Environmental DNA metabarcoding of fish communities in a small hydropower dam reservoir: a comparison between the eDNA approach and established fishing methods

Hai Li, Fang Yang, Ran Zhang, Shigang Liu, Zhijin Yang, Longshan Lin and Sunzhong Ye

ABSTRACT
Environmental DNA methods have emerged as a promising tool in fish diversity studies and fishery management in various aquatic ecosystems. However, eDNA metabarcoding of fish communities in small hydropower dam reservoirs has received limited attention. In this study, we tested whether eDNA metabarcoding was an appropriate approach for the characterization of fish communities and fishery stock assessment by comparing its results with combined capture-based fishing methods and hydroacoustic surveys, which involved species detection and abundance/biomass evaluation. Our results indicated that the species detection performance of eDNA metabarcoding was basically similar to that of traditional capture-based fishing gear. However, it was also noteworthy that the eDNA method failed to cover all species detected by capture-based method, although some additional species were found. Besides, although we observed a significant correlation between site occurrence and sequence abundance for fishes, an effort to quantitatively establish a correlation between eDNA sequence counts and fish abundance/biomass was unsuccessful. Therefore, our study suggested that eDNA metabarcoding was an important supplementary tool to traditional capture-based fishing methods for the investigation and biomonitoring of fish diversity in small hydropower dam reservoirs. Further studies on the mechanisms of eDNA production, persistence, transportation and degradation in reservoirs might facilitate the interpretation of fish abundance and biomass from eDNA data.

CONTACT
Longshan Lin linlsh@tio.org.cn Laboratory of Marine Biodiversity Research, Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, China; Sunzhong Ye ysz1@sina.com Key Laboratory of Cultivation and High-value Utilization of Marine Organisms in Fujian Province, Fisheries Research Institute of Fujian, Xiamen, China

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1. Introduction

Freshwater habitats constitute only approximately 0.8% of the global surface and host disproportionately high biodiversity with one-third of the recorded vertebrates, including approximately 40% of fish species, inhabiting freshwater environments (Nelson et al. 2016). However, freshwater biodiversity is also experiencing a rapid decline at high rates worldwide, and this loss is even higher than that in marine and terrestrial ecosystems, which is thought to have considerable impacts on not only freshwater ecosystem services and health (WWF 2014) but also the fishery industry and public (Zhang et al. 2020). It is widely acknowledged that biodiversity loss is closely related to a variety of anthropogenic factors (Barnosky et al. 2011). Among all the threats to freshwater biodiversity caused by human activities, damming is thought to be one of the major drivers of change (Consuegra et al. 2021, Dudgeon et al. 2006, Pelicice et al. 2017, Wang et al. 2021). Dams contribute to factors with direct economic benefits, such as farmland irrigation, energy supply, flood control and reservoir fisheries (Grill et al. 2019), but affect freshwater ecosystems by inducing alteration of local hydrology (Timpe and Kaplan 2017), deterioration of water quality (Thompson et al. 2020), degradation of ecological connectivity (Kuriqi et al. 2021), loss of biological diversity (Granzotti et al. 2018), and habitat fragmentation (Barbarossa et al. 2020) of freshwater species. Among all aquatic organisms, the habitat fragmentation impact caused by damming may be most significant for freshwater fishes, as dams block their migration routes and limit their dispersal capacity and habitat connectivity (Benejam et al. 2016, Carvajal-Quintero et al. 2017, Puijenbroek et al. 2019), affecting the composition of fish communities and fish stock; thus, freshwater fish biodiversity is being threatened by an increasing number of hydropower facilities.

Characterizing the species composition and occurrence of fish taxa is an integral component of both biodiversity conservation and fishery stock assessment in freshwater ecosystems. Traditional fish diversity monitoring and fishery assessment in freshwater environments rely mainly on capture-based approaches, such as gillnets and electrofishing, which either require a large effort or are invasive to targeted aquatic organisms. In addition, the identification of fish samples requires well-trained and highly experienced taxonomic experts (Nelson et al. 2016), who are becoming increasingly sparse in our era. The application of environmental DNA (eDNA) may provide an alternative solution to such scenarios in aquatic ecosystems. eDNA refers to the genetic material derived from organisms in the form of shed cells, decaying tissue, excreta, gametes, saliva, or other secretions (Hänfling et al. 2016, Sassoubre et al. 2016). The combination of versatile polymerase chain reaction (PCR) primers for fish species, such as MiFish (Miya et al. 2015), and high-throughput parallel DNA sequencing (HTS) has led to the development of a new method called eDNA metabarcoding, which has shown great potential in examining fish community composition in various artificial conditions (Kelly et al. 2014, Miya et al. 2015) and natural settings (Fujii et al. 2019, Li et al. 2019, Valentini et al. 2016, Zhang et al. 2020) and occasionally in assessing fish abundance and biomass (Hänfling et al. 2016, Takahara et al. 2012, Thomas et al. 2016). These eDNA metabarcoding-based analyses showed that the new method has the potential to be comparable or even superior to traditional techniques for characterizing fish diversity. Therefore, it might hold great promise as a useful way to evaluate the effects of hydropower dam reservoir construction on local fish diversity and fishery resources. Nevertheless, the performance of eDNA metabarcoding should be validated by testing and comparing its effectiveness with traditional approaches before this method is developed into a practical tool (Thomsen et al. 2012; Shaw et al. 2016) and applied in reservoirs associated with hydropower dam construction.
In this study, we chose a small reservoir associated with stream hydropower dam construction to validate the suitability of eDNA metabarcoding for assessing fish diversity and abundance/biomass in small reservoir configurations. The selected reservoir—Lantian Reservoir—is located in Anxi County, Fujian Province, China. It supports local commercial freshwater aquaculture by implementing both cage aquaculture and free aquaculture, with cultures of the main commercial fish species in southern China. Thus, the majority of fish species in the reservoir are known to us after inquiring local people and fish farmers. In addition, although the reservoir is quite small and a relatively species-poor system, its elongated main section stretches from west to east, forming many fjord-like bays along the shore and creating many fine-scale heterogeneous microenvironments, which may lead to distinct species occurrences. Moreover, the water flow upstream, midstream and downstream may maintain or enhance the connectivity between different fish communities. Therefore, the chosen reservoir is suitable for testing the performance of eDNA metabarcoding in a small reservoir associated with stream hydropower dam construction. Here, we examined the occurrence and abundance of fish species in a small hydropower dam reservoir by employing eDNA metabarcoding along with conventional gillnets, traps and additional hydroacoustic surveys. The objectives of our study were to (1) reveal the composition and occurrence of fish communities in reservoirs; (2) validate the suitability of eDNA metabarcoding under a small hydropower dam reservoir scenario; and (3) determine the role of eDNA metabarcoding in assessing the impacts of stream hydropower dam construction on local fish communities and in fishery stock assessments.

