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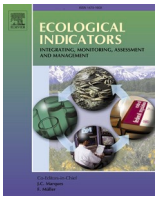

Unraveling fish diversity and assembly patterns in a temperate river: Evidence from environmental DNA metabarcoding and morphological data

Yue Shi [a], Shuping Wang [a], Xiaolong Lin [b], Hong Li [c,d], Aopu Li [a], Juntao Fan [a,*]

[a] State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China
[b] College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China
[c] Lancaster Environment Centre, Lancaster University, LA1 4YQ, UK
[d] UK Centre for Ecology & Hydrology, MacLean Building, Wallingford OX10 8BB, UK

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ABSTRACT

The loss of freshwater biodiversity has drawn greater attention to fish diversity and community assembly patterns. However, traditional survey methods, such as ground cages and electrofishing, may cause damage to fish communities and have become increasingly unsuitable for frequent and large-scale fish diversity surveys. In this study, environmental DNA (eDNA) metabarcoding and morphological data were both used to investigate the distribution and diversity of fish communities in the Taizi River basin, a temperate river in Northeast China, and the fish diversity and assembly patterns were investigated by using the null model. The results showed that a total of 7 orders, 17 families, 49 genera and 56 species were detected by the eDNA metabarcoding (6 orders, 15 families, 40 genera and 45 species) and morphological method (6 orders, 10 families, 31 genera and 34 species), and the eDNA method had a higher detection probability. Cypriniformes was detected most frequently, followed by Perciformes. Principal coordinate analysis revealed that fish communities in the Taizi River exhibited different spatial structures between the upper and lower reaches, with fish sensitive to environmental changes mostly found in the upper reaches and higher fish richness in the same area. The beta diversity in the upper reaches was higher than that in the lower reaches. The null model results showed the main factor that affected the distribution of fish in the Taizi River is the stochastic processes. Among the deterministic processes, the main environmental filter factors affecting fish community structure include total phosphorus, biochemical oxygen demand and temperature. We also confirm that eDNA metabarcoding has a higher detection rate compared to traditional survey methods, so it is feasible for freshwater ecosystem and fish resource monitoring. Therefore, the utilization of eDNA metabarcoding can effectively enhance monitoring efficiency and minimize interference with water bodies.

1. Introduction

Accelerating environmental change has led to unprecedented biodiversity loss across freshwater ecosystems. The monitoring and research of freshwater ecosystems and their biodiversity has become a hot topic in the field of ecology (Pereira et al., 2013). As the apex predators in the aquatic ecosystem, fish have a broad distribution and play a crucial role in the existence and diversity of other aquatic organisms. Freshwater fish make up one-fifth of the world's vertebrates and provide irreplaceable ecological functions and services. The study showed that more than 50 percent of the fish fauna in 2456 rivers around the world have been strongly influenced by human activities, noting that no river has been untouched by human impacts, with temperate rivers being the most affected (Su et al., 2021). Therefore, fish community structure and diversity are of great scientific significance for assessing the health of temperate river ecosystems (Zou et al., 2020).

The Taizi River, situated in the east of Liaoning Province, is a significant tributary of the Liao River and characterized by temperate climate. The Taizi River basin has a typical temperate continental monsoon climate. As the industrial and agricultural production base of Liaoning Province, Taizi River basin has become the most serious water shortage area in Liaoning province due to the double pressure of economic development and high population density. Because of its particularity, the investigation of fish diversity and analysis of community

* Corresponding author.
E-mail address: fanjt@craes.org.cn (J. Fan).

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assembly can provide scientific reference for the distribution and conservation of fish resources in other temperate rivers.

In the past, the principal techniques employed to investigate river biodiversity were physical identification methods, including morphology and acoustics (Cornell and Lawton, 1992; Hutchinson, 1959). However, due to their time-intensive and laborious nature, as well as the strict restrictions of fish conservation policies and measures in recent years. Their significant reliance on specialized expertise, these methods have progressively fallen short of meeting the demands of fish diversity research. And the fish species diversity obtained via morphological identification has often been underestimated (Snyder, 2003). With the development of molecular biology technology, based on the differences of specific gene sequences of different species, environmental DNA (eDNA) metabarcoding which takes short DNA sequences as taxa database to realize rapid and accurate species identification has been widely recognized and developed (Sanchez et al., 2022; Shan et al., 2020; Shaw et al., 2016). Compared with traditional methods, eDNA metabarcoding has higher sensitivity and accuracy (Afzali et al., 2020; Michael et al., 2019) and has been used in investigating the distribution of fish (Coutant et al., 2023; Evans et al., 2016; Laporte et al., 2022). But at present, there is no report of fish diversity survey in Taizi River basin by using eDNA metabarcoding. In most previous studies, morphological methods were used to investigate fish diversity in the Taizi River basin. In the previous fish investigation of Taizi River, the dominant species were *Rhynchocypris lagowskii*, *Zacco platypus*, *Nemacheilus toni* and *Abbottina rivularis* (Chong et al., 2022; Li et al., 2017; Wang et al., 2013a).

Fish community structure is affected by many environmental factors, such as river water quality, habitat quality, land use type and hydrological conditions, and is closely related to the ecological environment of the water area and riparian zone (Espínola et al., 2020; Wexler et al., 2011; Wright and Flecker, 2004). Traditionally, both stochastic and deterministic processes influence fish community structure modeling. Assuming that fish have the same adaptations, stochastic processes emphasize the importance of stochastic demographic processes and ecological drift in explaining patterns of species coexistence patterns (Matthews and Whittaker, 2014). By contrast, deterministic process typically involve non-random, niche-based ecological processes, including environmental filtering and mechanisms of various biological interactions mechanisms such as predation, parasitism, symbiosis, cooperation and competition (Cornell and Lawton, 1992; Hutchinson, 1959). The null model has its limitations, but by using the null model, we can understand whether deterministic or stochastic processes dominate changes between communities, allowing for a more comprehensive understanding of the composition and assembly patterns of fish communities (Dini-Andreote et al., 2015). Giam and Olden provide compelling evidence for non-random structure in most temperate stream fish communities and community assembly is governed largely by environmental filtering (Giam and Olden, 2016). And previous studies have shown that patterns of fish community assembly vary greatly in different aquatic ecosystems. Therefore, it is necessary to clarify whether fish assemblage in Taizi River is governed by stochastic or deterministic processes and to identify environmental factors that influence fish community diversity. So as to provide basic data and scientific basis for watershed ecological management decisions making (Fan et al., 2020; Wexler et al., 2011).

