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Research article

Towards a comprehensive assessment of ichthyofaunal diversity in the Yangtze River estuary: Leveraging environmental DNA technology and bottom trawl surveys

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ABSTRACT

The fishery resources in the Yangtze River Estuary (YRE) have declined drastically because of overfishing and environmental changes, leading to ecosystem degradation of the YRE, and bringing numerous rare fish species to the brink of extinction. As a new technology with great prospects for popularization and application, environmental DNA (eDNA) technology has been utilized and proven by many studies to have high potential in revealing the various species' biodiversity. In this study, we analyzed the species composition and diversity of the Yangtze River Estuary using a combination of eDNA technology and bottom trawling approaches, and later, the comparison of both methods. The results showed that combining eDNA technology and bottom trawling, 30 fish species from 7 orders and 11 families were identified. Among the 30 fish species, a total of six species of fish could be observed in catches from both methods. Perciformes were the most abundant and *Coilia mystus* was the dominant species. According to diversity indices, the eDNA technology reveals significant differences in fish community richness and diversity in the Yangtze River Estuary compared to the bottom trawl. In summary, the eDNA technology is feasible for monitoring fishery resources in the waters of the Yangtze River Estuary, thereby serving as a valuable supplementary tool for conducting comprehensive surveys in this region. Moreover, it holds significant implications and promising prospects for conserving the diverse ecosystem of the YRE in future conservation efforts.

1. Introduction

Assessing biodiversity is typically the first stage of research related to natural ecosystems. Fish diversity, as a key indicator for monitoring the health of aquatic ecosystems, holds significant importance in evaluating changes within these ecosystems [1]. In recent years, due to the escalating disturbances from human activities, the pre-existing aquatic ecosystems have suffered severe damage, leading to the continuous decline in the biodiversity levels of fish communities [2,3]. This and other effects underscore the urgency of

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biodiversity research globally.

Traditional sampling and monitoring technologies primarily encompass visual surveys, trawling investigations, and acoustic surveys. Among these, bottom trawl surveys can quantitatively estimate fish abundance and biomass, providing insights into population size structure, age at maturity, and physiological conditions [4,5]. However, influenced by factors such as net type, mesh size, and trawling speed, bottom trawling faces hurdles like high costs, environmental degradation, and inaccessibility to certain locations [6]. This method inherently possesses limitations and dependence on specific environmental conditions [7,8]. Consequently, environmental DNA (eDNA) has emerged as a non-invasive, rapid and cost-effective method for monitoring biodiversity [9].

eDNA refers to the genetic material obtained directly from environmental samples, encompassing water, soil, and air [10]. DNA captured from these samples undergoes preservation, extraction, amplification, sequencing, and classification, determining the biological distribution within the sampled environment eDNA technology. This approach commonly employs metabarcoding and quantitative polymerase chain reaction (qPCR) to identify target species and biological communities [11,12]. Compared to traditional methods, eDNA boasts benefits like reduced time consumption, lower costs, convenient sampling, minimal ecological harm, and the obviation of advanced ichthyological taxonomy knowledge, heralding it as a promising and innovative technology for biodiversity monitoring [13]. Many studies have juxtaposed eDNA outcomes with conventional methods. For instance, Zhou et al. [4] analyzed and compared fish diversity levels acquired through eDNA metabarcoding and bottom trawling across different seasons, discovering that the number of fish species detected through eDNA notably exceeded that from trawl captures. Similarly, Thomsen et al. [14] compared eDNA metabarcoding data of seawater samples from the Davis Strait continental slope off southwest Greenland with trawl capture data, finding a relatively favorable correlation between eDNA read abundance and trawling data. Czeglédi et al. [15] used eDNA metabarcoding to compare the consistency evaluation of fish community attributes using electrofishing (EF) and gill nets (GN). Research has shown that eDNA metabarcoding may be the best universal method for inferring fish communities. Therefore, eDNA can supplement traditional fishery surveys, offering a more comprehensive understanding of the impacts of human activities and other factors on community structure and diversity [16].

The Yangtze River Estuary, one of China's largest estuaries, located at the confluence of the Yellow Sea and East China Sea in the Yangtze River Delta, is a pivotal transition zone for material exchanges between terrestrial and marine ecosystems, boasting abundant fish resources and biological diversity [17]. As a high-productivity fishery ecosystem, the estuary also represents a vital source of China's fishery economy [18]. Influenced by the freshwater of the Yangtze River, the cold-water mass of the Yellow Sea, and the Kuroshio Current, the estuary provides pivotal habitats for fish spawning, foraging, and growth [19]. Yet, recent studies indicate that due to human overexploitation, water pollution, dam and shoreline construction, port shipping, and other factors, the ecosystem of the Yangtze River Estuary has been jeopardized, with many rare fish species facing extinction [18,20]. Hence, safeguarding fish diversity in the Yangtze River Estuary is imperative for ensuring the ecological health of the Yangtze River Basin and promoting sustainable development of China's freshwater fisheries [21].