2. Material and methods

2.1. Study area

A field survey and sampling were conducted in the water area of the Lantian Reservoir. The reservoir is located in the upstream basin of Changkeng Stream, Lantian County, Fujian Province, China. Its geographical location range is approximately 25° 09’ 28.86” N – 25° 08’ 42.62” N and 117° 51’ 40.45” E – 117° 53’ 55.15” E. At normal water levels of 592 m above sea level at altitude, the surface area of the reservoir region is 1.11 km², with a water storage capacity of 1.30 × 10⁷ m³. The hydropower dam of the Lantian reservoir is 42 m high, its crest length is 162 m, and the maximum water flow capacity is 12.02 m³/s. Dam project mainly focus on power generation while considering irrigation benefits. There are two turbines installed inside the hydropower dam, with a total power capacity of 25,000 (2 × 12,500) kW. Construction of the dam began in 1993, and it commenced generating power in 1995; the annual average power generation was 9.63 × 10⁷ kW-h. The geographic features in this reservoir region are typical narrow valleys. The climate in this region can be divided into four seasons: spring, summer, fall and winter. The trophic status of reservoir is classified as eutrophic (Editorial Committee 1998). The fish community of the reservoir is represented mainly by cyprinids (family Cyprinidae), with black carp Mylopharyngodon piceus, grass carp Ctenopharyngodon idella, silver carp Hypophthalmichthys molitrix, bighead carp Hypophthalmichthys nobilis, common carp Cyprinus carpio and golden carp Carassius auratus being the most abundant species.

2.2. Gill netting, trapping and hydroacoustic surveys

To establish a comprehensive reference background of species composition and stock status, we carried out gill netting, trapping, and hydroacoustic surveys on June 12–13, 2018.
Sampling in summer was chosen because fish distribution and habitat utilization were unlikely to be affected by spawning, and the feeding activity of fish was expected to be high (Vašek et al. 2009).

Fish were sampled by gill netting and trapping to capture fishes inhabiting deep water and nearshore areas, respectively. Gillnets and traps were made with distinct lengths, heights and mesh sizes (Table 1), which could capture fish groups with different body sizes. Three gillnet series containing 2 type I gillnets, 2 type II gillnets and 2 type III gillnets were deployed at sampling site A (25° 08' 53.11" N, 117°51' 40.87" E) downstream and sampling site B (25° 09' 17.64" N, 117°51' 56.09" E) upstream. The gillnets were deployed in a straight line roughly parallel to the shoreline over maximum depths (maximum depth approximately of 11 m upstream and 16 m downstream). The three gillnet series all were fished simultaneously for a total of 2 hours, anchoring on 8:00 p.m., June 12, 2018 and retrieving on 10:00 p.m., June 12, 2018. Two trap series containing 2 type I traps and 2 type II traps were deployed in the littoral zone of sampling site C (25° 09' 16.68" N, 117°51' 52.37" E) upstream, exposed to a 1.6 m depth for a total of 8 hours, from 10:30 p.m., June 12, 2018 to 6:30 p.m., June 13, 2018 (Figure 1). Fish catches were all removed from gillnets and traps after final exposition, and all fish samples were sorted by species and then transported to the laboratory on ice, where species identifications were confirmed by morphological examination. Information on the composition of fish species in the surveyed water was obtained by collections made with gillnets and trapping, and this information was used to assist in determining the identification and distribution of fishery resources from acoustic images.

Acoustic data were collected continuously between 8 p.m. and 11 p.m. on June 12, 2018, using a Simrad EK80 scientific echosounder (Kongsberg Maritime Inc., Kongsberg, Norway), with 120 and 200 kHz split-beam transducers mounted on the survey vessel. The acoustic equipment was calibrated following the standard procedure given in the Simrad ER80 reference manual before the cruise. Detailed echosounder settings are given in Table 2. Acoustic data were analyzed using Echoview 8.0 (Myriax Software Pty Ltd., Battery Point, TAS, Australia). The depth of analysis was set from 1.2 m below the transducer, to shield interference of navigation noise, to 0.25 m above the bottom, to eliminate the interference of echo signals from the bottom. The elemental distance sampling unit (EDSU) was set to 50 m. The fish species composition was considered the basis of the distribution for echo integration. The volume backscattering strength (Sv) threshold and target strength (TS) threshold were −70 dB and −66 dB, respectively. We quantified the echo integrator on the basis of the fish catch and estimated \( \rho_{i,a} \) the density of the \( i \)th species (inds/km²), and \( \rho_{i,b} \) the stock density of the \( i \)th species (kg/km²), by the \( \rho \) formula:

\[
\rho_{i,a} = C_i \times \frac{(NASC)}{(4 \times \pi \times \bar{\sigma} \times 1.852 \times 1.852)}
\]

\[
\rho_{i,b} = C_i \times \frac{(NASC)}{(4 \times \pi \times \bar{\sigma} \times 1.852 \times 1.852)}
\]

where \( C_i \) (%) is the percentage of the \( i \)th species caught in the surveyed water, NASC is the nautical area scattering coefficient in m²/nmi², \( \bar{\sigma} \) is the mean backscattering cross

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**Table 1. Information of capture-based fishing gears and associated samplings.**

| Sampling gear | Type I gillnet | Type II gillnet | Type III gillnet | Type I trap | Type II trap |
|---------------|----------------|----------------|-----------------|-------------|-------------|
| Length/m      | 150            | 150            | 150             | 10          | 10          |
| Height/m      | 15             | 10             | 10              | 0.4         | 0.4         |
| Mesh size/cm  | 14             | 14             | 8               | 2           | 1           |
| Number of net | 2              | 2              | 2               | 2           | 8           |
| Sampling time/h | 2              | 2              | 2               | 8           | 8           |
section in m², 1,852² is the conversion coefficient between nm² and m², and \( \bullet w_i \) is the mean body weight of the \( i \)th species in kg.

\[
\bar{\sigma} = \sum_{i=1}^{n} C_i 10^{\frac{z_i}{10}} \quad (3)
\]

\[
TS_i = 20 \times \log TL_i - 71.9 \quad (4)
\]

where \( n \) is the number of species involved in hydroacoustic estimation, \( TS_i \) is the TS of the \( i \)th species estimated in dB, and \( TL_i \) is the mean body length of the \( i \)th species estimated in cm.

### 2.3. Sampling sites and sample collection

Water samples were collected along the midline of the main water body of the reservoir by boat on 2:30 p.m. to 5:30 p.m. June 12, 2018, and 8:30 a.m. to 10:30 a.m. June 13, 2018. Ten sampling sites were set at intervals of 300–600 m, covering all sections of the reservoir (Figure 1 & Table 3). At each sampling site, three 0.8 L replicate water samples were collected in both the pelagic layer and bottom layer (except for sampling site 1, where the water depth was as shallow as approximately 2 m and only water sample of pelagic layer were collected) by submerging a Model 1010 2.5 L Niskin water sampler.
Finally, nineteen water samples were collected by hands, and the researchers wore sterile latex gloves to avoid direct contact with the water samples. Accurate sampling locations were recorded by a handheld global positioning system (GPS) device. In addition, a bottle of double-distilled H₂O (ddH₂O) was placed on ice and brought to each sampling site to serve as a sampling blank to check contamination during the on-site survey.

