eDNA metabarcoding as a promising conservation tool to monitor fish diversity in Beijing water systems compared with ground cages

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Abstract

Fish diversity, an important indicator of the health of aquatic ecosystems, is declining sharply due to water pollution, overfishing, climate change, and species invasion. Effective surveying and monitoring are prerequisites for protecting fish diversity. In this study, eDNA (environmental DNA) metabarcoding and ground cages were used to survey the fish diversity of the Chaobai and Beiyun Rivers in Beijing. Based on the two methods, the results showed that 40 species, belonging to 35 genera, 18 families, and six orders, were identified. The richness of fish identified by eDNA metabarcoding was higher than that captured by ground cages in both rivers. All fish captured by the ground cage method were recognized by eDNA metabarcoding, except *Squalidus wolterstorffi* and *Saurogobio dabryi*, which were captured only in ground cages. The correlation of relative abundance between the two methods was affected by the properties of the rivers, such as the flow rate. The relative abundance detected by eDNA metabarcoding was positively correlated with that of the ground cages in the Beiyun River, while no correlation was observed for the Chaobai River. Our results also suggest that the Chaobai River has higher fish diversity and a different population structure than the Beiyun River. The primary fish species of the Beiyun River are mainly pollution resistant. In addition to differences in natural factors, the development of urbanization is a major contributor to different community structures in the two Rivers. Our results show that eDNA metabarcoding as a new survey tool has great application prospects. It provides certain theoretical data and methodological references for the protection and management of river fish diversity.

1. Introduction

Fish species account for one-quarter of the world's vertebrates, and their diversity is an important indicator of aquatic ecosystem health (Zou et al., 2020). The 2020 Living Planet Index (LPI) shows that 3,741 monitored populations have declined by an average of 84%, equivalent to a 4% decline per year since 1970. Freshwater fish biodiversity is declining much faster than that in oceans or forests (Almond et al., 2020). This is due to various factors such as water pollution, overfishing, and species invasion (Beverton, 1991; Jackson and Head, 2020).

Beijing is located in the north of China and is mainly composed of five water systems belonging to the Haihe River System. There is rich fish diversity in Beijing, with more than 80 historical species (Chen et al., 2007; Gao, 1994; Wang, 1994). As reported in a study by Zhang et al., a total of 93 species were found in a survey and collation of fishes in and around Beijing (Zhang et al., 2011). However, those surveys are relatively dated. The methods used, such as sticky nets, hand-scattered nets, and obtaining specimens from fishermen, are traditional or destructive subversive (Chen et al., 2007; Wang, 1994). Therefore, the development of cost-effective, dependable, rapid, and harmless methods for monitoring and assessing fish diversity is crucial for the timely and effective management and conservation of fish in Beijing. The metabarcoding approach combined with eDNA is often used as a powerful tool to monitor and assess fish diversity (Aglieri et al., 2020; Shaw et al., 2016; Yamamoto et al., 2017; Zou et al., 2020).

Environmental DNA (eDNA) is a mixture of DNA from different species, such as microorganisms, animals, and plants. It includes the intracellular DNA emitted by the organism, as well as the extracellular DNA released into the environment after cell death by lysis (Rees et al., 2014a; Rees et al., 2014b; Shen et al.). Environmental DNA

technology is used to capture DNA from environmental samples, to preserve, extract, amplify, sequence, and classify it, and then determine the distribution of organisms in the sampled environment (Deiner et al., 2015). So far, eDNA has been used to monitor the status of endangered (Thomsen et al., 2012) or invasive (Miralles et al., 2019) species and to assess the biomass (Takahara et al., 2012) and diversity (Aglieri et al., 2020; Zou et al., 2020) of aquatic organisms, especially fish. However, in studies on fish diversity using the eDNA method, the water body is generally the ocean or a single river or lake (Altermatt et al., 2020; Stat et al., 2019). Few studies have investigated fish diversity in multiple rivers by eDNA and compared the community structure of different rivers in combination with urban development.

Beijing is one of the fastest growing urban areas in China (Fu et al., 2016). From 1978 to 2019, the urbanization rate of Beijing increased from 54.9 to 86.6%. Urbanization inevitably affects fish habitats through a reduction in the water area, aggravated pollution, and hydropower projects (Hao and Sun, 2021; Su et al., 2021). Urbanization is an essential trigger of species extinction and homogenization (Yan et al., 2016). Urbanization has been carried out unevenly in regional Beijing. The Beiyun River is located in the central plain, with the highest urbanization rate, and the water quality is the worst. The Chaobai River is located in the northern mountainous region, with a lower urbanization rate, and the water quality is the best in Beijing (Xiao et al., 2018). Urbanization also reduces fish diversity and tends to produce homogenization (Zhang et al., 2011).