Biodiversity, an intrinsic pivotal component within natural ecosystems, often presents monitoring challenges in aquatic ecosystems. eDNA is revolutionizing our capability to study biodiversity. While eDNA boasts numerous advantages, it cannot entirely supplant traditional technologies [22,23]. Concurrently, China's decade-long fishing ban to conserve Yangtze fishery resources has intensified monitoring challenges in the Yangtze River Basin [24,25]. Traditional trawlers may not be able to adapt to this policy, whereas eDNA, with its technical characteristics, may be better suited for such adaptation. Currently, studies employing eDNA for fish diversity investigations in the Yangtze River Estuary are scarce. Therefore, this study aims to assess the effectiveness of eDNA

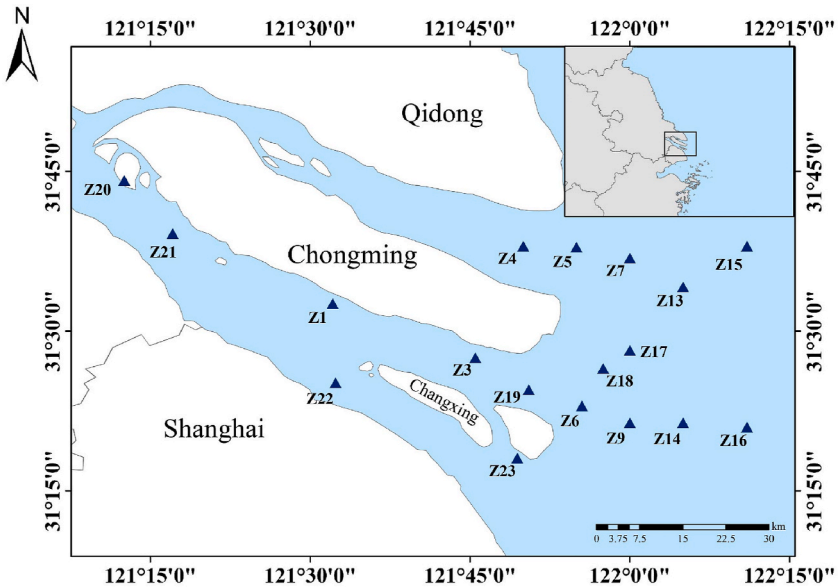

Fig. 1. eDNA and bottom trawl sampling sites in the Yangtze River Estuary and its adjacent waters.

technology in this particular water area. Given the estuary's unique "three tributaries, four mouths" configuration and trumpet-shaped features, exhibiting strong sea-land interactions, with water quality influenced by the freshwater influx from the Yangtze and the saline water from the Yellow Sea, leading to significant salinity disparities between the southern and northern branches and a highly intricate natural environment fostering rich fish resources [26,27]. This study collected eDNA and bottom trawl data from 18 sampling sites in and around the Yangtze River Estuary during the spring of 2023, comparing the abilities of the two methods in detecting fish community species composition and diversity. The present study aims to evaluate more accurately and objectively the applicability of eDNA technology in the Yangtze River Estuary, offering a robust scientific basis for future protection and assessment of biodiversity recovery status in this ecosystem.

2. Materials and methods

This survey was conducted from April 22 to May 25, 2023 (spring) in the Yangtze River Estuary and its adjacent waters. Both water sample collection and bottom trawl surveys were carried out simultaneously. A total of 18 sampling sites were established, as shown in Fig. 1.

2.1. Trawl sample collection and identification

The fishing vessel used for this survey was "Huchongyu11225". The net equipment was a single-ship outrigger trawl, with a net opening of approximately 6 m long and 1.8 m high. The net body was about 11 m long, with a mesh size of 2 cm, and included two codends with a minimum mesh size of 1 cm. The average boat speed was 2 knots, with an average trawling duration of 0.5 h. In total, there were 17 designated sites for bottom trawling (Site Z22 was not sampled as it was located in a navigation channel). Each site was sampled once during both the flood and ebb tides. All captured fish from the trawling were transported to the laboratory in a refrigerated state and then stored in a −20 °C freezer. Once thawed, fish species identification and biological measurements were conducted. Fish species were identified based on morphological characteristics, with the identification standards referring to the "Fish of the Yangtze River Estuary" compiled by Zhuang Ping et al. [28] and the" Fishes of Jiangsu Province " compiled by Ni Yong et al. [29].

2.2. Environmental DNA sample collection and processing

During eDNA sample collection at each station, surface, middle and bottom water samples were collected and subsequently evenly mixed together. A total of 15 sampling sites were established (No species were detected at sites Z4, Z5, and Z7). At each site, two 1000 mL mixed water samples were collected using sampling bottles and vacuum-filtered using a mixed fiber filter membrane with a diameter of 47 mm and a pore size of 0.45 μm. When sampling turbid water, the water should be pre-filtered or pre-treated to prevent clogging of the filter membrane, thereby accelerating the filtration rate. The equipment used for filtration was pre-disinfected by soaking in a diluted solution of 84 disinfectants (with an effective chlorine content of 0.3 %). Before filtering the experimental water samples, three blank water samples were filtered. The filtered water samples were collected in 1 L sterile, sealable wide-mouth bottles. Each filter membrane was used to filter 1000 mL, and the filter membrane obtained after filtration was placed in a 1.5 mL centrifuge tube and sealed. It was then stored in a −20 °C freezer with labels indicating the sampling site and time. The samples were frozen and sent to the laboratory as soon as possible. A negative control sample was set for every filtration at each sampling site.

