

Original Articles

Comparison of environmental DNA metabarcoding and bottom trawling for detecting seasonal fish communities and habitat preference in a highly disturbed estuary



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ABSTRACT

Environmental DNA (eDNA) metabarcoding has been used to study fish diversity in various aquatic ecosystems. However, studies on fish community structure in estuarine ecosystems have not been well corroborated by eDNA metabarcoding, and comparisons with bottom trawling are lacking. We used eDNA sequencing of mitochondrial 12S rRNA gene to investigate the fish species composition and relative abundance, community temporal and spatial variations, and community-environment relationship of the Pearl River Estuary during spring and autumn. Then, we compared these results with those obtained by bottom trawling. Results showed that eDNA metabarcoding detected more species (214 vs 90), genera (148 vs 69), families (67 vs 38), and a significantly greater number of species at each station. Results of nonmetric multidimensional scaling and permutational multivariate analysis of variance based on the Bray-Curtis dissimilarity index indicated that eDNA metabarcoding detected significant differences in fish communities between spring and autumn, which was similar to the bottom trawling results. eDNA metabarcoding revealed that the fish community differences increased with spatial distance among stations. However, when we compared results of the two methods using principal coordinates analysis, we observed discordance in the fish community differences among sites. eDNA metabarcoding may provide new insights into and a more detailed and comprehensive understanding of estuarine ecosystems. Additionally, eDNA metabarcoding revealed that salinity and temperature were closely linked to fish community composition in spring, and salinity and dissolved oxygen were closely associated with fish community composition in autumn. In conclusion, eDNA metabarcoding may represent an important supplementary method, or even replace current methods, to monitor and assess temporal and spatial variation of fish communities and infer the community-environment relationship, especially in estuarine ecosystems, which are difficult to sample using traditional methods.

1. Introduction

Estuaries are one of the prominent biodiversity hotspots, providing habitat, feeding grounds, recruitment and nursery areas for various fish species (Beck et al., 2001; Nicholson et al., 2008; Potter et al., 2015). However, as one of the planet's most threatened ecosystems, estuaries are dramatically threatened by environmental deterioration and a vast array of human-driven activities, such as global warming, agricultural and industrial pollution, habitat loss, and overfishing (Liu et al., 2021;

Mahoney and Bishop, 2017; McCall et al., 2021). Fish are essential components of aquatic ecosystems and play significant roles in directly providing goods and ecosystem services (Balvanera et al., 2006; Brummett et al., 2013) and in the ocean's biological pump (e.g., sequestering and mediating fluxes of carbon, phosphorus, and nitrogen) (Barbier et al., 2011; Beck et al., 2001; Grosholz, 2002; Oostdijk et al., 2022). Consequently, alterations in their community can be indicative of ecological regime shifts (Aschan et al., 2013; Michaela et al., 2013). Effective monitoring of fish communities is critical to the protection of

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estuarine ecosystems (Feld et al., 2010; Pettorelli et al., 2018).

Various methods have been applied to study fish communities, including bottom trawling (Farriols et al., 2017), electrofishing (Trumbo et al., 2016), gillnetting (Li et al., 2018), hand line fishing (Li et al., 2022b), underwater visual census (UVC) (Polanco Fernández et al., 2021), and acoustic measurement (Desidera et al., 2019). Among them, bottom trawling is one of the most common survey techniques for the estuary environment (Felicio et al., 2021; Li et al., 2018; Shan et al., 2010). However, this method has limitations; it has low sampling efficiency, and damages the seabed and benthic communities (Guyonnet et al., 2008; Hall-Spencer et al., 2002; Hiddink et al., 2011). Furthermore, bottom trawling heavily relies on specialized expertise of morphological identification, which is time-consuming and laborious (Afzali et al., 2021; Deiner et al., 2017).

The emergence of environmental DNA (eDNA) metabarcoding offers new possibilities for monitoring fish communities (Lamy et al., 2021). Previous studies showed that eDNA metabarcoding is emerging as a potentially powerful tool to assess fish communities in diverse habitats, including reservoirs (Boivin-Delisle et al., 2021; Li et al., 2022a), lakes (Czeglédi et al., 2021; Fonseca et al., 2022), rivers (Bylemans et al., 2018b; Laporte et al., 2022; Xie et al., 2021), estuaries (García-Machado et al., 2022; Jia et al., 2020; Zou et al., 2020; Ruan et al., 2022), coral reefs (Mathon et al., 2022; Oka et al., 2021), mangroves (Foster et al., 2020), and oceans (Afzali et al., 2021; Suter et al., 2021). eDNA is obtained from both free molecules (extracellular DNA) and free cells released by aquatic organisms from their skin cells, mucus, metabolic waste, decomposing corpses, and spawning products (Bista et al., 2017). The presence of aquatic animals can be determined by collecting eDNA from environmental samples from a habitat without direct observations or sampling of whole organisms (Bylemans et al., 2018a; Harper et al., 2021; Lamy et al., 2021). This method has been shown to have relatively high efficiency; for example, fish diversity determined by eDNA from 1 L of water was the same as or higher than fish diversity determined by trawling with one tow for 20 min (Stoeckle et al., 2020) or 3 h (Zou et al., 2020). Moreover, eDNA metabarcoding was found to be more efficient for quantifying fish species richness compared with trawling (Afzali et al., 2021; Stoeckle et al., 2020; Zou et al., 2020), UVC (Polanco Fernández et al., 2021), electrofishing (Czeglédi et al., 2021), and gillnetting (Czeglédi et al., 2021), mainly because eDNA more effectively detects smaller, less abundant species and species that are able to avoid capture.

Recent studies revealed that eDNA metabarcoding is both able to detect the presence of species and shows potential for reflecting ecological information. First, this technique can reflect the temporal and spatial distributions of local communities. For example, Laporte et al. (2022) showed that eDNA metabarcoding could be a conveyor belt of biodiversity information. Sales et al. (2021) revealed the space-time dynamics of neotropical fish communities using eDNA metabarcoding, and showed that fish community composition is dynamic in space and time. Cantera et al. (2021) tested how eDNA performed relative to UVC for evaluating beta diversity of marine communities and found that eDNA provided a more detailed picture of the main sources of spatial variation in both taxonomic richness and community turnover. eDNA can show the taxonomic diversity and functional diversity of communities; for example, Zhong et al. (2022) showed that eDNA recovered distinct variations of fish taxonomic and functional diversity by integrating ecological traits. Moreover, eDNA metabarcoding can also reveal the impact of environmental factors on fish communities (Berger et al., 2020; Czeglédi et al., 2021; Xie et al., 2021).

The Pearl River Estuary (PRE) region is among the most industrialized and densely urbanized regions worldwide. As a typical estuarine ecosystem (Kuang et al., 2021), fish biodiversity in this region has dramatically declined in recent decades because of anthropogenic disturbance (e.g., overfishing, channel drainage, and development of ports and sea-spanning bridges) and environmental change (e.g., ocean warming, pollutant content increase, and dissolved oxygen [DO]

decrease) (Hou et al., 2021; Zeng et al., 2019). However, because of the complex geographical location of the PRE (e.g., it has many waterways, a lot of marine engineering, and the large area of the Dolphin Nature Reserve), the use of traditional methods to study fish communities is usually limited and expensive. Therefore, many studies have begun to use efficient and nondestructive monitoring techniques to evaluate the health of aquatic ecosystems (Jia et al., 2020; Nagarajan et al., 2022; Zou et al., 2020).

To evaluate the use of eDNA compared with bottom trawling for studying fish communities in estuarine ecosystems, we conducted a study in the PRE. The main goals of this study were to: i) examine the similarities and differences in detecting species and ecological traits using eDNA metabarcoding and bottom trawling, ii) compare the congruency in characterizing space-time dynamics of fish communities between the two methods, and iii) compare the congruency in the relationship between the fish community and environmental factors revealed by the two methods.

2. Methods

2.1. Study area

The PRE, which is situated on the south coast of China and connects the Pearl River and the South China Sea, is a large subtropical and permanently open estuary (Kuang et al., 2021) (Fig. 1). The Pearl River is the second largest river in China and has an annual runoff exceeding $3.26 \times 10^{12} \text{ m}^3$ that flows into the PRE through several waterways (Editorial Committee, 2010). Additionally, runoff across the Pearl River Delta floodplain carries a large amount of nutrients to the PRE, which results in extremely high primary and secondary productivity of the PRE (Hou et al., 2021). The average temperature in spring is 24.70 °C, and the salinity is 9–34.13 ‰, while average temperature in autumn is 29.10 °C, and the salinity is 1–31.61 ‰. In addition, the islands ranged from east to west outside the estuary represent a strong natural barrier in the PRE. This makes the PRE a natural spawning and feeding ground for fish, and results in high fish diversity in the PRE.

