Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510380, China
* Correspondence: zshuli2009@126.com (S.Z.); shenyanjun@cqnu.edu.cn (Y.S.)
† These authors contributed equally to this work.

Abstract: Hydrological changes caused by dam construction are among the primary drivers of global freshwater biodiversity decline. To assess the current status of fish community diversity and examine the impacts of cascade hydropower development on fish diversity, this study employed environmental DNA (eDNA) technology from 2023 to 2024 to conduct seasonal surveys at 18 sampling sites across six river segments separated by five dams in the downstream section of the Fujiang River. The study aimed to uncover the temporal and spatial dynamics of fish diversity and community structure, as well as to analyze the influence of environmental factors on these patterns. The results identified 84 fish species spanning 60 genera, 19 families, and 7 orders, including 2 nationally protected species, 11 endemic species of the upper Yangtze River, and 13 alien species. The cascade dams were found to have significantly reduced fish diversity compared to historical records, with a marked decline in native species and a rise in alien species, contributing to the miniaturization and homogenization of fish communities. Environmental factor analysis revealed that chemical oxygen demand (COD), dissolved oxygen (DO), total dissolved solids (TDSs), electrical conductivity (EC), and reservoir formation time were significant drivers of fish community structure and diversity. This study provides essential baseline data on fish diversity under the influence of cascade hydropower development in the Fujiang River. It also offers valuable insights into the current status of fish resources and supports efforts in fish conservation and aquatic ecosystem management in the region.

Keywords: fish diversity; cascade dams; environmental DNA; lower reaches of the Fujiang River

Key Contribution: This study employed eDNA technology to conduct multi-temporal and multi-spatial sampling throughout all seasons in the downstream section of the Fujiang River (Chongqing section), revealing the response mechanisms of fish communities to hydrological changes under the influence of cascade dams.

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1. Introduction

River ecosystems not only provide habitats for diverse aquatic organisms but also play a vital role in linking and maintaining material cycling and energy flow between terrestrial and aquatic ecosystems through the transport of water, sediments, and nutrients [1]. Additionally, rivers serve as critical migration corridors for freshwater fish, supporting regional

ecosystem stability and biodiversity. This is especially significant in downstream river areas, which act as endpoints of river systems, bearing substantial amounts of nutrients and sediments while making significant contributions to ecological regulatory functions [2]. Therefore, maintaining the health of downstream river ecosystems is of paramount importance. However, with the growing global demand for energy, the construction of cascade dams has played a vital role in providing clean energy and regulating water resources. At the same time, it has significantly altered river systems by disrupting connectivity, slowing water flow, and changing the physicochemical properties of water, transforming dynamic flowing systems into relatively static reservoirs. These changes have had profound negative impacts on biodiversity and habitat conditions in downstream aquatic ecosystems [3].

Migratory fish rely on the natural flow of rivers to complete their reproductive and life cycles. The construction of cascade dams has interrupted their migration routes, leading to a gradual decline or even disappearance of these species in upstream and midstream waters, exacerbating biodiversity loss [4]. Changes in downstream habitats have also facilitated the spread of alien species adapted to stagnant or slow-moving water, gradually replacing native species and becoming dominant populations in reservoirs [5]. For instance, a study in Brazil demonstrated that the proportion of migratory fish significantly decreased with the construction of cascade dams, while non-migratory and alien species gradually dominated the ecosystem [6]. Similarly, in river basins such as the Yellow River and the Yangtze River, the construction of cascade dams has significantly reduced fish diversity and intensified ecosystem homogenization [7,8]. Thus, dams not only affect fish populations but also alter fish community composition, habitat structure, and biodiversity distribution patterns.

Assessing the impacts of dam construction on fish biodiversity relies on biodiversity monitoring. However, traditional fish monitoring methods, such as electrofishing, trawling, and trapping, while providing information on fish population abundance and distribution, are labor-intensive, time-consuming, and have significant limitations in detecting rare species, conducting large-scale sampling, and ensuring non-invasive environmental monitoring [9]. In recent years, environmental DNA (eDNA) technology has emerged as an efficient and non-invasive biodiversity monitoring tool, demonstrating immense potential in aquatic ecosystems [10]. By collecting trace DNA from water samples, eDNA reduces environmental disturbance and enhances the sensitivity of detecting low-abundance and rare species [11]. Applications in river basins such as the Yangtze River have revealed seasonal and spatial distribution changes in fish, offering finer-scale insights into community dynamics [12]. In the Pearl River wetlands and Himalayan river basins, eDNA monitoring has complemented traditional methods such as bottom trawling and electrofishing, accurately capturing changes in aquatic biodiversity, particularly in identifying rare species distribution patterns [13]. Furthermore, eDNA technology has proven effective in detecting the impacts of environmental disturbances on aquatic biodiversity, providing scientific support for long-term ecosystem monitoring and assessments of human activity [14]. Integrating traditional fishing methods with eDNA technology significantly improves the comprehensiveness and sensitivity of aquatic ecosystem monitoring, laying a foundation for accurately assessing the long-term impacts of dams on fish biodiversity [15].

The Fujiang River, the largest tributary on the right bank of the Jialing River in China, boasts diverse cultural and geographical features, natural landscapes, and rich biodiversity [16]. As a critical ecological buffer in the Jialing River basin, its aquatic biodiversity plays a pivotal role in regional ecological balance. Historical records indicate that the Fujiang River basin hosts a diverse aquatic ecosystem, providing essential habitats for numerous aquatic organisms [17–19]. However, existing studies on fish composition in this basin are often fragmented or outdated, lacking systematic investigation. The downstream Fujiang River has been divided into six isolated river segments due to the construction

of five dams. Previous studies indicate a significant decline in fish populations under the influence of cascade hydropower development [20]. Therefore, this study employs eDNA technology to reveal spatiotemporal dynamics of fish communities through multi-temporal and spatial sampling over the course of a year, elucidating changes in various fish populations along environmental gradients, with a focus on the spatiotemporal distribution of endangered and key protected species. Furthermore, the study systematically explores the responses of fish community structures in the downstream Fujiang River (Chongqing section) to hydrological changes, analyzing the impacts of environmental factors on fish composition and diversity in each reservoir area. This research aims to provide foundational data on the effects of cascade hydropower development on fish biodiversity and community structures while offering scientific support for ecological monitoring and fish biodiversity conservation in the Fujiang River basin.

2. Materials and Methods

2.1. Study Area

The Fujiang River is located between 30°42′–33°03′ N and 103°45′–105°43′ E, with a total length of 668 km (Figure 1). The study area encompasses the downstream Chongqing section of the Fujiang River, which has been divided into six isolated river segments by the construction of five dams (Figure 1). These segments include the Shuangjiang (SS) reservoir, Tongnan (ST) reservoir, Fujinba (SF) reservoir, Anju (SA) reservoir, Weituo (SW) reservoir, and the Weituo River confluence section (SWX). In each reservoir, three sampling sites were established, located at the upstream, midstream, and downstream areas of the reservoir. In total, 18 sampling sites were set up for this study (Figure 1, Table S1).