In this study, the eDNA metabarcoding technology and morphological method were used to monitor fish diversity and explore the assembly mechanism of fish community in Taizi River. The Alpha diversity index was calculated to evaluate the fish diversity in the basin, and the beta diversity of eDNA metabarcoding and morphological identification data was analyzed by principal coordinate analysis. The null model was used to study the assembly pattern of fish community in Taizi River. We discussed whether the fish communities in temperate rivers are dominated by stochastic or deterministic processes. Through redundancy analysis, the relationship between fish community and environmental filter factors was analyzed, and the influence of deterministic process on fish community structure was evaluated.

2. Materials and methods

2.1. Study area

Taizi River (122°23′E – 122°53′E, 40°28′N – 41°39′N) is the largest river in Benxi city, Liaoning Province, China. As shown in Fig. 1, the main land use types in the upper reaches are woodland and grassland. Under the influence of urbanization, the land use type downstream are dominated by urban land (Fan et al., 2018).

2.2. Ecological and environmental data collection

In October 2018, we sampled a total of 24 sampling sites, including 13 sampling sites in the mainstream and 11 sampling sites in the tributaries. The numbering and distribution of each sampling point are shown in Fig. 1. Considering the filtering time and transportation convenience, and referring to the sampling volume in previous studies of temperate rivers, we finally select a sampling volume of 1 L (Lacoursiere-Roussel et al., 2016; Shu et al., 2022). Surface water along the riverbank was collected for eDNA metabarcoding analysis by using sterile 1 L bottles at each site. The number of parallel samples was set to 3 to ensure the detection probability. 72 samples were collected from 24 sampling sites and all samples were filtered using 0.22 μm Millipore hydrophilic nylon membranes within 6 h. The membrane disc containing eDNA in water was placed in a 5 mL centrifuge tube, immediately frozen and stored at −20℃ until DNA extraction.

While collecting water samples, portable water quality analyzer was used to collect temperature, conductivity, pH, dissolved oxygen (DO) and other data at each station site. Morphological data were collected mainly by electrofishing method, and species information was identified after sample collection. The range of electrofishing area is 200 m upstream and downstream of the sampling point, a total of 400 m, and the duration is 30 min. The morphological identification was mainly by the researchers based on “Zoology of China” and “Zoology of Liaoning Province: Fishes”.

2.3. DNA extraction, polymerase chain reaction (PCR) amplification and high-throughput sequencing

DNeasy Blood & Tissue Kit was used to extract DNA from the membrane and all operations are carried out in the ultra-clean workbench.

The fish universal primers MiFish-U-F: 5 ’-GTCGGTAAAACTCGTGCCAGC-3′ and MiFish-U-R: 5 ’-CATAGTGGGGTATCTAATCCCAGTTTG-3′ was used for PCR amplification of environmental DNA samples (Miya et al., 2015). The amplification region of this primer was 12S rRNA, and the amplified DNA sequence length was about 163–185 bp (Wang et al., 2021a).

The PCR reaction procedure was: predenaturation at 95℃ for 3 min, denaturation at 94℃ for 20 s, annealing at 55℃ for 20 s, extension at 72℃ for 30 s, and finally extension at 72℃ for 5 min. The PCR product was diluted 10 times with deionized water as the template for the second amplification. Barcode sequence of 12 bases was added to the 5 ’end of the original primer as the primer for the second PCR amplification, and the other components remained unchanged. The two PCR amplification procedures remained unchanged. After two-step PCR amplification, PCR products were detected in 2 % agarose gel and no amplification was shown in filter blank or negative control. After amplification, the samples were purified by 2 % agarose gel electrophoresis and then double-ended sequencing was performed on Illumina Miseq sequencing platform (Sangon Biotech (Shanghai) Co., Ltd.).

