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Research Paper

Comparative evaluation of fish community diversity and stability in the Zhanjiang mangrove watershed using environmental DNA and centipede net sampling*

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Abstract Coastal and estuarine wetlands support high biodiversity and provide essential ecosystem services. As flagship blue-carbon ecosystems, mangroves and adjacent tidal flats store disproportionate carbon, making robust biodiversity monitoring foundational to blue-carbon conservation and management**.** However, the challenges associated with conventional fish surveys limit effective management. In this study, environmental DNA (eDNA) metabarcoding and centipede sampling were conducted at four stations within the Zhanjiang Gaoqiao mangrove-mudflat system to compare their performance in assessing community composition, co-occurrence structure, and network stability. Using both methods, 102 species (80 genera, 46 families) were identified. PERMANOVA and PCoA analyses indicated that species composition differed significantly between methods (P<0.01), while the mean detection rates did not differ (P>0.05). Species accumulation and rarefaction-extrapolation curves showed that sampling sufficiency had nearly reached saturation for both methods. In the co-occurrence networks, the key species were *Glossogobius giuris* in the centipede net network and *Periophthalmus modestus* in the eDNA network. The eDNA network was substantially larger, comprising 188 significant pairs compared to 16 in the centipede net network. Habitat weighting of key species differed by method: the eDNA dataset was more enriched in benthopelagic and pelagic-neritic guilds and had higher weights in estuary/brackish, coastal/nearshore, and freshwater categories. In contrast, the centipede net dataset was more demersal, targeting sandy/muddy bottoms and mangrove habitats. Stability analyses revealed higher robustness, lower vulnerability, and greater positive and negative cohesion for the eDNA network (P< 0.001), indicating stronger signals of coordination and turnover. Overall, eDNA metabarcoding and centipede net sampling provided complementary insights into species composition, network structure, and stability, offering a more comprehensive understanding of fish diversity. These results establish a blue-carbon-relevant biodiversity baseline for a typical estuarine mangrove district and support integrated molecular-conventional monitoring to guide habitat protection and restoration across mangroves and tidal flats.

Keyword: environmental DNA (eDNA); centipede net sampling; mudflat; fish diversity; ecological network; community stability

1 INTRODUCTION

Coastal ecosystems comprise a variety of nearshore habitats, including salt marshes, mangroves, and seagrass meadows, which provide essential functions

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such as habitat and nursery support, nutrient retention and water purification, coastal protection, and carbon sequestration (Cannicci et al., 2021; Lennon and Sealey, 2022; Zhao et al., 2024; Preston et al., 2025). Although mangroves occupy only about 0.5% of the global coastal area, they contribute approximately 10%–15% of carbon sequestration in coastal zones (Sasmito et al., 2020). Mangroves and other vegetated coastal habitats are collectively known as blue-carbon ecosystems (James et al., 2024). These ecosystems store large and long-lasting carbon stocks in both biomass and soils, giving them significant potential for climate mitigation (Duarte et al., 2013; Azman et al., 2023). Consequently, strengthening standardized biodiversity monitoring in mangrove-mudflat mosaics has become an integral component of blue-carbon management to protect carbon stocks while maintaining ecosystem functions (Mcleod et al., 2011). Additionally, these systems export substantial amounts of organic carbon and nitrogen to adjacent habitats, thereby supporting the food web structure and productivity in neighboring ecosystems (Cannicci et al., 2021). Fish exhibited high species richness within mangrove ecosystems (Marley et al., 2020). As integral components of ecosystem functioning, fish assemblage composition was closely linked to root architecture, providing opportunities for refuge, foraging, breeding, and nursery functions. Additionally, fish were widely used as bioindicators of ecological condition and restoration status (Hernández-Mendoza et al., 2022; Basyuni et al., 2025). Increasing and compounding pressures from human activities and climate change threaten coastal ecosystems and their capacity to buffer hazards (Paxton et al., 2024). Disturbance-driven mangrove degradation reduces the complexity and connectivity of nursery habitats (Arceo-Carranza et al., 2024; Preston et al., 2025), which in turn leads to declines in the richness and biomass of intertidal fish assemblages (Lennon and Sealey, 2022). Within mangrove ecosystems, mudflats were spatially limited yet supported macrobenthic communities with high diversity and biomass (Leung, 2015). These mudflats were critical to nutrient cycling, material exchange, food-web coupling, and ecological connectivity (Caswell et al., 2020; Cannicci et al., 2021; Meijer et al., 2021; Preston et al., 2025). Accordingly, sustained biodiversity monitoring in mangrove-tidal flat mosaics is crucial for informing restoration and management efforts.