2.3. Environmental DNA testing

2.3.1. DNA extraction

The DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) was used to extract eDNA. Specifically, the filter membrane obtained from water sample filtration was chopped, mixed with lysing solution, and then heated in a water bath. After centrifugation, the supernatant was transferred to a DNA centrifuge column. After washing the column with various buffer solutions, the elution buffer was added 1–2 times to obtain the eDNA sample. The quality of the extracted genomic DNA was then checked using 1 % agarose gel electrophoresis. DNA was extracted independently from each water sample's filter membrane. To assess potential contamination during the experiment, distilled water was set as a negative control. The remaining DNA solution was stored in a −80 °C freezer.

2.3.2. PCR amplification and high-throughput sequencing

Mifish-U universal primers (F: 5'-GTCGGTAAAACTCGTGCCAGC-3', Mifish-U R: 3'-GTTTGACCCTAATCTATGGGGTGATAC-5') were used for PCR amplification. The amplified region targeted the 12S rDNA, resulting in DNA sequences approximately 163–185 bp in length [30]. The overall PCR system was set at 25 μL, including various components in specified quantities. The amplification parameters included pre-denaturation at 95 °C for 5 min, followed by 55 cycles of 30 s at 95 °C, and 30 s at 60 °C. Amplification was performed using the ABI 2720 PCR machine. Negative controls were established to detect microbial contamination from the environment or reagents. Subsequently, any negative control group with band amplification was not used. Finally, paired-end sequencing of the library was performed on the Illumina MiSeq sequencing platform (Illumina).

2.4. Data analysis

Sequences obtained from sequencing were initially screened, and the DADA2 method was used for primer removal, quality

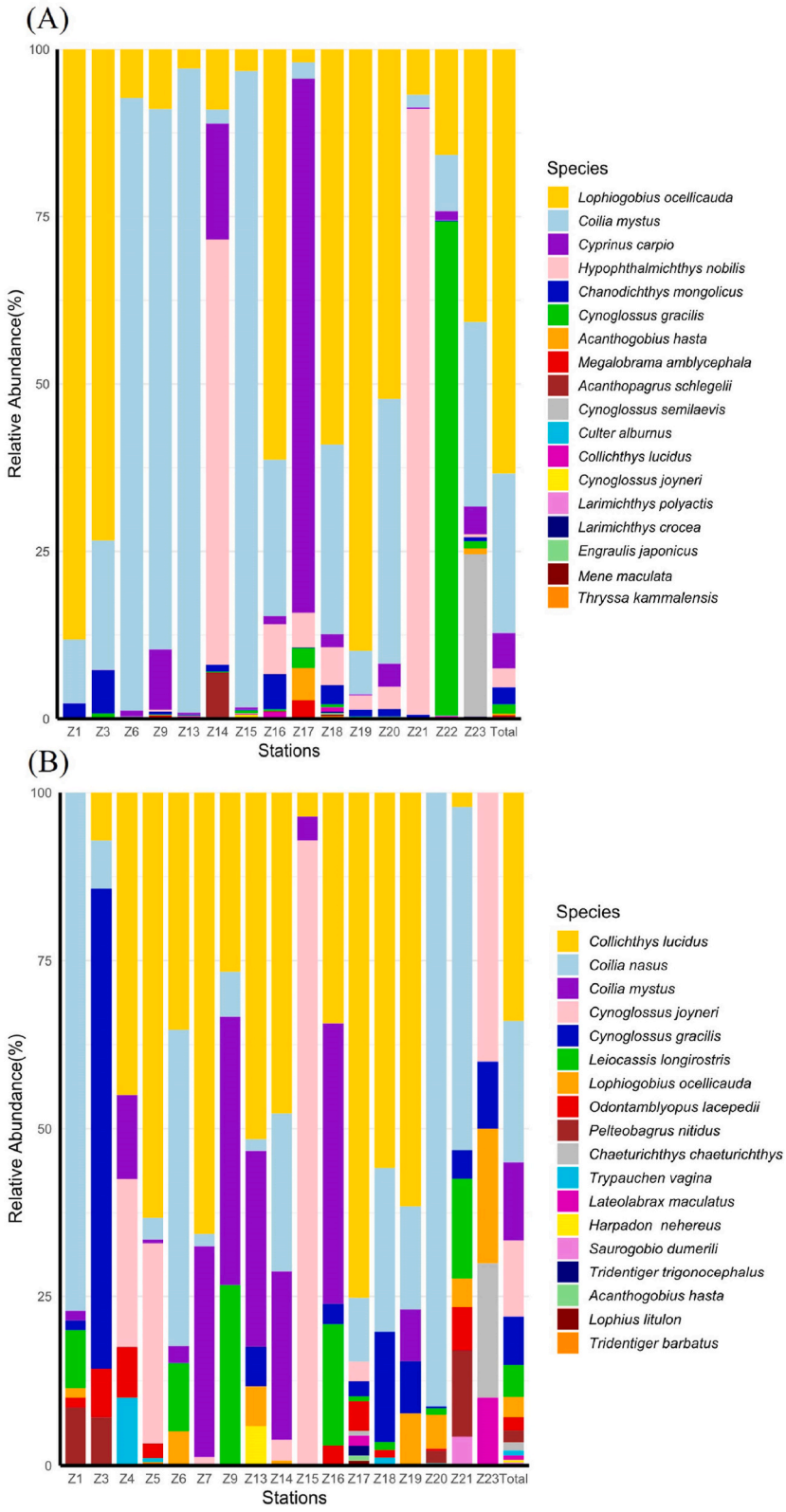

Fig. 2. Species composition at various sampling sites as detected by environmental DNA (eDNA) technology (A) and bottom trawling (B).