### 2.4. Edna extraction, amplification and sequencing

Each 0.8 L replicate water sample was filtered through a 0.45 μm Millipore Cellulose Esters Membrane Filter (RAWP04700; Merck Millipore) with a vacuum pump. Before filtration and between the water sample replicates, forceps and the inner surface of all the filtration units were treated with 10% bleach solution for 10 min to remove any traces of exogenous DNA and were then rinsed with ddH₂O three times to remove residual bleach. In addition, three blanks (0.8 L ddH₂O) were filtered alongside the field samples to control for potential contamination. All filters were stored individually in sterile 15 mL microcentrifuge tubes at −80°C until eDNA extraction.

eDNA extractions were conducted in a dedicated pre-PCR laboratory, where all facilities and instruments were routinely decontaminated with UV and bleach sterilization. Each filter triplicate that represented distinct water samples was cut into pieces as small as possible using sterile scissors and sonicated for 30 s at 60 Hz with sterile stainless steel beads using a tissuelyser (Tissuelyser-24; Shanghai Jingxin Experimental Technology). eDNA extraction was performed using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer’s instructions with slight modifications. A clean filter was also added to each batch of extractions to control for possible contamination during eDNA extraction. Elution of each final eDNA extract was performed in 200 mL of AE buffer. eDNA concentration was measured with the Quant-iT dsDNA HS Assay Kit and a Qubit 2.0 Fluorometer (Life Technologies, California, USA) and detected with a 1.5% agarose gel.

eDNA from all the water samples and blanks was subjected to PCR amplification with a MiFish-U primer pair targeting a hypervariable region (163–185 bp) of the mitochondrial 12S rRNA gene (Miya et al. 2015). PCR amplifications were carried out in a total volume of 25 μL, containing 5 μL eDNA extract diluted 10-fold, 0.2 μm each of forward and reverse primers, 0.4 μg/μL bovine serum albumin, and 1× Premix Ex Taq (Takara). The thermal cycle profile consisted of an initial 3 min denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 15 s, and extension at 72°C for 15 s, with a final extension at 72°C for 5 min. All the blanks and negative controls during sampling, filtration, extraction, and PCR were subjected to PCR amplification in triplicate along with the samples. A uniquely tagged primer pair was used for each

| Station | Longitude (°/E) | Latitude (°/N) | Date       | Time | Depth (Pelagic layer) (m) | Depth (Bottom layer) (m) |
|---------|----------------|---------------|------------|------|--------------------------|--------------------------|
| S1      | 117.8604       | 25.1564       | 2018/06/12 | 15:48| 1                         | —                        |
| S2      | 117.8632       | 25.1572       | 2018/06/12 | 16:04| 1                         | 8                        |
| S3      | 117.8670       | 25.1573       | 2018/06/12 | 16:22| 1                         | 10                       |
| S4      | 117.8747       | 25.1559       | 2018/06/12 | 16:43| 1                         | 10                       |
| S5      | 117.8787       | 25.1565       | 2018/06/12 | 17:01| 1                         | 10                       |
| S6      | 117.8837       | 25.1584       | 2018/06/12 | 17:18| 1                         | 15                       |
| S7      | 117.8703       | 25.1566       | 2018/06/13 | 8:58 | 1                         | 10                       |
| S8      | 117.8860       | 25.1556       | 2018/06/13 | 9:30 | 1                         | 15                       |
| S9      | 117.8883       | 25.1528       | 2018/06/13 | 10:00| 1                         | 15                       |
| S10     | 117.8908       | 25.1506       | 2018/06/13 | 10:20| 1                         | 10                       |
PCR. Replicate PCR products were combined and purified using an EasyPure PCR Purification Kit (TransGen Biotech) following the manufacturer’s protocols and used as templates for the generation of 12 × 8 dual-indexed amplicons in the second PCR, following the “16S Metagenomic Sequence Library Preparation” protocol (Illumina, California, USA) using the Nextera XT Index Kit (Illumina, California, USA).

Multiplexed PCR products were purified using a QIAquick Gel Extraction Kit, quantified by a Quant-iT dsDNA HS Assay Kit using a Qubit 2.0 Fluorometer (Life Technologies, California, USA). All the PCR products were subsequently pooled in equal amounts and the pooled library was checked for size and concentration using an Agilent 2100 bioanalyzer (Agilent Technologies, California, USA). The pooled, quantified library was adjusted to 4 nM and denatured following the Illumina MiSeq library denaturation and dilution guide. Sequencing was performed with the Illumina MiSeq platform (Illumina, California, USA) using the 2 × 300 paired-end protocol, and samples were demultiplexed based on barcode sequences. The raw sequences were deposited in SRA repository with the accession number PRJNA748176.

2.5. Bioinformatic analyses and sequence filtering

The overall quality of raw demultiplexed reads was verified with FastQC (Andrews 2013). Both the forward and reverse primers were removed. A maximum error rate of 20% was allowed for reads, while read pairs that did not contain primer pair sequences were discarded. Reads longer than 30 nucleotides were retained. Adapters and low-quality reads were removed to obtain clean reads from the raw Illumina sequencing data. Paired-end reads were merged with a minimum overlap of 10 nucleotides using FLASH (v1.2.7). Tags with an average Phred score lower than 10 were further filtered to eliminate chimeras using Trimmomatic (v 0.33). Effective tags were then clustered into operational taxonomic units (OTUs) using UCLUST (v 4.2) at a 99% similarity threshold. All the effective tags were mapped to the nonredundant nucleotide (NT) database downloaded from the GenBank database by using the Blastn tool. A query sequence was designated as belonging to a species if there was ≥ 99% sequence identity to the reference database barcode across the entire length of the amplicon, if a sequence from at least one other species within the same genus was available for comparison (and < 99% identical), and if the distribution of the species matched the published records or occurrence data we obtained by surveying local residents. As sequences displaying high identity (≥ 99%) to GenBank sequences were most likely to allow accurate species-level assignments, we applied this filter for taxonomic assignments. The assigned taxa were considered OTUs, and only fish OTUs were included in subsequent analyses.

2.6. Statistical analyses

Analyses were performed mainly in R v3.6.1 with the package vegan v2.3–5 (Oksanen et al. 2016). A species accumulation curve was constructed to evaluate the effect of the number of samples on the number of eDNA-detected fish OTUs using the function specaccum within the R package vegan. eDNA samples were categorized into two types according to the sampling depth, while fishing samples were grouped by sampling gear. The taxonomic composition of fish species and their relative abundances revealed by eDNA and established survey methods were exhibited by drawing species bar plots.

Correlations between the site occurrence and relative sequence abundances (natural logarithm [ln]-transformed) for all the detected fish species were assessed using Pearson’s
product-moment test, while correlations between the site occurrence rank and relative sequence abundances (natural logarithm [ln]-transformed) were assessed using Spearman’s rho test.

We compared the performance of the eDNA approach and established fishing survey methods in two ways. First, a Venn diagram was drawn to demonstrate the differences in the number of fish species identified by the eDNA and capture-based methods. Second, overall correlations between the log-transformed values of the number of reads obtained and the number of fish or biomass caught per species were explored with Pearson correlation coefficients, using a t test to determine whether the correlation coefficients were significantly different from zero; this analysis was performed in the R package Stats v0.1.0.

Fish species involved in the analyses were those detected by eDNA metabarcoding and (a) gillnet types I & II, (b) gillnet type III, (c) trap types I & II, (d) total fish catch, and (e) acoustic data.

To investigate the species-specific spatial pattern of fish communities revealed by eDNA metabarcoding, variation in the relative abundance of each fish species was shown by a heatmap using the function pheatmap within the R package pheatmap. Nonmetric multidimensional scaling (NMDS) was also implemented to visually compare the fish community dissimilarity among the eDNA samples using the R package vegan.