Monitoring fish diversity in coastal wetlands has traditionally relied on both active and passive capture methods, as well as visual or video techniques. Active capture methods include electrofishing, seine netting, and trawling, while passive capture methods encompass minnow traps, Windermere traps, fyke nets, and gill nets (Mehdi et al., 2021). Sampling gears differ in many respects, and the choice of gear can introduce unanticipated biases into population and community assessments. Gears such as beam trawls and gillnets lend themselves to extended deployments, can cover nighttime or cryptic activity periods, and allow the collection of individual-level biological data after retrieval (Golpour et al., 2022; Philpott et al., 2025). However, nets are selective, exhibiting preferences based on fish body size, swimming speed, and behavior (Klein and McCormick, 2023). Towed gears such as beam trawls are appropriate for standardized transect sampling, but their deployment in complex habitats is limited by factors such as season, tide, water level, and current velocity (Philpott et al., 2025). By comparison, the centipede net, used as an unbaited passive interception gear, can be deployed rapidly in mangrove mudflats with dense root systems and well-defined tidal creeks, causes minimal disturbance, and intercepts tidally shuttling mobile fishes with high efficiency. It often yields higher species detection and a complementary catch relative to other gears, and is therefore well-suited for routine monitoring and supplementary sampling in structurally complex habitats, such as mangroves (Scholten, 2024). Overall, a single gear rarely captures the full breadth of the community in complex habitats, and multiple, complementary methods are usually required to reduce bias (Hammerl et al., 2024).

Environmental DNA (eDNA) metabarcoding has emerged as a powerful complement to traditional survey methods, alongside recent advances in visual, acoustic, and tagging techniques (Xin et al., 2024; Bacheler, 2025). As a non-invasive approach, eDNA metabarcoding infers community composition by detecting short DNA fragments shed into the environment, followed by DNA extraction, amplification, high-throughput sequencing, and taxonomic assignment (Xin et al., 2024). Compared to capture- or observation-based surveys, eDNA generally offers greater sensitivity and efficiency and has been widely applied in fish biodiversity studies (Zou et al., 2020; Shen et al., 2022; Gibson et al., 2023; Qiu et al., 2023; Hammerl et al., 2024). However, known limitations include primer bias (Macher et al., 2023), observation and detection

biases (Shaffer et al., 2025), uncertainties arising from DNA transport and degradation, and the context-dependent relationship between sequence read counts and organism abundance or biomass. Despite these challenges, eDNA's high sensitivity enables the detection of threatened, nocturnal, or cryptic taxa that are often missed by conventional methods, while also reducing costs, increasing throughput, and avoiding the need for lethal sampling (Rourke et al., 2022; Ruan et al., 2022).

Comparative studies of fish assemblages indicate that different traditional gears often exhibit moderate to high concordance in species detection (Golpour et al., 2022). Simultaneously, eDNA and conventional surveys are complementary and are frequently used together to provide corroborative evidence for estimates of abundance or biomass (Rourke et al., 2022). In mangrove and intertidal systems, many investigations have combined traditional nets or visual techniques with molecular methods to analyze community composition and diversity. The joint application of eDNA metabarcoding alongside netting or visual sampling has increased detection rates and offered complementary insights into community structure, highlighting the growing importance of integrating molecular and conventional monitoring for comprehensive biodiversity assessments. For instance, nets have been combined with acoustic and video methods to survey fish along natural and modified estuarine shorelines, providing a robust foundation for cross-method comparisons (Smith et al., 2021). Other studies have integrated multiple traditional gears with water-column eDNA sampling in temperate reservoirs to compare community composition across methods (Golpour et al., 2022). In a mangrove-seagrass-coral reef continuum in Hainan, China, gill-net sampling combined with eDNA barcoding showed that eDNA identified greater taxonomic diversity, whereas gill nets more effectively resolved habitat-specific community differentiation and provided size and biomass data essential for ecological interpretation (Qiu et al., 2023).