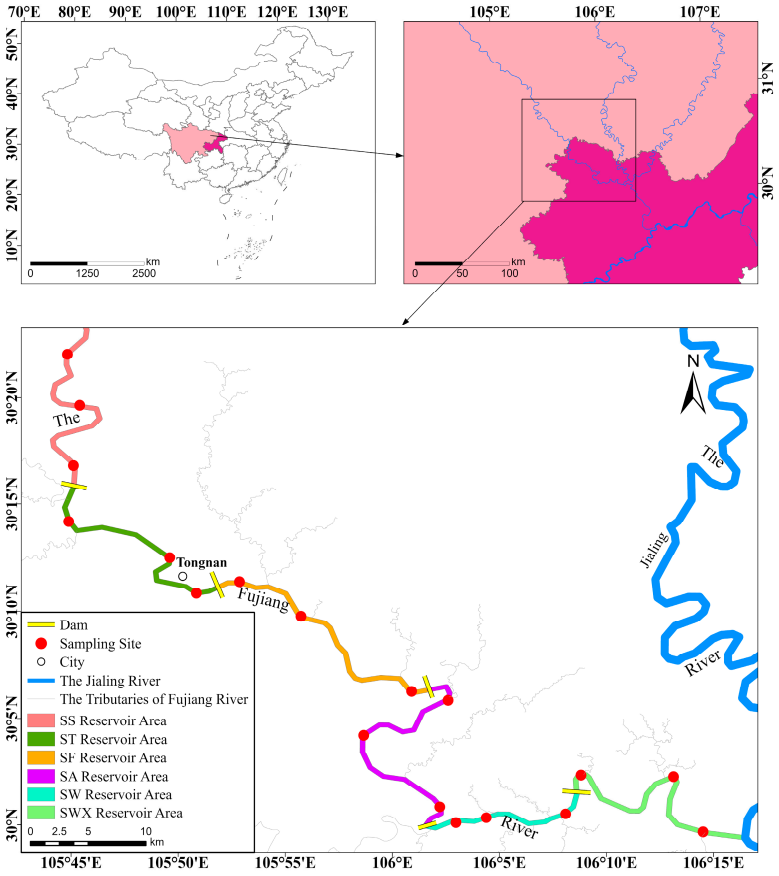

Figure 1. Information on sampling locations by ArcGIS 10.8. Refer to the Methods section for an explanation of the acronyms in this figure.

2.2. eDNA Water Sample Collection and Environmental Factor Detection

Water samples were collected in May 2023, September 2023, and January 2024. At each sampling site, 3 L of mixed water samples were collected using an acrylic water sampler from the upstream, midstream, and downstream sections of each reservoir. In total, 1 L was collected from the upper, middle, and lower water layers of each section and then mixed. Subsequently, water samples from the three sampling sites within the same reservoir segment were combined to create a composite sample. From this composite sample, 6 L of water was evenly divided into three replicates, with each 2 L portion used as one replicate for eDNA enrichment. All sampling equipment was sterilized with a 10% bleach solution before use and rinsed twice with double-distilled water (ddH_2O) [21]. The collected water samples were refrigerated and transported to the laboratory within 24 h. Samples were then filtered using a vacuum pump through 0.45 μm mixed cellulose ester membranes (Whatman, Maidstone, UK) for eDNA concentration. To prevent cross-contamination, all equipment was disinfected and rinsed with double-distilled water before and after filtering each sample. A negative control was included during each filtration process to assess potential external DNA contamination. Finally, the filtered membrane was stored at −80 °C in a freezer for subsequent DNA extraction.

In this study, seven water quality parameters were measured. pH was determined using a LichenpH-10 pen tester. Temperature and dissolved oxygen (DO) levels were measured using a HANNA HI98193 portable dissolved oxygen meter. Chemical oxygen demand (COD), electrical conductivity (EC), and total dissolved solids (TDS) were measured using a YSI (6600) water quality analyzer. Transparency was measured using a 20 cm diameter JCT-8 Secchi disk. Stable factors, such as elevation, reservoir formation time, and reservoir length, were determined through single measurements. Periodic variables, such as water temperature, dissolved oxygen (DO), pH, and electrical conductivity, were derived from the average of multiple measurements taken in May, September, and January, with one measurement conducted during each period (a total of three measurements).

2.3. eDNA Amplification and Sequencing

Total DNA was extracted from the filters using Qiagen PowerWater DNA Isolation Kits. The quality of the extracted environmental DNA (eDNA) was assessed through 1% agarose gel electrophoresis. DNA extraction was performed separately for each sample, and a blank filter was prepared as a negative control. The extracted DNA samples were stored at −20 °C for subsequent PCR amplification.

PCR was performed using the tele02 primers (tele02-F: 5′-AAA CTC GTG CCA GCC ACC-3′; tele02-R: 3′-GGG TAT ACT TAT CCC ACT TTG-5′) for the amplification of mitochondrial 12S rRNA [22]. PCR was carried out using TransStart Fastpfu DNA Polymerase in a 20 μL reaction system: 4 μL of 5× FastPfu Buffer, 2 μL of dNTPs (2.5 mM), 0.4 μL of FastPfu Polymerase, 2–5 μL of template DNA (10 ng), 0.8 μL of each primer (10 μM), and ddH_2O to a final volume of 20 μL. The PCR program was as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. Samples were then held at 10 °C. To evaluate potential contamination during PCR amplification, ddH_2O was used as a template for the negative control. Each sample underwent three replicate amplifications, and the resulting products were pooled. The DNA bands were detected using 2% agarose gel electrophoresis. The PCR products were subsequently gel-purified and sent to a commercial company for high-throughput sequencing on the Illumina NovaSeq 6000 platform, employing a paired-end sequencing approach, where both ends of each DNA fragment were sequenced. The length of each read is 150 base pairs (bp).

2.4. Historical Data Collection and eDNA Reference Database Construction

Based on the recent literature and survey data [17,18,20], we compiled a historical catalog of fish species in the mainstem of the Fujiang River, comprising 108 species belonging to 7 orders, 18 families, and 74 genera. The 12S rRNA and mitochondrial genome sequences of all freshwater fish species available in the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/nuccore/?term=, accessed on 27 March 2024) were used as the reference database for eDNA annotation in this study.