filtering, denoising, merging, and chimera removal. Clustering analysis was performed based on a sequence similarity ≥97 %, resulting in Operational taxonomic units (OTUs). OTU representative sequences were matched and classified against the NCBI database (https://www.ncbi.nlm.nih.gov/) [31]. Manually proofread, the annotation results and screen out the OTU of non-fish and exogenous fish. If the query sequence matches a local species with consistency ≥97 %, then the sequence matches that species, but if the query sequence only matches non-local species with consistency ≥97 %, then the query sequence matches non-local species [32]. The classification matching results can be found in Fishbase (https://www.fishbase.org)and the corresponding OTU abundance table can

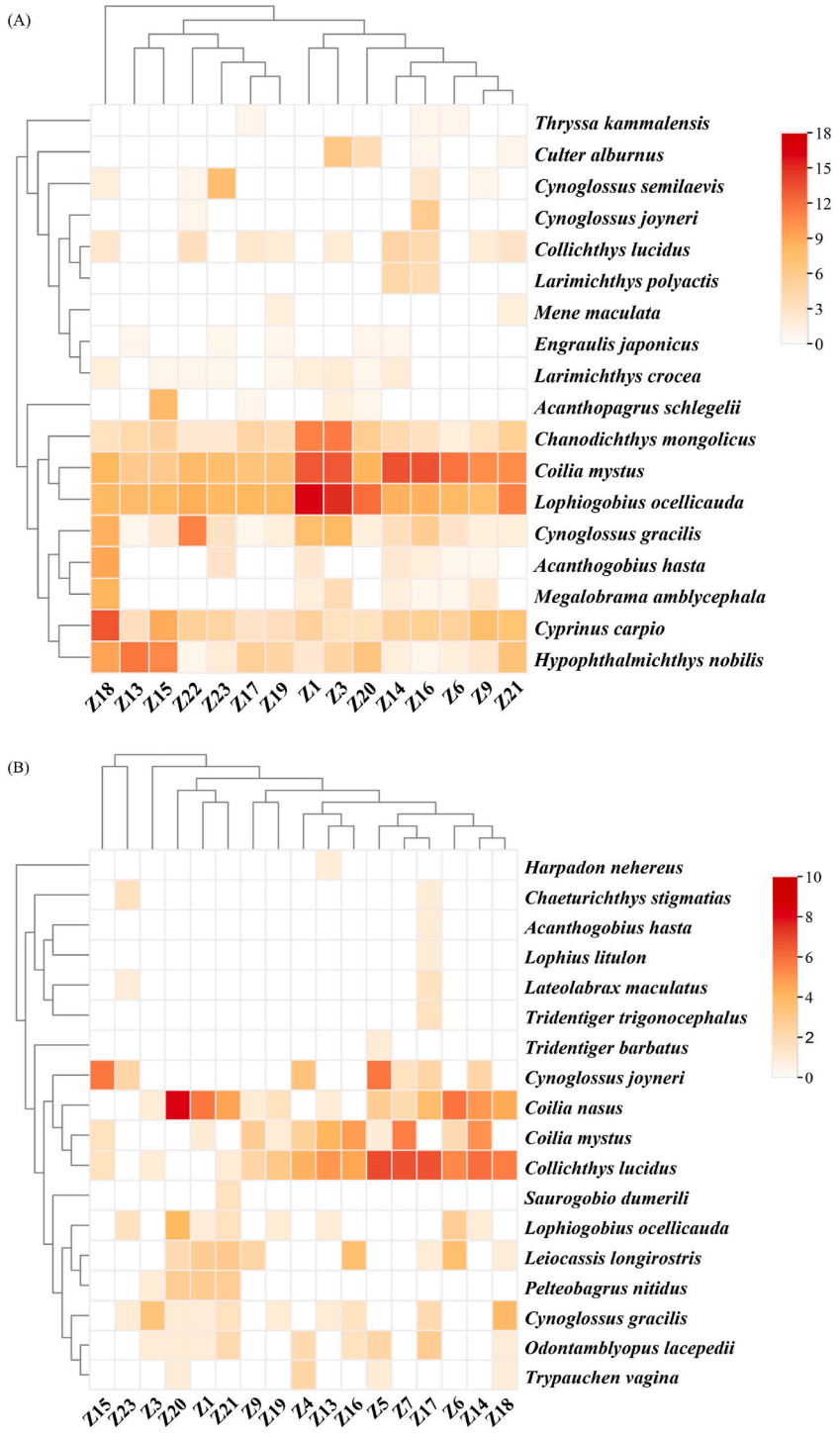

Fig. 3. Interactive heatmap of fish composition in the Yangtze Estuary based on eDNA technology (A) and bottom trawl (B).

be obtained.