2.2. Bottom trawling survey

Bottom trawling was performed at nine sites in the PRE in both September 2020 (autumn) and April 2021 (spring) (Fig. 1). To reduce the impact of fish activities on the spatial variation of community structure, all sampling work was completed within 4 days. Bottom trawling was not conducted at site S10 (Fig. 1) because of the poorly suited bottom structure. Each site was dragged during the day with a target duration of 30 min at 3 knots with a net (cod-end mesh size, 3 cm; net mesh size, 5 cm; upper strand length of net, 36 m; full-length of net, 50 m). The fish samples obtained by bottom trawling were maintained on ice, transported to the lab, and preserved in a –20 °C freezer. Fish morphological identification to the species level was based on an identification key described by Wu (2021).

2.3. eDNA sample collection

eDNA sample collection was conducted at 10 sites and in parallel with trawling (Fig. 1). In total, 15 L of mixed water samples were collected at each sampling site, which included 5 L of surface water (underwater 1 m), 5 L of middle water (10 m underwater; no middle water was taken when the water depth was <10 m), and 5 L of bottom water (1 m from the bottom). The mixed water samples were stored in water buckets. The collected water was subsequently filtered on-site within an hour through a 0.45-µm cellulose acetate membrane (Jing-jin, China) with a vacuum peristaltic pump (Auto-Science, China). Then, one membrane was used to filter 1 L water samples, and a total of 6 L water were filtered at each site. Additionally, a negative filtration control with ultrapure water was filtered. Finally, the membranes were

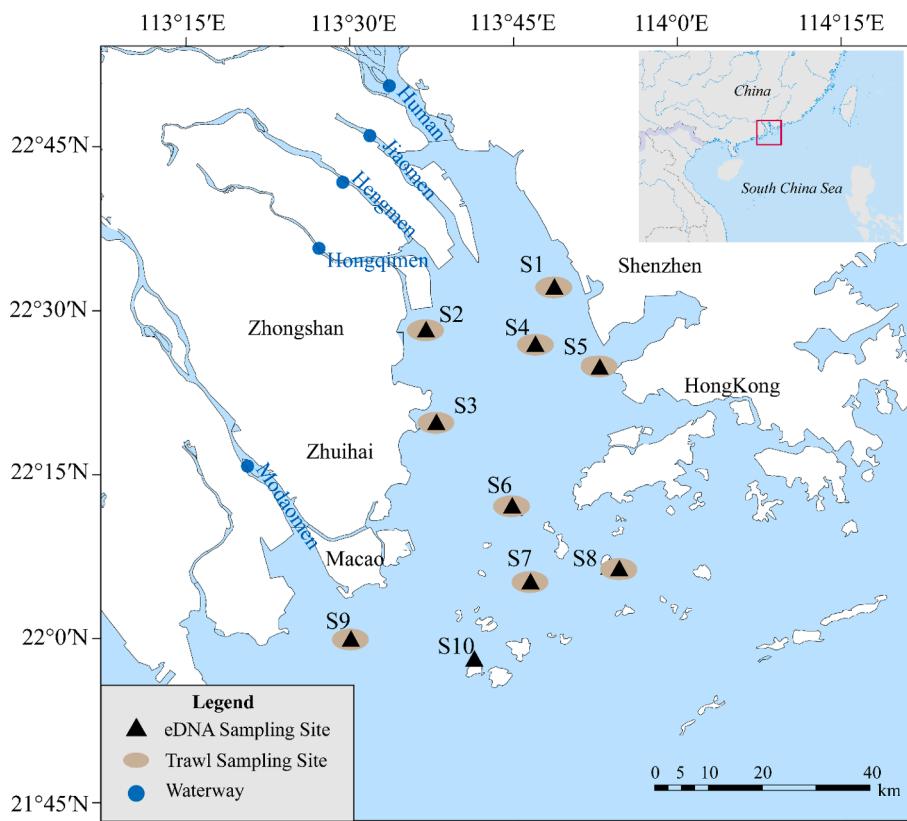


Fig. 1. Map of sampling sites in the Pearl River Estuary.

placed in a freezing tube (Biofount, China) and immediately stored in liquid nitrogen. Before sampling at each station, a water harvester, water kettles, and filtration equipment were disinfected with 5% sodium hypochlorite and rinsed with sterile water (Ji et al., 2022).

2.4. Measurement of environmental variables

While collecting eDNA samples, we monitored six environmental variables at each sampling site. For bottom water, DO, salinity, pH, and temperature were measured in situ with a YSI multiparameter probe (YSI Incorporated, USA). Depth was measured by shipboard automatic identification system, and transparency was measured by Secchi disc (Supplementary Table S2).

2.5. DNA extraction and purification

eDNA metabarcoding experiments were conducted in a dedicated laboratory at South China Sea Fisheries Research Institute that is specialized for eDNA studies and follows important decontamination routines, including use of UV light, DNA decontaminant solution (Molecular BioProducts™ DNA AWAY™), a UV hood, and isolated pre- and post-PCR rooms.

DNA was extracted using a Marine Animal Tissue Kit (Tiangen, China) and following the manufacturer's protocol with some modifications. One membrane (1 L of water per membrane) for each sample was cut into pieces, ground, and mixed. Then, it was soaked in 600 μ L of ATL buffer and 60 μ L of proteinase K. Incubation with this mixture was performed at 56 °C for 2.5 h. Finally, we washed the filters in the mixture and performed elution in 100 μ L of AE buffer. Filtration blanks and negative controls were coextracted alongside the samples and were subjected to the same protocol as the samples. The DNA concentration was determined using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, USA) and detected in a 2.0% agarose gel. No data or bands

were observed for the filtration blanks or negative controls.

Metabarcoding of the mitochondrial 12S rRNA gene (approximately 172 bp; hereafter called the MiFish sequence) was completed using the MiFish-U/E primer set. MiFish-U/E was derived from the MiFish-U and MiFish-E primers designed by Miya et al. (2015). We replaced the different bases in the two primers with merged bases to better amplify the eDNA of cartilaginous and bony fishes. 12S rRNA was amplified using specific primers (MiFish-U/E-F: 5'- GTCGGTAAWCTCGTC-CAGC-3'; MiFish U/E-R: 5'- NNNNNNNNNNNNCATGTGGGTATCTAATCCYAGTTG-3') with a 12-bp barcode. PCRs were conducted in a 50- μ L volume that included 25 μ L of 2 \times Pro Taq Master Mix (Accurate Biology AG, China), 1 μ L of each primer (10 μ M), 3 μ L of DNA template and 20 μ L sterile distilled H₂O. Amplification occurred as follows: initial denaturation at 98 °C for 30 s, denaturation at 98 °C for 10 s, annealing at 60 °C for 30 s, and extension at 72 °C for 20 s; 35 cycles were performed with a final extension at 72 °C for 6 min. The PCR products were detected in a 1% agarose gel. Samples with bright main strip between 150 and 200 bp could be used for further experiments. None of the filtration blanks or negative controls showed amplification and were not sequenced in subsequent experiments. No amplification was obtained for eDNA from site S4. Therefore, site S4 was removed from subsequent analyses.

2.6. Library preparation and sequencing

PCR products were mixed in equidensity ratios according to GeneTools 4.03.05.0 analysis software (SynGene, USA). Then, mixture of PCR products was purified with E.Z.N.A. Gel Extraction Kit (Omega, USA). Sequencing libraries were generated using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) following the manufacturer's recommendations, and index codes were added. The library quality was assessed on a Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific, USA). Finally, the library was sequenced on

an Illumina Nova6000 platform and 250-bp paired-end reads were generated (Guangdong Magigene Biotechnology Co., Ltd. Guangzhou, China).

2.7. Bioinformatics

Fastp was used to control the quality of the raw data by sliding window (-W4 -M 20) (Chen et al., 2018). The primers were removed using Cutadapt software according to the primer information at the beginning and end of the sequence to obtain the paired-end clean reads (Martin, 2011). Paired-end clean reads were merged using usearch-fastq mergepairs according to the relationship of the overlap between the paired-end reads. When there was an at least 16-bp overlap, the read was generated from the opposite end of the same DNA fragment, the maximum mismatch allowed in the overlapping region was 5 bp, and the spliced sequences were called raw tags (Edgar, 2010). Fastp was used to control the quality of the clean tags by sliding window (-W 4 -M 20). The tags were clustered into operational taxonomic units (OTUs) using UPARSE with a 97% threshold (Edgar, 2013). During the clustering, usearch removed the chimera sequences and singleton OTUs at the same time, and produced an OTU abundance table (Edgar, 2010).