2.5. Bioinformatics Analysis and Processing

The raw reads (accession numbers: PRJNA1195328) were obtained from the Illumina platform and filtered using Trimmomatic v.0.36 to remove low-quality reads [23], such as those shorter than 100 bp or with trailing base quality scores below 20. Paired-end reads that passed quality control were merged into single sequences using FLASH, based on the overlapping regions between reads [24]. Low-quality sequences were further filtered using Usearch to obtain high-quality reads, which were then clustered into Molecular Operational Taxonomic Units (MOTUs) to generate representative sequences. Sequences were clustered based on a sequence identity threshold of $\geq$97%, ensuring that sequences classified as the same MOTU are sufficiently similar, representing the same or closely related species. These sequences were classified using Blastn and the Uclust algorithm to search the NCBI nucleotide database, excluding alignments to non-freshwater species. MOTUs with an identity value of $\geq$97% and an E-value of $\leq 10^{-5}$ were retained, and those classified as the same species were merged for downstream analysis [25]. Based on historical data, MOTUs corresponding to fish species from the Fujiang River basin were manually curated, retaining only fish MOTUs with sequence counts >10 across all samples for analysis [26].

2.6. Fish Diversity Analysis

Random subsampling of reads from each sample was performed using QIIME v.1.9.0 [27]. To ensure comparability across all eDNA samples, the data were normalized to the smallest actual sequence count among all samples [28]. This approach involved setting the minimum sequence abundance across all samples as the extraction depth, and randomly subsampling sequences from each sample to achieve a uniform total sequence abundance across all samples.

Alpha diversity was used to assess species diversity within each sample. Indices such as the Pielou evenness index, Shannon index, Simpson index, and Coverage were calculated using the Mothur software (v.1.35.1, http://www.mothur.org, accessed on 1 June 2024). The results for the Shannon index, Simpson index, and evenness index were visualized using boxplots to display the variability among samples.

Beta diversity was used to evaluate dissimilarities in fish community composition across different sampling regions. This analysis was divided into two independent components: spatial turnover and species richness [29]. The overall beta diversity was quantified based on the Sørensen index, the turnover component of the Sørensen index (β_{-3}), the richness component (β-rich), and the Jaccard similarity index. The quantification of beta diversity as the similarity of sampled sections was performed using the following formulas:

$$\beta_{\text{sor}} = \frac{b + c}{2a + b + c}$$

$$\beta_{-3} = \frac{\min(b, c)}{2a + b + c}$$

$$\beta_{\text{rich}} = \frac{|b - c|}{2a + b + c}$$

$$I = \frac{a}{b + c + a}$$

In the calculation of diversity indices, a represents the number of species shared between two sampling sites, while b and c represent the species unique to the first and second sampling sites, respectively. The Sørensen index ranges from 0 to 1, with 0 indicating no shared species and 1 indicating that all species are shared between two sampling sites. I represents the Jaccard similarity index, with values of 0.75–1 indicating very high similarity, 0.5–0.75 moderate similarity, 0.25–0.5 low similarity, and 0–0.25 very low similarity.

Fish species were categorized based on habitat preference, water column strata, spawning type, and feeding habits. Statistical differences in alpha diversity indices that satisfied tests for homogeneity of variance and normal distribution were analyzed using One-Way ANOVA. For data that did not meet these assumptions, the non-parametric Kruskal–Wallis test for independent samples was applied. The minimum level of statistical significance was set at $p = 0.05$.

2.7. Environmental Factor Correlation Analysis

Decision curve analysis (DCA) indicated that the length of the first axis was 3.43; thus, redundancy analysis (RDA) was selected to examine the relationships between fish communities and environmental factors. Environmental factors were categorized into stable factors and periodic variables, and RDA analyses were conducted separately for each type. This approach allowed us to investigate the influence of different environmental factor categories on fish community structure and diversity.

3. Results

3.1. Fish Composition Based on eDNA

PCR amplification was performed on 54 samples using universal primers. After quality control, filtering, BLAST alignment, and manual refinement, a total of 84 fish species were identified, belonging to 7 orders, 19 families, and 60 genera. These included 2 nationally protected fish species, 11 endemic species of the upper Yangtze River, and 13 alien species. Among these, 76 species were observed during the breeding period (May), 71 species during the feeding period (September), and 53 species during the overwintering period (January) (Figure 2).

The eDNA results revealed that the order Cypriniformes accounted for the largest proportion of species, comprising 71.43% (N = 60), followed by Siluriformes at 15.48% (N = 13) and Perciformes at 9.52% (N = 8), based on the total samples. Figure 2A illustrates the composition of fish sequence abundance at the order level across different sampling sites and seasons. At the family level, Cyprinidae was the most abundant, representing 69.05% of all species, followed by Bagridae at 9.52%. Additionally, only Cyprinidae and Xenocyprididae were detected at all sampling sites (Figure 2B).

Over the past 20 years, traditional methods have identified 108 fish species belonging to 7 orders, 18 families, and 74 genera (Table S2). A comparison between eDNA and traditional survey results showed that both methods detected 43 species, with a coverage rate of 39.81% (Figure 3). Both approaches consistently identified a higher abundance of Cyprinidae, Bagridae, and Cobitidae species. However, the number of nationally protected species and endemic species of the upper Yangtze River detected by the eDNA method was lower than that detected by the traditional method in the past, while the number of alien species detected was higher with the eDNA method (Figure 3).