3. Results

3.1. NGS data analyses and overall taxonomic composition

Illumina sequencing runs generated a total of 1.59 Gb raw data, from which approximately 7.6 million paired-end reads were obtained. A total of 1.5 million clean tags were retained for downstream analyses after a quality check and filtering. A total of 77,460 clean tags were assigned to each sample, with 79,548 clean tags per sample on average (Table 4). Negative control PCRs generated few or no reads, suggesting that insignificant contamination was observed during library preparation and Illumina sequencing. Moreover, the species accumulation curve indicated that fish OTUs increased rapidly and

| Sample ID | Pair-ended reads | Raw tags | Clean tags | Effective tags | Average length (bp) | GC (%) | Q20 (%) | Q30 (%) | Effective (%) |
|-----------|------------------|----------|------------|----------------|---------------------|--------|---------|---------|---------------|
| S01P      | 399,322          | 345,841  | 80,809     | 323,237        | 186                 | 48.27  | 99.22   | 97.87   | 80.95         |
| S02D      | 399,371          | 342,880  | 80,187     | 320,749        | 188                 | 49.52  | 99.47   | 98.47   | 80.31         |
| S02P      | 400,141          | 342,186  | 79,964     | 319,857        | 183                 | 47.64  | 99.57   | 98.69   | 79.94         |
| S03D      | 400,546          | 334,388  | 77,460     | 309,840        | 200                 | 50.20  | 99.38   | 98.28   | 77.35         |
| S03P      | 399,581          | 337,160  | 78,744     | 319,979        | 190                 | 49.33  | 99.51   | 98.59   | 78.83         |
| S04D      | 398,539          | 345,570  | 81,308     | 325,233        | 181                 | 49.92  | 99.23   | 97.85   | 81.61         |
| S04P      | 400,109          | 341,962  | 79,470     | 317,883        | 202                 | 50.97  | 99.30   | 98.07   | 79.45         |
| S05D      | 400,626          | 340,455  | 79,363     | 317,455        | 211                 | 48.55  | 99.25   | 97.99   | 79.24         |
| S05P      | 400,089          | 334,908  | 78,319     | 313,278        | 180                 | 49.84  | 99.63   | 98.88   | 78.30         |
| S06D      | 400,193          | 336,464  | 77,524     | 310,099        | 193                 | 49.36  | 99.42   | 98.35   | 77.49         |
| S06P      | 400,285          | 340,328  | 79,254     | 317,017        | 181                 | 49.90  | 99.65   | 98.93   | 79.20         |
| S07D      | 400,057          | 342,736  | 79,766     | 319,064        | 216                 | 50.47  | 99.18   | 97.79   | 79.75         |
| S07P      | 400,473          | 341,998  | 79,869     | 319,476        | 181                 | 49.52  | 99.60   | 98.80   | 79.77         |
| S08D      | 399,957          | 339,240  | 79,200     | 316,803        | 188                 | 49.95  | 99.54   | 98.65   | 79.21         |
| S08P      | 400,337          | 344,744  | 80,346     | 321,387        | 196                 | 48.79  | 99.45   | 98.46   | 80.28         |
| S09D      | 399,888          | 350,157  | 78,739     | 314,959        | 226                 | 52.12  | 99.07   | 97.53   | 78.76         |
| S09P      | 399,888          | 357,810  | 81,471     | 325,886        | 245                 | 53.84  | 98.95   | 97.22   | 81.49         |
| S10D      | 400,172          | 353,944  | 81,336     | 325,345        | 228                 | 51.58  | 99.10   | 97.62   | 81.30         |
| S10P      | 399,894          | 336,603  | 78,283     | 313,135        | 199                 | 48.69  | 99.40   | 98.32   | 78.30         |

Table 4. Statistic information of Illumina sequencing of environmental DNA samples.
reached an asymptote after approximately eight samples (Figure 2). Therefore, our sequence results reliably reflected the amplification of eDNA in each sample.

A total of 24 fish OTUs were recovered from the dataset, and all the OTUs were successfully assigned at the species level. Among these OTUs was one that was assigned to *Oncorhynchus masou*, which is a nonnative cold-water species. Thus, this OTU was excluded from further analyses. In addition, OTUs assigned as identical species were merged in subsequent biodiversity analyses. In total, eDNA metabarcoding from 19 samples identified 14 species that belonged to 13 genera, 4 families, and 3 orders, with Cyprinidae and Cypriniformes being the most common family and order, respectively (Figure 3 & Table 5).

### 3.2. Comparison with established fishing methods

Fish catches from type I & II gillnets included 8 species, among which *H. nobilis* had the highest abundance and weight; fish catches from type III gillnets included 10 species, among which *O. niloticus* contributed the greatest abundance and weight; and fish catches from type I & II traps included five species, among which *Hemiculter leuciscus* was the predominant species in terms of both abundance and weight. The occurrence of all these fish species is well known in local watersheds. Due to the differences in the type, mesh size and deployment time of sampling gear, the composition of fish stocks in the surveyed waters was standardized according to the abundance and biomass of individuals in the per-unit cross-sectional area (m²) and per-unit period of time (h) of each sampling site.

![Sample-based species accumulation curve of fish OTU recovered by eDNA metabarcoding.](image)
The stock abundance and stock biomass per sampling unit were then estimated. *H. leuciscus*, *Rhinogobius giurinus*, *O. niloticus*, *Chanodichthys ilishaeformis* and *H. nobilis* were the most abundant species in terms of abundance, while *H. nobilis*, *O. niloticus*, *H. molitrix*, *C. idella* and *C. carpio* were the dominant species in terms of biomass (Table 6). When the datasets derived from eDNA metabarcoding and capture-based fishing methods were combined, 19 fish species from 18 genera, 7 families and 4 orders were identified, among which 11 species were detected by both eDNA metabarcoding and established fishing methods, while 3 and 5 species were recovered by eDNA metabarcoding and established fishing methods alone, respectively (Figures 3 and 4). The species with the highest abundance and greatest biomass were all characterized by eDNA metabarcoding.
An overall correlation was not found between the number of sequencing reads per species and the corresponding fish abundance or fish biomass across the various capture-based fishing methods, total fish catch, and acoustic data; however, a significant and robust positive correlation was found between the number of sequencing reads and fish abundance in trap types I & II ($R^2 = 0.94$, $p < 0.01$) (Figure 5).

### 3.3. Correlations between site occurrence and sequence abundances of fish species

The actual values of species occurrence and relative sequence abundances (ln-transformed) of the eDNA-detected fish species displayed generally positive and extremely significant correlations (Pearson’s product moment tests, $p < 0.01$). The ranks of species occurrences and of relative sequence abundances of the fish species also exhibited generally positive and extremely significant correlations (Spearman’s rho tests, $p < 0.01$) (Figure 6). *C. idella*, *H. nobilis*, *H. leucisculus*, and *H. molitrix* were among the most dominant fish taxa in all the samples, both by sequence abundance and site occurrence.

### 3.4. Spatial patterns of fish communities

Fish communities seemed to be distinct between sampling layers, as revealed by the NMDS ordination plot (Figure 7). Moreover, a heatmap of the relative sequence abundances of individual fish taxa exhibited a clear vertical pattern of fish communities between the pelagic layer and bottom layer for some fish species, such as *C. carpio*, *M. piceus*, *C. idella* and *H. nobilis*. Most fish taxa showed a high relative abundance in a minority of samples and an even distribution in the majority of samples, indicating a horizontal heterogeneous pattern of fish communities (Figure 8).