To compare fish diversity and relative abundance with eDNA metabarcoding and ground cages, fish diversity was surveyed in the Chaobai and Beiyun Rivers at different sampling sites in Beijing. This paper focused on three research objectives: (1) to present the results of two methods of surveying fish composition, diversity, and relative abundance in two water systems; (2) to compare fish diversity collected by ground cages and eDNA metabarcoding, highlight the potential of the latter, and analyze the correlations between the results of the two methods; and (3) to compare the diversity and community structure of the two river systems and analyze the reasons for diversity differences.

2. Methods

2.1. Sampling sites

Five sites were surveyed in the Chao and Bai rivers, which belong to the Chaobai River. The five sampling points were in the Beiyun, Wenyu, and Qinghe rivers, which are part of the Beiyun River; sampling was conducted in September 2020 (Figure 1). The ground cage and eDNA metabarcoding methods were applied at each sampling point. Need to declare: the experimental protocol referred *Laboratory animal—Guideline for ethical review of animal welfare*. ICS 65.020.30, and all experiments were performed in accordance with relevant. And the study is reported in accordance with ARRIVE guidelines

2.2. eDNA and ground cage sample collection

Surface water samples of 1 L were collected and mixed in a sterile, sealable brown plastic bottle and preserved at 4 °C for transport to the laboratory. Three duplicate samples from a single site were collected for eDNA analysis. Then, the samples were filtered through a 0.45 µm nylon filter membrane with a vacuum peristaltic pump. The glass funnel and filter head were thoroughly disinfected with 10% sodium hypochlorite before use.

The filter membranes were immediately placed into sterile 2 mL plastic scintillation tubes at each test point and stored at -20 °C for the experiments. At each site, filtration blanks were established by filtering 1 L of distilled water in the same way to check for contamination during field collection.

Ground cages were used as the traditional method to contrast with the eDNA method. Three cages with a length of 2 m were placed at each sampling point, and fish food was placed in the cages. The distance between the cages was about 3 m, and the storage time was 24 h to maximize the probability of detecting fish species. The eDNA water sample collection and fish acquisition by ground cages at each site were conducted simultaneously. This approach ensured that differences in fish assemblages determined between methods did not result from fish migration. After the capture with ground cages, the fish samples were stored on ice and transported to the laboratory within 10 h. To avoid potential bias in species assessment, identification was performed in a timely manner, and abundance at each site was recorded.

2.3. DNA extraction and metabarcoding

The eDNA extraction experiment was completed at the Molecular Biology Laboratory of the Chinese Academy of Environmental Sciences, where all utensils are routinely decontaminated with UV light. The experimental consumables and instruments required for extraction were cleaned with disinfectant or autoclaved. DNA from the water was extracted using a Qiagen DNeasy Tissue and Blood DNA extraction kit (Qiagen, Venlo, the Netherlands) following the manufacturer's protocol with some modifications. The filter membrane obtained by 1 L water suction filtration was cut and placed in a 2 mL centrifuge tube. Then, 720 µL of ATL tissue lysis buffer reagent and 80 µL of proteinase K were added to the tube and mixed by shaking. Incubation of this mixture was performed at 56 °C for 3 h. Next, the samples were divided into two groups, and an 800 µL mixture of 400 µL AL lysis buffer + 400 µL anhydrous alcohol was added. Then, 500 µL of AW1 wash buffer and 500 µL of AW2 wash buffer were added to each tube and the tubes were centrifuged. Finally, 50 µL of AE buffer was added to each elution column, which was placed in a 37°C water bath for 10 min before centrifuging. Filtration blanks and negative controls were co-extracted alongside the samples and were subjected to the same protocol. DNA was detected using 1.0% agarose gel. No data or bands were observed for the filtration blanks or negative controls.

Metabarcoding was performed in duplicate on each DNA extract with the primers MiFish-F (5'-GTCGGTAAACTCGTGCCAGC-3') and MiFish-R (5'-CATAGTGGGGTATCTA ATCCCAGTTTG-3') (Miya et al., 2015), which target the 12S rDNA region (~180 bp) of the mitochondrial genome, to identify fish species. Three PCR replicates were performed for each sample, the negative control and filtrate blanks. A two-step PCR protocol was adopted in this study. To determine the volume of DNA that should be added to the PCR reaction, the first round of amplification was performed using a Qubit 3.0 DNA Assay Kit for precise quantification of genomic DNA. The PCR assay volume was 30 µL and included 15 µL 2×Hieff® Robust PCR Master Mix, 1 µL of the forward and reverse primers, and 10–20 g of DNA template; ddH₂O was added to 30 µL. For all samples, the first-step PCR was performed as follows: 94 °C for 2 min, followed by 30 cycles of 98 °C for 5 s, 50 °C for 10 s, and 72 °C for 10 s, with a final extension at 72 °C for 5 min. In the second-step PCR, Illumina bridge PCR-compatible primers were introduced into the sterile PCR tube (200 µL) to prepare the same reaction system as in the first-step PCR. The reaction solution was gently blown or stirred by pipette and briefly centrifuged to the bottom of the tube. The PCR procedure was as follows: 95 °C for 3 min, followed by 5 cycles of 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. After the two-step PCR

amplification, the PCR products were detected using 1.0% agarose gel. None of the filtration blanks or negative controls exhibited amplification.