In this study, clustering analysis based on species composition was conducted on trawling and eDNA technology data using R 4.3.2 software to reveal differences in fish composition between sampling stations. The vegan package was utilized to calculate alpha diversity indices associated with both methods, including the Chao1 index (S_{chao1}) [33], species number (S), Shannon index (H) [8], and Simpson index (D) [33], to comprehensively evaluate fish richness and diversity among stations. The calculation methods are as follows:

$$S_{Chao1} = S + \frac{F_1(F_1 - 1)}{2(F_2 + 1)} \tag{1}$$

Where S is the total number of OTUs in the sample, and F_1、 F_2 represent the number of OTUs containing only one and two sequences, respectively.

$$H = -\sum_{i=1}^{S} P_i \, ln(P_i) \tag{2}$$

$$D = 1 - \sum_{i=1}^{S} P_i^2 \tag{3}$$

Where P_i is the proportion of the total sequences contained in the i th OTU relative to the total sequences across all OTUs in the sample, and S represents the total number of OTUs in the sample. The "pheatmap" package in R was used to generate a heatmap of species composition at each eDNA sampling site and to conduct Cluster analysis based on Bray-Curtis distance.

3. Results

3.1. Analysis of fish species composition based on eDNA technology and bottom trawling

The compiled operational taxonomic units (OTUs) were annotated against the NCBI database. After manually excluding non-piscine and allochthonous fish OTUs, 18 species of fish from the Yangtze River Estuary were identified, spanning 4 orders, 7 families, and 16 genera. Specifically, these includes the Perciformes (33.33 %), Cypriniformes (33.33 %), Clupeiformes (16.67 %), and Pleuronectiformes (16.67 %). Bottom trawling investigations revealed fish from 7 orders, 9 families, and 15 genera, yielding a total of 18 fish species. At the order level, the predominant categories were the Perciformes (50.00 %), followed by Clupeiformes (11.11 %), Pleuronectiformes (11.11 %), and Siluriformes (11.11 %) (Supplementary Table 1).

The predominant fish species in the Yangtze River Estuary as determined by eDNA analysis were *L. ocellicauda*, *C.mystus*, *C. carpio*, and *H. nobilis*. These species exhibited a higher sequence richness across the 15 sampled sites compared to other fish. Conversely, the predominant species identified via bottom trawling were *C. lucidus*, *Coilia nasus*, *C. mystus*, and *C. joyneri*, with these species demonstrating a higher overall abundance across the 17 sites (Fig. 2).

3.2. Diversity analysis of fish species

Supplementary Table 2 and Supplementary Table 3 list the alpha indices reflecting fish community richness and diversity in the Yangtze River Estuary. The Shannon index and Simpson index are used to represent fish diversity. The Shannon index derived from the eDNA technology ranges from 0.20 to 1.36, and the Simpson index ranges from 0.07 to 0.70. Both indices follow a similar distribution trend, with Z23 being the most diverse site and Z14 the least diverse. From bottom trawl data, the Shannon index ranges from 0.31 to 1.55, while the Simpson index ranges from 0.14 to 0.79. Their distribution trends generally align, with Z21 being the most diverse site and Z15 the least diverse. The Chao1 and species number are used to represent fish richness. In this study, the Chao1 index obtained via eDNA technology ranges from 8.00 to 17.00, and the species number ranges from 7.00 to 14.00. Both indices exhibit similar distribution trends, with site Z16 showing high Chao1 and species number, while Z13 has lower richness. From bottom trawl data, the Chao1 index ranges from 3.00 to 13.00, and the species number ranges from 3.00 to 11.00. Their distribution trends are also aligned, with Z17 showing high Chao1 and species number, while site Z15 has lower richness.

A cluster analysis of fish in the Yangtze River Estuary was conducted, and heatmaps of species composition and abundance were generated for both eDNA and bottom trawling methods. The cluster analysis of samples by different sites using the eDNA technology (Fig. 3A) shows that Z9 and Z21 are the most similar. From the species clustering perspective, *C. lucidus* and *L. polyactis* clustered together first and showed high relative abundance at site Z14. *E. japonicus* and *L. crocea* were similarly distributed across sites. The cluster analysis of samples by different sites using the bottom trawling method (Fig. 3B) indicates that Z14 and Z18 are the most similar, while site Z15 shows the most distinct species composition compared to Z14 and Z18. From the point of view of species clustering, *L. longirostris* and *P. nitidus* clustered together first and distributed similarly among different sites.

3.3. Comparison of environmental DNA technology and bottom trawling

Using both the eDNA technology and bottom trawl net method, a total of 30 species of fish from 7 orders, 11 families, and 24 genera

were detected at 18 sampling sites in the Yangtze River Estuary. The number of fish species identified using the eDNA technology and those caught with bottom trawl nets are illustrated in Fig. 4. Between both methods, 6 species (20 %) were commonly detected, namely, *C. mystus*, *A. hasta*, *C. lucidus*, *L. ocellicauda*, *C. joyneri*, and *C. gracilis*.