### 4. Discussion

#### 4.1. Comparison of eDNA metabarcoding and established fishing methods for species detection

Many comparative studies between eDNA metabarcoding and established fishing methods have been conducted in various aquatic habitats, such as aquariums (Chen et al. 2021, Evans et al. 2016, Kelly et al. 2014), streams (Bagley et al. 2019, Cantera et al. 2019), rivers
Table 6. Statistic information of fish catch by established fishing methods.

| Scientific name       | Genus       | Family     | Order     | Type I & II gillnet | Type III gillnet | Type I & II trap | Total catch | Hydroacoustic survey |
|-----------------------|-------------|------------|-----------|---------------------|------------------|------------------|-------------|----------------------|
|                       |             |            |           | biomass (kg) | abundance (ind.) | biomass (kg) | abundance (ind.) | biomass (kg) | abundance (ind.) | biomass (kg) | abundance (ind.) |
| *Carassius auratus*   | *Carassius* | *Cyprinidae* | *Cypriniformes* | 0           | 0                | 17.48       | 54                | 0            | 0                | 17.48       | 54                |
|                       |             |            |           | 0           | 0                | 17.48       | 54                | 0            | 0                | 17.48       | 54                |
| *Chanodichthys erythropterus* | *Chanodichthys* |            |           | 0           | 0                | 1.71        | 13                | 394.8        | 14               | 396.51      | 27               |
|                       |             |            |           | 0           | 0                | 1.71        | 13                | 394.8        | 14               | 396.51      | 27               |
| *Cinnthus mitgala*    | *Cinnthus*  |            |           | 9.28        | 3                | 4.96        | 3                 | 0            | 0                | 14.24       | 6                |
|                       |             |            |           | 9.28        | 3                | 4.96        | 3                 | 0            | 0                | 14.24       | 6                |
| *Ctenopharyngodon idella* | *Ctenopharyngodon* |            |           | 7.82        | 3                | 35.46       | 12                | 0            | 0                | 43.28       | 15               |
|                       |             |            |           | 7.82        | 3                | 35.46       | 12                | 0            | 0                | 43.28       | 15               |
| *Cyprinus carpio*     | *Cyprinus*  |            |           | 0           | 0                | 28.78       | 16                | 0            | 0                | 28.78       | 16               |
|                       |             |            |           | 0           | 0                | 28.78       | 16                | 0            | 0                | 28.78       | 16               |
| *Hemiculter leucisculus* | *Hemiculter* |            |           | 0           | 0                | 0           | 0                 | 745.2        | 36               | 745.2       | 36               |
|                       |             |            |           | 0           | 0                | 0           | 0                 | 745.2        | 36               | 745.2       | 36               |
| *Hypophthalmichthys molitrix* | *Hypophthalmichthys* |            |           | 24.5        | 7                | 19.12       | 29                | 0            | 0                | 43.62       | 36               |
|                       |             |            |           | 24.5        | 7                | 19.12       | 29                | 0            | 0                | 43.62       | 36               |
| *Hypophthalmichthys nobilis* | *Hypophthalmichthys* |            |           | 501         | 147              | 16.32       | 16                | 0            | 0                | 517.32      | 163              |
|                       |             |            |           | 501         | 147              | 16.32       | 16                | 0            | 0                | 517.32      | 163              |
| *Mylopharyngodon piceus* | *Mylopharyngodon* |            |           | 0           | 0                | 1.54        | 1                 | 0            | 0                | 1.54        | 1                |
|                       |             |            |           | 0           | 0                | 1.54        | 1                 | 0            | 0                | 1.54        | 1                |
| *Pseudorasbora parva* | *Pseudorasbora* |            |           | 0           | 0                | 0           | 0                 | 100.7        | 9                | 100.7       | 9                |
|                       |             |            |           | 0           | 0                | 0           | 0                 | 100.7        | 9                | 100.7       | 9                |
| *Spinibarbus holandi* | *Spinibarbus* |            |           | 3.44        | 4                | 0           | 0                 | 3.44         | 4                | 3.44        | 4                |
|                       |             |            |           | 3.44        | 4                | 0           | 0                 | 3.44         | 4                | 3.44        | 4                |
| *Clarias fuscus*      | *Clarias*   | *Clariidae* | *Siluriformes* | 0           | 0                | 8.68        | 2                 | 0            | 0                | 8.68        | 2                |
|                       |             |            |           | 0           | 0                | 8.68        | 2                 | 0            | 0                | 8.68        | 2                |
| *Ictalurus punctatus* | *Ictalurus* | *Ictaluridae* |           | 12.16       | 4                | 0           | 0                 | 12.16        | 4                | 12.16        | 4                |
|                       |             |            |           | 12.16       | 4                | 0           | 0                 | 12.16        | 4                | 12.16        | 4                |
| *Oreochromis niloticus* | *Oreochromis* | *Cichlidae* | *Perciformes* | 1.06        | 1                | 72.82       | 190               | 107.5        | 2                | 181.38      | 193              |
|                       |             |            |           | 1.06        | 1                | 72.82       | 190               | 107.5        | 2                | 181.38      | 193              |
| *Perca fluviatilis*   | *Perca*     |            |           | 0.078       | 1                | 0           | 0                 | 0.078        | 1                | 0.078       | 1                |
|                       |             |            |           | 0.078       | 1                | 0           | 0                 | 0.078        | 1                | 0.078       | 1                |
| *Rhinogobius giurinus* | *Rhinogobius* | *Gobiidae* |           | 0           | 0                | 0           | 0                 | 54           | 25               | 54          | 25               |
|                       |             |            |           | 0           | 0                | 0           | 0                 | 54           | 25               | 54          | 25               |

Note: Fish species, whose scientific names were written in bold, were those detected by both captured-based fishing methods and eDNA metabarcoding.
(Goutte et al. 2020, Pont et al. 2018), lakes (Fujii et al. 2019, Hänfling et al. 2016, Li et al. 2019), ponds (Valentini et al. 2016), estuaries (Zhang et al. 2019, Zou et al. 2020), and oceans (Fraija-Fernandez et al. 2020, Thomsen et al. 2016). Most of these studies demonstrated that eDNA methods have a similar or even better performance than capture-based fishing methods in terms of species detection. In our study, the number of fish species revealed by eDNA metabarcoding was basically comparable to that revealed by various types of sampling gear; the main aquaculture fish species were recovered, and the predominant species, both in terms of abundance and biomass, in the capture-based fishing methods were detected. Furthermore, several fish species that were not found in fish catches were characterized; these species were also recorded in local fishery literature (Editorial Committee 1984). However, it was also noteworthy that eDNA metabarcoding did not cover all fish species detected by conventional fishing methods, and conventional sampling gear recorded more exclusive fish species than the eDNA method did. There are several potential explanations for our failure to detect a few fish species by eDNA metabarcoding, such as the setting of the sampling sites (Hänfling et al. 2016, Zhang et al. 2020), versatility of metabarcoding primers (Takeuchi et al. 2019), quality and completeness of the reference database (Callahan et al. 2017), and bias of PCR against low-abundance sequences (Ficetola et al. 2015, Hänfling et al. 2016, Li et al. 2018). Nevertheless, the time and labor required for eDNA methods are still less than those required for traditional fishing methods, as eDNA metabarcoding simply involves water sampling, while established fishing methods require more personnel and incur greater costs (Dejean et al. 2011, Yamamoto et al. 2017). In addition, various types of sampling gear were needed to ensure that distinct groups of fishes were sampled due to the inherent bias of all established fishing methods (Kubečka et al. 2009).

![Figure 4. Fish species detected by capture-based fishing methods and eDNA metabarcoding.](image-url)
Figure 5. Relationship between log$_{10}$-transformed values for the number of reads and abundance /biomass from all fish species found by both eDNA method and capture-based methods, total fish catches and hydroacoustic assessment. Shaded area represents the 95% confidence interval of the linear regression.
Several species were found inhabiting the reservoir, including *Anguilla rostrata*, *Coptodon zillii* and *O. acutipinnis*. Both *A. rostrata* and *C. zillii* are established alien fish species that were introduced abroad and widely farmed in China (Luo et al. 2019). Although *C. zillii* was not among the fish caught by the sampling gears, its relative abundance in several water samples was quite high. In contrast, the relative abundance of *O. niloticus*, a sibling species of *C. zillii*, was low in all the samples, even though this tilapia species was abundant in the fish caught with sampling gear. Therefore, we suggested that the *O. niloticus* individuals caught with the sampling gear might be *C. zillii* and

**Figure 6.** Correlations between site occurrence and sequence abundances for fish species detected by eDNA metabarcoding. Correlations are calculated based on actual numbers (sequence abundance data are natural logarithm [ln]-transformed; a) and rank (b) of relative sequence abundance and site occurrence. Each dot represents a unique fish species.