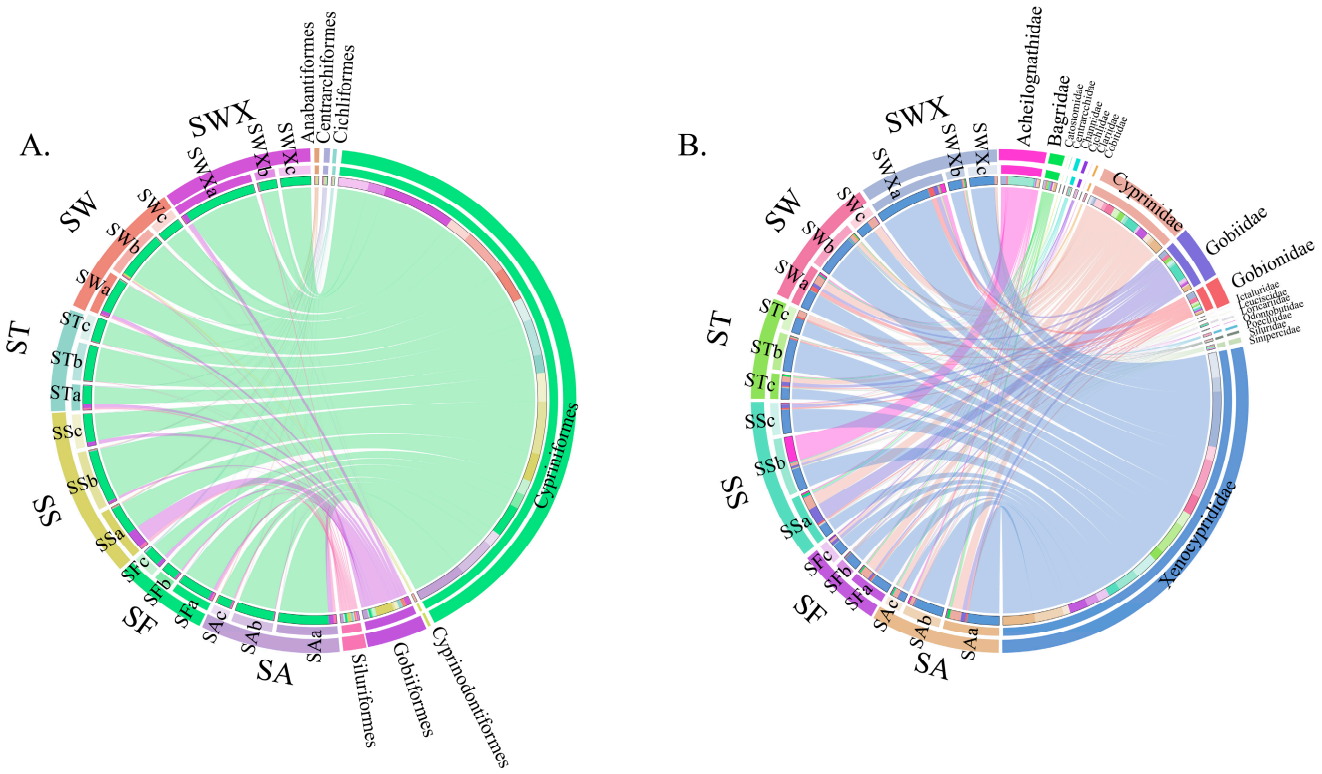

Figure 2. Circos species relationship map for each sample, showing species composition: (**A**) represents species composition at the order level, and (**B**) represents species composition at the family level; a represents the fish collected in May; b represents the fish collected in September; c represents the fish collected in January.

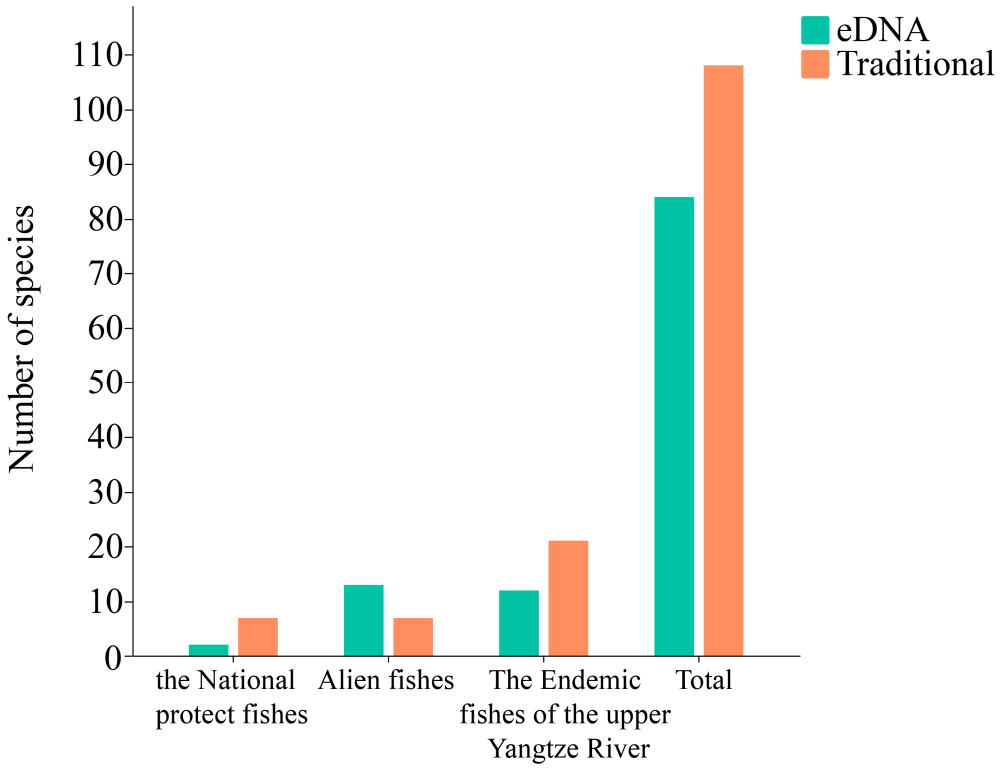

Figure 3. Comparison of the number of fish species detected by the traditional method and the eDNA method.

3.2. Variations in Fish Species Diversity Patterns Across Different Reservoirs

Based on species composition, 41 fish species were shared among all reservoirs in the downstream Chongqing section of the Fujiang River, accounting for 48.81% of the total species identified (Table S3). Additionally, the ecological composition of species across reservoirs was highly consistent, with adhesive-egg-laying, benthic, lentic-adapted, and omnivorous fish dominating all areas, while lower mesopelagic fish, eurytopic fish, and limnophilic fish also played significant roles (Figure 4(A1,B1,C1,D1)). The ecological composition based on relative sequence abundance analysis indicated that eurytopic fish exhibited strong adaptability and abundance across all sampling sites. Carnivorous fish were particularly abundant at most sites, especially at SS. Benthic and mid-lower-layer fish showed higher abundance at SA and SWX, while adhesive-egg-laying fish and those with specialized spawning modes were more abundant at most sites, particularly at SA and SWX. Overall, eurytopic fish, carnivorous fish, benthic fish, and adhesive-egg-laying fish accounted for the largest proportions, while limnophilic fish and omnivorous fish also played important ecological roles. In contrast, rheophilic fish, demersal spawners, and pelagic spawners had the lowest proportions (Figure 4(A2,B2,C2,D2)). The Shannon, Simpson, and Pielou evenness indices exhibited similar trends across all reservoirs. The SW reservoir had the highest evenness index, while SWX had the highest Shannon and Simpson indices. The ST reservoir showed the lowest values for all three indices (Figure 5). However, no significant differences were observed among sampling sites ($p > 0.05$). The coverage rate for each sample was close to 1 (Table S4), indicating that the sequencing depth effectively captured all species present in the samples.