2.4. High-throughput sequencing and bioinformatics analysis

The PCR products were utilized to construct libraries, followed by high-throughput sequencing using the Illumina MiSeq platform (Sangon Biotech (Shanghai) Co., Ltd.). Paired-end sequence data (PE reads) were obtained by utilizing PEAR (v 0.9.8) to combine with tags based on overlaps (Zhang et al., 2014). Then, the samples were identified and distinguished according to the barcode tag sequence. To obtain clean reads, the data were filtered by Fastp (v 0.21.0) (Chen et al., 2018) to eliminate adapter contamination and reads with low quality. USEARCH (v 11.0.667) (Edgar, 2010) was used to perform OTU (operational taxonomic unit) clustering of the optimized sequences of each sample according to 97% similarity, and chimeras were filtered out. All optimized sequences were mapped to each representative OTU sequence using USEARCH to obtain an OTU abundance table. The taxonomic assignment of OTU sequences was mapped to the fish gene database downloaded from the Mitochondrial Genome Database of Fish (MitoFish) (Sato et al., 2018) website (<http://mitofish.aori.u-tokyo.ac.jp/download.html>) using the Blastn tool. Sequences were designated as belonging to a species if there was $\geq 99\%$ sequence identity to the MitoFish barcode database from across the entire length of the amplicon. An OTU was categorized into another OTU if a sequence could not be assigned to a species. If a sequence could be assigned to several species and the species belonged to the same genus, the species were screened in combination with historical fish species in Beijing (Du et al., 2019; Wang, 1984; Zhang et al., 2011). Finally, OTUs representing each taxon were matched using TaxonKit (v 0.8.0.) (Shen and Ren, 2021).

The eDNA data were compared with the data obtained by ground cages for the species richness table, and species and sequence abundance data were standardized to the proportions of the total sample. The relative abundance of species was calculated from the OTU abundance table for each taxonomic rank and the species count table. The vegan package (<https://rdrr.io/cran/vegan/man/vegan-package.html>) of R (v4.1.0) (<https://www.r-project.org/>) was used to calculate the fish richness of each sample, and a t-test was used to determine whether there were significant differences between methods. Spearman correlation coefficients between the relative abundance of species obtained by the two methods were calculated. The beta diversity of the two water systems was sorted by non-metric multidimensional scaling (NMDS) based on the Bray–Curtis to determine the differences in species distribution between the two water systems.

3. Results

3.1. Fish diversity

In total, 50 fish OTUs from 30 samples were generated; 38 fish species from 33 genera, 18 families, and six orders were identified by eDNA metabarcoding, and 711 fish captured with ground cages were identified as 19 species from 19 genera, six families, and two orders. Fish species obtained by the two methods covered 40 species in 35 genera, 18 families, and six orders (Table 1). Compared with the ground cage results, higher numbers of species, genera, families, and orders were detected by eDNA metabarcoding, with a higher capture rate. In total, 17 species (42.5%) were detected by both eDNA metabarcoding and ground cages, and 21

species (52.1%) were identified by eDNA metabarcoding alone. *Squalidus wolterstorffi* and *Saurogobio dabryi* (5.0%) were caught by ground cages exclusively (Figure 2).

Table 1
Sequence numbers of fish detected by eDNA and caught by ground cages at 10 sites in Beijing.

Order	Species	eDNA(Sequence)			Traditional (Number)		
		Chaobai	Beiyun	Total	Chaobai	Beiyun	Total
Beloniformes	<i>Oryzias latipes</i>	1073	3008	4081	0	0	0
Cypriniformes	<i>Abbottina rivularis</i>	924	2301	3225	19	6	25
	<i>Acheilognathus macropterus</i>	46	874	920	4	2	6
	<i>Carassius auratus</i>	26614	165549	192163	0	0	0
	<i>Chanodichthys erythropterus</i>	84	39	123	3	0	3
	<i>Cobitis melanoleuca</i>	1726	0	1726	0	0	0
	<i>Cyprinus carpio</i>	9189	3151	12340	0	0	0
	<i>Gnathopogon strigatus</i>	140	0	140	0	0	0
	<i>Hypophthalmichthys molitrix</i>	640	0	640	0	0	0
	<i>Squalidus wolterstorffi</i>	0	0	0	5	0	5
	<i>Hypophthalmichthys nobilis</i>	21193	357	21550	0	0	0
	<i>Misgurnus anguillicaudatus</i>	762	42882	43644	4	15	19
	<i>Cobitis granoci</i>	1393	2415	3808	1	0	1
	<i>Saurogobio dabryi</i>	0	0	0	4	0	0
	<i>Opsariichthys uncirostris</i>	2440	39	2479	1	0	1
	<i>Paramisgurnus dabryanus</i>	3399	643	4042	99	3	102
	<i>Pseudorasbora parva</i>	5265	42561	47826	36	158	194
	<i>Rhodeus ocellatus</i>	758	14452	15210	1	17	18
	<i>Rhynchocypris lagowskii</i>	24493	177	24670	11	0	11
	<i>Sarcocheilichthys soldatovi</i>	2890	393	3283	5	0	5
	<i>Ctenopharyngodon idella</i>	299	332	631	0	0	0

