Chishui River fish diversity and its driving factors An environmental DNA study

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Abstract

Freshwater biodiversity is facing a great crisis due to the looming threat of biodiversity loss. Some important target areas are difficult to survey owing to their accessibility, making them susceptible to data deficiencies. In this study, we surveyed 52 sites using environmental DNA techniques to investigate fish biodiversity in the Chishui River Basin of the Yangtze River. A total of 96,031 valid fish sequences were read, resulting in the identification of 77 species belonging to six orders, 62 genera, and 18 families. The dominant orders were Cypriniformes, Siluriformes, and Perciformes. Among the identified fishes, 71 were native and six were exotic, with the native fishes including 16 endemic fishes from the upper reaches of the Yangtze River. The upstream and downstream Shannon-Wiener indices differed significantly, the Shannon-Wiener and richness indices of the tributaries in the upstream section were significantly higher than those of the tributaries in the downstream section, and we found that the Datong River is the most diverse secondary tributary of the Chishui River. Among the environmental factors in the Chishui River Basin, altitude and electrical conductivity had the greatest influence on fish diversity (P <0.01), whereas human factors had little effect. Our findings highlight the application of environmental DNA technology to modern biodiversity surveys and illustrate that the Chishui River Basin is primarily affected by environmental factors at this stage. However, continuing efforts are needed to protect freshwater biodiversity, and additional research is required to better understand the complex interplay between human activity and environmental factors.

Chishui River fish diversity and its driving factors: An environmental DNA study

Running title: Chishui River fish diversity

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ABSTRACT

Freshwater biodiversity is facing a great crisis due to the looming threat of biodiversity loss. Certain important target areas are difficult to survey owing to their accessibility, making them susceptible to data deficiencies. In this study, we surveyed 52 sites using environmental DNA techniques to investigate fish biodiversity in the Chishui River Basin of the Yangtze River. A total of 96,031 valid fish sequences were read,

resulting in the identification of 77 species belonging to six orders, 62 genera, and 18 families. The dominant orders were Cypriniformes, Siluriformes, and Perciformes. Among the identified fishes, 71 were native and six were exotic, with the native fishes including 16 endemic fishes from the upper reaches of the Yangtze River. The upstream and downstream Shannon-Wiener indices differed significantly, the Shannon-Wiener and richness indices of the tributaries in the upstream section were significantly higher than those of the tributaries in the downstream section, and we found that the Datong River is the most diverse secondary tributary of the Chishui River. Among the environmental factors in the Chishui River Basin, altitude and electrical conductivity had the greatest influence on fish diversity (P < 0.01), whereas human factors had little effect. Our findings highlight the application of environmental DNA technology to modern biodiversity surveys and illustrate that the Chishui River Basin is primarily affected by environmental factors at this stage. However, continuing efforts are needed to protect freshwater biodiversity, and additional research is required to better understand the complex interplay between human activity and environmental factors.

Keywords: Chishui River, environmental DNA, environmental factors, fish biodiversity, human activities, influencing factors

INTRODUCTION

Despite extensive conservation measures, biodiversity loss is unavoidable, and freshwater biodiversity is currently in crisis (Reid et al., 2018; Cooke et al., 2021). The loss of freshwater biodiversity far exceeds that in terrestrial and marine environments (Tickner et al., 2020). Furthermore, freshwater fishes have gone extinct globally at a faster rate than other vertebrates, with freshwater species declining by 83% since 1970 (Dias et al., 2017; Harrison et al., 2018).

Fish diversity has received increasing attention as a result of the global biodiversity crisis. Fish are important consumers in the aquatic food chain (Villéger et al., 2017) and play a significant role in aquatic ecosystems (Su et al., 2021). Changes in the structural composition and distribution of fish communities can reflect the stability and health of aquatic ecosystems (Weigand et al., 2019). Unfortunately, China's large population base, rapid economic development, and increased resource exploitation have resulted in serious threats to aquatic biodiversity, particularly fish diversity (Hu et al., 2009; Limburg et al., 2011; Chen, 2017).

The Yangtze River is China's longest river, spanning 6300 km and originating in the Qinghai-Tibetan Plateau before flowing into the East China Sea. According to Ye et al. (2011) and Liu et al. (2021), the Yangtze River has diverse climatic conditions and high habitat heterogeneity. It has one of the most diverse areas for fish species in the Palearctic region with 416 fish species, 178 of which are endemic to the Yangtze Basin (Ye et al., 2011). Unfortunately, the fish population in the Yangtze River has significantly declined in recent decades, with over 90 fish species now endangered or seriously threatened. This decline is primarily due to human activities such as overfishing, habitat loss, climate change, and the introduction of invasive alien species (Blanchet et al., 2010; Ye et al., 2011; Liu et al., 2019; Chen et al., 2020; Su et al., 2021). Iconic species, such as *Psephurus gladius* and *Tenualosa reevesii*, are now considered functionally extinct (Zhang et al., 2020a; Zhang et al., 2020b).