To make the OTU match species as accurately as possible, manual inspection and classification-specified refinement were carried out (He et al., 2022; Zhong et al., 2022). First, OTUs with total reads >10 (Berger et al., 2020; Consuegra et al., 2021) as they could be caused by sequencing artifacts or sample misidentification (Laporte et al., 2021; Schnell et al., 2015) were retained. Second, for each site, any OTU that appeared in at least two replicates was considered present, and OTU reads for each site were merged from three replicates. Otherwise, the OTUs at each site were set to 0. Third, previously published research showed that the maximum intraspecific genetic distance of fish in the PRE was 0.017 (Jiang et al., 2022). Therefore, we chose 98.3% as a minimum similarity threshold of “species”. When the sequence identity between an OTU and NCBI database barcode was $\geq 98.3\%$, the OTU was designated as belonging to a species, the OTU was retained, and OTUs that were not fish were removed. Finally, if an OTU sequence matched a native species with $\geq 98.3\%$ similarity, the OTU was defined as a native species. However, if the OTU sequence matched multiple native species or non-native species with $\geq 98.3\%$ similarity, the OTU was removed. The taxonomic information of each species annotated from OTUs was obtained on FishBase (<https://www.fishbase.org>).

2.8. Ecological traits of fish communities

To assess the differences in ecological guild composition of the two methods of detection, fish species were classified according to the following ecological traits reported by the Fish Database of Taiwan (<https://fishdb.sinica.edu.tw/>) and FishBase: (a) habitat: benthic or pelagic-neritic and (b) body size: small (typical size < 10 cm), small and medium (10–20 cm), medium (20–40 cm), and large (>40 cm) (Supplementary Table S1).

2.9. Statistical analysis

To evaluate the consistency of the two methods for detecting the ecological characteristics of fish, Chi square tests were conducted in SPSS 28.0. To assess possible correlations among overlapping species detected by the two methods, reads (OTUs), abundance (individual number of species), and biomass (weight of species in a single bottom trawl survey) were expressed as total percent by season and converted to logarithm base 10. Linear regression was then performed. ANOVA was used to compare the number of species detected by the two methods at each site in SPSS 28.0.

The Shannon-Wiener diversity index was performed to evaluate the differences in seasonal changes of fish communities detected by the two methods. For further investigation, the raw datasets from bottom

trawling and eDNA metabarcoding were standardized by the Hellinger transformation (Laporte et al., 2022). The following analysis was performed in R 4.2.0 (R core team, 2022). Then pairwise compositional distances between sites were calculated using the Bray-Curtis distances using the R package vegan (function: “vegdist”) (Dixon, 2003). A non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities was used for the ordination between the spring and autumn dataset using the R package vegan (function: “metaMDS”) (Dixon, 2003). The significance of those results was tested with permutational multivariate analysis of variance (PERMANOVA) using the R package vegan (function: “adonis2”) (Dixon, 2003). We used linear regressions to model the relationship between Bray-Curtis distances and geographic distances between sites (m) for each season in OriginPro 2022b (OriginLab Corporation, USA). Principal coordinate analysis (PCoA) was performed with the Bray-Curtis dissimilarity metric to analyze spatial patterns in the fish communities using the R package stats (function: “cmdscale”) (R core team, 2022).

The environmental factors datasets were natural-log transformed using log1p, and the Hellinger transformation standardized the abundance datasets. Following data normalization, we conducted redundancy analysis (RDA) to examine and visualize the fish communities-environment relationships using the R package vegan (function: “rda”) (Dixon, 2003). The R^2 was then adjusted using the R package vegan (function: “RsquareAdj”) (Dixon, 2003). The R package vegan (function: “envfit”) was used to test the significance of each environmental factor (after 999 random permutations). Differences were considered significant when $p < 0.05$.

3. Results

After bioinformatics filtering, we obtained 3,157,303 reads for fish from the 54 samples (three samples each from the nine sampling stations collected during two seasons). In total, 214 fish species were identified by eDNA metabarcoding and distributed across 67 families and 148 genera (Supplementary Table S1). Overall, 10,430 individuals were caught by bottom trawling at nine stations in the spring and autumn seasons, and 90 fish species from 38 families and 69 genera were identified (Supplementary Table S1).

3.1. Fish composition and relative abundance

In total, 229 fish species belonging to 156 genera and 70 families were identified when combining both eDNA metabarcoding and bottom trawling information and 75 fish species (32.75%) were identified by both methods (Fig. 2a). For eDNA metabarcoding, the 10 most abundant species were *Sardinella lemuru*, *Stolephorus chinensis*, *Collichthys lucidus*, *Harpodon nehereus*, *Nuchequula nuchalis*, *Thryssa dussumieri*, *Ambassis gymnocephalus*, *Psenopsis anomala*, *Odontamblyopus rebecca*, and *Pampus argenteus*. These accounted for 67.47% of the total number of reads assigned to species (Fig. 2b). For bottom trawling, the 10 most abundant species were *Nuchequula nuchalis*, *Ilisha elongata*, *Sardinella lemuru*, *Ambassis gymnocephalus*, *Thryssa kammalensis*, *Coilia mystus*, *Leiognathus ruconius*, *Trichiurus lepturus*, *Stolephorus indicus*, and *Thryssa dussumieri*, which accounted for 76.89% of the total catch (Fig. 2c).

For eDNA metabarcoding data, the fish compositions in both seasons were dominated by fish that belonged to benthic habits (74.87% in spring, 75.59% in autumn) and that had a small or medium body size (37.70% in spring, 40.94% in autumn) (Fig. 3). Similarly, for bottom trawling, the fish compositions of both seasons were dominated by fish that belonged to benthic habits (68.85% in spring, 72.73% in autumn) and that had a small or medium body size (49.18% in spring, 46.15% in autumn) (Fig. 3). Between the seasons, there were no significant differences between the ecological traits of the fish communities detected by the two methods (Chi square test, $p > 0.05$). For the 140 fish species detected by eDNA metabarcoding exclusively, there were slightly different results. The majority of fish were small-sized in spring (Fig. 3).