Order	Species	eDNA(Sequence)			Traditional (Number)		
		Chaobai	Beiyun	Total	Chaobai	Beiyun	Total
	<i>Zacco platypus</i>	31034	1243	32277	0	0	0
	<i>Hemiculter leucisculus</i>	618	1145	1763	289	0	289
Perciformes	<i>Micropterus salmoides</i>	0	219	219	0	0	0
	<i>Rhinogobius cliffordpopei</i>	0	176	176	0	0	0
	<i>Channa argus</i>	4962	20092	25054	2	0	2
	<i>Lateolabrax maculatus</i>	0	32	32	0	0	0
	<i>Macropodus chinensis</i>	366	505	871	5	0	5
	<i>Micropercops swinhonis</i>	7263	61546	68809	9	0	9
	<i>Odontobutis potamophila</i>	90	5620	5710	0	8	0
	<i>Rhinogobius brunneus</i>	1365	424	1789	3	1	4
	<i>Rhinogobius similis</i>	3012	1037	4049	0	0	0
	<i>Siniperca chuatsi</i>	770	204	2773	0	0	0
Salmoniformes	<i>Hypomesus olidus</i>	260	0	260	0	0	0
	<i>Oncorhynchus mykiss</i>	1647	0	1647	0	0	0
Siluriformes	<i>Ictalurus punctatus</i>	0	85	85	0	0	0
	<i>Silurus asotus</i>	6842	514	7356	0	0	0
	<i>Tachysurus fulvidraco</i>	3552	3871	7423	0	0	0
	<i>Tachysurus ussuriensis</i>	1393	32	1425	0	0	0
Synbranchiformes	<i>Monopterus albus</i>	259	136	395	0	0	0

Six orders were detected by eDNA metabarcoding. Cypriniformes and Perciformes were the dominant orders, with a sequence abundance of 75.73 and 20.10%, respectively, of the total sequence abundance. Siluriformes was the most common order, accounting for 2.99% of all sequences. Beloniformes (0.75%), Salmoniformes (0.35%), and Synbranchiformes (0.07%) were relatively rare orders. The dominant families in Cypriniformes were Cyprinidae (87.1%) and Cobitidae (12.90%) (Figure 3a). The less abundant orders contained only 1–2

species, such as *Oryzias latipes* in Beloniformes, *Hypomesus olidus* and *Oncorhynchus mykiss* in Salmoniformes, and *Monopterus albus* in Synbranchiformes (Table 1). Only two orders, Cypriniformes (93.18%) and Perciformes (6.82%), were captured using the ground cage method. Cypriniformes was also composed of Cyprinidae (82.14%) and Cobitidae (17.86%) (Figure 3b). Among them, the dominant species were *Hemiculter leucisculus* and *Pseudorasbora parva* in Cyprinidae and *Paramisgurnus dabryanus* in Cobitidae (Table 1).

3.2. Comparison and relationship between eDNA metabarcoding and ground cages

At the genus level, the diversity obtained by eDNA metabarcoding ($n = 33$) was higher than that obtained by ground cages ($n = 19$). The genera with high relative abundance based on eDNA metabarcoding were *Carassius* (35.28%), followed by *Micropercops* (12.63%) (Figure 5). The genera with high relative abundance captured by ground cages were *Hemiculter* (40.65%) and *Pseudorasbora* (27.28%) (Figure 5).

The fish richness at each site detected by eDNA metabarcoding (average = 15.10 (SD = 7.19)) was significantly higher than that detected with ground cage fishing (average = 4.60 (SD = 2.37); Welch two-sample t-test, $T = 4.1603$, $df = 10.941$, $p < 0.05$). The fish richness identified using eDNA metabarcoding in the Chaobai River ($n = 34$) and the Beiyun River ($n = 18$) was higher than that identified with ground cages (Figure 4). The difference was significant for the Beiyun River (Welch two-sample t-test, $t = 1.93$, $df = 4.00$, $p < 0.05$; Figure 4) but was not significant for the Chaobai River (Welch two-sample t-test, $t = 1.93$, $df = 4.00$, $p = 0.13$).