**Figure 7.** Nonmetric multidimensional scaling analysis of pelagic fish and demersal fish groups revealed by eDNA metabarcoding.

Several species were found inhabiting the reservoir, including *Anguilla rostrata*, *Coptodon zillii* and *O. acutipinnis*. Both *A. rostrata* and *C. zillii* are established alien fish species that were introduced abroad and widely farmed in China (Luo et al. 2019). Although *C. zillii* was not among the fish caught by the sampling gears, its relative abundance in several water samples was quite high. In contrast, the relative abundance of *O. niloticus*, a sibling species of *C. zillii*, was low in all the samples, even though this tilapia species was abundant in the fish caught with sampling gear. Therefore, we suggested that the *O. niloticus* individuals caught with the sampling gear might be *C. zillii* and
misidentified as its sibling species. The genus *Opsariichthys* is an endemic cyprinid genus that is widely distributed in East Asia (Lin et al. 2016), including Fujian Province in southern China. Although we did not capture any *Opsariichthys* individuals in our sampling gear, we did observe a large number of *Opsariichthys* groups foraging in the near-shore waters after we collected water samples. Therefore, we assumed that inappropriate sampling gear and eDNA sampling site settings might be the reason why we failed to capture any *O. acutipinnis* and *A. rostrata* individuals.

In sum, our study demonstrated that the performance of eDNA metabarcoding was basically similar to that of traditional survey methods for fish species surveillance in small reservoirs. Due to the inability of eDNA metabarcoding to record all fish species revealed by established fishing methods; however, it cannot completely replace traditional capture-based methods at present. Even so, eDNA metabarcoding has been proven to be an

**Figure 8.** Heatmap of relative sequence abundances of fish species characterized by eDNA metabarcoding. Samples are named according to the sampling sites and associated layers, among which “P” represents for pelagic layer, “B” represents for benthic layer.
important supplementary approach to traditional capture-based fishing methods when applied to characterize the fish diversity of small hydropower dam reservoirs.

4.2. Quantitative assessment of fish community composition by eDNA data

Quantitative assessment of fish communities is an intriguing research focus that may facilitate rapid and accurate fish monitoring and fisheries management. Accordingly, eDNA analyses should be developed from quantitative species detection toward quantitative estimation of species numbers or biomass estimates, which relies on the assumption that eDNA concentration or sequence counts are correlated with fish abundance or live biomass (Hansen et al. 2018). Although some studies have confirmed that a linear relationship between eDNA and abundance/biomass (Doi et al. 2015, Evans et al. 2016, Fraija-Fernandez et al. 2020, Klymus et al. 2015, Salter et al. 2019) exists in experimental aquaria, ponds and oceans, a similar relationship is expected to be either less significant (Schmelzle and Kinziger 2016, Thomsen et al. 2016, Yamamoto et al. 2016) or lacking (Spear et al. 2015) in other cases. As a result, it remains disputed whether correlations exist between eDNA metabarcoding sequence counts and fish abundance or biomass (Zhang et al. 2020). Our study also failed to confirm a solid tight relationship between eDNA sequence counts and fish abundance or biomass under most scenarios. In fact, the relationship between eDNA and the biomass or abundance of fish species could be obscured by various factors, including the size structure of the local population (Maruyama et al. 2014), hydrodynamics, water chemistry and fish physiology (Zhang et al. 2020). Specifically, inevitable biases introduced during sampling, primer binding, subsequent PCR amplification (Li et al. 2018) and high-throughput sequencing (Hänfling et al. 2016) were thought to be the most crucial factors determining the complex nature of the relationship between eDNA and biomass or abundance. Therefore, the direct usage of sequence count data might be unsuitable; instead, site occupancy models were developed to address various biases and uncertainties (Hänfling et al. 2016). In this study, we observed a consistent significant relationship between occupancy and relative sequence abundance as well as occupancy rank and sequence abundance rank. Our results indicated that both sequence counts and occupancy data were tightly correlated and thus equally effective at estimating relative abundance under comprehensive spatial sampling with integrated sampling gear. Similar significant correlations between site occupancy and sequence abundance have also been found in fish communities of lentic systems (Hänfling et al. 2016, Zhang et al. 2020). Altogether, our results suggested that although eDNA metabarcoding data may not be a favorable proxy for estimating fish abundance and biomass, they have the potential to provide quantitative information on fish community compositions in addition to being a sensitive and effective tool for species detection, which may facilitate and benefit further studies of ecosystem dynamics, conservation efforts and fishery management.

4.3. Spatial patterns of fish communities inferred from eDNA data

Although the relationships between eDNA concentrations or sequence abundances and fish population occurrences remain obscured and might be confounded by various biological factors and local environmental settings, such as temperature (Jo et al. 2019), pH, and UV-B radiation (Strickler et al. 2015), a positive significant correlation between eDNA data and observed species has been revealed in several aquatic ecosystems. For example, the eDNA concentrations of common carp (C. carpio) were found to be closely
related to their spatial distribution in small lakes (Eichmiller et al. 2014), while spatial concordance was observed between the relative abundance of eDNA and distribution trends illustrated by visual surveys of marine vertebrates (Port et al. 2016). Therefore, spatial distribution patterns of fish species determined by eDNA sequencing are likely to be feasible and credible.

In general, fish communities in small ponds and lakes tend to be homogenous (Evans et al. 2017, Thomsen et al. 2012) due to the relative stability of the habitat configuration (Zhang et al. 2020) and the mixture of water, which is driven by wind-facilitated water flow; in contrast, fish assemblages in large lentic systems are more likely to be heterogeneous (Hänfling et al. 2016), which could be explained by the complicated and confounding effects of water size, hydrodynamics and fish ecology (Zhang et al. 2020). In our study, the spatial distribution of fish communities in reservoirs revealed by eDNA showed a heterogeneous pattern that contrasted with the fish distribution pattern of small lentic systems described above. The Lantian Reservoir is a small artificial lake; however, its elongated drainage basin indicated that it might share some of the complicated and confounded effects of large lakes. In addition, the elongated nature of the lake system created more variable habitat features and formed many microenvironments for distinct fish groups. Furthermore, the upstream, midstream and downstream cage culture sites that harbored different commercial fish species may have contributed to the heterogeneous fish distribution.

We observed a hierarchical distribution for some species; for example, *C. carpio* and *M. piceus* were most likely to inhabit the bottom layer. These vertical habitat preferences were consistent with the ecology of these fishes (Editorial Committee 1984). Although inconsistencies existed between the observed habitat occurrences and ecology of some other fishes, such as *C. idella* and *H. nobilis*, which occurred in the pelagic layer and bottom layer, respectively, there are several possible reasons for this pattern. For example, pelagic fishes may descend to the cool bottom layer if the temperature is too high, while demersal fishes may move to the pelagic layer for forage when food and oxygen in the bottom layer are insufficient. The rest of the fish species did not show similar significant layer-related distributions, which might be the result of complex and confounding effects of fish behavior, such as vertical movement for cooling, food and oxygen; the existence of diverse microenvironments suitable for different fish species; and water flow dynamics.