The Chinese government implemented several measures to address biodiversity loss in the Yangtze River. These included a seasonal fishing moratorium in the Yangtze River Basin from to 2002–2016 and a one-year fishing ban on 332 aquatic natural reserves in the Yangtze River Basin, including the Chishui River, from 2017 to 2018 (COMA, 2019).

The Chishui River is a first-class tributary of the upper reaches of the Yangtze River, with a total length of 436.5 km and a watershed area of 21,010 km². The main stream and major tributaries remain free of hydraulic facilities, rendering the river close to its natural state. The Chishui River supports a diverse and abundant fish population, with 143 fish species belonging to eight orders and 24 families. Since 2017, the Chishui River has been under a 10-year fishing ban, as mandated by the Ministry of Agriculture and Rural Affairs of the People's Republic of China (2016). As of 2020, this ban has been extended to all major natural water bodies in the Yangtze River, as per the State Council of China (2018). Despite this ban, the fish community in the basin has changed significantly over the past decade (Liu et al., 2021). However, the

dominant drivers of these changes remain unclear. Therefore, it is critical to investigate the factors that have contributed to changes in the fish communities of the Chishui River.

Using traditional methods to conduct systematic survey monitoring during the fishing ban period has been challenging. As a result, the development of new technologies to monitor fish diversity is critical for conservation. Environmental DNA (eDNA) is a non-invasive technology for detecting and identifying species based on DNA released into the environment. Because of its efficiency and speed, it has become an increasingly popular tool for large-scale aquatic biological surveys (Cowart et al., 2020).

There is no consensus on the factors influencing fish diversity and composition. Natural factors such as climate and geography are frequently thought to influence large-scale fish distribution patterns (Collen et al., 2014; Henriques et al., 2017). However, as human societies have developed, human drivers such as overfishing, exotic species introduction, and urbanization have played an increasing role in shaping fish diversity (Xiong et al., 2015; Geist and Hawkins, 2016; Chen et al., 2020). Given the pressures associated with the China's rapid socioeconomic growth and changes in ecosystem environments on a spatial scale, determining the contributions of human and natural factors to fish diversity and community composition is crucial.

In this study, we utilized the eDNA method to assess the fish community composition of the entire Chishui River Basin and analyzed the response of fish diversity to drivers in waters dominated by different drivers. We used fish taxonomic units (OTU) to examine the alpha and beta diversities of fish at 52 sites in the Chishui River and tested the relationship between fish and different environmental and human factors to determine the drivers of fish community characteristics.

MATERIALS AND METHODS

Field sampling

The Chishui River Basin in China is located on the border between the Yunnan and Guizhou Plateau and the Sichuan Basin. Within its distinct geographical context, this area has diverse topography, soil types, various vegetation types, and rich biodiversity. A survey was conducted in September 2021 covering an altitude range from 179–1433 m. A total of 52 sampling points were selected based on the geographical characteristics of the Chishui River Basin and the composition of fish fauna, with the goals of accessibility and maximal coverage of different habitats. The sampling points were distributed throughout five main tributaries along the Erdao, Tongzi, Guling, Datong, and Xishui rivers. The sampling points were divided across three reaches: 14 points in the upstream reach (nos. 1–14), 20 in the middle (nos. 15–34), and 18 in the downstream (nos. 35–52). The sampling point locations are shown in Fig 1.

To obtain accurate data, GPS was utilized to determine altitude, a handheld velocity radio (HZBP, Beijing, China) was used to measure flow velocity, and an infrared rangefinder (Trueyard SP1500H, Nevada, USA) was used to measure river width. A portable chlorophyll sensor (YSI6920V2-2, Ohio, USA) was used to record the chlorophyll a concentration in the water, and a multiparameter water quality analyzer (YSI6920V2-2, Ohio, USA) was used to measure dissolved oxygen (DO), pH, electrical conductivity (EC), and water temperature. The Chishui River Basin survey encompassed a wide range of altitudes and sampling points, providing valuable data on the diverse topography, soil types, vegetation types, and biodiversity within the area.