The relative abundance in the Beiyun River identified by eDNA metabarcoding showed a significant Spearman correlation with the relative abundance detected by ground cages in BY2, BY3, BY4, and BY5 (Figure 6). However, no significant correlation at the BY1 sample site was observed ($p = 0.07$; Figure 6). In the Chaobai River, the relative abundance obtained by ground cages at CB1 and CB3 had a significant correlation with that detected by eDNA metabarcoding. The abundance obtained by the two methods showed a positive correlation at CB2 ($p = 0.53$) and CB4 ($p = 0.4$), but the correlation coefficient was not significant. There was a negative correlation coefficient for CB5 (Figure 6). Thus, the correlation of relative abundance obtained by the two methods in the Beiyun River was stronger than that in the Chaobai River.

3.3. Diversity of the Chaobai and Beiyun Rivers

The fish richness was higher in the Chaobai River (36 species: 34 based on eDNA metabarcoding, 18 based on ground cages) than in the Beiyun River (33 species: 33 based on eDNA metabarcoding, 8 based on ground cages) (Table 1). For the species detected by eDNA, the most abundant in the Chaobai River was *Zacco platypus* (18.41%), followed by *Carassius auratus* (15.79%), *Rhynchocypris lagowskii* (14.53%), and *Hypophthalmichthys nobilis* (12.57%). *Carassius auratus* (44.02%) was the most abundant species detected in the Beiyun River, followed by *Micropercops swinhonis* (16.37%), *Misgurnus anguillicaudatus* (11.40%), and *Pseudorasbora parva* (11.32%) (Table 1).

The distribution of the top 10 most abundant species in the two rivers is shown in Figure 7. For species caught by ground cages, the most abundant in the Chaobai River was *Hemiculter leucisculus* (57.80%), followed by

Paramisgurnus dabryanus (19.80%), *Pseudorasbora parva* (7.20%), and *Abbottina rivularis* (3.80%). The most abundant species in the Beiyun River was *Pseudorasbora parva* (75.24%), followed by *Rhodeus ocellatus* (8.09%), *Misgurnus anguillicaudatus* (7.14%), and *Odontobutis potamophila* (3.81%) (Table 1).

Beta diversity showed a certain separation of the fish communities between the Chaobai and Beiyun Rivers by both the eDNA metabarcoding and ground cage method (Figure 7). However, the distribution of samples in the Beiyun River was more concentrated, while samples in the Chaobai River were more scattered, and the distance between CB5 and the other sample points was very far.

4. Discussion

Fish are an important component of aquatic ecosystems and have a significant impact on the balance of an ecosystem. Fish can also be used as an indicator to evaluate the biological integrity of water bodies (Karr, 1981). The application of nondestructive techniques to investigate fish diversity is becoming an inevitable trend, which allow the results of a study to be meaningful. In some studies on fish diversity in Beijing (Du et al., 2019; Zhang et al., 2011), the findings showed a gradual decline due to the influence of human activities. In this study, we present proof that eDNA can be used for monitoring fish diversity in such water bodies; the species richness from the eDNA survey was higher than that from the traditional method. This also proves that the fish community structure of Beiyun River in urban is relatively homogeneous compared with Chaobai River in mountain.

4.1 Fish diversity

A total of 40 species were detected in this study (Table 1). All of these fish were recorded in the historical survey conducted in Beijing (Du et al., 2019; Zhang et al., 2011). Dominant groups belonged to Cypriniformes, Cyprinidae, and Cobiaceae (Figure 3). Our result is consistent with that of Zhang et al., who showed that the relative abundance of Cypriniformes, Perciformes, Cyprinidae, and Cobitidae was 72.0, 11.8, 68.0, and 12.0%, respectively (Zhang et al., 2011), indicating that the dominant fish species in Beijing have not changed in the past decades.

There were 17 fish species obtained by both methods (Figure 2). Among them, *Pseudorasbora parva*, *Abbottina rivularis*, *Hemiculter leucisculus*, *Paramisgurnus dabryanus*, *Rhodeus ocellatus*, *Carassius auratus*, *Opsariichthys uncirostris*, and *Micropercops swinhonis* are common species in eastern China and are the dominant species investigated in research (Zhang and Zhao, 2013; Zhang et al., 2011). *Cobitis granoci* and *Rhynchocypris lagowskii* have adapted to the cold climate in the north. However, it is worth noting that *Odontobutis potamophila* is generally distributed in the middle and lower reaches of the Yangtze River. This species was not recorded in the historical survey results of *Beijing Fishes, Amphibians and Reptiles* (1994) until recently (Di et al.; Du et al., 2019). Such fish may have been introduced due to south-to-north water diversion or may have been released by citizens. *Carassius auratus*, *Opsariichthys uncirostris*, and other fish are economic species. However, *Pseudorasbora parva*, *Abbottina rivularis*, *Macropodus chinensis*, and other small non-economic fish species exist and also play an important role in the ecosystem and are an important food source for some birds (Chen et al., 2007).