The average Shannon index, Simpson index, Chao1 index, and species number obtained using eDNA technology were 0.73, 0.36, 9.93, and 11.09, respectively. Using the bottom trawling method, the average Shannon index, Simpson index, Chao1 index, and species number were 1.05, 0.55, 7.58, and 5.76, respectively. The bottom trawling method significantly outperformed the eDNA technology in Shannon and Simpson indices ($P < 0.05$; Fig. 5A and B). In contrast, the eDNA technology showed significantly higher Chao1 and species number than the bottom trawling method ($P < 0.05$; Fig. 5C, $P < 0.001$; Fig. 5D). In summary, there are significant differences in species abundance and diversity between the two methods.

4. Discussion

4.1. Species composition of fishes in the Yangtze River estuary based on eDNA technology and bottom trawling

The Yangtze River Estuary is China's largest estuarine ecosystem and serves as a vital spawning, feeding, and nursery ground of both economic and ecological significance [34]. Due to a prolonged fishing ban currently in place in the estuary, traditional sampling methods face challenges in assessing fishery resources without compromising the ecological environment [35]. In our study at the estuary, using eDNA technology, we identified 18 species of fish comprised of 4 orders, 7 families, and 16 genera. Both the Perciformes and Cypriniformes orders were the most represented, each having 6 species. In contrast, bottom trawling detected 18 fish species spread across 7 orders, 9 families, and 15 genera, with the *Perciformes* order being predominant. Our sampling was conducted during the spring, which coincides with the spawning season of many species in the Perciformes and Cypriniformes orders. Consequently, the distribution of fish species was predominantly from these two orders, aligning with the findings of Zhang et al. [36] and Liu et al. [37]. Stoeckle et al. [8] indicated that the species composition detected by eDNA technology and trawling methods are generally consistent. Furthermore, these methods were found to be largely consistent in terms of fish diversity, seasonality, and relative abundance. On the other hand, Albonetti et al. [38] revealed that eDNA barcoding detected a larger number of species than trawling, and it exhibited higher sensitivity in detecting rare species. In our study, both eDNA technology and bottom trawling methods detected a total of 30 fish species, spanning 7 orders, 11 families, and 24 genera across 18 stations in the Yangtze River Estuary. Among these, 6 species were commonly detected by both methods, accounting for 20 % of the total fish count. The remaining species unique to each method each comprised 40 % of the total. Although both methods detected an equivalent number of fish species, species such as the *E. japonicus* and the *T. kammalensis* both mid to upper layer small fish were exclusively detected by eDNA, whose existence has been confirmed by previous investigations in the Yangtze Estuary. These species are challenging to capture using bottom trawling. On the other hand, species like *M. amblycephala*, *A. schlegelii*, and *C. semilaevis* mainly inhabit muddy or rocky shallow sea environments, which are hard for trawling nets to reach [39,40]. Hence, the study revealed the limitation of bottom trawling in capturing small and elusive fish species. In contrast, eDNA technology effectively overcomes these limitations, detecting species that trawling misses, and is more environmentally friendly with minimal impact [41]. The fish species detected by eDNA technology reflect their actual presence in the

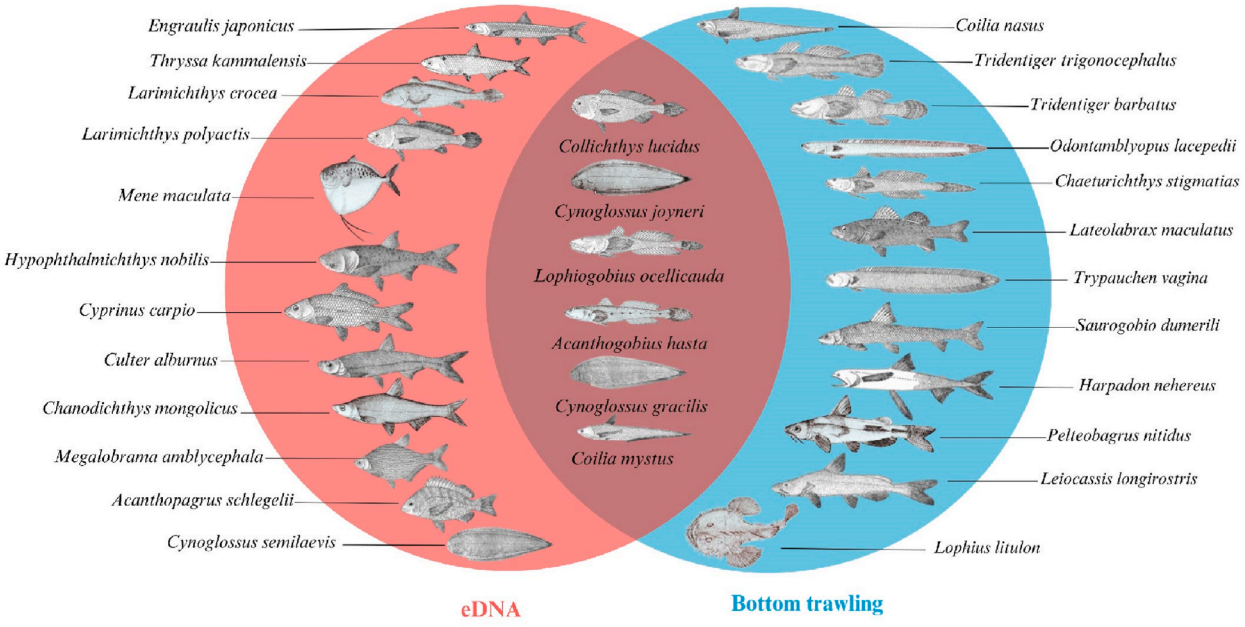

Fig. 4. Comparison of Fish Species Identified by eDNA Technology and Bottom Trawling.