Chishui River human Footprint (FPT) data were obtained from the human footprint map of the area (https://datadryad.org/stash/dataset/doi:10.5061/dryad.052q5) (Venter et al., 2016). Additional-ly, Chishui River land-cover data from high-resolution (10 m) land-cover maps were obtained from http://data.ess.tsinghua.edu.cn/fromglc10_2017v01.html. Based on the land use types used in a previous fish study (Zhang Q et al., 2022) and considering the characteristics of this study area, we selected three land types; farmland, forest, and impervious surface data were acquired using ArcGIS 10.6. Values were extracted from an area of 1 km radius around each sampling location, and each value was calculated as the proportion of the relevant land cover type to the total terrestrial area, excluding water areas. The land cover values at each sampling site were averaged over three locations. In subsequent analyses, we used generalized additive

DNA extraction and sequencing

A DNeasy Blood & Tissue Kit (Qiagen, Düsseldorf, Germany) was used to extract DNA. Given the low content and concentration of fish DNA in the water, the number of elution cycles was increased from two to four to enhance the concentration of the extracted DNA according to the manufacturer's instructions. To determine the amount of DNA to be added to the PCR, a Qubit3.0 DNA detection kit (Invitrogen, California, USA) was used to accurately quantify genomic DNA. The MiFish PCR primers were as follows: forward: 5'-GTCGGTAAAACTCGTGCCAGC-3' and reverse: 5'-CATAGTGGGGTATCTAATCCCAGTTTG-3'. Each PCR reaction consisted of 15 mL 2x Hieff(r) Robust PCR Master Mix (Yeasen, Shanghai, China), 1 μ L forward Bar-PCR primer, 1 μ L reverse Primer, 10–20 ng PCR product, Illumina bridge PCR-compatible primers, and 9–12 μ L H₂O, for a total volume of 30 μ L. The PCR reaction conditions were as follows: 94 °C for 3 min; 5 cycles at 94 °C for 30 s, 45 °C for 20 s, and 65 °C for 30 s; 27 cycles at 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s; 72 °C for 5 min; and then maintained at 10 °C. A PCR negative control was prepared using ddH2O as the template to assess for potential contamination. Three replicates were performed for each sample during PCR amplification and the PCR products were mixed. PCR products were detected by 2% agarose gel electrophoresis. Shenggong Bioengineering Co., Ltd. (Shanghai, China) spliced the PCR products, and high-throughput sequencing was conducted using the Illumina NovaSeq 6000 (Illumina, California, USA) sequencing platform.

Bioinformatics

After obtaining valid sequences from all samples, the quality of the sequence fragments was evaluated, and fragments < 100 bp in length were discarded. Based on their overlapping relationships, paired fragments were spliced into sequences. Finally, high-quality sequences for each sample were obtained by splitting them based on the barcode and primer sequences, with the sequence direction corrected according to the positive and negative barcodes and primer orientations.

Operational taxonomic unit (OTU) clustering was performed using MitoFish (http://mitofish.aori.utokyo.ac.jp/) and NCBI (https://www.ncbi.nlm.nih.gov/) databases as data sources. To create a custom database, all fish data from the Chishui River were retrieved and downloaded. To ensure data integrity, the scope of the searches was expanded for comparison with the entire NCBI database. After comparison and annotation, the corresponding OTU and species abundance tables were obtained.

Statistical analyses

Statistical analyses were performed based on the results of the OTU clustering and species composition analyses. Sequences with an OTU threshold < 10 were discarded. OTUs with an identity value of [?] 99% and an E-value of [?] 10^{-5} were screened and the OTUs from the same species were combined. If an OTU could not be compared at the species level, statistics were created at the next level, such as genus or family. Microsoft Excel was used to determine the proportion of valid sequences for each fish sample.

The Shannon-Wiener diversity index (H), Pielou uniformity index (J), richness index, and Simpson Dominance Index (D) were used to assess community diversity. To explore the differences or similarities in community composition between different groups of samples, the number of OTUs was used to calculate the Bray-Curtis distance measure matrix for nonmetric multidimensional scaling (NMDS), permutational multivariate analysis of variance (PerMANOVA), and analysis of similarities (ANOSIM). Additionally, the relationship between environmental factors and community composition was investigated using canonical correspondence analysis (CCA). Pearson correlation analyses were used to investigate the influence of environmental and human factors on the species richness index.