The Sørensen beta diversity index ($\beta_{sør}$) for the Fujiang River basin was 0.849, with inter-reservoir values ranging from 0.790 to 0.894 (Table S5), suggesting high overall similarity in fish species composition among the six reservoirs, albeit with some degree of variability. β Diversity Contribution Analysis indicated that richness differentiation ($\beta_{rich} = 0.6982$) was the primary contributor to beta diversity, with a larger magnitude than spatial turnover ($\beta_{-3} = 0.1509$). This highlights that differences in species richness among reservoirs played a more significant role in driving overall changes in species composition, while spatial turnover was less influential. Additionally, the Jaccard similarity index values for all reservoirs exceeded 0.65, confirming a high degree of similarity across reservoirs. Principal Coordinates Analysis (PCoA) of overall species similarity based on Sørensen indices ($\beta_{sør}$) indicated that, despite some differences among reservoirs in the Fujiang River basin, there was a trend of increasing similarity (Figure 6A). Further analysis of spatial turnover (Figure 6B, β_{-3}), richness differentiation (Figure 6C, β-$_{rich}$), and Jaccard similarity indices (Figure 6D) corroborated this trend, emphasizing the role of species richness in shaping fish diversity patterns across reservoirs. In addition, the sampling points form three clusters under the four indices (β_{sor}, Jaccard, β_{-3}, β-$_{rich}$): SF, SW, and SA form one group, SS and ST form another group, and SWX is separated from the other sampling points, indicating certain differences between the sampling points. In Figure 6D, SA, SF, and SWX form a loose cluster, possibly because the distance between SW and SS is relatively close. Additionally, the ST sampling point is clearly different from the other sampling points, but statistical analysis did not show significant differences ($p > 0.05$).

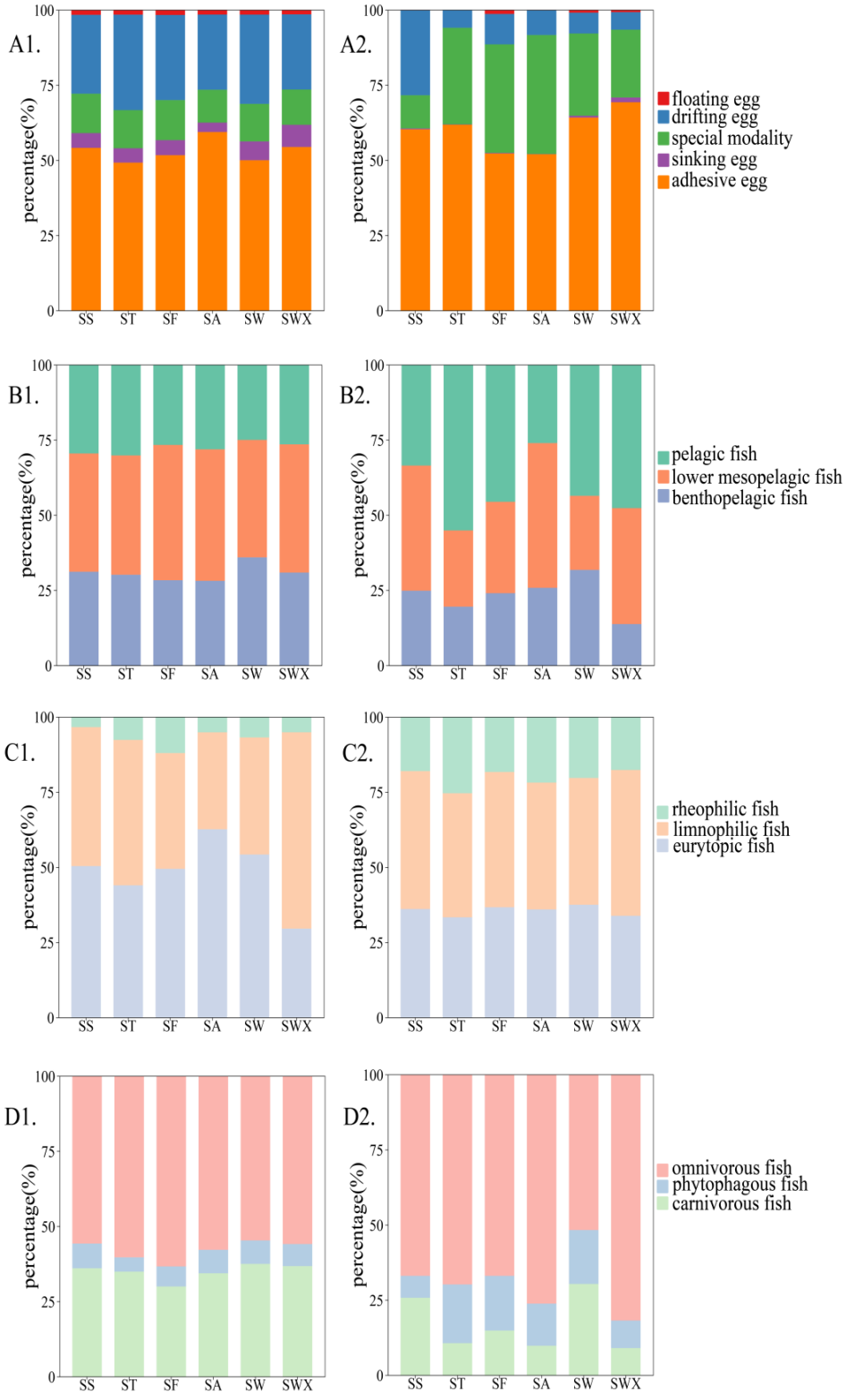

Figure 4. The composition of the ecological types in each sampling section: (**A1**) spawning types based on the number of species; (**B1**) habitat water layer based on the number of species; (**C1**) habitat water velocity based on the number of species; (**D1**) food type based on the number of species; (**A2**) spawning types based on the number of relative abundance of sequences; (**B2**) habitat water layer based on the number of relative abundance of sequences; (**C2**) habitat water velocity based on the number of relative abundance of sequences; (**D2**) food type based on the number of relative abundance of sequences.

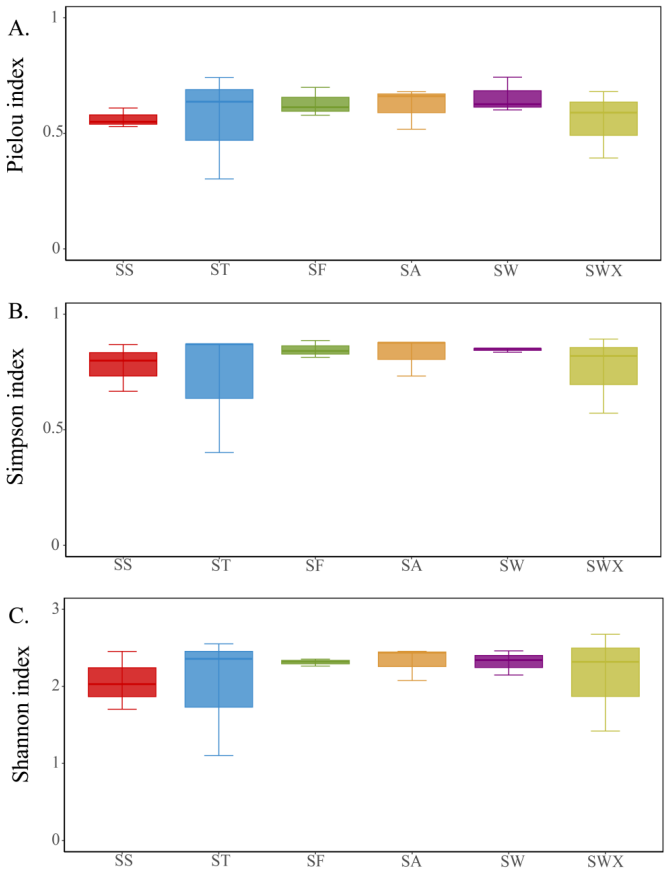

Figure 5. The boxplots showing the alpha diversity index for each sampling section: (**A**) is the Pielou index; (**B**) is the Simpson index; (**C**) is the Shannon index.