4.2 Richness differences between Rivers

The results of the two methods showed that the fish abundance in the Chaobai River was higher than that in the Beiyun River (Table 1), and this was also shown in the study conducted by Du et al. (Du et al., 2019). The fundamental reason lies in the development of urbanization. Rapid urbanization leads to increased human activities, river pollution, and channel hardening, which have all contributed to the decline in the quality of fish's natural habitat (Hao and Sun, 2021; Walters et al., 2005; Zhang et al., 2011). The ecological health of the water is generally worse in the Beiyun River in the center of Beijing city (Hu et al.) compared with the Chaobai River with less human interference.

Obvious spatial differences in the fish community structure of the two rivers were also found. Apart from the interference of human activities, there is also the influence of natural factors such as water temperature and altitude (Kadye et al., 2008; Smith and Kraft, 2005). Differences in habitats lead to differences in community structure (Blabolil et al., 2021; Xie et al., 2021). The Beiyun River is dominated by *Pseudorasbora parva*, *Misgurnus anguillicaudatus*, and *Carassius auratus*, low-altitude pollution-tolerant species, while the Chaobai River is dominated by *Zacco platypus*, *Rhynchocypris lagowskii*, and *Opsariichthys uncirostris*, high-altitude low-temperature species. Moreover, the fish abundance of the samples in the Beiyun River was relatively uniform, and the difference in fish abundance among the samples in the Chaobai River was relatively large (Figure 7). It may be that the sample sites of the Chaobai River span a larger distance and the spatial heterogeneity is more prominent, resulting in obvious differences in diversity between sampling points. However, this does not affect the result that the fish richness of the Chaobai River was higher than that of the Beiyun River.

4.3 eDNA metabarcoding as a transformative tool for assessing fish diversity in rivers

Most of the traditional methods that have been used are harmful to fish, such as ultrasonic fish devices, hanging nets, and hand-cast nets (Chen et al., 2007; Du et al., 2019; Zhang and Zhao, 2013). eDNA metabarcoding has been shown in many studies (Pont et al., 2018; Qu et al., 2020; Yamamoto et al., 2017) to be feasible and efficient for monitoring fish diversity. In the present study, eDNA metabarcoding identified more fish species than ground cages (Table 1, Figure 3&5) in both of the water systems (Figure 6). eDNA can not only detect conventional species but can also easily identify species with relatively lower richness. For example, *Hypomesus olidus*, *Chanodichthys erythropterus*, and other fish species were detected by eDNA metabarcoding only. eDNA has been used for the detection of endangered and rare species (Harper et al., 2019; Laramie et al., 2015; Xu et al., 2018). Although eDNA failed to identify all fish species (such as *Squalidus wolterstorffi* and *Saurogobio dabryi* in this study), its high detection rate makes it well positioned to become a mainstream monitoring method. It also overcomes the limitations of traditional survey methods and reduces fish pressure and habitat destruction.

Many studies have compared eDNA results with those from traditional methods and have suggested a correlation between them (Evans et al., 2015; Ushio et al., 2018), but the specific relationship is not currently clear. In our study, the positive correlation between the results of the two methods was high in the Beiyun River but irregular in the Chaobai River. We can infer that this was determined by the characteristics of the river. Compared with lakes and reservoirs, rivers have temporal and spatial variability (Altermatt et al., 2020; Civade et al., 2016). In addition, DNA can persist for several days in water (Barnes et al., 2014), so the species detected

by eDNA metabarcoding may not originate from this habitat (the upper stream) (Shaw et al., 2016; Shogren et al., 2018; Zhao et al., 2021). However, the Chaobai is a mountainous river with high flow velocity. The Beiyun River is basically static in the urban area; the results detected by eDNA basically originated from the sampling points themselves and were highly correlated with those from traditional methods. Therefore, in the investigation of river fish diversity by eDNA metabarcoding, the characteristics of the river should also be considered, especially in the study of some river segments.

5. Conclusions

eDNA metabarcoding is a developing trend in aquatic biodiversity investigation because of its wide recognition ability. This study used eDNA metabarcoding and ground cages to investigate fish diversity in two river systems in Beijing. A total of 40 fish species were surveyed at 10 sites, and eDNA metabarcoding identified 38 species. The results showed that compared with the ground cage method, eDNA metabarcoding recorded higher fish abundance. The correlation between the detected relative abundance of the two methods was different in the two water systems due to the influence of river characteristics. These findings shown the feasibility of eDNA metabarcoding for the investigation of fish diversity in Beijing rivers and provide new reference data for the management and protection of Beijing river fish. The survey results also suggest that the diversity in mountain rivers is higher than that in urban rivers, indicating that urbanization reduces river fish diversity. The fish community structures of the two rivers were also different. Although eDNA metabarcoding cannot identify the biological characteristics of organisms, it is an important tool for aquatic biodiversity monitoring.