All analyses were conducted using R v2.5.6, with the vegan package used for NMDS, PerMANOVA, ANOSIM, and diversity index calculations. The Pacman package was used for the Pearson correlation analysis of environmental factors, whereas diversity index boxplots were generated using the Amplicon package. ANOVA

was performed using Perim (v9.1.0) software. The GAMs were implemented using the function "gam" with the default settings in the R package "mgcv."

RESULTS

Fish composition in the Chishui River

A total of 96,031 valid fish sequences were read, resulting in the identification of 77 species from six orders, 62 genera, and 18 families (Table 1 and Fig 2a). The dominant orders were Cypriniformes, Siluriformes, and Perciformes, accounting for 87.87%, 8.23%, and 3.44% of the total sequence abundance, respectively. Cyprinidae and Botiidae were the dominant families, representing 71% and 13.52% of the total sequence abundance, respectively, while Bagridae accounted for 7.34% of the total sequence abundance.

The upstream fish consisted of 38 species belonging to 33 genera in 14 families from two orders. Mesomigratory fish included 45 species from 36 genera in 10 families from five orders. Downstream fish consisted of 54 species from 45 genera in 15 families from seven orders (Fig 2a and b, and Fig 3a– c). An increasing gradient was observed from upstream to downstream. Notably, 17 species were found in the upper, middle, and lower reaches: *Pseudorasbora parva*, *Xenocypris davidi*, *Schizothorax prenanti*, *Zacco platypus*, *Sinogastromyzon sichangensis*, *Misgurnus anguillicaudatus*, *Carassius auratus*, *Ctenopharyngodon idella*, *Homatula potanini*, *Rhinogobius giurinus*, *Platysmacheilus exiguous*, *Pseudobagrus ussuriensis*, *Rhodeus ocellatus*, *Rhodeus sinensis*, *Gambusia affinis*, *Acrossocheilus monticola*, and *Hemiculterella sauvagei*.

The Shannon-Wiener index of the Chishui River differed significantly between upstream and downstream regions (Fig 4a), with the downstream index being higher than the upstream index (P < 0.05). However, there was no statistically significant difference in the richness index. Community composition also varied between the upstream and downstream regions (PerMANOVA=0.86, P < 0.05; Fig 4b), but the difference between the middle and adjacent reaches was not significant.

A variance analysis of the diversity index and community analyses of the main streams and tributaries was also conducted. There were no significant differences between the groups. We further analyzed the differences in diversity in the upper, middle, and downstream tributaries. The Shannon-Wiener and richness indices of the upstream tributaries were significantly higher than those of the downstream tributaries (P < 0.05; Fig 5a). We also observed significant differences in the community composition between the upstream and downstream tributaries (PerMANOVA=0.72, P < 0.05; Fig 5b). The midstream tributaries show partial similarities to both the upstream and downstream tributaries.

We also explored the specific diversity and community differences among the identified tributaries by conducting a grouping ANOVA and community analysis. The results showed that the richness index of the Datong River was significantly higher than that of the other tributaries (P < 0.05; Fig 6a), indicating that it had the highest level of species diversity among all tributaries. Fish community analysis showed no significant differences between the secondary and fifth tributaries (Fig. 6b).

Fish diversity responds to environmental variables and anthropogenic activities

The CCA results showed that the explanatory variance was 4.06% (P = 0.001) for the first axis and 3.1% (P = 0.004) for the second axis. These results indicate that the first axis significantly separated the environmental variables from different groups based on fish communities (Fig. 7). The ranking results revealed that altitude, river width, dissolved oxygen, flow rate, and pH had substantial effects on community composition.

Finally, we conducted a Pearson analysis of the environmental variables and species richness index. The results showed a significant positive correlation between water temperature (TE) and fish richness (P = 0.027; Fig. 8). However, there was a significant negative correlation between altitude (ASL), electrical conductivity (EC), and fish species richness (P = 0.0018 and P = 0.0081, respectively).

Analysis of the data using a generalized additive model revealed that the impact of FPT and land use on fish species richness was negligible. However, it is important to mention that the percentage of impervious area, which is commonly used as an indicator of urbanization, had some effect on fish species richness. Specifically,

fish species richness was higher at lower percentages of impervious area, but the increase was not statistically significant (Fig. 9).