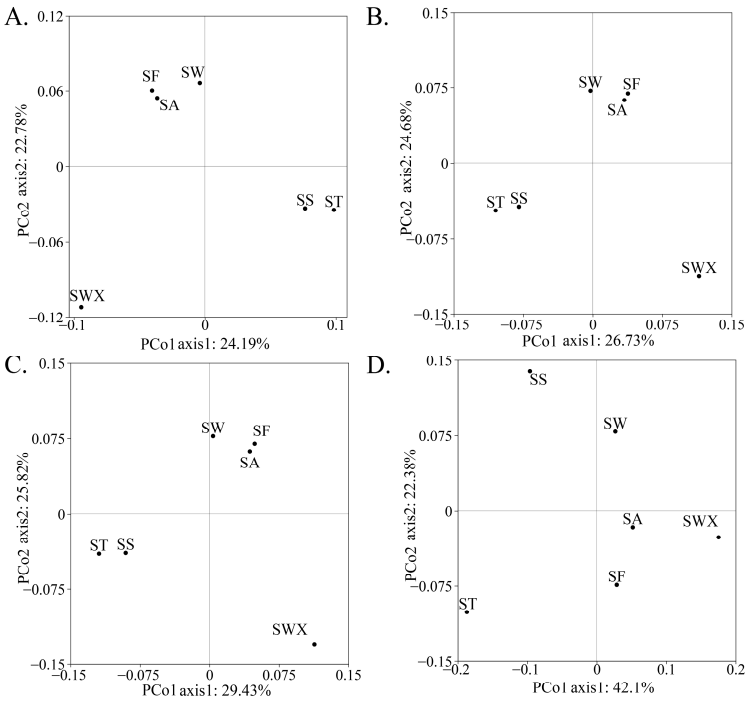

Figure 6. The PCoA for the beta diversity indexes: (**A**) is the Sørensen index (β_{sor}); (**B**) is the Jaccard similarity index; (**C**) is the richness (β-$_{rich}$) components of the Sørensen index; (**D**) is the turnover (β_{-3}) components of the Sørensen index.

3.3. The Response of Fish Diversity Patterns to Environmental Factors

In the redundancy analysis (RDA) of stable environmental factors (Figure 7A), the mean attitude and length of the reservoirs had some effect on SA and ST, but had a minor impact on SS, and these effects were not statistically significant ($p > 0.05$). In contrast, the reservoir formation time had a significant impact on SF, SW, and SA, especially on the structure of the fish community, which was statistically significant ($p = 0.009$).

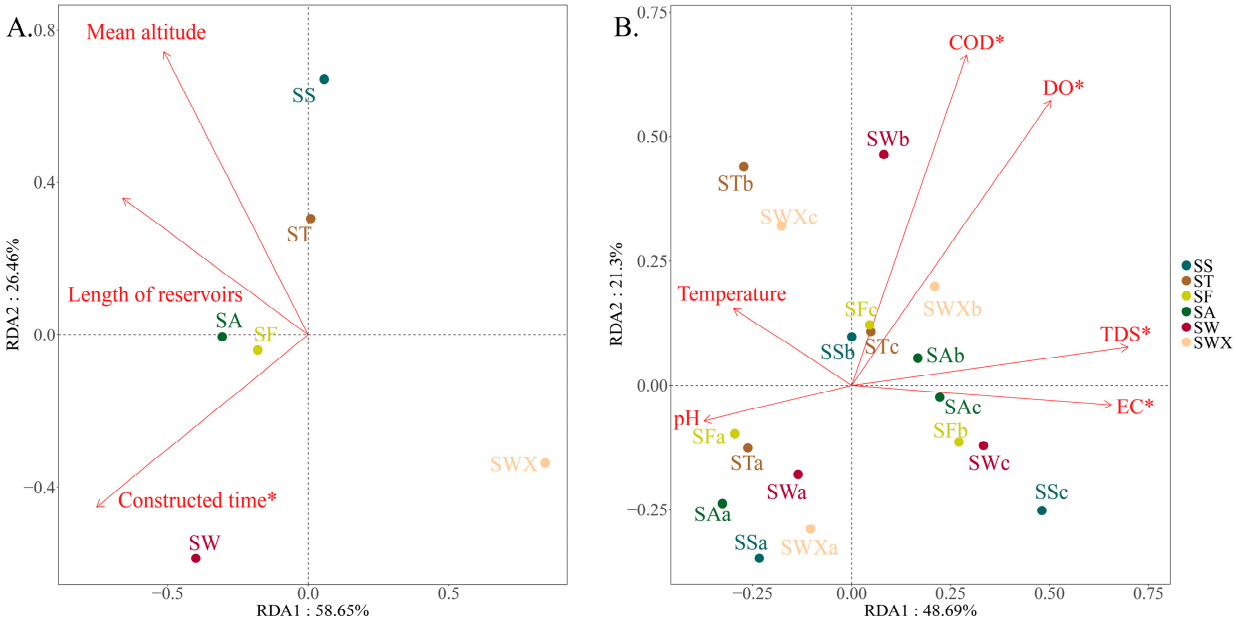

Figure 7. The RDA showing the correlation between environmental factors and fish communities: (**A**) is stable-type factors; and (**B**) is cyclical variable factors. In (**B**), a stands for spring; b represents autumn; c indicates winter. The environmental factors marked with * have a statistically significant impact on fish communities ($p < 0.05$).

In the RDA of periodic environmental factors (Figure 7B), it was found that chemical oxygen demand (COD, $p = 0.011$), and dissolved oxygen (DO, $p = 0.002$) significantly affected the fish community, particularly in the autumn (September). COD and DO had a positive influence on SAb, SWXb, STc, SFc, SSb, and SWb, while they had a negative influence on SFa, STa, SWa, SAa, SSa, and SWXa. In winter (January), total dissolved solids (TDS, $p = 0.022$) and electrical conductivity (EC, $p = 0.046$) also had significant effects on the fish community. In spring (May), pH had a very significant influence on SFa, STa, SWa, SAa, SSa, and SWXa, although it did not reach statistical significance ($p < 0.05$).