Declarations

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Author Contributions Statement

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References

1. Aglieri G, Baillie C, Mariani S, et al., 2020.Environmental DNA effectively captures functional diversity of coastal fish communities. *Molecular Ecology*.30,3127– 3139.
2. Almond R, Grooten M, Peterson T. Living Planet Report 2020-Bending the curve of biodiversity loss: World Wildlife Fund, 2020.
3. Altermatt F, Little CJ, Mächler E, et al., 2020.Uncovering the complete biodiversity structure in spatial networks: the example of riverine systems. *Oikos*.129,607-618.
4. Barnes MA, Turner CR, Jerde CL, et al., 2014.Environmental Conditions Influence eDNA Persistence in Aquatic Systems. *Environmental Science & Technology*.48,1819-1827.
5. Beverton R, 1991.Fish resources, threats and protection. *Netherlands Journal of Zoology*.42,139-175.
6. Blabolil P, Harper LR, Ricanova S, et al., 2021.Environmental DNA metabarcoding uncovers environmental correlates of fish communities in spatially heterogeneous freshwater habitats. *Ecological Indicators*.126.
7. Chen S, Zhou Y, Chen Y, et al., 2018.fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*.34,884-890.
8. Chen W, Hu D, Fu B. Research on Biodiversity of Beijing Wetland: Science Press, 2007.
9. Civade R, Dejean T, Valentini A, et al., 2016.Spatial representativeness of environmental DNA metabarcoding signal for fish biodiversity assessment in a natural freshwater system. *PloS one*.11,e0157366.
10. Deiner K, Walser J-C, Mächler E, et al., 2015.Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation*.183,53-63.
11. Di Y, Zhang L, Ye Z, et al.Distribution of fish communities and its influencing factors in the Nansha and Beijing sub-center reaches of the Beiyun River. *Acta Scientiae Circumstantiae*.41,156-163.
12. Du L, Xu J, Li Y, et al., 2019.Fish Community Characteristics and Spatial Pattern in Major Rivers of Beijing City. *Research of Environmental Sciences*.32,447-457.
13. Edgar RC, 2010.Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*.26,2460-2461.
14. Evans NT, Olds BP, Renshaw MA, et al., 2015.Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. *Molecular Ecology Resources*.16,29-41.
15. Fu M, Xiao N, Zhao Z, et al., 2016.Effects of Urbanization on Ecosystem Services in Beijing. *Research of Soil and Water Conservation*.23,235-239.
16. Gao W. Beijing Vertebrate Key: Beijing Publishing House, 1994.
17. Hao L, Sun G, 2021.Impacts of urbanization on watershed ecohydrological processes: progresses and perspectives. *Acta Ecologica Sinica* 41,13-26.
18. Harper LR, Griffiths NP, Lawson Handley L, et al., 2019.Development and application of environmental DNA surveillance for the threatened crucian carp (*Carassius carassius*). *Freshwater Biology*.64,93-107.

19. Hu X, Zuo D, Liu B, et al. Quantitative Analysis of the Correlation between Macrobenthos Community and Water Environmental Factors and Aquatic Ecosystem Health Assessment in the North Canal River Basin of Beijing. *Environmental Science*.1-12.
20. Jackson S, Head L, 2020. Australia's mass fish kills as a crisis of modern water: Understanding hydrosocial change in the Murray-Darling Basin. *Geoforum*.109,44-56.
21. Kadye WT, Magadza CHD, Moyo NAG, et al., 2008. Stream fish assemblages in relation to environmental factors on a montane plateau (Nyika Plateau, Malawi). *Environmental Biology of Fishes*.83,417-428.
22. Karr JR, 1981. Assessment of Biotic Integrity Using Fish Communities. *Fisheries*.6,21-27.
23. Laramie MB, Pilliod DS, Goldberg CS, 2015. Characterizing the distribution of an endangered salmonid using environmental DNA analysis. *Biological Conservation*.183,29-37.
24. Miralles L, Parrondo M, Hernandez de Rojas A, et al., 2019. Development and validation of eDNA markers for the detection of *Crepidula fornicata* in environmental samples. *Marine Pollution Bulletin*.146,827-830.
25. Miya M, Sato Y, Fukunaga T, et al., 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *Royal Society Open Science*.2,150088.
26. Pont D, Rocle M, Valentini A, et al., 2018. Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Scientific Reports*.8,10361.
27. Qu C, Stewart KA, Clemente-Carvalho R, et al., 2020. Comparing fish prey diversity for a critically endangered aquatic mammal in a reserve and the wild using eDNA metabarcoding. *Scientific Reports*.10,16715.
28. Rees HC, Bishop K, Middleditch DJ, et al., 2014a. The application of eDNA for monitoring of the Great Crested Newt in the UK. *Ecol Evol*.4,4023-4032.
29. Rees HC, Maddison BC, Middleditch DJ, et al., 2014b. REVIEW: The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*.51,1450-1459.
30. Sato Y, Miya M, Fukunaga T, et al., 2018. MitoFish and MiFish Pipeline: A Mitochondrial Genome Database of Fish with an Analysis Pipeline for Environmental DNA Metabarcoding. *Molecular Biology and Evolution*.35,1553-1555.
31. Shaw JLA, Clarke LJ, Wedderburn SD, et al., 2016. Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. *Biological Conservation*.197,131-138.
32. Shen M, Xiao N, Lu L, et al. Methods and Application of Environmental DNA Detection of Fish. *Journal of Hydroecology* 1-16.
33. Shen W, Ren H, 2021. TaxonKit: A practical and efficient NCBI taxonomy toolkit. *Journal of genetics and genomics*.48,844-850.
34. Shogren AJ, Tank JL, Egan SP, et al., 2018. Water Flow and Biofilm Cover Influence Environmental DNA Detection in Recirculating Streams. *Environmental Science & Technology*.52,8530-8537.
35. Smith TA, Kraft CE, 2005. Stream fish assemblages in relation to landscape position and local habitat variables. *Transactions of the American Fisheries Society*.134,430-440.