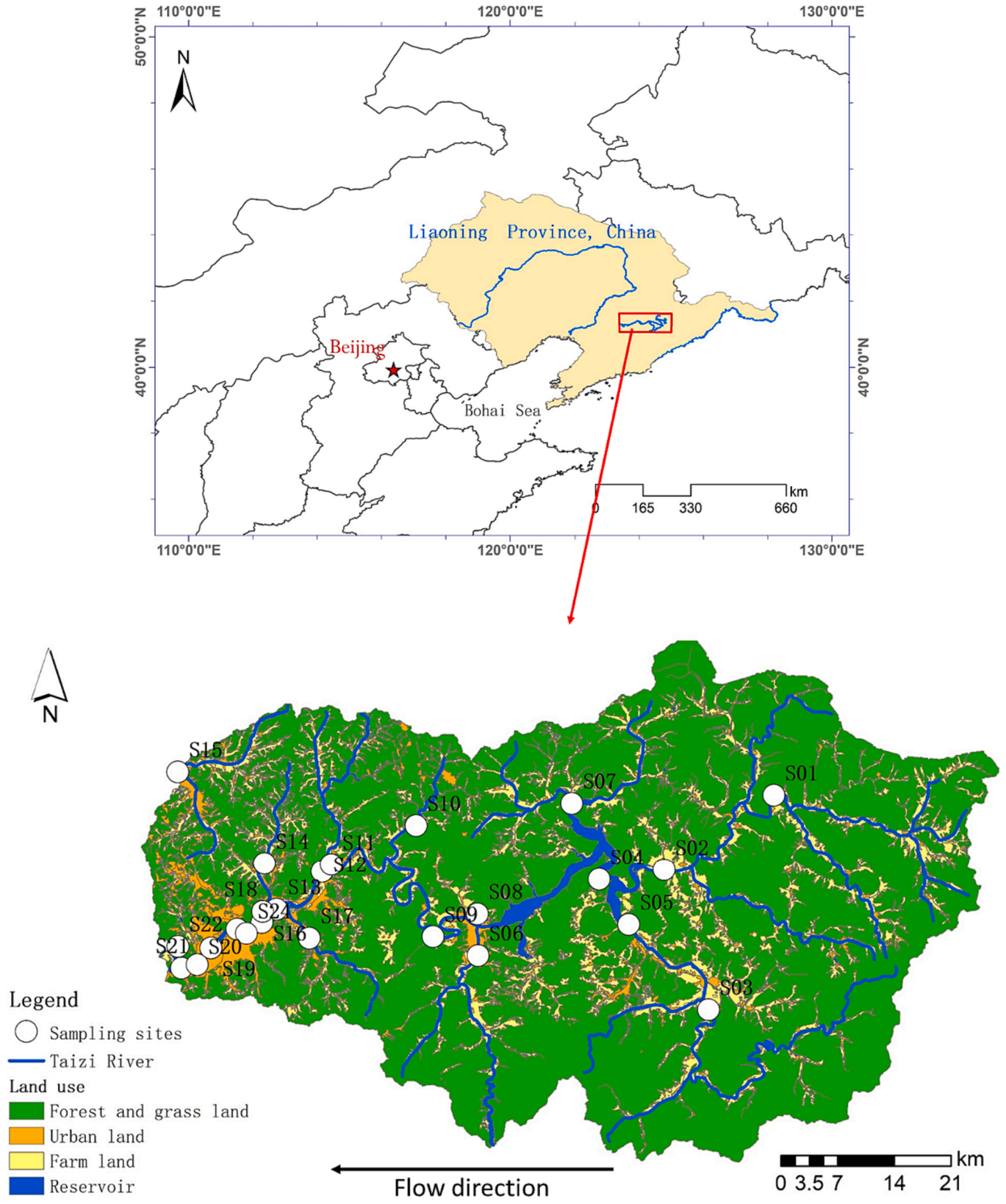

Fig. 1. Distribution map of 24 sampling sites and different land use types in Taizi River Basin.

2.4. Bioinformatics analysis and statistical analysis

The sequences were processed by method of DADA2, including Primers, quality filtering, splicing and chimerism were removed. The remaining sequences were grouped according to ≥ 97 % by QIIME2 software. The clustering result was called Operational Taxonomic Units (OTUs). The Brocc annotation algorithm was used to conduct species taxonomy annotation for the obtained OTUs sequences. The annotation

results were manually reviewed to eliminate non-fish information, and OTUs with an identity value of $\geq$ 97 % and a value of $\leq 10^{-5}$ were selected for comparison with fish. The OTUs that had the same species identification result were merged. The obtained OTUs were sequentially compared with Mitofish database (Wataru et al., 2013) and GenBank database (W et al., 2022).

The relative abundance of species sequences is calculated from the number of species and sequences at different taxonomic levels. Subsequent analysis was conducted based on the species sequence abundance of each sampling point. The Shannon index was calculated using the vegan and ggplot2 packages in the R software and was used to compare the Alpha diversity of the two methods in the test area (Lagkouvardos et al., 2017). In order to compare spatial distribution differences of fish communities in detection of various sampling sites, the vegan package of R software is used to perform Principal coordinates analysis (PCoA) based on Bray-Curtis distance.

To characterize the phylogenetic community composition within each sampling site, beta nearest taxonomic index (βNTI) and Raup-Crick Matrix (RC_{bray}) were used to establish the mechanisms of change or transformation of phylogenetic and taxonomic diversity (Ni et al., 2021). RC_{bray} can provide some information on the degree to which pairwise communities are more different (or more similar) than expected by chance (Chase et al., 2011; Stegen et al., 2015). βNTI is used to indicate the extent to which changes between two communities are affected by deterministic or stochastic factors. βNTI can be calculated through R software's picante package. RC_{bray} is calculated from the Bray-Curtis matrix when $|\beta NTI|<2$. RC_{bray} values range from −1 to 1. βNTI greater than 2 indicates coexisting taxa are more closely related than expected by chance (phylogenetic clustering), which is interpreted as a variable selection of deterministic processes. βNTI less than −2 indicates coexisting taxa are more distantly related than expected by chance (phylogenetic overdispersion), which is interpreted as a homogeneous selection of deterministic processes (Stegen et al., 2012). If βNTI is between −2 and 2, it suggests that the observed differences in phylogenetic composition are the result of stochastic processes. $|\beta NTI| < 2$ means that the replacement of a group of community by dispersal limitation, homogenizing dispersal and undominated control of the processes. $|\beta NTI| < 2$ and $RC_{bray} < 0.95$ can be interpreted as being influenced by undominated processes, including weak selection, weak dispersal, diversification and drift. $RC_{bray} > 0.95$ indicates that the community turnover is mainly influenced by dispersal limitation, while $RC_{bray} < -0.95$ indicates that the community composition is mainly controlled by homogenizing dispersal (Dini-Andreote et al., 2015). The formula for calculating βNTI is as follows:

$$\beta NTI = (\beta MNTD_{obs} - \beta MNTD_{null}) / sd(\beta MNTD_{null}),$$

$$\beta MNTD = 0.5 \left[\sum_{ik=1}^{nk} f_{ik} \min(\Delta_{ikjm}) + \sum_{im=1}^{nm} f_{im} \min(\Delta_{imjk})\right], \quad \text{where}$$

$\beta MNTD_{obs}$ is observed βMNTD, $\beta MNTD_{null}$ are null values of βMNTD, and sd indicates the standard deviation of the $\beta MNTD_{null}$ distribution. And f_{ik} is the relative abundance of OTU i in community k, n_k is the number of OTUs in k, min (Δ_{ikjm}) is the minimum phylogenetic distance between OTU i in community k and all OTUs j in community m min ($\Delta_{im\,jk}$). We quantified βNTI for all pairwise comparisons, using a separate null model for each comparison.

To understand the relationship between fish communities and environmental factors. Detrended correspondence analysis (DCA) was conducted using the vegan package in R correspondence analysis. The first 4 axes of Axis length were all less than 3. Therefore, Redundancy analysis (RDA) was used to analyze environmental factors (Dixon, 2003).

3. Results and discussion

3.1. Biodiversity and community structure derived from morphological data

A total of 3514 fish individuals were collected in the Taizi River basin, which belong to 8 orders, 10 families, 33 genera and 34 species. Among them, *Carassius auratus*, *Abbottina rivularis* and *Zacco platypus* were found frequently in the sampling sites. By contrast, *Acheilognathus chankaensis*, *Pungtungia herzi*, *Paracheilognathus himantegus* and *Hemibarbus maculatus* were found only once between different sampling sites. The relative abundance of fish at each point is shown in Fig. 2.