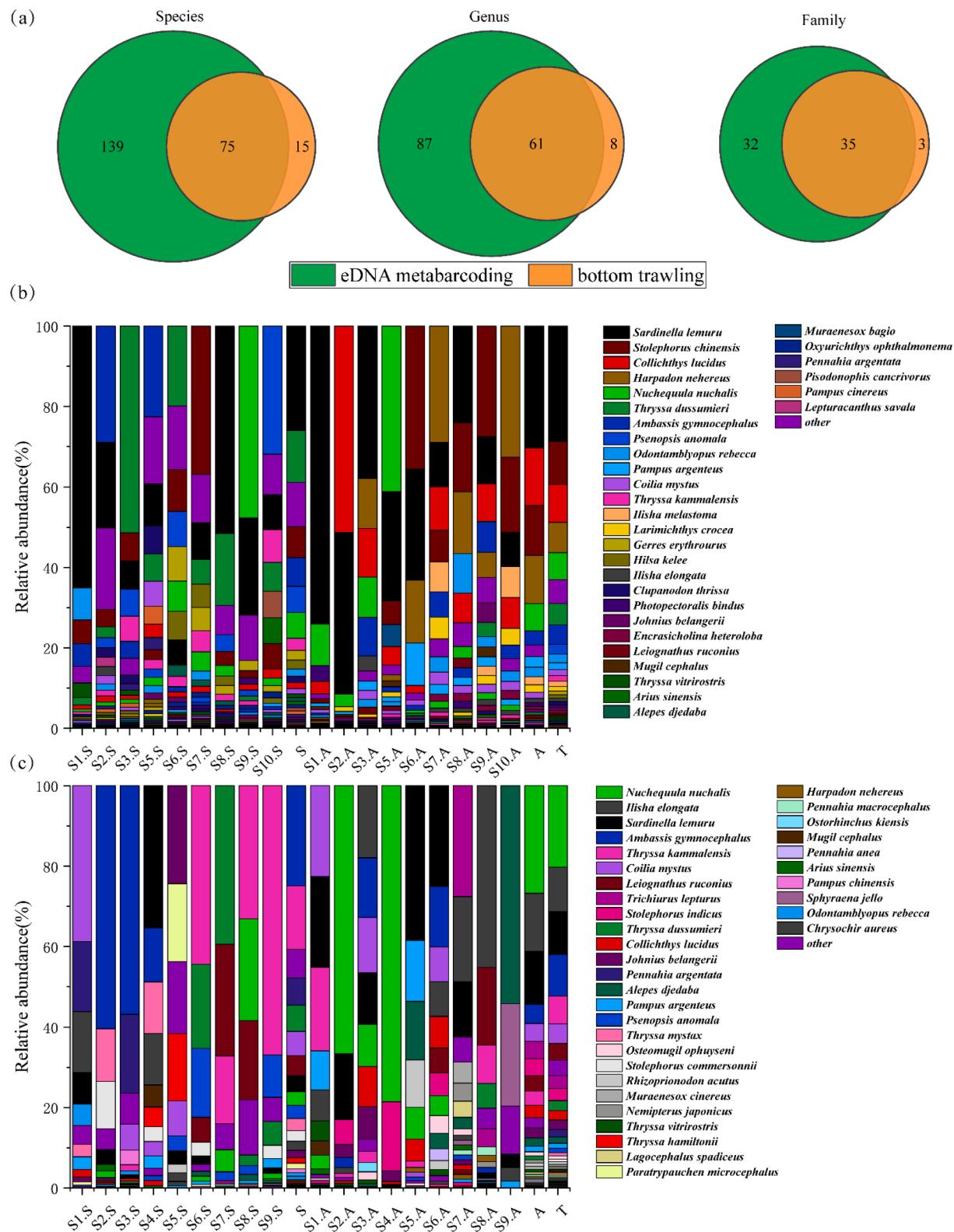


Fig. 2. Differences in fish species, genera, and families detected by eDNA metabarcoding and bottom trawling methods (a). Relative abundance (%) of the fish at the species level with eDNA metabarcoding (b) or bottom trawling (c) at every sampling site. Species with a relative abundance lower than 0.3% were included in “other” (b, c). The relative abundance in the spring, autumn, and both seasons are indicated by S, A, and T, respectively.

Moreover, we compared OTU reads from eDNA metabarcoding and abundance/biomass from bottom trawling for the 75 co-detected species on log-log plots scaled as percent values (Fig. 4). There were significant correlations between reads and abundance (spring: $p < 0.001$; autumn:

$p < 0.001$), and between reads and biomass (spring: $p < 0.001$; autumn: $p < 0.001$). Additionally, the abundance data (spring: $R^2 = 0.41$; autumn: $R^2 = 0.31$) showed stronger correlation with the amount of reads than the biomass data (spring: $R^2 = 0.31$; autumn: $R^2 = 0.28$).

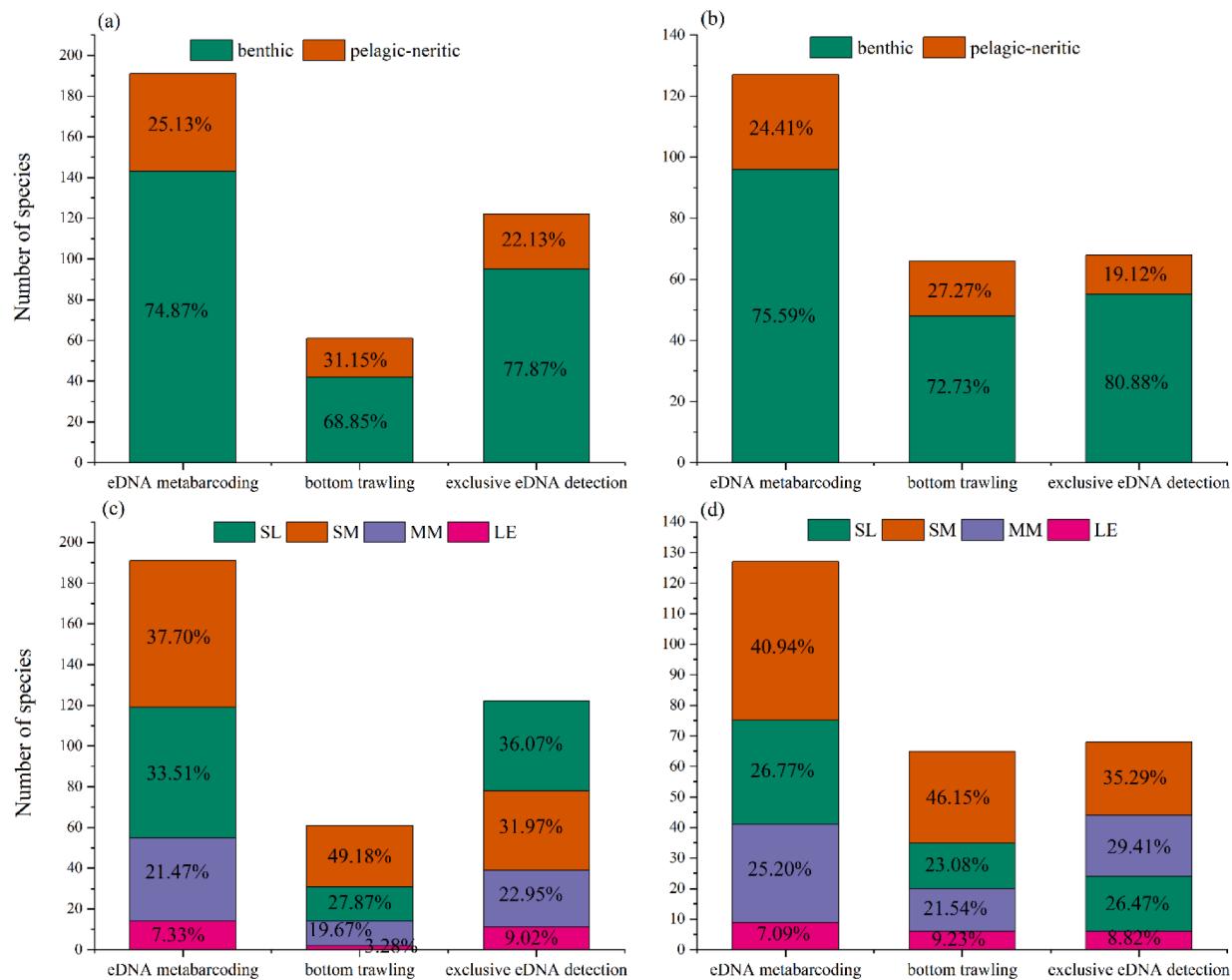


Fig. 3. Comparison of different methods for detecting the composition of fish ecological traits. Exclusive eDNA detection revealed what fish ecological traits were only detected via eDNA metabarcoding. (a) Species composition by habitat in spring; (b) species composition by habitat in autumn; (c) species composition by body size in spring; (d) species composition by body size in autumn. Body size: Small (SL), small and medium (SM), medium (MM), and large (LE).

3.2. Seasonal variation

The number of fish species detected at each sampling site by eDNA metabarcoding in both the spring (average = 157.55, SD = 13.77) and autumn (average = 103.11, SD = 4.93) was significantly greater (Mann-Whitney-Wilcoxon Test, $W = 45$, $p < 0.001$) than that detected by bottom trawling (spring: average = 19.33, SD = 4.09; autumn: average = 20.11, SD = 8.95) (Fig. 5a). eDNA metabarcoding (ANOVA: $p = 0.065$) and bottom trawling (ANOVA: $p = 0.804$) showed that there was no significant difference in fish diversity between spring and autumn based on the Shannon-Wiener diversity index (Fig. 5b).

The NMDS analysis revealed remarkable seasonal variations in the assemblages of fish communities in the PRE (Fig. 5c; Fig. 5d). The PERMANOVA results confirmed that significant differences existed in the composition of communities between spring and autumn, and this was detected by both eDNA metabarcoding ($R^2 = 0.257$, $p = 0.01$) and bottom trawling ($R^2 = 0.12$, $p = 0.008$) (Supplementary Table S3). This indicates that fish species showed apparent variability between spring and autumn, whereas fish abundance did not.