DISCUSSION

Nondestructive technical monitoring and investigations are gaining increasing attention (Weigand et al., 2019; Tsuji et al., 2020). This is especially important in watersheds with a high concentration of rare fish species (Chen et al., 2020; Liu et al., 2020), where disturbance and damage to fish communities must be considered (Su et al., 2021), and high costs may be incurred (Zhang et al., 2022). The Chishui River Basin has implemented a 10-year fishing ban that has been in place for four years (Yu et al., 2022). Although fish diversity monitoring studies in the basin have continued (Liu et al., 2019; Liu et al., 2022), Liu et al., 2021; Yu et al., 2022), traditional survey methods have been used (Liu et al., 2021), and the impact of human activities on fish after the ban has not been assessed (Yu et al., 2022). Because fish diversity data are only available through a single method, fish conservation efforts in the Chishui River Basin may be limited. To address this issue, we utilized the eDNA method to investigate the fish diversity in the Chishui River Basin. Our findings were consistent with traditional survey methods, and we demonstrated that human activities in the basin have a lower impact on fish. This study highlights the potential benefits of using nondestructive monitoring methods for fish conservation and biodiversity surveys, particularly in areas with rare fish species and high levels of human activity.

Species composition of fish in the Chishui River

In this study, we conducted a comparative analysis of 77 fish species, accounting for 54% of the species studied (Liu et al., 2021). Of the 77 species, 72 were known fish species, including seven medium exotic fish species and five new fish species. Our findings revealed that Cypriniformes, Siluriformes, and Perciformes were the dominant orders (Figure 1b), which is consistent with the results of previous studies (Liu et al., 2021). Additionally, our results indicated that Cyprinidae, Balitoridae, and Bagridae were the dominant families in the Chishui River, which is consistent with earlier studies (Liu et al., 2020; Liu et al., 2021).

The five new fish species in the Chishui River include: Acipenser sinensis, Schizothorax prenanti, Euchiloglanis davidi, Rhynchocypris lagowskii, and Cirrhinus molitorella .A. sinensis is a migratory fish (Boscari et al., 2022) that was once commonly found in the Yangtze River Basin. In the present study, we detected 142 OTUs of A. sinensis near a river estuary. Although Chinese sturgeons are known to be released and bred in the upper reaches of the Yangtze River, the presence of A. sinensis in the Chishui River is likely due to human influence rather than a wild population (Yang et al., 2023).

S. prenanti , another new fish species found in the Chishui River, is typically distributed in the upper reaches of the Yangtze, Guizhou, and Sichuan Rivers. We believe that its presence in the Chishui River is due to its occurrence in the surrounding areas. E. davidi , R. lagowskii , and C. molitorella were also identified. These fish species were confirmed through interviews with local fish experts as individual catches were reported in the area.

Notably, the eDNA technology used in this study to detect fish species can only identify species sequences. Thus, although the presence of these fish species in the Chishui River was confirmed, further research is needed to determine their abundance, distribution, and potential impacts on the ecosystem.

Seven alien species were identified: Ictalurus punctatus ,Clarias gariepinus , Gambusia affinis , Rhynchocypris oxycephalus , Barbatula nuda , Cirrhinus molitorella , andMicropterus salmoides . Of these species, R. oxycephalusand B. nuda have demonstrated strong adaptability to the Chishui River environment, establishing wild populations and occupying certain ecological niches. However, this could threaten the ecological balance of the river (Tang et al., 2021).

I. punctatus is a fast-growing carnivorous fish with a broad range of food habits that primarily feeds on aquatic insects and small fish. Under certain conditions, it may pose a greater threat to rare juveniles or other fish species. Similarly, *M. salmoides* and *C. gariepinus*, both carnivorous fish with strong environmental adaptability, have the potential to prey on the juveniles of local fish species and force indigenous species to

switch habitats through interspecific competition (Zhang et al., 2022; Zhao et al., 2020). This ultimately affects the distribution of these species (Li et al., 2016).

The omnivorous G. affinis has been reported to consume a large number of eggs and juveniles of local fish species in invaded areas, leading to a significant decline in their populations (Goodsell and Kats, 1999).

Comparison of different spatial fish communities

Noticeable differences were observed in the fish species composition between the upper and lower reaches of the Chishui River Basin, as shown in Figure 2(b). However, no significant differences were observed between upstream and downstream reach. Nonetheless, there were significant differences between the upstream and downstream tributaries, indicating that disparities between the upstream and downstream tributaries could be attributed to the fish communities in the tributaries. Studies have revealed that fish diversity is associated with river area (Oberdorff et al., 2019) and habitat heterogeneity (Hugueny et al., 2010). Therefore, habitat heterogeneity may be a more influential factor for community differences. Furthermore, in the comparison of transverse gradients, it was discovered that the DaTong River exhibited the highest level of α diversity among all tributaries. This finding differs from the results of traditional survey studies conducted at the same position on the Xishui River (Liu et al. 2020). We believe that the topography and hydrological conditions of the DaTong River tend to impede traditional surveys, leading to an underestimation of tributary diversity. Conversely, the gentle flow speed and easy accessibility of the Xishui River facilitated a tributary survey which was more comprehensive. Therefore, the status of the DaTong River should be elevated to the same level as that of the Xishui River.