The dominant species in the Taizi river are *Rhynchocypris lagowskii*, *Carassius auratus*, *Zacco platypus*, *Rhodeus lighti* which accounted for 22.8 %, 14.0 %, 12.6 % and 10.2 % of total fish, respectively. Among them, *Carassius auratus* is omnivorous fish. It is distributed in the water bodies of riparian zone mainly cultivated land and construction land. *Rhynchocypris lagowskii* and *Cobitis granoei* are typically sensitive species (Li et al., 2017). They are strict to water quality and are often found in clear streams. Indigenous fish and clean fish that are sensitive to environmental changes are mainly distributed in the middle and upper reaches of the Taizi River, such as *Lampetra morii*, which is listed in the "National List of Protected Animals" (Li et al., 2017). The *Hemibarbus maculatus* was found in S04. It is one of the most expensive medium-sized fish in fresh water. In the upstream (e. g. S05, S06, S08 and S09), the *Pungitius sinensis* was found. It was listed in the "Red List of Endangered Species of the International Union for Conservation of Nature" (IUCN ver 3.1, 2013) as a vulnerable species (VU). Among the rare species collected this time, *Pungitius sinensis*, *Lampetra morii* and *Huigobio chinssuensis* were primarily found in water bodies located within riparian zones dominated by forest land (Wang et al., 2016). In the riparian zone of agricultural land and construction land mainly downstream sites such as S15 and S24, much *Pseudorasbora parva* and *Carassius auratus* was observed. *Carassius auratus* and *Pseudorasbora parva* exhibit a preference for habitation in shallow waters with low flow velocity and abundant vegetation, and demonstrate exceptional ecological adaptability to a range of environmental conditions, even some polluted water bodies. No fish were found at the downstream sampling site S19, which may be related to the fact that the sampling site located in the urban area of Benxi and was greatly affected by human activities. The water environment is harsh, the habitat type is relatively single, fish and other aquatic organisms are extremely rare. At the sampling site S19 which is situated in the urban area of Benxi and is subject to substantial anthropogenic pressure, no fish species were observed. The poor water environment and relatively singular habitat type have led to an extreme rarity of fish and other aquatic organisms at this particular site.

The Alpha diversity index, as presented in Table 1, is an effective tool for characterizing the fish species diversity within the Taizi River. One of the primary advantages of employing the Alpha diversity index is its ability to effectively capture the spatial dynamics of diversity trends (Yang et al., 2013). The Alpha diversity index comprises various indicators, such as species richness, diversity, and evenness. Notably, the index values of fish species found differed significantly across diverse sampling sites, suggesting a complex and heterogeneous distribution of fish communities within the Taizi River ecosystem. Based on morphological data, the average Shannon Wiener index was 1.37, the average Simpson index was 0.62 and the average Pielou index was 0.64 by morphological data. High observed species index was observed at S02, S05, S11, S18 and S23. The Shannon diversity index was higher in S02, S05, S16, S23 and S24. Overall, according to three ecological indicators, fish diversity in the Taizi River was lower than in previous surveys (Wang et al., 2013a). It may be due to the large contingency in morphological sampling, which fail to reflect the fish composition truly and comprehensively.

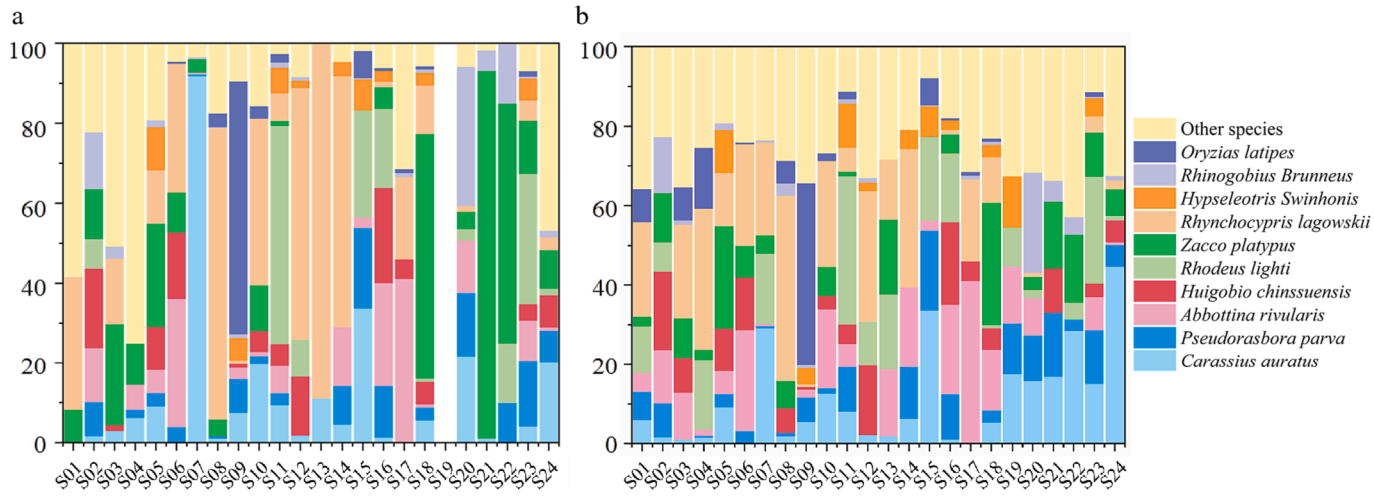

Fig. 2. Fish relative abundance derived from morphological data (a) and eDNA (b).

Table 1
Alpha diversity index of fish at each site derived from morphological data.

	Richness	Shannon	Simpson	Pielou
S01	5.00	1.33	0.69	0.83
S02	13.00	2.24	0.88	0.87
S03	8.00	1.55	0.73	0.75
S04	7.00	1.07	0.48	0.55
S05	13.00	2.20	0.86	0.86
S06	9.00	1.60	0.75	0.73
S07	8.00	0.41	0.15	0.20
S08	10.00	0.97	0.44	0.42
S09	11.00	1.37	0.58	0.57
S10	10.00	1.73	0.76	0.75
S11	14.00	1.69	0.68	0.64
S12	12.00	1.32	0.57	0.53
S13	2.00	0.35	0.20	0.50
S14	8.00	1.24	0.57	0.60
S15	9.00	1.62	0.76	0.74
S16	12.00	1.92	0.82	0.77
S17	7.00	1.34	0.69	0.69
S18	14.00	1.49	0.60	0.57
S19	0.00	0.00	1.00	0.00
S20	9.00	1.76	0.78	0.80
S21	5.00	0.36	0.15	0.22
S22	4.00	1.11	0.59	0.80
S23	13.00	2.06	0.83	0.80
S24	13.00	2.04	0.83	0.80

3.2. Biodiversity and community structure derived from eDNA metabarcoding

A total of 6875 operational taxonomic units (OTUs) were observed at 24 sampling sites using eDNA metabarcoding. After the removal of marine fish and fish not adapted to temperate regions, 1847 OTUs were obtained belonging to 8 orders, 11 families, 43 genera and 48 species of fish. It is confirmed that 12S rRNA gene sequencing can indicate species composition of fish. In this study, MiFish-U universal primer was selected to amplify the 12S rRNA gene region, which has been proved to have better performance than other competing primers in previous study (Bylemans et al., 2018; Shu et al., 2021). The eDNA metabarcoding detected *Leuciscus Waleckii, Saurogobio dabryi, Megalobrama skolkovii, Ctenopharyngodon idella, Aristichthys nobilis* and *Cottus Poecilopus* that were not found by morphological data. Among the species mentioned, *Leuciscus Waleckii, Saurogobio dabryi*, and *Cottus Poecilopus,* previous investigations in the Taizi River had identified their presence, but these species were not detectable based on morphological investigation. Overall, eDNA metabarcoding has demonstrated more accurate detection probabilities.