3.4. Fish Species Distribution and the Expansion Trend of Alien Fish Species

This study detected two nationally protected fish species (2.38%), *Schizothorax prenanti* and *Myxocyprinus asiaticus*, along with 11 endemic species of the upper Yangtze River (13.09%) (Table S3). However, the number of most protected and endemic species declined compared to the results of traditional surveys conducted in the past (Figure 3). Traditional surveys conducted over the past 20 years indicated the presence of 7 nationally protected species (6.79%), 7 alien species (6.48%), and 22 endemic species of the upper Yangtze River (20.38%) in the Fujiang River (Table S2).

In the current survey, 84 fish species were detected, including 71 native species and 13 alien species, which accounted for 15.48% of the total species. Among these, five alien species, including *Pseudorasbora parva*, *Pseudohemiculter dispar*, *Ictalurus punctatus*, *Phoxinus lagowskii*, and *Megalobrama amblycephala*, were also identified in traditional surveys. Notably,

the proportion of alien species increased compared to previous surveys (Figure 3), with some alien species even emerging as dominant groups (Table S3).

4. Discussion

4.1. Changes in Fish Diversity in the Downstream of the Fujiang River

The current fish ecological composition in the downstream Fujiang River is primarily dominated by adhesive-egg-laying, benthic, lentic-adapted, and omnivorous fish species. Changes in hydrological conditions have had a significant impact on fish populations and community diversity. Although the Fujiang River flows through hilly and low mountainous areas with relatively gentle terrain overall, regional variations in riverbed gradients have shaped its unique geomorphological characteristics. Historical surveys primarily recorded benthic, rheophilic, adhesive-egg-laying, and carnivorous fish species [18]. However, this study found that lentic-adapted fish and eurytopic fish have gradually become dominant, with rheophilic fish species detected at only half of their previous levels, showing a clear trend of change. The study also revealed that, compared to historical data, the proportion of mid- to lower-layer fish has increased [17]. The transformation of rivers into reservoirs has led to significant hydrological changes, with riverine habitats shifting to lacustrine environments. Adhesive-egg-laying fish, which require specific substrate conditions for spawning, have lost their habitats, resulting in significant population declines or even local extinctions [30]. The study also found reductions in carnivorous fish and increases in omnivorous fish. Migratory fish, once dominant in the basin, have experienced significant declines, while the proportion of non-migratory fish has increased. The construction of dams has altered fish community structures by obstructing the reproduction and distribution of large migratory species, while creating favorable habitats for smaller omnivorous species, which now play a prominent role in reservoir fish communities [5,31]. Among the reservoirs, *Opsariichthys bidens* exhibited the highest relative sequence abundance, establishing itself as a dominant species. Additionally, small fish such as *Carassius auratus*, *Xenocypris fangi*, and *Xenocypris yunnanensis* showed high sequence abundance in several reservoirs. This suggests a trend toward miniaturization of fish communities in the downstream Fujiang River. Reservoir formation has significantly reduced water flow, altered temperature and dissolved oxygen levels, and decreased habitat heterogeneity, thereby affecting fish biodiversity and community structure [32].

The results of Jaccard (0.616) and Sørensen (0.708) indices revealed high consistency in fish species composition across reservoirs for different ecological types. The alpha diversity indices showed no significant differences among reservoirs, further indicating pronounced homogeneity in fish community diversity across the region. This phenomenon is likely driven by habitat fragmentation and environmental convergence caused by dam construction. Studies have shown that dams alter hydrological characteristics and ecological connectivity, providing favorable environments for adaptable alien species while threatening populations of native endemic species, leading to significant declines [4]. Furthermore, the presence of reservoirs has been shown to increase the similarity of fish communities within the basin, contributing to the homogenization of species diversity [33]. This underscores the profound impacts of dam construction on biodiversity patterns in river basins.

The primary factor influencing Sørensen beta diversity (β_{sor}) was species richness differentiation (β-$_{rich}$), indicating that differences in species richness among reservoirs drive community composition changes more than species turnover (i.e., replacement of different species). This finding aligns with the widespread dispersal of alien species across reservoirs. High abundance and consistency of alien species in reservoirs reduce the contribution of native species to community structure, lowering species turnover rates. This low turnover

and high similarity in species composition further confirm the trend toward biotic homogenization induced by dam construction. Studies have shown that environmental changes caused by dams allow highly tolerant alien species to dominate habitats, exacerbating community homogenization [34]. Strengthening ecological conservation measures in the Fujiang River basin is thus crucial for maintaining regional biodiversity [35].

4.2. Impact of Environmental Factors on Fish Diversity

Fish community structure is significantly influenced by environmental factors at various spatial and temporal scales, with the sensitivity of fish communities to these factors varying depending on regional and ecological conditions. Key factors such as elevation, flow velocity, dissolved oxygen (DO), and pH affect fish diversity by altering habitat conditions [36,37]. In this study, RDA results revealed that periodic environmental factors had a significant impact on fish community structure in the downstream Fujiang River. During the feeding season, chemical oxygen demand (COD) and dissolved oxygen (DO) were the primary factors influencing fish community structure. Elevated COD indicates active decomposition of organic matter in the water, which can lead to rapid depletion of DO, thereby restricting the survival and activity of oxygen-demanding fish species. This finding aligns with previous studies that highlighted the role of COD and DO in regulating fish metabolism and distribution [38]. In winter, total dissolved solids (TDS) significantly influenced fish communities. Higher TDS levels can alter the osmotic balance of the environment, affecting the physiological state and adaptive behavior of fish, which indirectly impacts their growth and habitat distribution [39]. Moreover, changes in TDS can indirectly affect fish community structure by influencing the diversity and abundance of planktonic organisms. For instance, high TDS concentrations can reduce certain zooplankton populations, diminishing food resources for fish and consequently affecting their growth and distribution [40].

Research has shown that different habitat types have a significant impact on the composition of fish communities, with high-quality habitats often supporting more stable and diverse community structures [41]. However, the RDA results in this study showed that while elevation and reservoir length had some influence on fish communities in specific reservoirs, their overall effect was not statistically significant. This phenomenon may be due to the limited range of elevation changes within the Fujiang River basin, which reduces its influence on fish diversity. Previous studies have shown that high-altitude areas are typically characterized by lower water temperatures and higher flow velocities. For species with low cold tolerance, these environmental factors may affect their distribution [42]. In addition, reservoir length may affect fish communities by altering habitat scale and resource distribution. However, in the Fujiang River basin, this effect may be masked by changes in water quality and hydrological conditions [43]. In this study, the formation time of the reservoirs significantly impacted the fish community's diversity. This may be because, after years of operation, the fish communities in the SF, SA, and SW reservoirs have gradually adapted to the new ecological conditions, resulting in a stable structure and functional characteristics that align with their habitats, thereby influencing fish diversity and community dynamics [44].