Therefore, in the long term, standardized monitoring of fish assemblages across mangroves and their tidal-flat habitats is essential to support blue-carbon stewardship, guide ecological restoration and habitat management, and establish conservation priorities. Integrated monitoring that combines molecular tools with standardized sampling gear can connect biodiversity indicators to carbon-rich habitats and detect ecotonal changes, thereby prioritizing actions that sustain fish diversity and sedimentary carbon stocks. To comprehensively characterize fish diversity and community structure in the Gaoqiao mangrove-tidal flat wetland, surveys were conducted at four sites using both centipede net sampling and eDNA metabarcoding in parallel. An integrated systems framework was employed, encompassing three levels: community composition, interspecific interactions, and community dynamics and stability. Specifically, at the composition level, the two methods were compared in terms of species detection capacity, relative abundance, frequency of occurrence, detection rate, and sampling sufficiency under equal sample sizes. Next, for interactions, co-occurrence networks were constructed to identify key hub species, and habitat allocation was visualized by integrating water-column strata and distribution environments. Finally, for dynamics and stability, topological resilience and patterns of co-occurrence versus mutual exclusion were characterized based on network robustness, vulnerability, and cohesion. In this way, centipede net sampling and eDNA methods were systematically evaluated to assess their consistency and complementarity across community differences, network structure, and stability metrics. This study aimed to quantify the complementary detection capabilities and potential methodological biases in mangrove-estuarine fish assemblages, as well as to identify hub species exhibiting markedly different responses and their niche preferences. The findings aim to provide methodological support and empirical foundations for the design of long-term monitoring and the integration of multi-source data in mangrove wetlands, along with quantitative evidence to inform habitat management and the designation of conservation priorities.

2 MATERIAL AND METHOD

2.1 Sampling area and site

Zhanjiang Mangrove National Nature Reserve (ZMNNR), the largest coastal wetland reserve in China (Wang et al., 2013), is located along the northwest of the Leizhou Peninsula and the northeastern coast of the Beibu Gulf. It spans about 2.03×10^8 m^2, with roughly 7.23×10^7 m^2 covered by mangrove forest. Our research area lies within the core zone of the ZMNNR (20°14′N–21°35′N, 109°40′E–110°35′E), situated in Gaoqiao Town, Zhanjiang, Guangdong, China. The waterways draining the Zhanjiang Mangrove Forest discharge into Yingluo Harbor, which is encircled by dense mangrove stands that form an intertidal ecosystem

at the confluence of saline and freshwater. The maze of tidal creeks and intricate tidal channels creates ideal conditions for the establishment and growth of mangrove roots, as well as critical habitat for diverse marine organisms (Li et al., 2016). Tidal fluctuations are irregular over diurnal cycles, with an average tidal range of 2.53 m and a maximum of approximately 6.25 m (Wang et al., 2013). The region experiences a mean annual temperature of 23.8 °C, a mean annual precipitation of 1 704 mm, and a mean annual relative humidity of 81.8% (Wang et al., 2019a). The region's warm and humid climate offers conducive growing conditions for a diverse array of mangrove species, with the dominant taxa comprising *Aegiceras corniculatum*, *Bruguiera gymnorrhiza*, *Avicennia marina*, and *Rhizophora stylosa* (Wang et al., 2019b).

Four sites were selected in the summer (August 2024) in the mangrove mudflat waters of Zhanjiang mangrove for fish diversity surveys using unbaited centipede net capture and eDNA metabarcoding (Fig.1). At each site, five centipede nets (10 m each; 50 m total) were deployed and retrieved once per tidal cycle, and this deployment-retrieval sequence was repeated on three consecutive days to yield three replicate samples per site. For eDNA