Fish diversity responds to environmental variables and anthropogenic activities

In this study, we found that changes in distribution and diversity in the Chishui River Basin were predominantly influenced by environmental factors. The results of our CCA showed that altitude had a significant impact on upstream fish richness, resulting in a significant decrease in fish species richness with increasing altitude. Similarly, conductivity significantly affected downstream fish richness (Chen et al., 2020). Our findings demonstrated a significant decrease in fish species richness with increasing conductivity. Previous studies have highlighted the importance of these two factors in affecting fish species richness. The altitude difference between the upper and lower reaches of the Chishui River can reach 1180 m, leading to changes in water temperature and flow rate. These changes resulted in noticeable differences in upstream, middle, and downstream fish habitats, leading to variations in fish species composition. For instance, *Sinocrossocheilus labiatus* other fish adapted to cold-water environments were only found in the upstream basin in this study, leading to specific fish species upstream that exhibited significant differences from those downstream.

Human activity in the downstream basin is more intensive than in the upstream reaches, resulting in increased water pollution and impurities. As a result, most fish are intolerant of this environment (Bertora et al., 2022), leading to a decrease in fish species. However, tolerant fish species, such as *Zacco platypus*, have shown an increase in population numbers (Yeom et al. 2007; Morosawa 2011). In summary, our research showed that environmental factors such as altitude and conductivity play a significant role in the distribution and diversity of fish species in the Chishui River Basin.

This study found that the impact of anthropogenic activities, specifically the use of fertilizers, pesticides, and land, on the study area was not significant. Based on our analysis, we believe that this result can only be considered valid under the assumption that the intensity of anthropogenic activity in the Chishui River Basin has not yet reached the threshold affecting fish diversity. Additionally, human influencing factors, such as fishing intensity, disappeared after the implementation of the fishing ban. This policy has led to fish in the Chishui River Basin being primarily affected by environmental factors, which is similar to the natural succession stage and conducive to the community reconstruction process (Liu et al. 2021). In summary, the results of this study suggest that the current environmental conditions in the Chishui River Basin are favorable for community reconstruction.

Fish survey limitations based on eDNA methods

Although the eDNA method has had a significant impact on the field of ecology, it faces some limitations as a developing science (Goldberg et al., 2016). One area of contention is whether the number of sequences obtained through eDNA analysis can be used as a proxy for the number of species identified in traditional surveys (Deiner et al., 2016; Zhang et al., 2022). This is a crucial consideration because the accuracy of the eDNA data relies on this assumption.

Another challenge is the reliability and accuracy of the species comparison databases used in eDNA studies. The credibility of the new records generated using this method is directly related to the quality of the database. However, the eDNA method alone cannot verify the accuracy of this database, which can affect the scientific rigor and reliability of the results.

Finally, there is the issue of whether eDNA results accurately reflect the fish species present at the sample site. DNA can originate from organisms located several kilometers away, which can lead to erroneous results (Deiner et al., 2016). This raises concerns about the representativeness of the eDNA data and underscores the importance of careful sampling.

CONCLUSIONS

Aquatic biodiversity surveys are increasingly adopting fast and nondestructive eDNA methods. This study aimed to demonstrate the diversity and endemicity of fish in the Chishui River by identifying 77 fish species at 52 sites. Fish assemblages in the river displayed significant variation along its longitudinal gradient, with notable differences between the upstream and downstream areas. The driving factors behind this phenomenon were analyzed, indicating that the flow rate and altitude were the most significant environmental factors. In contrast, human factors did not significantly influence the distribution of fish species. Overall, this study highlights the feasibility of using eDNA methods to investigate the Chishui River Basin and provides valuable reference data for the management and protection of fish resources in the upper reaches of the Yangtze River.

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Data Accessibility and Benefit Sharing

Raw sequence data and information on nucleotide tagging have been deposited the NCBI repository, Accession number is PRJNA999606.

Conflict of Interest

The authors declare no competing interests.