3.3. Spatial variation

The compositions of fish communities varied by geographical area. Results from the linear models showed that a distance-decay pattern of similarity was found in the fish communities by eDNA metabarcoding (spring: $p < 0.05$, slope = 2.48E-6; autumn: $p < 0.001$, slope = 7.37E-6)

(Fig. 6a) and bottom trawling (spring: $p < 0.01$, slope = 5.62E-6, $R^2 = 0.21$; autumn: $p < 0.01$, slope = 5.01E-6, $R^2 = 0.17$) (Fig. 6b) in spring and autumn. Species composition dissimilarity of the communities significantly increased with geographical distance. Moreover, the increasing slope of the relationship obtained with the eDNA data was smaller than the slope obtained with bottom trawling data.

PCoA further showed spatial differences of fish communities at different sampling sites. In spring, there were differences between the two methods in fish community clustering. All eDNA samples were more dispersed (Fig. 7a), but some bottom trawling samples were more highly clustered (e.g., S2 and S3; S1 and S4; S6, S7 and S8) (Fig. 7c). In autumn, the similarity of fish community structure between sites changed. Moreover, there were similarities between sample sites S1 and S8 detected by bottom trawling (Fig. 7b), but the eDNA metabarcoding results showed there was a large amount of variation between sites S1 and S8 (Fig. 7d).

3.4. Influence of environmental factors on fish community assemblages

When using eDNA metabarcoding data, the RDA results showed that the explanatory variables accounted for 81.2% of the total variance (adjusted variation was 24.7%) and 79.0% of the total variance (adjusted variation was 16.1%) in spring and autumn community structures. Additionally, the fish community composition was significantly linked to salinity (envfit: $R^2 = 0.87$, $p = 0.001$) and temperature (envfit: $R^2 = 0.67$, $p = 0.045$) in spring (Fig. 8a) and linked to salinity

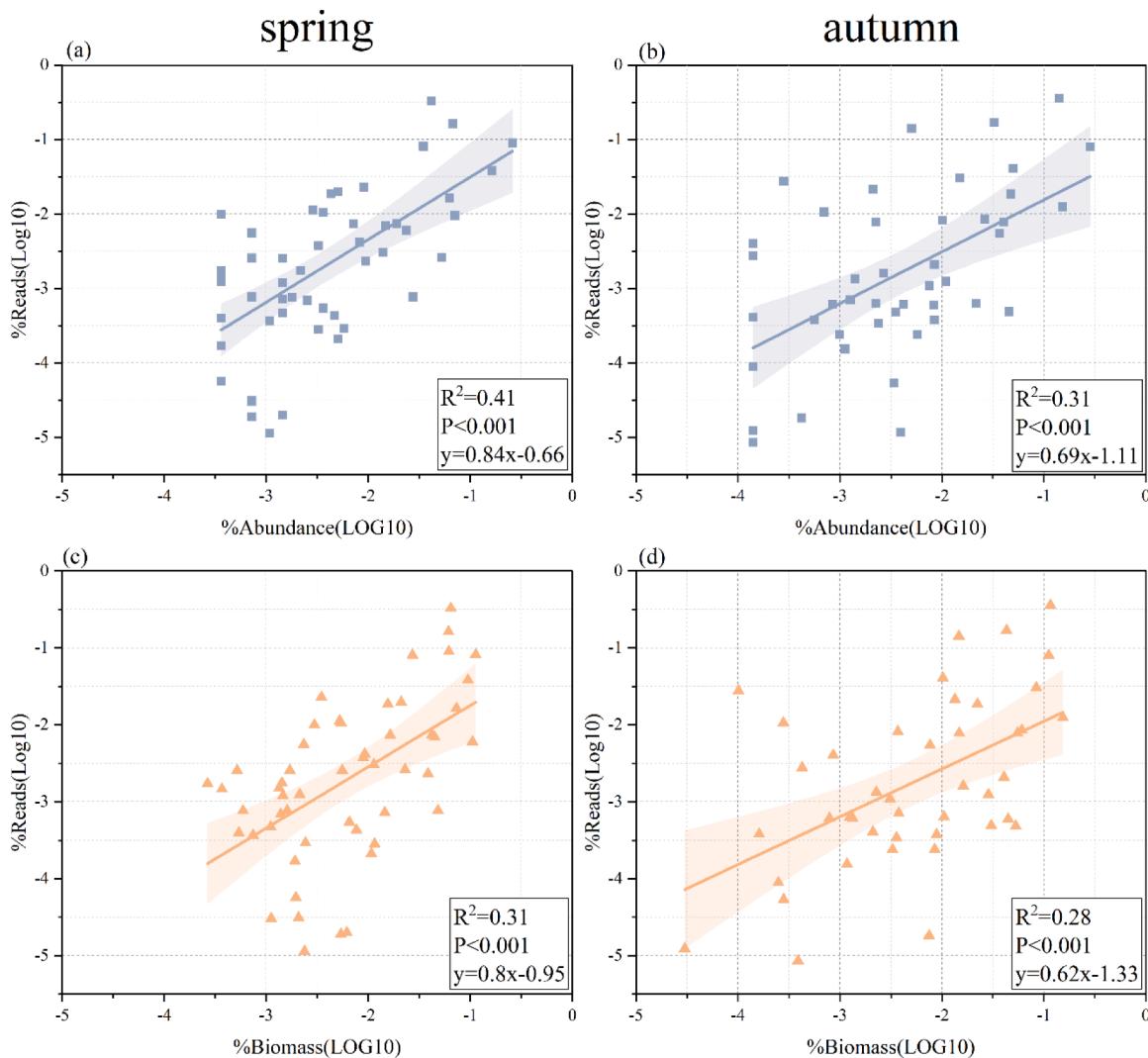


Fig. 4. Correlations between eDNA reads percentage and fish abundance (a, b) or fish biomass (c, d) for overlapping species detected by eDNA metabarcoding and bottom trawling on a log-log scale. The solid lines and 95% confidence intervals (shaded areas) were fitted by linear regression.

(envfit: $R^2 = 0.85$, $p = 0.007$) and DO (envfit: $R^2 = 0.65$, $p = 0.031$) in autumn (Fig. 8b). For bottom trawling, the RDA explained 87.3% of the total variance (adjusted variation was 11.2%) and 88.3% of the total variance (adjusted variation was 17.8%) in spring and autumn, and pH (envfit: $R^2 = 0.98$, $p = 0.001$), temperature (envfit: $R^2 = 0.90$, $p = 0.001$), and salinity (envfit: $R^2 = 0.83$, $p = 0.022$) were the key variables in determining the characteristics of the fish community in spring (Fig. 8c). However, no significant correlations were observed between environmental factors and the relative abundance of fish in autumn (Fig. 8d).

4. Discussion

In this study, the applicability of eDNA metabarcoding in studying of fish community structure in estuarine ecosystems was evaluated by comparison with bottom trawling data. Our results revealed that eDNA metabarcoding had similar effectiveness for studying ecological traits, seasonal differences of fish communities, the distance-decay of similarity, and community-environment relationship, but had superior species detection rates. Additionally, there were correlations of eDNA data with abundance and biomass. We also highlighted that eDNA metabarcoding is reliable for monitoring fish diversity, and increases our power to detect the temporal and spatial variation of fish communities, and understand the interaction between fish communities and

environmental factors.

4.1. eDNA metabarcoding is an effective tool for detecting fish diversity

Species richness is the most fundamental variable of community structure (Czeglédi et al., 2021). In this study, we found that eDNA revealed by far more fish species per site than bottom trawling. Additionally, eDNA captured more fish diversity at both the genus and family levels than bottom trawling, and this is also consistent with the findings of previous studies (Afzali et al., 2021; Aglieri et al., 2021; Zhong et al., 2022). The number of fish species identified using eDNA metabarcoding (214) was 2.38 times that of the number of fish species identified with bottom trawling (90). However, the number of species detected by eDNA was only similar to (Stoeckle et al., 2020) or slightly more than (Afzali et al., 2021; Cantera et al., 2021; Oka et al., 2021; Zou et al., 2020), but not more than twice that detected by traditional methods in previous research. The difference in our study may result from the rich species diversity in the PRE. Li et al. (2000) showed that 542 fish species appeared in the PRE waters, of which 287 species appeared in the estuary and offshore.