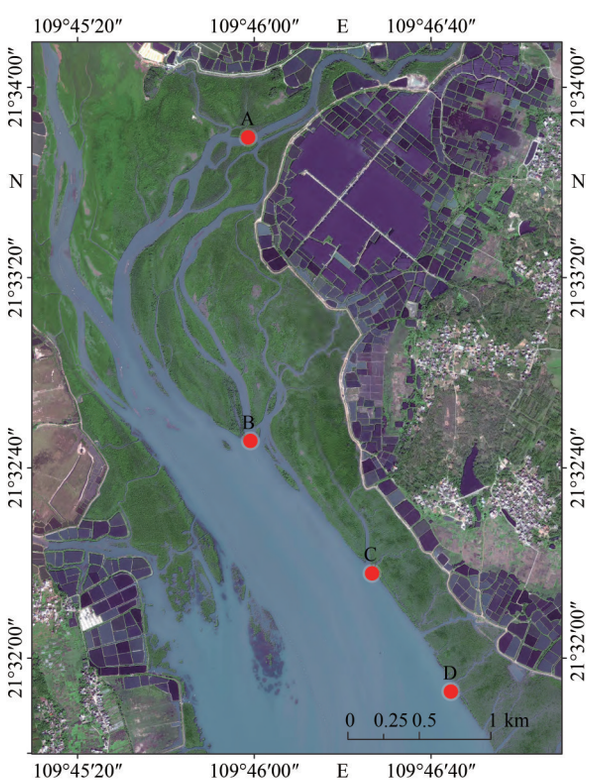

Fig.1 Map of sampling sites in the ZMMNR, Gaoqiao Town, Zhanjiang, Guangdong, China

sampling, four separate 1.5-L surface water samples were obtained at each site and immediately filtered through 0.22-μm Supor®-PES membrane (Pall Corporation, Port Washington, NY, USA) using a vacuum peristaltic pump (Auto-Science, Tianjin, China). The membrane filters were then placed into sterile 5-mL cryotubes, flash-frozen in liquid nitrogen, and stored at -80 °C until DNA extraction and sequencing were performed.

2.2 Centipede net capture for fish diversity analysis

Centipede nets are among the few gear types that can be used directly within mangrove areas; owing to their small size and pliable design, they conform to complex substrates and can be deployed around mangrove roots and other structures (Tse et al., 2008). They are more readily used than traditional sampling gear within complex mangrove root systems (Faunce and Serafy, 2006; Wang et al., 2009). Owing to their small cross-section and flexible design, centipede nets can adapt to soft substrates with abundant roots and wrap around mangrove support roots, reducing fish entanglement and facilitating escape during immersion. Many studies have used centipede nets to collect fish in mangrove settings (Zhang et al., 2020b; Jiang et al., 2022; Wang et al., 2026). The centipede net used in this study was a prism-shaped gear constructed from netting (35 cm wide, 25 cm high, 10 m long, 8.5-mm mesh) supported by 23 rectangular iron frames (35 cm×25 cm) spaced at 40-cm intervals (Supplementary Fig.S1). At each site, five nets were connected head-to-tail and treated as one sampling unit; deployments were repeated on three consecutive days, yielding three replicate units per site. Nets were set parallel to the shoreline at high tide and hauled after one full tidal cycle the following day. The total sampling effort comprised 12 units (60 nets). The fish samples collected with centipede nets were kept on ice and promptly transported to the laboratory. In the lab, specimens were taxonomically identified to the species level based on external morphological characters, and their abundances were recorded. Initial identifications were made on the basis of external morphology and then cross-referenced with previous Gaoqiao community studies (Lei et al., 2022; Zhao et al., 2022a; Wang et al., 2023) and species information (distribution, habitat, diet, conservation status, body size) obtained from FishBase (https://www.fishbase.se/search.php, accessed on 18 August, 2024), the

World Register of Marine Species (WoRMS, https://www.marinespecies.org/index.php, accessed on 18 August, 2024), and the Fish Database of Taiwan, China (https://fishdb.sinica.edu.tw/chi/home.php, accessed on 18 August, 2024). In instances where morphological traits were inadequate for precise identification or where specimens were particularly diminutive, muscle tissue was collected for COI barcoding to verify species identity, using the primers FishF1 (5′-TCAACCAACCACAAAGACATTGGCAC-3′) and FishR1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) to amplify a 655-bp fragment (Xing et al., 2018). Subsequently, the data were systematically compiled and examined to generate comprehensive documentation of species' morphological characteristics and community structure metrics for the Gaoqiao mangrove fish assemblage.