Author Contributions

N.N.G.:Conceptualization;Methodology;Formal analysis;Investigation;Resources;Data Curation;Writing— Original Draft;Visualization.N.W.X.: Conceptualization;Supervision; Writing—Review and Editing; Project administration;Funding acquisition.X.Q.G.: Investigation;Resources; Data Curation.M.S.: Investigation;Resources; Data Curation.X.X.S.: Investigation;Resources.J.S.L.: Project administration. All authors contributed critically to the drafts and gave final approval for publication.

FIGURES



Figure 1. Fish sampling locations in the Chishui River Basin.



Figure 2. Fish composition in the upper, middle, and lower reaches of the Chishui River Basin. (a) The number of fish species in different river segments. (b) The composition of fish families in different river segments.



Figure 3. Composition of fish genera in the (a) upstream, (b) middle, and (c) downstream river sections.



Figure 4. Differences in fish communities and comparison of biodiversity in different reaches of the Chishui River. (a) Biodiversity indices of the upper (U), middle (M), and downstream (D) regions. (b) Differences in fish communities in the Chishui River Basin.



Figure 5. Differences in fish communities and biodiversity comparison among tributaries of different sections in the Chishui River Basin. (a) Tributary biodiversity index. (b) Difference in fish communities in the tributaries of the Chishui River Basin. UT: Upstream tributaries, MT: Midstream tributaries, DT: Downstream tributaries.



Figure 6. Differences in fish communities and biodiversity among the major and small. (a) The biodiversity indices between groups. (b) Fish community differences between tributaries.



Figure 7. CCA exploring the relationship between species and measured environmental variables.



Figure 8. Correlation between fish richness and environmental factors in the Chishui River Basin.



Figure 9. GAMs showing the relationship between the proportion of different land use types (farmland, forest, and impervious surface) and fish abundance in the Chishui River Basin.

Species	Feeding habits	Feeding habits	Feeding habits	Habit chara
Acipenseriformes				
鲟科Acipenseridae				
1. Acipenser sinensis	carnivorous	carnivorous	bottom	bottom
Cypriniformes				
Cyprinidae				
2. Opsariichthys bidens	carnivorous	carnivorous	middle and upper class	middle and
3.Zacco platypus	omnivorous	omnivorous	middle and lower class	middle and
4. Ctenopharyngodon idella	herbivorous	herbivorous	middle and lower class	middle and
5. Rhynchocypris lagowskii	herbivorous	middle and upper class	middle and upper class	middle and
6. Rhynchocypris oxycephalus*	carnivorous	upper class	upper class	upper class
7. Chanodichthys dabryi dabryi	carnivorous	middle and upper class	middle and upper class	middle and
8. Culter alburnus	carnivorous	middle and upper class	middle and upper class	middle and
9. Hemiculter tchangi	omnivorous	middle and lower class	middle and lower class	middle and
$10. Hemiculterella\ sauvagei$	omnivorous	middle and upper class	middle and upper class	middle and
$11.Megalobrama \ pellegrini$	omnivorous	middle and lower class	middle and lower class	middle and
12. Megalobrama skolkovii	omnivorous	middle and lower class	middle and lower class	middle and
13.Pseudobrama simoni	herbivorous	middle and lower class	middle and lower class	middle and