The Alpha diversity index (Fig. 3) was obtained from eDNA metabarcoding to characterize the diversity of fish species in Taizi River. The observed species index of most upstream sites (e. g. S01, S02 and S05) was higher than that of downstream sites (e. g. S20, S21, S22 and S23), but the Shannon index was lower than that of downstream sites, indicating poor distribution uniformity of fishes in the upstream.

3.3. Comparison of morphological data and eDNA metabarcoding

The results showed that a total of 9 orders, 12 families, 48 genera and 53 species (Table S1) were detected by the eDNA metabarcoding (8 orders, 11 families, 43 genera and 48 species) and morphological method (8 orders, 10 families, 33 genera and 34 species), and the eDNA method had a higher detection probability (Fig. 4). Cypriniformes was detected most frequently, followed by Perciformes. Among them, 29 species of fish were identified by the two methods, accounting for 54.72 % of the total species, such as *Carassius auratus*, *Pseudorasbora parva*, *Abbottina rivularis*, *Oryzias latipes*. The unique species captured by eDNA metabarcoding mainly included *Gobio cynocephalus, Culter alburnus, Cultrichthys erythropterus, Megalobrama skolkovii* and *Cottus Poecilopus*. And the unique species captured by morphology data included *Lampetra morii, Ladislavia taczanowskii, Aphyocypris chinensis, Sarcocheilichthys nigripinnis* and *Hemibarbus maculatus.*

Investigations based on morphological data failed to collect most species detected based on eDNA metabarcoding data. Environmental DNA metabarcoding had picked up species such as *Cottus Poecilopus*, *Cultrichthys erythropterus* and *Leuciscus Waleckii* found in previous fish surveys in the Taizi River, while they were not observed in morphological data. This is because eDNA metabarcoding can provide information on more taxa that are difficult to identify through morphology, resulting in a more comprehensive reflection of fish richness and diversity. However, it is worth noting that eDNA metabarcoding technology may also overestimate some low-abundance groups. This could be attributed to the fact that organisms DNA fragments can persist in the river for up to a month, and fish DNA fragments in the water can spread to adjacent sampling sites through water currents (Li et al., 2020). In contrast, morphological data can only reveal fish information in the immediate sampling area.

However, it should be noted that for some species, the diversity level obtained through eDNA metabarcoding technology was lower than that obtained through morphological identification. For instance, *Lampetra morii* and other fish that were not detected through eDNA metabarcoding were primarily due to the following reasons: a) The absence of a local fish database of Taizi River, due to the lack of comprehensive DNA sequences in the reference database used for analysis, the general

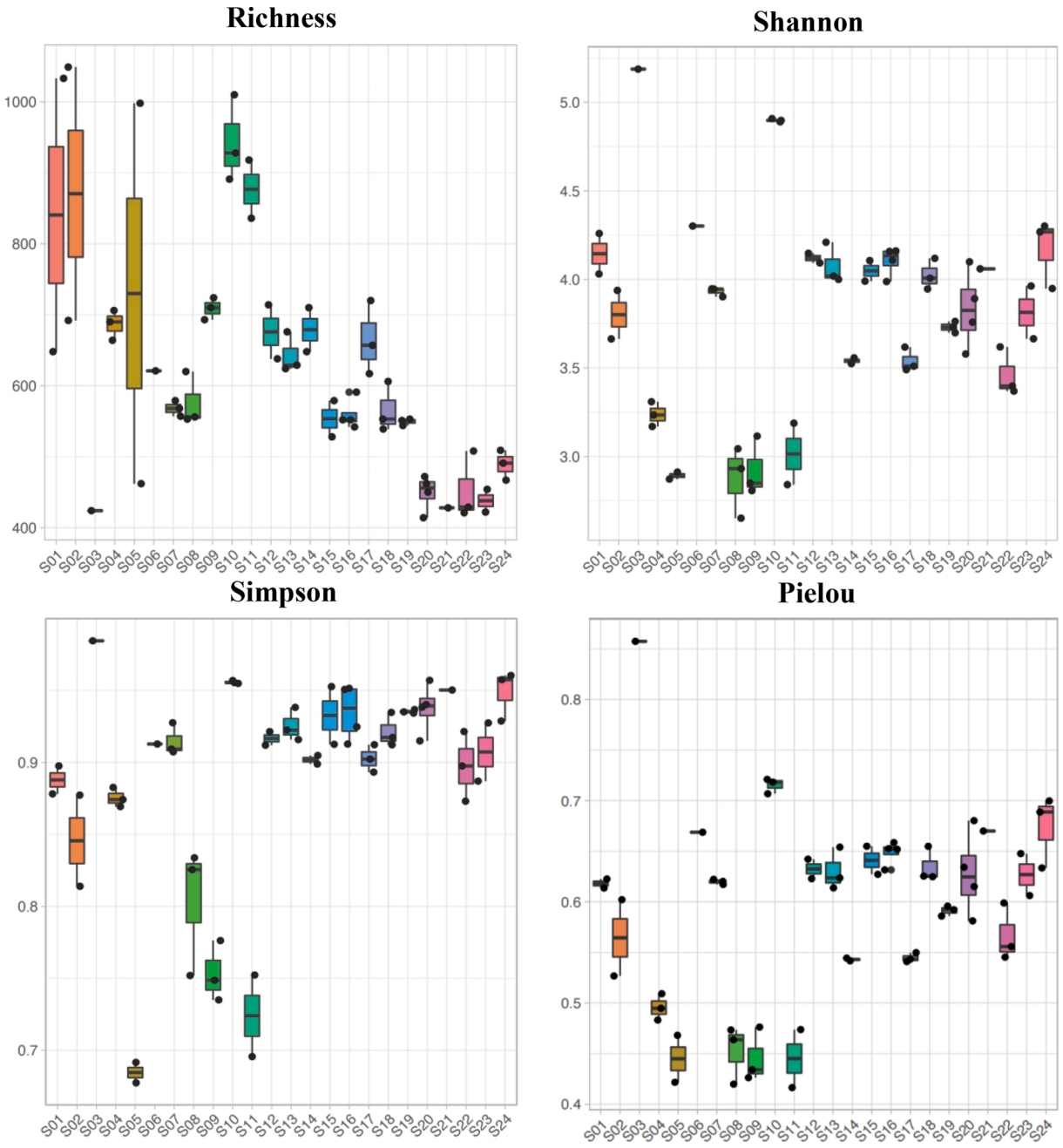

Fig. 3. Alpha diversity index of fish at each site derived from eDNA.