36. Stat M, John J, DiBattista JD, et al., 2019. Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Conservation Biology*.33,196-205.
37. Su G, Logez M, Xu J, et al., 2021. Human impacts on global freshwater fish biodiversity. *Science*.371,835-838.
38. Takahara T, Minamoto T, Yamanaka H, et al., 2012. Estimation of Fish Biomass Using Environmental DNA. *Plos One*.7,e35868.
39. Thomsen PF, Kielgast JOS, Iversen LL, et al., 2012. Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*.21,2565-2573.
40. Ushio M, Sakurai S, Yamanaka H, et al., 2018. Quantitative monitoring of multispecies fish environmental DNA using high-throughput sequencing. *Metabarcoding and Metagenomics*.2,e2329.
41. Walters DM, Freeman MC, Leigh DS, et al. Urbanization effects on fishes and habitat quality in a southern Piedmont River basin. *Effects of Urbanization on Stream Ecosystems*. 47, 2005, pp. 69-85.
42. Wang H. *Beijing Fish Records*: Beijing Publishing House, 1984.
43. Wang H. *Beijing Fish and Amphibians and Reptiles*: Beijing Publishing House, 1994.
44. Xiao N, Gao X, Li J, et al. *Evaluation and Conservation Measures of Beijing Biodiversity*: China Forestry Publishing House, 2018.
45. Xie R, Zhao G, Yang J, et al., 2021. eDNA metabarcoding revealed differential structures of aquatic communities in a dynamic freshwater ecosystem shaped by habitat heterogeneity. *Environmental research*.201,111602.
46. Xu N, Zhu B, Shi F, et al., 2018. Monitoring seasonal distribution of an endangered anadromous sturgeon in a large river using environmental DNA. *Science of Nature*.105,62.
47. Yamamoto S, Masuda R, Sato Y, et al., 2017. Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Scientific Reports*.7,40368.
48. Yan B, Xiao N, Qi Y, et al., 2016. Effects of Urban Development on Soil Microbial Functional Diversity in Beijing. *Research of Environmental Sciences*.29,1325-1335.
49. Zhang C, Zhao Y. *Fishes in Beijing and adjacent areas, China*: Science Press, 2013.
50. Zhang C, Zhao Y, Xing Y, et al., 2011. Fish species diversity and conservation in Beijing and adjacent areas. *Biodiversity Science*.19,597-604.
51. Zhang J, Kobert K, Flouri T, et al., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*.30,614-620.
52. Zhao B, van Bodegom PM, Trimbos K, 2021. The particle size distribution of environmental DNA varies with species and degradation. *The Science of the total environment*.797,149175.
53. Zou K, Chen J, Ruan H, et al., 2020. eDNA metabarcoding as a promising conservation tool for monitoring fish diversity in a coastal wetland of the Pearl River Estuary compared to bottom trawling. *Sci Total Environ*.702,134704.

Figures

Figure 1

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Figure 2

Number of fish species identified by eDNA (eDNA metabarcoding) metabarcoding and ground cages (17 taxa were detected by both methods, 21 taxa were detected only by eDNA, and two taxa were detected only by ground cages).

Figure 3

Relative abundance of fish at the order level detected by the two methods and relative abundance of occurrence at the family level corresponding to Cypriniformes.

Figure 4

Richness detected at each site using eDNA metabarcoding (red) or ground cage fishing (blue). Differences between the methods were determined using t-tests (number of species followed a normal distribution), Chaobai River $P > 0.05$, Beiyun River $P < 0.05$.

Figure 5

Relative abundance (%) of fish detected at each sampling point at the genus level by (a) eDNA metabarcoding and (b) ground cages.

Figure 6

Correlation between the relative abundance of fish detected by eDNA metabarcoding and captured by ground cages.

Figure 7

Community variation based on Bray–Curtis dissimilar non-metric multiscale (NMDS) sorting: the left panel is based on the results of eDNA metabarcoding, the right panel is based on ground cage data.