Species	Feeding habits	Feeding habits	Feeding habits	Habit chara
14.Xenocypris davidi	omnivorous	middle and lower class	middle and lower class	middle and
15. Xenocypris microlepis	herbivorous	middle and lower class	middle and lower class	middle and
16. Aristichthys nobilis	planktonic	middle and upper class	middle and upper class	middle and
17. Hypophthalmichthys molitrix	planktonic	upper class	upper class	upper class
18. Rhodeus ocellatus	herbivorous	middle and upper class	middle and upper class	middle and
19. Rhodeus sinensis	herbivorous	middle and upper class	middle and upper class	middle and
20. Abbottina rivularis	omnivorous	bottom	bottom	bottom
21. Coreius heterodon	omnivorous	bottom	bottom	bottom
22. Hemibarbus labeo	benthic	middle and lower class	middle and lower class	middle and
23. Hemibarbus maculates	benthic	middle and lower class	middle and lower class	middle and
24. Platysmacheilus exiguous	omnivorous	bottom	bottom	bottom
25. Platysmacheilus nudiventris	carnivorous	middle and lower class	middle and lower class	middle and
26.Pseudorasbora parva	omnivorous	upper class	upper class	upper class
27. Rhinogobio typus	omnivorous	bottom	bottom	bottom
28. Rhinogobio cylindricus	omnivorous	bottom	bottom	bottom
29.Saurogobio dabryi dabryi	omnivorous	bottom	bottom	bottom
$30. Squalidus \ argentatus$	omnivorous	bottom	bottom	bottom
31. Carassius auratus	omnivorous	middle class	middle class	middle clas
32. Cyprinus carpio	omnivorous	bottom	bottom	bottom
33. Procypris rabaudi	omnivorous	bottom	bottom	bottom
34. Acrossocheilus monticola	omnivorous	upper class	upper class	upper class
35. Acrossocheilus yunnanensis	omnivorous	bottom	bottom	bottom
36.Percocypris pingi	carnivorous	middle and upper class	middle and upper class	middle and
37. Spinibarbus sinensis	omnivorous	middle and upper class	middle and upper class	middle and
38.Bangana rendahli	herbivorous	bottom	bottom	bottom
39. Cirrhinus molitorella*	herbivorous	middle and upper class	middle and upper class	middle and
40. Garra imberba	omnivorous	bottom	bottom	bottom
41. Pseudogyrinocheilus procheilus	herbivorous	bottom	bottom	bottom
42. Sinocrosssocheilus labiatus	herbivorous	bottom	bottom	bottom
43. Schizothorax kozlovi	omnivorous	bottom	bottom	bottom
$44. Schizothorax \ prenanti$	omnivorous	bottom	bottom	bottom
Noemacheilidae				
45.Barbatula nuda*	omnivorous	bottom	bottom	bottom
46.Homatula potanini	carnivorous	bottom	bottom	bottom
47. Homatula variegatus	carnivorous	bottom	bottom	bottom
Cobitidae				
$48. Misgurnus \ anguillicau datus$	omnivorous	bottom	bottom	bottom
$49. Paramisgurnus\ dabryanus$	omnivorous	bottom	bottom	bottom
Botiidae				_
50.Botia superciliaris	carnivorous	bottom	bottom	bottom
Balitoridae			-	_
51. Lepturichthys fimbriata	omnivorous	bottom	bottom	bottom
52. Metahomaloptera omeiensis omeiensis	omnivorous	bottom	bottom	bottom
53. Sinogastromyzon sichangensis	herbivorous	bottom	bottom	bottom
54. Sinogastromyzon szechuanensis	herbivorous	bottom	bottom	bottom
Siluriformes				
Amblycipitidae		•	•	
55.Liobagrus marginatus	carnivorous	bottom	bottom	bottom
Sisoridae		1	1	
56. Euchiloglanis davidi	omnivorous	bottom	bottom	bottom

Species	Feeding habits	Feeding habits	Feeding habits	Habit chara
57. Glyptothorax sinensis	carnivorous	bottom	bottom	bottom
Siluridae				
58. Silurus meridionalis	carnivorous	bottom	bottom	bottom
59. Silurus asotus	carnivorous	bottom	bottom	bottom
Clariidae				
60. Clarias gariepinus*	omnivorous	bottom	bottom	bottom
Bagridae				
61. Hemibagrus macropterus	omnivorous	bottom	bottom	bottom
62. Leiocassis crassilabris	carnivorous	bottom	bottom	bottom
63. Leiocassis longirostris	carnivorous	bottom	bottom	bottom
64. Pelteobagrus eupogon	omnivorous	bottom	bottom	bottom
65.Pelteobagrus fulvidraco	omnivorous	bottom	bottom	bottom
66.Pelteobagrus vachellii	carnivorous	bottom	bottom	bottom
67. Pseudobagrus brevicaudatus	carnivorous	bottom	bottom	bottom
68. Pseudobagrus truncatus	omnivorous	bottom	bottom	bottom
69. Pseudobagrus ussuriensis	carnivorous	bottom	bottom	bottom
Ictaluridae				
70. Ictalurus punctatus*	carnivorous	bottom	bottom	bottom
Synbranchiformes				
Synbranchidae				
71. Monopterus albus	carnivorous	bottom	bottom	bottom
Perciformes				
Percichthyidae				
72. Siniperca chuatsi	carnivorous	bottom	bottom	bottom
Odontobutidae				
73. Odontobutis potamophila	carnivorous	upper class	upper class	upper class
Gobiidae				
74. Rhinogobius cliffordpopei	carnivorous	bottom	bottom	bottom
75. Rhinogobius giurinus	carnivorous	bottom	bottom	bottom
Centrarchidae				
76. Micropterus salmoides*	carnivorous	middle and lower class	middle and lower class	middle and
Cyprinodontiformes				
Poeciliidae				
77. Gambusia affinis*	omnivorous	upper class	upper class	upper class

* for exotic species and for fish endemic to the upper reaches of the Yangtze River.