Moreover, bottom trawling often shows instantaneous fish composition. Previous experiments showed that eDNA persisted for 72 h–21 days post-species removal from water (Barnes et al., 2014; Pilliod et al., 2014; Troth et al., 2021). The high sensitivity of eDNA methods allow

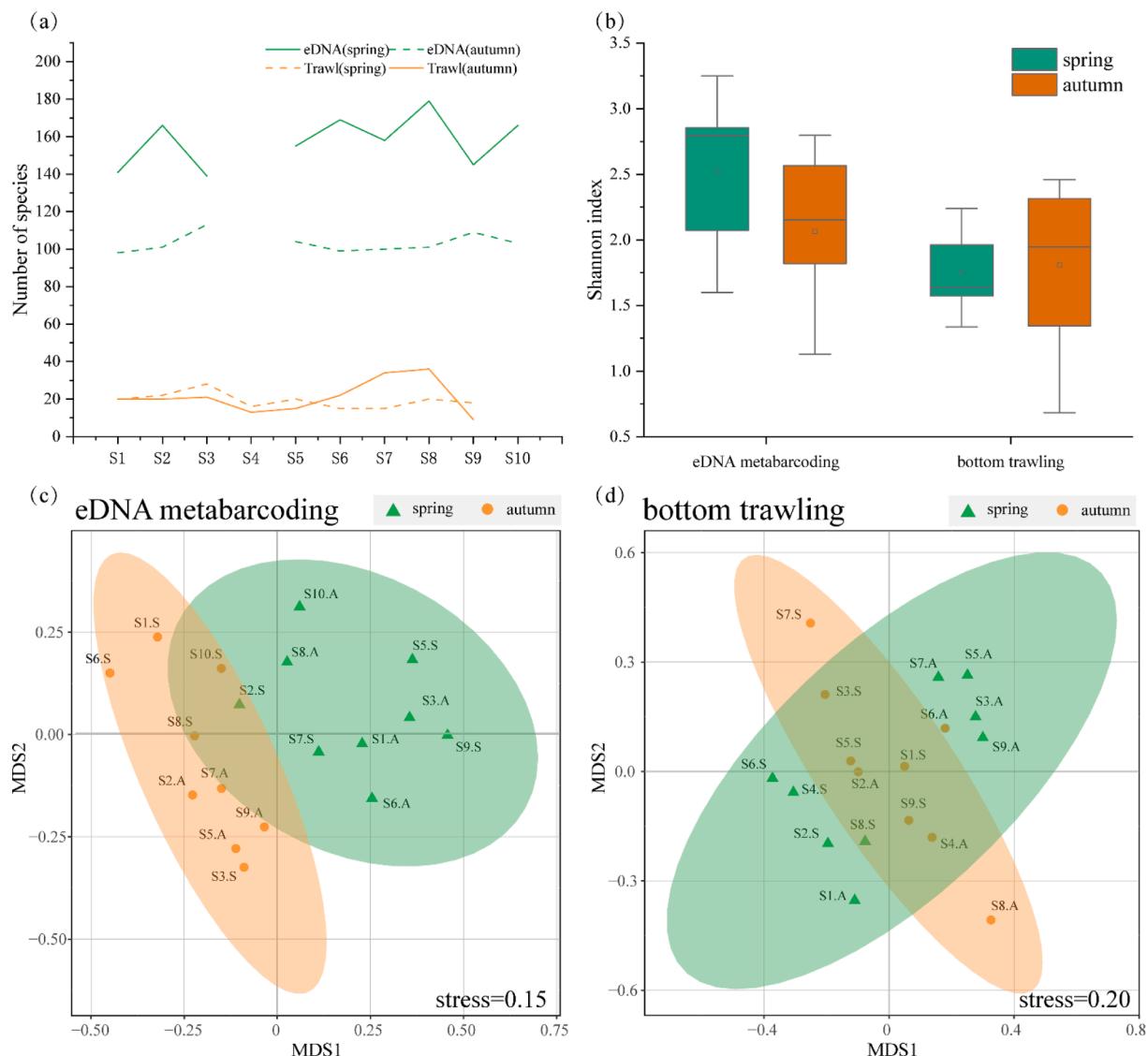


Fig. 5. The number (a) and α diversity (Shannon-Wiener diversity index) (b) of fish species per site detected by eDNA metabarcoding and bottom trawling. NMDS ordination of the data sets for eDNA metabarcoding (c) and bottom trawling (d), and the sites in the spring and autumn are indicated by S and A, respectively.

extremely low concentrations of DNA to be detected (Furlan et al., 2016). Thus, eDNA metabarcoding often shows biodiversity over a certain period of time. Therefore, a single eDNA metabarcoding result may contain multiple effects by bottom trawling method.

In addition, this study showed that eDNA metabarcoding had both a high species detection rate, and the ecological traits of the detected fish communities were consistent with those obtained by bottom trawling (Fig. 3). When considering fish species detected by eDNA metabarcoding alone, there were some differences. The highest proportion of fish were small-sized, which may have been missed using bottom trawling. Because the mesh size of the net in this study was 3 cm, it was difficult to capture small-sized fish, which may underestimate fish diversity. Czeglédi et al. (2021) found that eDNA outperformed both electrofishing and gillnetting-based surveys for detecting taxa with various traits, such as benthic species and very active species, similar to those of the present study. These findings indicate that eDNA metabarcoding is an effective tool for detecting fish diversity.

The assessment of fish diversity includes both the presence/absence of species and the abundance of species (Bylemans et al., 2018a; Bylemans et al., 2018b; Sales et al., 2021). Previous studies showed that there were positive relationships between abundance/biomass and eDNA concentration, but were mainly conducted in an experimental

indoor setting (Lacoursière-Roussel et al., 2016) or freshwater environments (Doi et al., 2017; Wilcox et al., 2016). However, this is not the case for read data produced by high-throughput sequencing. Skelton et al. (2022) showed hyper-abundant species largely drove the correlations between read numbers and the numerical abundance of the detected species, and this might be due to metabarcoding primer and PCR bias. Therefore, this aspect must be handled with caution. In this study, eDNA data were also correlated with abundance and biomass obtained by bottom trawling (Fig. 4). Our results may provide a basis for further studies evaluating the contribution of the correlation between the reads number and the abundance of fish in estuarine ecosystem.

4.2. eDNA metabarcoding could be applied to monitor the seasonal variation of fish communities in estuarine ecosystems

Understanding seasonal changes in the composition of biological communities is vital for protecting biodiversity (Li et al., 2022c; Zou et al., 2020). Recent studies have shown that eDNA metabarcoding can be effectively applied to monitor the seasonal variation in estuarine fish composition (Jia et al., 2020; Milhau et al., 2021; Sigsgaard et al., 2017). In the present study, the Shannon-Wiener diversity index showed that fish species composition between the seasons did not significantly differ

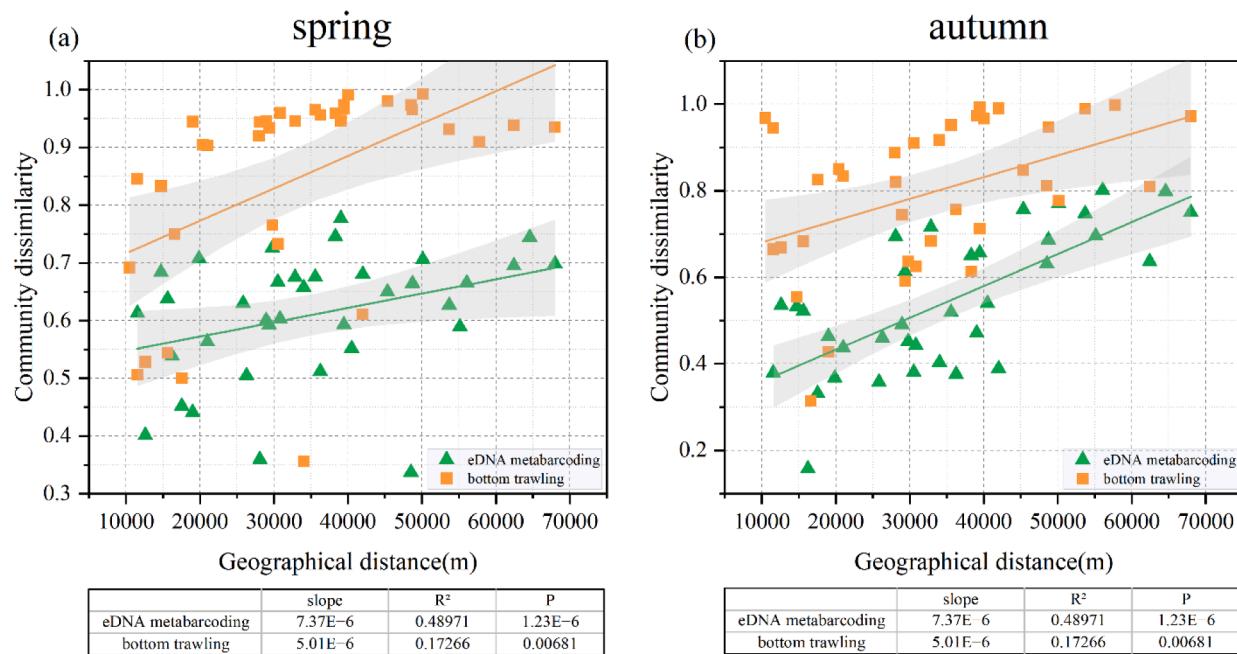


Fig. 6. Correlations between community dissimilarities (Bray-Curtis distances) and geographic distances. The solid lines and 95% confidence intervals (shaded areas) were fitted by linear regression. (a) Distance-decay in spring detected with eDNA metabarcoding (green triangles) and bottom trawling (yellow squares); (b) Distance-decay in autumn detected with eDNA metabarcoding (green triangles) and bottom trawling (yellow squares). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