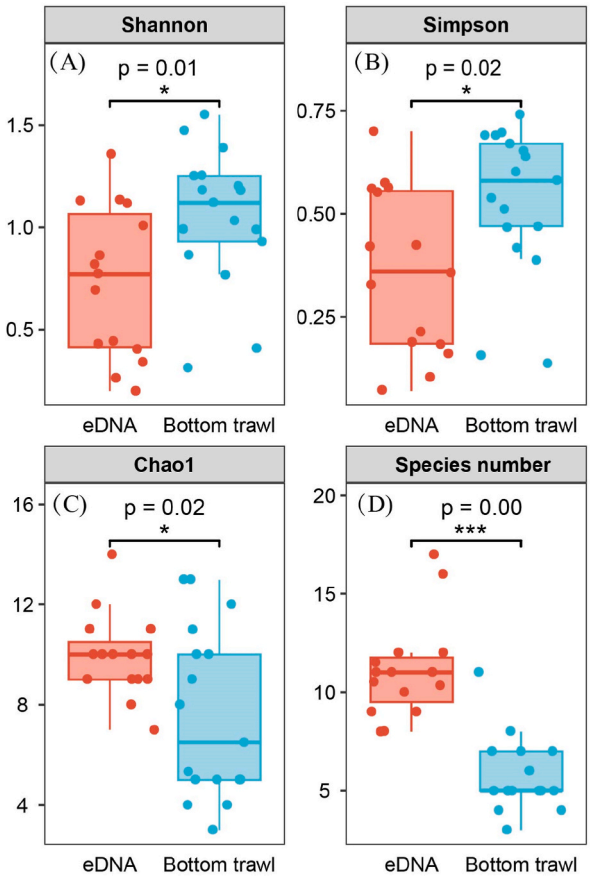

Fig. 5. Alpha Diversity of Fish Species in the Yangtze River Estuary Based on Shannon Index (A), Simpson Index (B), Chao 1 Index (C) and species number (D); $*p < 0.05$, $***p < 0.001$.

area to some extent. However, it should be noted that factors such as the stability and degradation rate of eDNA may lead to false positive or false negative results for some species. Meanwhile, due to the incompleteness of reference sequence databases and limitations inherent in mitochondrial DNA sequences, traditional trawling surveys may be more reliable in certain cases compared to environmental DNA [42]. Furthermore, both methods identified several relatively rare species, such as *L. crocea*, *C. nasus*, and *C. mystus*. This discovery is of paramount significance for the conservation of fishery resources in the Yangtze River Estuary and its adjacent marine areas. Meanwhile, compared to the 35 fish species collected in the southern Yangtze River Estuary during spring using a single-wing trawl by Li et al. [43], our study found 30 species using bottom trawl and eDNA technology, which is relatively close in count, and the 4 dominant fish species obtained by bottom trawling in this study are also among the top 5 dominant fish species obtained by Li et al. However, eDNA technology is quite different from the other, which may be due to the fact that this research site is around Chongming Island, while its research is in the waters near the sea on the southeast side of Changxing Island. At the same time, it may have a certain relationship with typhoons. In 2022, under the influence of typhoons such as "Xuan Lanuo" on No. 11 and "Meimei" on No. 12, there will be a strong phenomenon of saltwater inpouring in the north branch of the Yangtze River Estuary, and the inpouring mainly occurs between November and April of the following year. The path is that saltwater from the north branch of the outer sea invades to the south branch, raising the salinity of its waters, which may exceed the salinity of some fish. This makes the area inhospitable to fish, resulting in fewer species [44].

As evident from Fig. 2A, population of the *L. ocellicauda* species was more abundant at sampling sites Z1, Z3, and Z19. These sites are situated to the south of Chongming Island, towards the inner part of the Yangtze River Estuary. *L. ocellicauda*, typically found in estuarine and nearshore temperate waters, corresponds to this pattern [43]. The sites Z6, Z9, Z13, and Z15 had a considerable proportion of *C. mystus*, which might be attributed to its semi-anadromous behavior [45]. Every year, from late April (with a spawning peak from May to July), this species spawns in the brackish waters of the Yangtze River Estuary [46]. Additionally, habitat choices for fish and eDNA degradation rates are influenced by several environmental factors like temperature, pH, chlorophyll, ultraviolet rays, and bottom sediments [47,48]. In our study, three sampling sites (Z4, Z5, and Z7) did not detect any fish species. We postulate that this could be due to the aforementioned environmental factors affecting the samples. Thus, a more in-depth investigation is warranted to understand the relationship between fish diversity in the Yangtze River Estuary and environmental determinants.