2.3 eDNA laboratory procedure and bioinformatic analysis

2.3.1 eDNA extraction and PCR amplification

For total eDNA extraction, eDNA captured on the filters was recovered using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, GA, USA) following the manufacturer's protocol. In this study, the amplification of eDNA barcodes by PCR from each DNA extract was conducted using the MiFish-F (5′-GTTGGTAAATCTCGTGCCAGC-3′) and MiFish-R (5′-CATAGTGGGGTATCTAATCCTAGTTTG-3′) primers. These primers targeted a ~180-bp fragment of the mitochondrial 12S rRNA gene. Thermal cycling was performed as follows: initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s; and a final extension at 72 °C for 10 min. Each sample was amplified in triplicate in 20-μL reactions using TransStart FastPfu DNA Polymerase, with each reaction containing 4 μL of 5× FastPfu Buffer, 2 μL of 2.5-mmol/L dNTPs, 0.8 μL of each primer (5 μmol/L), 0.4 μL of FastPfu polymerase, and 10 ng of template DNA. The PCR products were resolved on agarose gels, and target bands were excised and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, CA, USA).

2.3.2 Library preparation and high-throughput sequencing

Following agarose gel extraction and purification, the quantification of PCR products was conducted using a Qubit® 3.0 fluorometer (Life Technologies, USA). Subsequently, twenty-four uniquely indexed amplicon libraries were then amalgamated in equimolar amounts to ensure balanced sequencing depth across all samples. The pooled fragments were ligated to Y-shaped adapters, and self-ligated adapter dimers were removed via magnetic bead cleanup. Adapter-ligated molecules were enriched by low-cycle PCR, and double-stranded DNA was denatured to single strands by treatment with 0.1-mol/L NaOH to produce the final single-strand library. Libraries were quality-checked and sequenced on the MGI DNBSEQ-G99 platform (MGI, Shenzhen, China) in PE300 mode following the manufacturer's standard protocols.

2.3.3 Bioinformatic and statistical analyses

The raw paired-end FASTQ files were first demultiplexed by sample barcodes using fastq-multx to generate per-sample R1/R2 files. Reads were then quality-filtered and trimmed with Trimmomatic (Bolger et al., 2014), and primer sequences were removed with Cutadapt, and the cleaned R1/R2 reads were then merged with FLASH. Within QIIME 2, chimeric sequences were removed to obtain high-quality, non-chimeric reads, which were subsequently denoised with DADA2 to produce representative sequences and a feature table (Callahan et al., 2016; Bolyen et al., 2019). The representative sequences were exported in FASTA format and aligned locally against the NCBI nt database using BLASTN to retrieve the best-matching taxid for each ASV. Taxonomic lineages were then obtained from the NCBI taxonomy using both the ete3 Python package (NCBITaxa module) and TaxonKit, and the two annotation sets were merged to maximize completeness across ranks (Shen and Ren, 2021). For ASVs that remained unassigned after the above workflow, additional searches were performed against NCBI GenBank (nt) and MitoFish, retrieving up to ten top hits from each database; taxonomic assignments were confirmed after comparative screening of the alignments and metadata. To further validate the eDNA-identified species, the resulting species list was cross-checked against historical records from the study basin and our findings from traditional investigation methods (unbaited centipede net). These steps minimized misannotation and false positives and ensured the reliability and accuracy of the dataset used for downstream analyses of fish community diversity and structure. Finally, the consolidated taxonomic annotations were joined with the ASV abundance table to generate a species-labeled ASV matrix, which served as the basis for

downstream analyses of fish community diversity and structure.

2.4 Community structure, sampling sufficiency, and network stability analysis

2.4.1 Taxonomic composition and occurrence frequency

Data processing and analysis were performed using R (version 4.4.3). Unless otherwise specified, all graphics were generated with *ggplot2* and its extensions, *ggraph* and *ggalluvial*. Raw data were curated and preprocessed. The Venn diagram was generated with the *VennDiagram* package to quantify the overlap and differences in species detection between eDNA sequencing and centipede net surveys. To examine community composition, the occurrence frequency and relative abundances at the order level were summarized across all samples. Order-level occurrence frequency was calculated in two steps: first, for each sampling unit, the proportion of counts assigned to a given order relative to that unit's total counts was computed; second, only sampling units with nonzero totals were retained, and the arithmetic mean of these per-sample proportions was taken and reported as a percentage to represent the occurrence frequency of that order (Bessey et al., 2020).