based on eDNA metabarcoding and bottom trawling data. The change of biodiversity in the PRE is largely affected by the runoff of the Pearl River (Yin and Harrison, 2008). The runoff of the Pearl River discharge is greatest in July and lowest in January, and our sampling time was between two peak discharge months. This might have led to the lack of significant difference in α diversity of fish communities between spring and autumn (Yin and Harrison, 2008).

Previous studies have shown that there was significant seasonal variation in fish assemblage composition (Huang et al., 2018; Kuang et al., 2021; Li et al., 2000). Here, the seasonal difference of fish composition was also detected by eDNA metabarcoding, and the correlation was stronger than that detected by bottom trawling (Fig. 5). This may be because more species were detected in spring than in autumn, and the 10 most abundant species in spring were somewhat different from those in autumn (Fig. 2b) (Supplementary Table S1). Seasonal variation determined by eDNA metabarcoding was similar and even more pronounced than that obtained by bottom trawling, which demonstrated the utility of eDNA metabarcoding for monitoring seasonal variation of fish communities in estuarine ecosystems.

4.3. eDNA metabarcoding reveals the spatial distribution of fish communities in estuarine ecosystems

Studying the spatial variation in species composition is important for understanding ecosystem functioning, biodiversity conservation, and ecosystem management (Legendre et al., 2005; Stewart-Koster et al., 2007). However, in summer, we cannot use bottom trawling to investigate the fish diversity in the PRE because of the existence of the summer fishing moratorium (May to August) (Wang et al., 2015). Therefore, eDNA metabarcoding, for which sampling is not limited by law because it is non-destructive, may become an alternative method for discriminating spatial variations of fish communities.

However, the PRE is strongly influenced by hydrodynamics (tides, river discharges, coastal currents, and mesoscale eddies) and the high material exchange capacity limits the spatial differences that can be detected by eDNA. Therefore, eDNA data may be unsuitable for describing spatial patterns of fish communities (Pan et al., 2014; Wong

et al., 2003). However, when using eDNA metabarcoding in this study, the similarity of fish communities in the PRE was highest between nearby sampling sites, and declined with increasing distances between sampling sites; this is consistent with the distance attenuation theory in ecology (Nekola and White, 1999) (Fig. 6). Thus, eDNA metabarcoding could be used to display spatial distributions of signals of fish communities, even in estuarine ecosystems with turbulent water exchange and mixing.

In previous research, eDNA metabarcoding was mostly used to depict the formation of biomes identified in different habitats. For example, in comparisons between wetlands, reservoirs, and rivers (Xie et al., 2021), and between rivers, urban areas, and agricultural areas (Ji et al., 2022). In this study, all sampling sites were in the same habitat, and eDNA metabarcoding also showed differences in fish communities at various sites (Fig. 7). In addition, eDNA metabarcoding showed inconsistent results with bottom trawling in the spatial distribution of fish communities. This may provide new research insights because bottom trawling results are not necessarily completely accurate. For example, the two methods had different results for the fish communities in sites S1 and S8, and the eDNA metabarcoding results seemed to be more reasonable because of the long spatial distance between these two sites. This finding demonstrates that there are complex spatial changes in the fish communities in the PRE that can be caused by various factors [e.g., environmental change (Zhou et al., 2019), migration (Chapman et al., 2012), human activities (Hook et al., 2001)]. Therefore, the addition of eDNA method may provide more insights for future research.

4.4. eDNA metabarcoding reveals relationships between fish communities and environmental factors in estuarine ecosystems

Environmental factors explain the characteristics and changes of fish communities to a great extent (Diao et al., 2022; Wang et al., 2017). Previous studies have shown that DO, salinity, and transparency were the main variables that influence the spatial-temporal dynamics of fish in the PRE (Kuang et al., 2021; Zhou et al., 2019). Here, eDNA methods showed that salinity is critical environmental factor affecting fish communities in spring (Fig. 8a), whereas the key environmental factor of

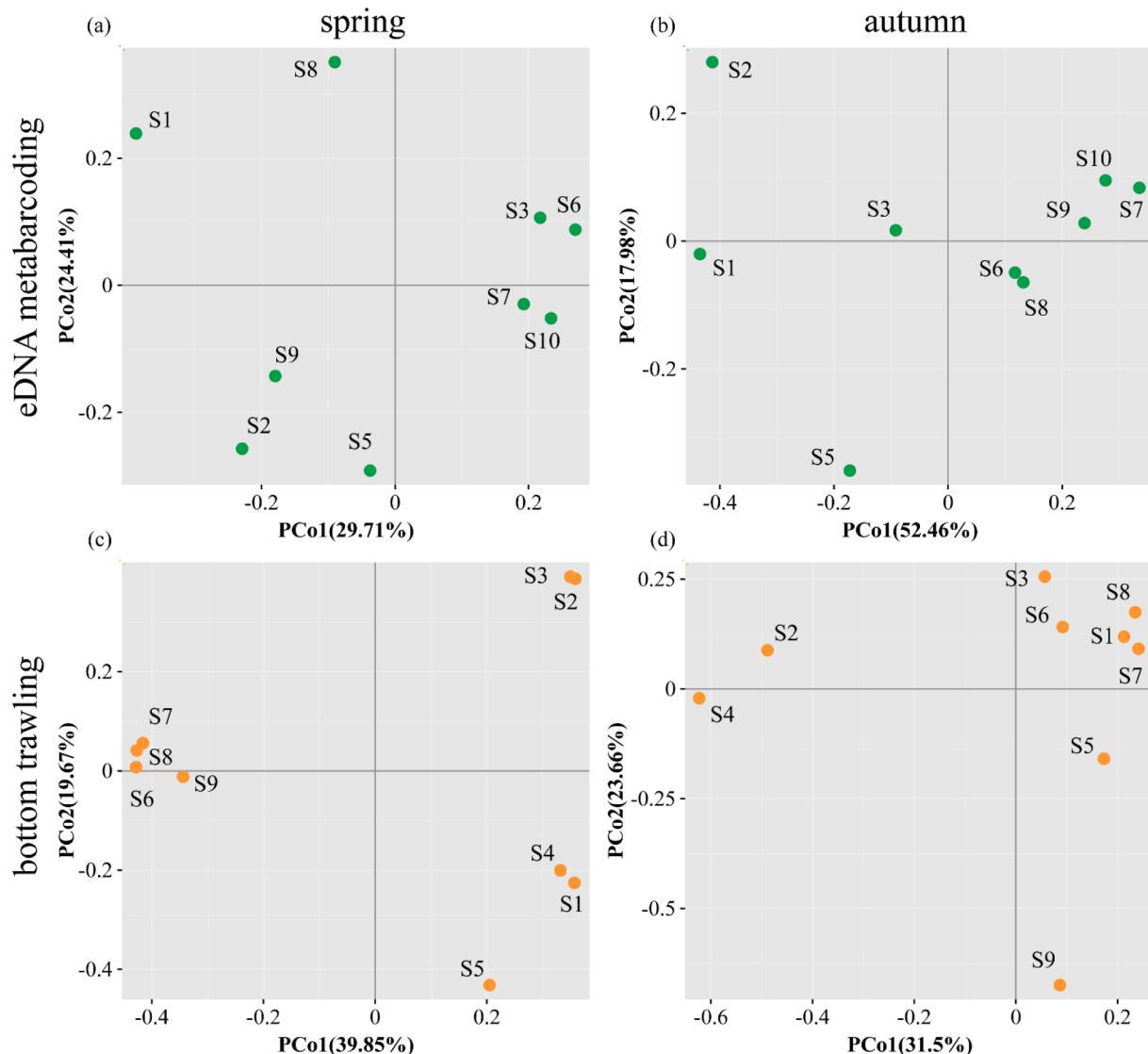


Fig. 7. Spatial variation in community structure among sites based on PCoA ordinations detected by eDNA metabarcoding (a, spring; b, autumn) and bottom trawling (c, spring; d, autumn).