5. Conclusions

As one of the most promising molecular approaches in aquatic biodiversity research and biomonitoring, eDNA has demonstrated great potential and robust power for determining species composition and revealing information about fish communities under various scenarios, including both artificial and natural environments. Our eDNA metabarcoding analyses on water samples collected from the Lantian Reservoir successfully recovered most fish species revealed by conventional capture-based methods. Both the eDNA method and the capture-based method recorded several exclusive fish species that were missed by each other, with the number of species revealed by the conventional fishing method being greater than that of eDNA metabarcoding. Even so, the suitability of eDNA metabarcoding for characterizing fish communities in small reservoirs is still being validated. Though we failed to establish a quantitative relationship between eDNA and fish biomass under most scenarios, a consistent significant relationship between occupancy and relative sequence abundance as well as occupancy rank and sequence abundance rank was observed. Taken together, these results suggest that eDNA metabarcoding can play a
potentially important role in evaluating the impacts of stream dam construction on local fish communities and might assist small reservoir fishery stock assessments to some extent. However, it will not replace traditional capture-based fishing methods or hydroacoustic approaches until we can fully characterize the complicated and confounding effects that various biological factors and environmental variables have on eDNA in small reservoirs. Further studies on the impacts of fish physiology, hydrodynamics, and environmental factors on the production, persistence, transportation and degradation of eDNA in reservoirs are urgently needed to facilitate our interpretation of local fish abundance and biomass from eDNA data.

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**Author contributions**

Hai Li: Investigation, Conceptualization, Data curation, Writing-original draft preparation, Validation.
Fang Yang: Data curation, Writing-original draft preparation, Validation. Ran Zhang: Methodology, Investigation, Visualization. Shigang Liu: Data curation, Software, Writing- reviewing and editing. Zhijin Yang: Methodology, Investigation. Longshan Lin: Supervision, Conceptualization, Resources. Sunzhong Ye: Methodology, Investigation, Data curation, Supervision, Conceptualization, Resources.

**Disclosure statement**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**References**

Andrews S. 2013. Babraham Bioinformatics -FastQC A Quality Control tool for High Throughput Sequence Data.
Bagley M, Pilgrim E, Knapp M, Yoder C, Domingo JS, Banerji A. 2019. High-throughput environmental DNA analysis informs a biological assessment of an urban stream. Ecol Indic. 104:378–389.
Barbarossa V, Schmitt RJP, Huijbregts MAJ, Zarfì C, King H, Schipper AM. 2020. Impacts of current and future large dams on the geographic range connectivity of freshwater fish worldwide. Proceedings of the National Academy of Sciences of the United States of America. 117:3648–3655. Epub 2020/02/06.
Barnosky AD, Matzke N, Tomiya S, Wogan GOU, Swartz B, Quental TB, Marshall C, McGuire JL, Lindsey EL, Maguire KC, et al. 2011. Has the Earth’s sixth mass extinction already arrived? Earth’s sixth mass extinction already arrived? Nature. 471(7336):51–57.
Benejam I, Saura-Mas S, Bardina M, Solà C, Munné A, García-Berthou E. 2016. Ecological impacts of small hydropower plants on headwater stream fish: from individual to community effects. Ecol Freshw Fish. 25(2):295–306.
Callahan BJ, Mcmurdie PJ, Holmes SP. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME Jisme J. 11(12):2639–2643.
Cantera I, Cillerós K, Valentini A, Cerdan A, Dejean T, Iribar A, Taberlet P, Vigouroux R, Brosse S. 2019. Optimizing environmental DNA sampling effort for fish inventories in tropical streams and rivers. Sci Rep. 9(1):3085.
Carvajal-Quintero JD, Januchowski-Hartley SR, Maldonado-Ocampo JA, Jézéquel C, Delgado J, Tedesco PA. 2017. Damming fragments species ranges and heightens extinction risk. Conservation Letters. 10(6):708–716.
Chen J, Chen Z, Liu S, Guo W, Li D, Minamoto T, Gao T. 2021. Revealing an invasion risk of fish species in Qingdao underwater world by environmental DNA metabarcoding. J Ocean Univ China. 20(1): 124–136.

Consuegra S, O’Rorke R, Rodriguez-Barreto D, Fernandez S, Jones J, Garcia de Leaniz C. 2021. Impacts of large and small barriers on fish assemblage composition assessed using environmental DNA metabarcoding. Sci Total Environ. 790:148054.

Dejean T, Valentini A, Duparc A, Pellier-Cuit S, Pompanon F, Taberlet P, Miaud C. 2011. Persistence of environmental DNA in freshwater ecosystems. PLoS One. 6(8):e23398.

Doi H, Uchii K, Takahara T, Matsuhashi S, Yamanaka H, Minamoto T. 2015. Use of droplet digital PCR for estimation of fish abundance and biomass in environmental DNA surveys. PLoS One. 10(3): e0122763.

Dudgeon D, Arthington AH, Gessner MO, Kawabata Z-I, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard A-H, Soto D, Stiassny MLJ, et al. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol Rev Camb Philos Soc. 81(2):163–182.

Editorial Committee. 1998. Anxi Yearbook (in Chinese). HongKong, China: Chinese International Press.

Editorial Committee. 1984. Fishes in Fujian Province (in Chinese). Fuzhou, China: Fujian Science and Technology Press.

Editorial Committee. 1998. Anxi Yearbook (in Chinese). HongKong, China: Chinese International Press.

Eichmiller JJ, Bajer PG, Sorensen PW. 2014. The relationship between the distribution of common carp and their environmental DNA in a small lake. PLoS One. 9(11):e112611.

Evans NT, Olds BP, Renshaw MA, Turner CR, Li Y, Jerde CL, Mahon AR, Pfrender ME, Lamberti GA, Lodge DM. 2016. Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. Mol Ecol Resour. 16(1):29–41.

Evans NT, Shirey PD, Wieringa JG, Mahon AR, Lamberti GA. 2017. Comparative cost and effort of fish distribution detection via environmental DNA analysis and electrofishing. Fisheries. 42(2):90–99.

Ficetola GF, Pansu J, Bonin A, Coissac E, Giguët-Covex C, De Barbà M, Gielly L, Lopes CM, Boyer F, Pompanon F, et al. 2015. Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. Mol Ecol Resour. 15(3):543–556.

Fraiia-Fernandez N, Bouqueixues MC, Rey A, Mendibil I, Cotano U, Irigoien X, Santos M, Rodriguez-Ezpeleta N. 2020. Marine water environmental DNA metabarcoding provides a comprehensive fish diversity assessment and reveals spatial patterns in a large oceanic area. Ecol Evol. 10(14):7560–7584.

Fujii K, Doi H, Matsuoka S, Nagano M, Sato H, Yamanaka H. 2019. Environmental DNA metabarcoding for fish community analysis in backwater lakes: a comparison of capture methods. PLoS One. 14(1): e0210357.

Gouette A, Molbert N, Guerin S, Richoux R, Rocher V. 2020. Monitoring freshwater fish communities in large rivers using environmental DNA metabarcoding and a long-term electrofishing survey. J Fish Biol. 97(2):444–452.

Granottzoti RV, Miranda LE, Agostinho AA, Gomes LC. 2018. Downstream impacts of dams: shifts in benthic invertivorous fish assemblages. Aquat Sci. 80(3):1–14.

Grill G, Lehner B, Thieme M, Geenen B, Tickner D, Antonelli F, Babu S, Borrelli P, Cheng l, Crochetiere H, et al. 2019. Mapping the world’s free-flowing rivers. Nature. 569(7755):215–221.