2.4.2 Community variation and sampling adequacy

To compare differences in community structure between the two methods, a permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarities was initially performed to assess between-group variation. Simultaneously, a test for homogeneity of multivariate dispersions (PERMDISP) was conducted to determine whether within-group dispersion could influence the PERMANOVA results. Following these analyses, principal coordinates analysis (PCoA) was employed to visualize the separation among samples based on community composition. Because Bray-Curtis dissimilarities can yield negative eigenvalues, PCoA was performed using the wcmdscale function (*vegan* package) with a constant-addition correction to reduce geometric distortion and ensure interpretable axes and explained variance. To compare the detection capabilities of the two methods, detection rates were calculated as the proportion of species detected in each sample relative to the total number of species detected by the method. These rates were then averaged within each method, and differences between the two methods were assessed using an unpaired Wilcoxon rank-sum test. Finally, to evaluate sampling completeness and the potential presence of undetected species, species accumulation curves were constructed. Additionally, sample completeness curves and sample-size-based rarefaction/extrapolation curves were generated using the *iNEXT* package (Hsieh et al., 2016). Rarefaction and extrapolation were conducted for Hill numbers (q=0, 1, 2) using the *iNEXT* default abundance-based framework, with extrapolation extended to twice the observed total abundance for each method. Diversity was expressed as Hill numbers, with q=0 corresponding to species richness, q=1 to the exponential of the Shannon index, and q=2 to the inverse Simpson index (Chao et al., 2014). Ninety-five percent confidence intervals were obtained via bootstrap resampling implemented in *iNEXT*.

2.4.3 Molecular ecological network construction and stability analysis

For network construction, data wrangling and subsetting were performed using *dplyr*, retaining only taxa/ASVs detected in at least 40% of the samples. Pairwise Spearman rank correlations were computed with *psych* and adjusted by the Benjamini-Hochberg procedure to obtain the correlation matrix R and adjusted P matrix. Based on the eDNA $|R|$ matrix, a random matrix theory (RMT) (Deng et al., 2012) cutoff τRMT was determined with *RMThreshold* by iteratively increasing the similarity threshold and evaluating the eigenvalue spacing distribution until the transition from random to non-random structure was detected; the resulting τRMT was then applied to both datasets for consistent edge selection. Undirected edges were defined for pairs with $|R_{ij}|\geq$τRMT and adjusted $P_{ij}<0.05$. Edge sign (positive/negative) and $|R_{ij}|$ were stored as edge attributes. Network diagrams were then produced from the resulting adjacency matrices. Global topology and node-level properties were computed using igraph, including the number of nodes and edges, mean degree, global clustering coefficient, mean shortest-path length, degree/betweenness/closeness centralization, as well as modularity.

In the stability analysis, the constructed *igraph* objects were used to evaluate network robustness, vulnerability, and cohesion. First, robustness was assessed through repeated random-failure simulations, which performed 500 Monte Carlo iterations per network, with 50% of nodes uniformly removed. Network performance under random attack was quantified by the proportion of nodes remaining in

the largest connected component after removal (Gu et al., 2023). For each network, the distribution of this proportion across iterations was taken as the robustness metric and used for between-method comparisons. Second, vulnerability was evaluated within the global efficiency framework: overall network efficiency was computed, and each node was subsequently removed one at a time. The resulting decrease in efficiency was used to characterize the impact of node removal on connectivity (Albert et al., 2000). Finally, cohesion was analyzed by decomposing the correlation matrix into positive and negative components. For each species, positive and negative connectivities were calculated and then aggregated to generate sample-level positive and negative cohesion indices (Herren and McMahon, 2017). Given the different dimensions of the two correlation matrices, the threshold sensitivity of the network was evaluated by reconstructing the network at different absolute correlation thresholds ($|R_{ij}|\geq 0.4$, 0.5, 0.6, 0.7, and 0.8; adjusted $P_{ij}<0.05$). Robustness, vulnerability, and cohesion were recalculated for each cutoff value, and the robustness, vulnerability, and cohesion of different methods were compared using the Wilcoxon rank-sum test.