Fig. 4. Comparison of fish numbers identified by eDNA metabarcoding and morphology at family, genus and species classification levels.

database is not always suitable for eDNA analysis in a specific region (Wang et al., 2021b). b) Environmental factors such as temperature, solar radiation and pH value affect the persistence of eDNA in water. c) Errors in the process of DNA extraction, PCR amplification and subsequent sequencing analysis. Because this experiment uses universal primers for fish, there may be some inaccuracies in the identification of certain species. In the process of PCR, the low affinity between the primer and the primer binding site causes the sequence to fail to be amplified, resulting in the failure to identify those species. This could be the reason why *Lampetra morii* and other fish were not detected in the eDNA metabarcoding (Elbrecht and Leese, 2017; Thomsen et al., 2016).

3.3.1. Alpha diversity comparison

From comparison of the three ecological index values derived from eDNA metabarcoding and morphology data (Fig. 5), it can be seen that Shannon Wiener index of fish at each sampling point was significantly higher than that of the morphological data. This means that eDNA metabarcoding can reflect fish richness and evenness more comprehensively. Firstly, the Pielou evenness index calculated by morphological data changes greatly at each point, indicating the calculation of community composition and biodiversity by morphological data is more accidental. Secondly, eDNA metabarcoding can provide more taxa information that is difficult to be identified by morphology, especially at the lower classification level. Therefore, the richness and Shannon Wiener index obtained by eDNA metabarcoding are greater than those obtained by morphology at most sampling sites.

3.3.2. Beta diversity comparison

Principal coordinate analysis (PCoA) was used to analyze the beta diversity of fish composition in different samples (Fig. 6). PCoA reflects the difference between multiple sets of data on a two-dimensional coordinate plot, where the axes are the two eigenvalues that best reflect the variance. Therefore, samples with similar compositions were clustered in the PCoA diagram. Morphological data were used to analyze fish beta diversity (Fig. 6a). PCoA results showed that fish species in Taizi River basin had high beta diversity. There were significant differences in fish species composition and community structure at different sampling sites.

PCoA was used to analyze the beta diversity of OTU composition at different points to reflect the differences among samples (Fig. 6b). There was little difference in the fish community composition between the six upstream sites S01, S02, S04, S05, S09, and S11. Moreover, the downstream sites S15, S18, S20, S23, and S24 almost overlap in the PCoA diagram, indicating that their fish community structure was similar. Therefore, the beta diversity of upstream sites was significantly higher than that of downstream sites. On the one hand, it may be due to the close distance between downstream sampling sites, and the DNA of sampling sites may come from a larger spatial scale due to the migration and diffusion of biological debris. Fish DNA fragments in water may be spread to adjacent sampling sites through water flow (Merkes et al., 2014), and one sampling site is likely to detect species from another. On the other hand, multiple PCR amplification may amplify the DNA of some fish, even if the DNA concentration of the species left in the water body is extremely low (Jeunen et al., 2019), so this may eventually result in a very similar fish community structure at downstream sampling sites. This would lead to errors in fish distribution results and a reduction in beta diversity (Leduc et al., 2019).

In conclusion, eDNA metabarcoding can quickly and accurately reflect the diversity and distribution characteristics of dominant fish groups (Semmouri et al., 2021). However, to achieve the most comprehensive understanding of fish diversity, a combination of eDNA metabarcoding and morphological identification is necessary. Firstly, while eDNA metabarcoding results can reveal sequence abundance, traditional survey methods can provide valuable information on fish morphology. Morphological changes in fish are often regarded as evidence of heavy metal contamination, which cannot be assessed through eDNA alone (Yanko et al., 1998). Secondly, eDNA metabarcoding often relies on traditional survey results. The final annotation results are often compared and screened with morphological data and historical fish survey data. Therefore, when eDNA metabarcoding or morphological data is used alone to assess the impact of external disturbances on aquatic ecosystems, the results obtained may be inconsistent. In conclusion, to achieve a more comprehensive understanding of fish diversity in aquatic ecosystems, traditional morphological methods should be used as a supplementary tool to eDNA metabarcoding when necessary (Yanko et al., 1998; Zhou et al., 2022).

3.4. Stochastic and deterministic processes in determine the distribution of fish communities

The results show that most of the βNTI values at the 24 sampling sites are between −2 and 2 (Fig. 7a). The βNTI results strongly confirmed that deterministic processes had less effect on community assembly than stochastic processes between sampling sites, but the fish communities at sampling sites S07, S08 and S14 were significantly affected by deterministic factors (i.e. environmental filtration and biological interaction factors) (Pecuchet et al., 2016). When $|\beta NTI| < 2$, Raup-Crick was calculated to determine which random processes regulated phylogenetic turnover under the null model. The results showed that |Raup-Crick| was less than 0.95 at most sampling sites (Fig. 7b), indicating that fish turnover was observed to be dominated by undominated processes. It includes weak selection, weak dispersal, diversification and drift, etc. (Chase et al., 2009). However, the importance of stochastic processes in influencing community structure has received far less attention, mainly

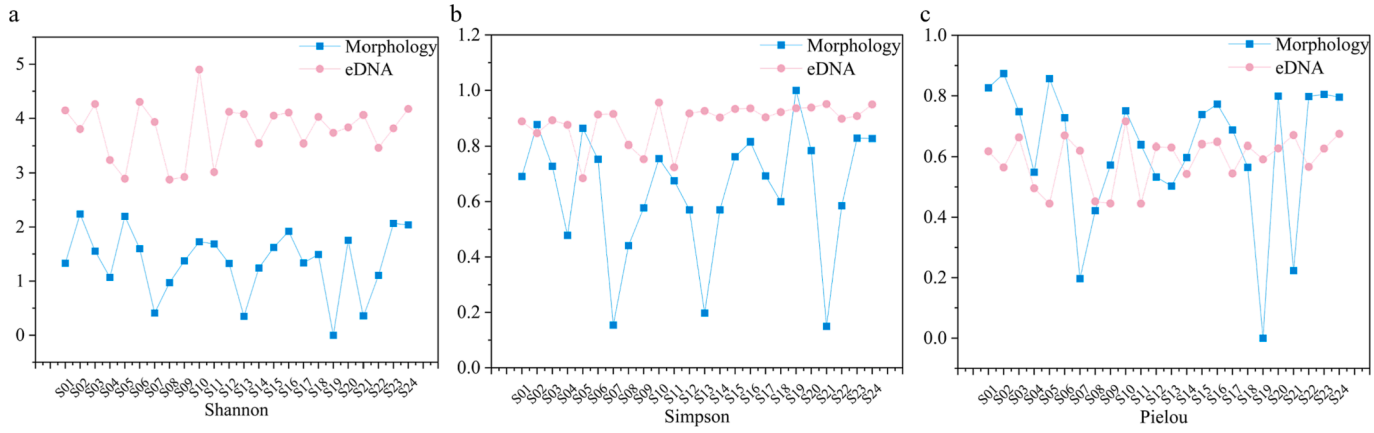

Fig. 5. Comparison of alpha diversity index derived from eDNA metabarcoding and morphology identification data.