Furthermore, the eDNA technology identified 9 non-native species to the Yangtze River Estuary. They include *Encrasicholina*

heteroloba, *Saurogobio dabryi*, *Argyrosomus japonicus*, *Gasterochisma melampus*, *Siganus fuscescens*, *Tachysurus nitidus*, *Repomucenus ornatipinnis*, *Gymnoscopelus nicholsi*, and *Parupeneus barberinus*. The presence of these species might be attributed to the residual materials from fishing vessels originating from other waters or bird migration patterns where fecal matter containing DNA of non-native fish species is introduced. Contamination during laboratory processes might also be a contributing factor [49].

4.2. Analysis of fish diversity in the Yangtze River estuary based on eDNA technology and bottom trawling

Currently, numerous researchers are comparing eDNA data with traditional sampling methods to evaluate the potential of eDNA technology in terms of diversity. Differences between biodiversity metrics obtained through eDNA and traditional methods are inevitable. Generally, the eDNA technology captures a broader range of taxa than traditional methods. This study separately lists the alpha diversity indices for eDNA and bottom trawling methods, with the Shannon and Simpson indices used to represent fish diversity, while the Chao1 and species number indicate fish richness. When comparing these four indices, the Shannon and Simpson indices were significantly higher using the bottom trawling method, whereas the Chao1 and species number were significantly higher with the eDNA technology. This contrasts with the results of Wang et al. [49], who studied fish diversity using eDNA technology in the Yangtze River Estuary. The discrepancy may be due to our sampling sites being more concentrated, with low water levels in the northern branch and inner side of Chongming Island during this survey, making it difficult for boats to access and collect samples via bottom trawl or water sampling. Their survey in 2021 allowed for sampling in the northern branch. Compared to Wang et al. [49], the larger mesh size of our nets made it challenging to capture smaller fish due to net selectivity. Similar research by He et al. [50] showed that the average taxonomic richness of fish calculated at the site level (alpha diversity) using eDNA was lower than that obtained through trawl data. Moreover, because of the incomplete reference sequence databases and limitations of mitochondrial DNA sequences, traditional trawl surveys may be more effective than eDNA technology for certain fish species and taxa. In this study's bottom trawl sampling results, some fish species were not detected by eDNA. Additionally, some OTUs could only be annotated to the family level or higher, making it challenging to directly compare eDNA results with trawl data. For a more straightforward comparison between the two methods, these OTUs were excluded from the comparison with the trawl survey. On the other hand, bottom trawl fishing often shows the immediate composition of fish species. Experiments have shown that eDNA can persist for 72 h to 21 days after the species is removed from the water. The high sensitivity of the eDNA method allows for the detection of extremely low DNA concentrations, meaning eDNA typically reflects biodiversity over a period of time. However, with the bottom trawling method, a single eDNA metabarcoding result might contain multiple effects [51].

In recent years, the environmental DNA (eDNA) technology has played a significant role in monitoring fish species diversity and has garnered increasing attention. In this study, both eDNA technology and bottom trawling methods were employed to survey the fish diversity in the Yangtze River Estuary. A total of 30 fish species were detected at all sampling sites by both methods combined. Specifically, 18 species were detected by eDNA technology, and 18 by the bottom trawling method, with 6 species identified by both technologies. eDNA technology can detect fish species that are challenging to identify through conventional methods. However, it is worth noting that although eDNA technology offers many advantages in fishery resource surveys, it cannot entirely replace traditional methods due to its inherent limitations. The eDNA method only collects information about the presence or absence of the target species. It does not provide any information on factors such as life stage, reproduction or fitness of the species, so it can be used as a good auxiliary tool for collaborative investigations [10]. The utilization of eDNA technology can reduce the impact of conventional detection in the Yangtze River Estuary ecosystem. When combined, the two approaches can maximize the number of detected species, provide a reliable assessment of species composition and fish diversity, and reduce associated sampling costs, time, and effort. To enhance the applicability and accuracy of eDNA technology in specific estuarine environments, improvements in eDNA collection and analysis methods should be focused on in future research. This includes adapting techniques to different environmental conditions and conducting comparative studies with other monitoring methods to validate their reliability. Moreover, the potential of eDNA technology in studying the seasonal variations of species composition and biodiversity in the Yangtze River Estuary is immense. Further research in this direction can shed light on the spatiotemporal distribution patterns within fish communities and their relationships with key environmental factors. This will benefit the protection of fish resources, the stability of the ecosystem, and the sustainable development of fisheries in the region. In summary, eDNA analysis serves as an effective tool for assessing fish diversity in the Yangtze River Estuary. It offers a valuable complementary approach to conventional surveys in the region, holding significant implications and promising prospects for future biodiversity conservation in the Yangtze River Estuary.

Ethics statement

The study was reviewed and approved by the ethics committee of scientific research office, shanghai ocean university (SHOU-DW-2023-097, 2022-11-30).

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Shuo Lyu: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Jianfeng Tong:** Writing – review

& editing, Validation, Supervision, Investigation, Data curation. **Jianhui Wu:** Methodology, Investigation. **Xuefang Wang:** Methodology, Investigation, Formal analysis. **Xiaoyu Geng:** Investigation, Data curation. **Chunxia Gao:** Investigation, Data curation. **Yin Wang:** Writing – review & editing, Formal analysis.

Declaration of competing interest

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

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Appendix A. Supplementary data

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