Hanshling B, Lawson Handley L, Read DS, Hahn C, Li J, Nichols P, Blackman RC, Oliver A, Winfield IJ. 2016. Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. Mol Ecol. 25(13):3101–3119.

Hansen BK, Bekkevold D, Clausen LW, Nielsen EE. 2018. The sceptical optimist: challenges and perspectives for the application of environmental DNA in marine fisheries. Fish Fish. 19(5):751–768.

Jo T, Murakami H, Yamamoto S, Masuda R, Minamoto T. 2019. Effect of water temperature and fish biomass on environmental DNA shedding, degradation, and size distribution. Ecol Evol. 9(3):1135–1146.

Kelly RP, Port JA, Yamahara KM, Crowder LB. 2014. Using environmental DNA to census marine fishes in a large mesocosm. PLoS One. 9(1):e86175.

Klymus KE, Richter CA, Chapman DC, Paukert C. 2015. Quantification of eDNA shedding rates from invasive bighead carp Hypophthalmichthys nobilis and silver carp Hypophthalmichthys molitrix. Biol Conserv. 183:77–84.

Kubecka J, Hohausová E, Matěna J, Peterka J, Amarasinghe US, Bonar SA, Hateley J, Hickley P, Suuronen P, Tereshenko V, et al. 2009. The true picture of a lake or reservoir fish stock: a review of needs and progress. Fish Res. 96(1):1–5.

Kuriqi A, Pinheiro AN, Sordo-Ward A, Bejarano MD, Garrote L. 2021. Ecological impacts of run-of-river hydropower plants—current status and future prospects on the brink of energy transition. Renewable Sustainable Energy Rev. 142:110833.
Li J, Hatton-Ellis TW, Lawson Handley L-J, Kimbell HS, Benucci M, Peirson G, Hänfling B, Paiva V. 2019. Ground-truthing of a fish-based environmental DNA metabarcoding method for assessing the quality of lakes. J Appl Ecol. 56(5):1232–1244.

Li J, Lawson Handley LJ, Read DS, Hänfling B. 2018. The effect of filtration method on the efficiency of environmental DNA capture and quantification via metabarcoding. Mol Ecol Resour. 18(5):1102–1114.

Lin H-D, Kuo P-H, Wang W-K, Chiu Y-W, Ju Y-M, Lin F-J, Hsu K-C. 2016. Speciation and differentiation of the genus Opsariichthys (Teleostei: Cyprinidae) in East Asia. Biochem Syst Ecol. 68:92–100.

Luo D, Wei H, Chaichana R, Yang D, Gu D, Mu X, Xu M, Yang Y, Jin S, Hu Y. 2019. Current status and potential risks of established alien fish species in China. Aquatic Ecosystem Health & Management. 22(4):371–384.

Maruyama A, Nakamura K, Yamanaka H, Kondoh M, Minamoto T. 2014. The release rate of environmental DNA from juvenile and adult fish. PLoS One. 9(12):e114639.

Miya M, Sato Y, Fukunaga T, Sado T, Poulsen JY, Sato K, Minamoto T, Yamamoto S, Yamanaka H, Araki H, et al. 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. R Soc Open Sci. 2(7):150088.

Nelson JS, Grande TC, Wilson MVH. 2016. Fishes of the World. 5th ed.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O’Hara RB, Simpson GL, Solymos P, Stevens M, Wagner H. 2016. Vegan: Community Ecology Package. R package version 2.3-5.

Pelicice FM, Azevedo-Santos VM, Vitule JRS, Orsi ML, Lima Junior DP, Magalhães ALB, Pompeu PS, Petrere M, Agostinho AA. 2017. Neotropical freshwater fishes imperilled by unsustainable policies. Fish Fish. 18(6):1119–1133.

Port JA, O’Donnell JL, Romero-Maracinni OC, Leary PR, Litvin SY, Nickols KJ, Yamahara KM, Kelly RP. 2016. Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. Mol Ecol. 25(2):527–541.

Puijenbroek PV, Buijse AD, Kraak M, Verdonschot P. 2019. Species and river specific effects of river fragmentation on European anadromous fish species. River Res Appl. 35(1):68–77.

Shaw JLA, Clarke LJ, Wedderburn SD, Barnes TC, Weyrich LS, Cooper A. 2016. Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. Biol Conserv. 197:131–138.

Spear SF, Groves JD, Williams LA, Waits LP. 2015. Using environmental DNA methods to improve detectability in a hellbender (Cryptobranchus alleganiensis) monitoring program. Biol Conserv. 183:38–45.

Strickler KM, Fremier AK, Goldberg CS. 2015. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. Biol Conserv. 183:85–92.

Takeuchi A, Sado T, Gotoh RO, Watanabe S, Tsukamoto K, Miya M. 2019. New PCR primers for metabarcoding environmental DNA from freshwater eels, genus Anguilla. Sci Rep. 9(1):7977.

Thomas AC, Deagle BE, Eveson JP, Harsch CH, Trites AW. 2016. Quantitative DNA metabarcoding: improved estimates of species proportional biomass using correction factors derived from control material. Mol Ecol Resour. 16(3):714–726.

Thomsen PF, Kielgast J, Iversen LL, Møller PR, Rasmussen M, Willerslev E. 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS One. 7(8):e41732.
Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MT, Orlando L, Willerslev E. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Mol Ecol. 21(11): 2565–2573.

Thomsen PF, Moller PR, Sigsgaard EE, Knudsen SW, Jorgensen OA, Willerslev E. 2016. Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. PLoS One. 11(11):e0165252.

Timpe K, Kaplan D. 2017. The changing hydrology of a dammed Amazon. Sci Adv. 3(11):e1700611.

Valentini A, Taberlet P, Miaud C, Civade R, Herder J, Thomsen PF, Bellemain E, Besnard A, Coissac E, Boyer F, et al. 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol Ecol. 25(4):929–942.

Vasek M, Kubečka J, Čech M, Draštik V, Matěňa J, Mrkvíčka T, Peterka J, Prchalová M. 2009. Diel variation in gillnet catches and vertical distribution of pelagic fishes in a stratified European reservoir. Fish Res. 96(1):64–69.

Wang J, Ding C, Tao J, Jiang X, Heino J, Ding L, Su W, Chen M, Zhang K, He D. 2021. Damming affects riverine macroinvertebrate metacommunity dynamics: Insights from taxonomic and functional beta diversity. Sci Total Environ. 763:142945.

Yamamoto S, Masuda R, Sato Y, Sado T, Araki H, Kondoh M, Minamoto T, Miya M. 2017. Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. Sci Rep. 7(1):40368.

Yamamoto S, Minami K, Fukaya K, Takahashi K, Sawada H, Murakami H, Tsuji S, Hashizume H, Kubonaga S, Horiuichi T, et al. 2016. Environmental DNA as a ‘Snapshot’ of fish distribution: a case study of Japanese Jack Mackerel in Maizuru Bay, Sea of Japan. PLoS One. 11(3):e0149786.

Zhang H, Yoshizawa S, Iwasaki W, Xian W. 2019. Seasonal fish assemblage structure using environmental DNA in the Yangtze Estuary and its adjacent waters. Front Mar Sci. 6

Zhang S, Lu Q, Wang Y, Wang X, Zhao J, Yao M. 2020. Assessment of fish communities using environmental DNA: effect of spatial sampling design in lentic systems of different sizes. Mol Ecol Resour. 20(1):242–255.

Zou K, Chen J, Ruan H, Li Z, Guo W, Li M, Liu L. 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. The Science of the Total Environment. 702:134704.