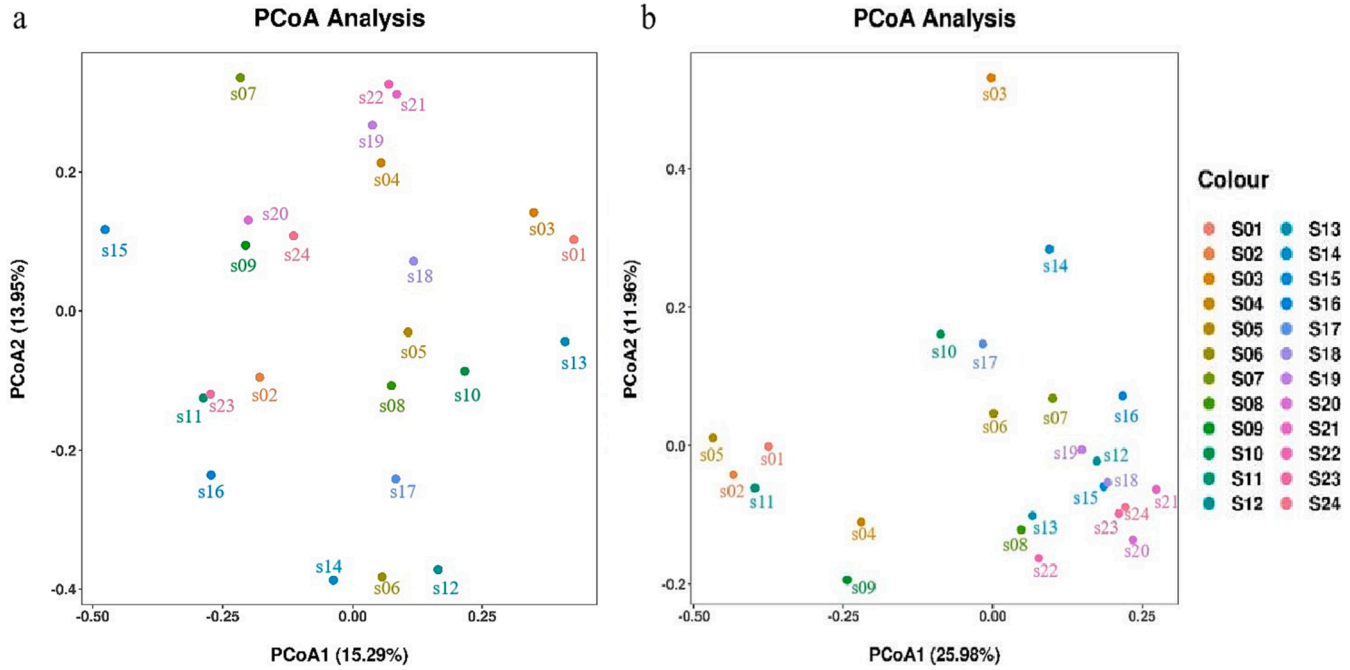

Fig. 6. PCoA diagram of beta diversity of all samples based on morphological data (a) eDNA metabarcoding (b).

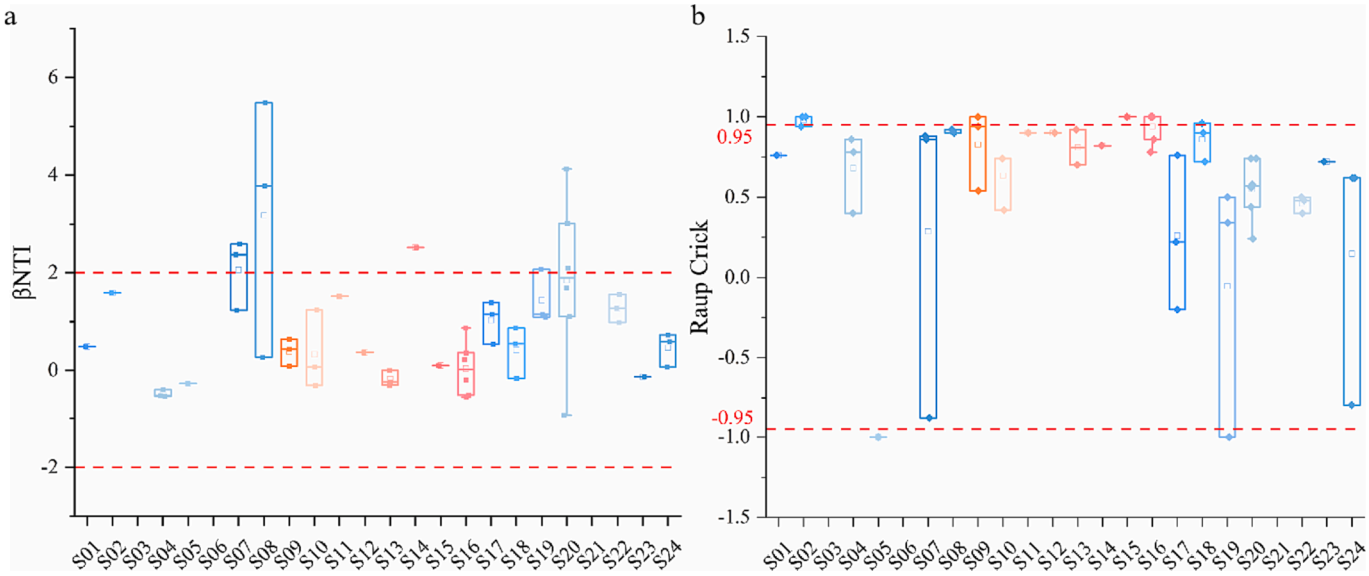

Fig. 7. Values of βNTI (a) and Raup-Crick (b) at 24 sampling sites in Taizi River.

because of the difficulty in defining stochasticity and the methods used to represent it (Strange et al., 1993). Giam and Olden provided compelling evidence for non-random structure in most temperate stream fish communities and community assembly is governed largely by environmental filtering (Giam and Olden, 2016). By contrast, the null model results showed the main factor that affected the distribution of fish in the Taizi River is the stochastic processes. This discrepancy may be attributed to the smaller scope of the Taizi River basin studied in our research. Nonetheless, our findings provide reliable evidence for understanding assembly processes in fish communities inhabiting small-scale, temperate rivers.