autumn was DO (Fig. 8b). Because estuarine water environment could be affected by river discharge, tides, wind stress, and turbulent mixing, the salinity of the PRE is also in a state of flux (Lai et al., 2015). Additionally, the northwestern PRE is affected by the freshwater of the Pearl River, and forms a large salinity gradient in the PRE that runs from the northwest, with riverine water, to the southeast, with more saline coastal water (Huang et al., 2017a; Huang et al., 2017b). Thus, site S2 was affected by the freshwater of Hongqimen, and showed a very different fish community structure from site S8 (Fig. 7). Additionally, nutrient enrichment in the estuary has become a societal issue because the input of excess organic matter can trigger eutrophication and phytoplankton blooms. Therefore, low DO concentrations in bottom water further alters fish community distributions (Breitburg et al., 2003; He et al., 2014; Tao et al., 2021).

However, the environmental factors detected by the two methods differed, and pH also significantly affected the fish community in spring based on bottom trawling (Fig. 8c). pH affects the physiological behavior of fish (Copatti et al., 2019) and toxicity of contaminants [e.g., ammonia (Eddy, 2005), copper sulfate (Carvalho and Fernandes, 2006)], which consequently affect fish distributions. Previous research has shown that eDNA had high degradation rates in neutral or acidic pH (Strickler et al., 2015). In this study, the water quality of the sampling

sites was weakly alkaline, which may result in pH not having a significant effect on the fish communities when using eDNA methods. Additionally, some species that inhabit specific habitats or their true abundance probably undetected by the traditional method, and the incompleteness of abundance data will make them less sensitive to an environmental stressor (Cilleros et al., 2019; Ji et al., 2022). This likely played a role in the lack of significance among autumn environment factors using bottom trawling (Fig. 8d). This demonstrated the utility of eDNA metabarcoding for identifying the key environmental factors underlying estuarine fish distributions. Moreover, both methods showed that the fraction of total variance in species abundance explained by environmental influences was not as high after correction, and thus more factors (e.g., overfishing, pollution, maritime transport) need to be considered in future estuarine biodiversity studies.

5. Conclusions

This study compared the similarities and differences between eDNA metabarcoding and bottom trawling for studying species richness of fish, space-time dynamics of fish communities, and community-environment relationships in the PRE. Compared with bottom trawling, eDNA metabarcoding detected more families, genera, and species, and expanded

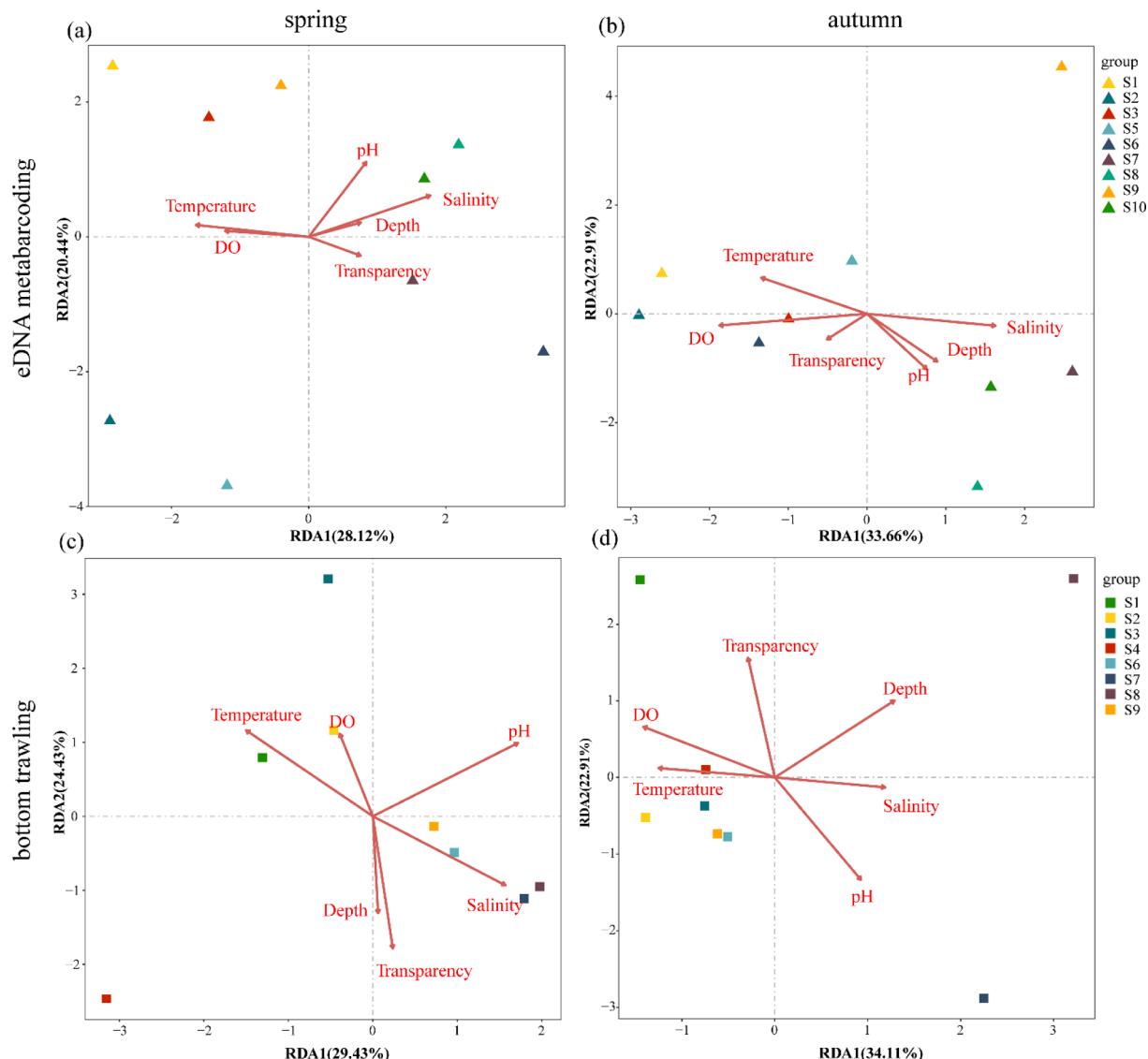


Fig. 8. Influence of environmental factors on fish community assemblages. RDA plots depict the fish communities and environmental factors for spring (a) and autumn (b) based on eDNA metabarcoding data, and depict the fish communities and environmental factors for spring (c) and autumn (d) based on bottom trawling data.

our current knowledge of biodiversity in the PRE. eDNA metabarcoding can detect the relatively complete ecological characteristics of fish communities, whereas the detection of small-sized fish seems to be limited by the mesh size of fishing nets in traditional methods. On seasonal variation, eDNA metabarcoding showed similar results to bottom trawling and there were no significant differences in α diversity of fish communities between spring and autumn. eDNA metabarcoding showed spatial differences among the fish communities, which may lead to new insights for studying estuarine fish communities. In addition, eDNA metabarcoding can also reveal the impact of environmental factors on fish communities, even if there is a difference from the results of traditional methods, which may be because environmental factors also affect the degradation rate of eDNA. In conclusion, this study shows that eDNA metabarcoding, which is environmentally friendly and convenient for sampling, advances the ability to assess the structure of fish communities in estuarine ecosystems.

CRediT authorship contribution statement

Peiwen Jiang: Investigation, Methodology, Formal analysis,

Visualization, Writing – original draft. **Shuai Zhang:** Investigation, Data curation, Validation, Writing – review & editing. **Shannan Xu:** Resources, Validation, Writing – review & editing. **Pengli Xiong:** Investigation, Formal analysis. **Yiting Cao:** Investigation, Visualization. **Zuozhi Chen:** Methodology, Funding acquisition, Validation, Writing – review & editing. **Min Li:** Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Data accessibility

The datasets analyzed during the current study are available in the NCBI Sequence Read Archive repository under accession number PRJNA876386.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2022.109754>.

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