4.3. Spatial Distribution and Relative Abundance of Native and Alien Fish Species

The results of this study indicate a declining trend in the diversity of native and rare protected fish species in the downstream Fujiang River, while the abundance of alien fish species has significantly increased. In recent years, the expansion of alien fish populations has posed an increasing ecological threat to native fish communities in the Fujiang River basin by competing for ecological niches and invading habitats. Environmental changes

induced by dam construction have allowed more adaptable alien fish species to occupy ecological niches more easily, while native fish species, particularly those highly dependent on flowing water, face survival pressures [45]. Additionally, dams reduce water turbidity, weakening predation pressure from native carnivorous fish on alien species, thereby enhancing the competitive advantage of alien fish [46]. Studies have also found that the number of alien fish species has significantly increased in the upper Yangtze River's cascade dam areas, while native fish diversity has significantly declined, indicating that dam construction has intensified the survival challenges for native fish [47].

The presence of alien fish species in the Fujiang River basin is primarily attributed to human activities, including escapes from aquaculture and intentional introductions [18]. These alien species often outperform native fish in adaptability and reproductive capacity. Research has shown that escapes from aquaculture systems have significantly facilitated the global spread of alien fish into natural water bodies, where they competitively occupy ecological niches in new environments [48]. Furthermore, certain alien fish species have been intentionally introduced to control aquatic organisms, such as mosquitofish for controlling mosquito larvae. However, due to insufficient regulation, these species often invade freshwater ecosystems extensively [49].

4.4. Limitations of eDNA Technology

Although eDNA technology is widely applied in aquatic ecosystems due to its sensitivity and non-invasive nature, its detection efficacy is influenced by environmental conditions. Factors such as water flow rate, temperature, pH, and sediment content determine the stability and concentration of eDNA in water. High flow rates accelerate eDNA diffusion and degradation, reducing the concentration of target species' eDNA and affecting detection sensitivity [50]. In static water environments, such as lakes, eDNA signals can exhibit spatial and temporal fluctuations due to seasonal variations, potentially leading to inaccurate detection [51]. Temperature significantly impacts eDNA preservation, with high temperatures accelerating degradation and low temperatures aiding preservation [52]. Although eDNA concentration is often used to infer species biomass, environmental disturbances can cause variations in concentration, affecting abundance estimates [53]. Additionally, the relationship between eDNA concentration and biomass becomes complex due to multiple factors in natural environments, reducing the accuracy of data interpretation [54]. In dynamic water bodies such as rivers, high flow rates can cause eDNA signals of upstream species to spread downstream, resulting in "false positive" detections [55]. Moreover, eDNA can only detect species presence and cannot provide information on individual numbers, size, age, or health status, limiting its application in population dynamics and ecological assessments [56]. Chemical inhibitors in polluted environments may also reduce eDNA extraction efficiency, especially in contaminated water bodies [57]. Therefore, integrating eDNA methods with traditional approaches provides a more comprehensive understanding of species diversity and ecological characteristics in specific regions [58].

4.5. Strategies to Mitigate the Impact of Cascade Hydropower Development on Fish Diversity

Fish populations in the Fujiang River face significant challenges, as aquatic ecosystems are highly sensitive to natural changes and human activities. To balance hydropower development with species conservation, ensure the sustainable development of hydropower, and promote the long-term reproduction of fish populations, this study proposes the following conservation measures:

Restoring natural river flow is crucial. Flow variation not only affects fish habitats but also alters interspecies competition, profoundly impacting fish communities [59]. The construction of dams in the Fujiang River has disrupted the original natural flow patterns.

Removing unnecessary dams and restoring natural river channels can improve ecological connectivity and provide more suitable habitat conditions for fish species.

Establishing natural reserves is an important means of conserving river biodiversity. Studies have shown that reserves can effectively reduce human disturbances and maintain biodiversity [60]. In the Fujiang River basin, Sørensen index analysis indicates that species richness differences are critical to community changes among reservoirs. It is recommended to prioritize establishing reserves in areas with high species richness and diverse habitats to maintain community stability and reduce the spread of alien species.

Fish passage facilities can effectively mitigate the obstruction of migratory fish by dams, promote population recovery, and ensure gene flow. Studies show that these facilities have significant effects on improving fish dynamics and restoring populations [61]. Artificial propagation technology is an important strategy for restoring populations of endangered and endemic fish species. By utilizing artificial propagation and stock enhancement, population numbers can be significantly increased in the short term, alleviating the negative effects of habitat loss [62]. Endemic fish species in the upper Yangtze River have achieved significant recovery through artificial propagation, but the technology still needs to be optimized to improve breeding efficiency and ensure genetic diversity.

Integrated ecological regulation through optimizing reservoir operations and simulating natural flow patterns supports fish reproduction and habitat maintenance while mitigating the negative impacts of dams [63]. In the Fujiang River basin, optimizing water allocation during key seasons through joint ecological scheduling will not only help support fish reproduction, but also enhance ecological connectivity between reservoirs.

5. Conclusions

The fish community composition in the downstream Chongqing section of the Fujiang River has undergone significant changes due to the impact of cascade hydropower development. These changes have resulted in the dominance of lentic-adapted fish, omnivorous fish, benthic fish, and adhesive-egg-laying species. The study findings indicate a decline in native fish diversity, an increase in alien invasive species, and an intensified homogenization of fish species composition among different reservoir areas. Results from the redundancy analysis (RDA) reveal that COD, DO, TDS, and EC significantly influence fish community diversity across seasons, while the duration of reservoir formation also exerts a notable effect on community diversity. Furthermore, the non-invasive eDNA method has proven to be an effective supplementary tool for fish monitoring in the basin. When combined with traditional methods, it enhances monitoring efficiency.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fishes10020043/s1; Table S1: Coordinates of Sampling Sites; Table S2: List of fish detected by traditional methods; Table S3: List of fish detected by eDNA methods; Table S4: Summary values of diversity metrics and environmental factors of sampling sites; Table S5: Beta diversity index.

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Institutional Review Board Statement: This study did not involve any live animals, and fish species were obtained by collecting river water samples for environmental DNA analysis.

Informed Consent Statement: Not applicable.

Data Availability Statement: The reference sequences are available on NCBI (https://dataview.ncbi.nlm.nih.gov, accessed on 7 December 2024) Sequence Read Archive (SRA) database under the following accession numbers: PRJNA1195328.

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