We used RDA analysis to determine the ability of environmental variables to explain changes in fish community composition. The deterministic processes affecting community structure include land use factors, water environmental quality factors and so on. For instance, water temperature can affect the rate of fish metabolism, while salinity can impact respiratory metabolism and digestive function. Water depth can significantly influence the growth, development, and distributions of swimming organisms (Wang et al., 2013b; Wang and Wu, 2015). Aquatic plants are known to provide habitats for fish and plant photosynthesis leads to higher dissolved oxygen content compared to open water, thereby encouraging fish to occupy such habitats (Maes et al., 2007). Notably, high dissolved oxygen and complex habitats offer shelter for fish as well as an abundance of food organisms, in addition to accelerating water material circulation and improving water quality. In a related study on the Taizi River, Li et al highlighted sediment index, dissolved oxygen, and electrical conductivity as environmental variables influencing fish community structure and diversity in the area (Li et al., 2017).

The results of redundancy analysis (RDA) based on eDNA

metabarcoding and morphological data showed that total phosphorus, biochemical oxygen demand (BOD_5) and temperature were the main environmental factors affecting the fish community in the Taizi River basin. The RDA axis explained 30.93 % of the differences in fish community structure (Fig. 8). The *Carassius auratus*, *Rhodeus lighti* and *Pseudorasbora parva* showed a clear positive correlation with the concentrations of total phosphorus (TP), ammonia nitrogen (NH_3-N), and BOD_5 and almost no correlation with pH. *Carassius auratus* prefers to live in the bottom layer of water and has a strong adaptability to hypoxic water. Furthermore, most of these species are omnivorous, with some preferring to feed on aquatic plants. TP and total nitrogen (TN) are essential nutrient elements for the growth of aquatic plants, and some fish feed on aquatic plants, so TP and TP affect the distribution of fish to a certain extent. The distribution of *Rhynchocypris lagowskii*, *Abbottina rivularisis* and *Huigobio chinssuensis* positively correlated with DO and negatively correlated with water temperature (T) and TN. It has been shown that the majority of *Rhynchocypris lagowskii* live in rivers with low water temperature and clear water quality. *Zacco platypus* distribution was positively correlated with T but had little correlation with TP and NH_3-N.

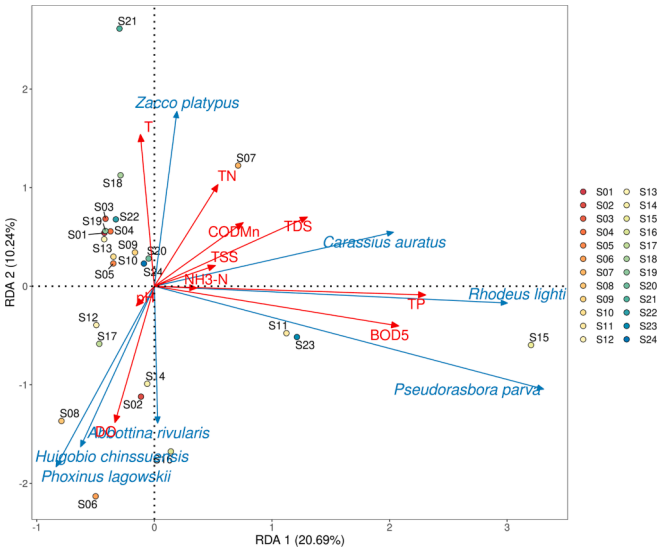

Fig. 8. The relationship between fish community and environmental factors.

4. Conclusion

Morphological data and eDNA metabarcoding were used to investigate the distribution and diversity of fish in the Taizi River basin. Fish sensitive to environmental changes mostly distributed in the upper reaches, where the fish richness was higher. The dominant specie was *Rhynchocypris lagowskii*, which tended to live in clean water. The lower reaches were affected by urbanization, and the fish community was less. The dominant species were *Carassius auratus* and *Abbottina rivularis*. A total of 8 orders,12 families and 48 genera 53 species were detected by the eDNA metabarcoding (7 orders, 11 families, 43 genera and 48 species) and morphological method (7 orders, 10 families, 33 genera and 34 species). Environmental DNA metabarcoding has a higher probability of detecting fish, which can reflect the richness and uniformity of fish more comprehensively. The integration of eDNA and morphology can enhance the precision of fish diversity assessment. PCoA analysis revealed that fish communities in Taizi River exhibited different spatial structures between the upper and lower reaches, with fish sensitive to environmental changes mostly found in the upper reaches and higher fish richness in the same area. The beta diversity in the upper reaches was higher than that in the lower reaches and beta diversity calculated based on morphology was greater than that of eDNA.

The null model results strongly support the significant effect of stochastic processes on fish community assembly, and undominated processes dominate fish replacement, including weak selection, weak diffusion, diversification, and drift. The result of RDA determined the ability of environmental variables to explain changes in fish community composition, and the environmental filter factors affecting community structure included TP, BOD_5 and T in the basin. The *Carassius auratus*, *Rhodeus lighti* and *Pseudorasbora parva* was positively correlated with the concentrations of TP, NH_3-N, and BOD_5. The distribution of *Rhynchocypris lagowskii*, *Abbottina rivularisis* and *Huigobio chinssuensis* positively correlated with DO and negatively correlated with T and TN. *Zacco platypus* distribution was positively correlated with T but had little correlation with TP and NH_3-N. This indicates that fish community assembly in temperate rivers is influenced by both deterministic and stochastic processes.

CRediT authorship contribution statement

Yue Shi: Conceptualization, Methodology, Visualization, Investigation, Writing – original draft, Writing – review & editing. **Shuping Wang:** Methodology, Investigation, Writing – review & editing. **Xiaolong Lin:** Investigation, Writing – review & editing. **Hong Li:** Investigation, Writing – original draft. **Aopu Li:** Investigation, Writing – original draft. **Juntao Fan:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.111111.

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