To elucidate the ecological relationships among key species, taxa were selected based on significance thresholds derived from network analysis. Authoritative ichthyological and biodiversity databases were consulted to retrieve and standardize the vertical habitat (demersal, benthopelagic, pelagic-neritic) and habitat class (intertidal/tidal flats, mangrove, estuary/brackish, coastal/nearshore, lagoon, reef/hard-bottom, sandy/muddy bottom, seagrass/vegetated, freshwater [rivers/lakes]). Species names and trait fields were cleaned and harmonized using the *janitor* package. Associations among key species, their ecological traits, and detection methods were then visualized using alluvial plot implemented with *ggalluvial* and *ggplot2*.

3 RESULT

3.1 Community diversity and compositional structure of fish assemblages

The eDNA metabarcoding and centipede net sampling techniques were applied to assess fish diversity within the Gaoqiao mangrove ecosystem in Yingluo Bay. A total of 102 fish species were identified, of which eDNA identified 83 species belonging to 25 orders, 39 families, and 68 genera, while centipede nets captured 31 species from 11 orders, 19 families, and 26 genera (Table 1). Twelve species were shared by both methods (Supplementary Fig. S2); 71 species (69.61% of the total) were detected only by eDNA, and 19 species (18.63%) were captured only by centipede nets (Fig.2). Relative abundance and within-method rank were compared for the 12 species detected by both methods (Table 2). Among these shared taxa, *Planiliza macrolepis* ranked first in both eDNA (25.13% of total reads) and centipede net catches (44.16% of total individuals). *Sillago sihama* also ranked among the most abundant species in both datasets (eDNA: 5.30%, rank 5; centipede net: 4.67%, rank 6). In contrast, several shared species exhibited pronounced rank discordance between methods, with high relative read abundance in eDNA but low representation in centipede net catches. For example, *Acentrogobius caninus* ranked third in eDNA (7.82%) but was much lower in centipede net sampling (0.41%, rank 19). Conversely, some taxa were relatively abundant in centipede net catches but contributed comparatively few reads in eDNA, including *Acanthopagrus latus* (centipede net: 5.38%, rank 3; eDNA: 0.54%, rank 24) and *Butis koilomatodon* (centipede net: 2.23%, rank 9; eDNA: 0.57%, rank 23). Overall, these comparisons reveal substantial rank discordance between methods for several shared taxa, suggesting that read-based and catch-based abundance patterns may reflect different aspects of the assemblage.

High-throughput sequencing produced a total of 1 792 616 reads. Following the denoising process, 273 amplicon sequence variants (ASVs) were obtained, of which 1 309 844 reads were assigned to fish. A total of 166 fish ASVs were annotated, of which 82 were resolved to the species level, and one was assigned only to the genus level (*Bregmaceros*, family Bregmacerotidae) (Supplementary Table S1). At the order level, eDNA exhibited the highest species richness in Cypriniformes (22 species), followed by Gobiiformes (17 species) and Clupeiformes (7 species) (Supplementary Table S1). The Mugiliformes contributed the most abundant relative read abundance (399 833 reads; 30.53%), primarily represented by *Planiliza macrolepis*. The Tetraodontiformes ranked second (255 393 reads; 19.50%), dominated by *Takifugu alboplumbeus* (Fig.3). Centipede net sampling yielded 985 individuals representing 31 species. At the order level, the highest species richness was observed in Gobiiformes (12 species), followed by Perciformes

Table 1 Comparison of fish taxa detected in the Gaoqiao Mangrove Reserve based on eDNA metabarcoding and centipede net trapping

Order	Family			Genus			Species		
	Centipede net	eDNA	Total	Centipede net	eDNA	Total	Centipede net	eDNA	Total
Acanthuriformes	0	1	1	0	2	2	0	2	2
Acipenseriformes	0	1	1	0	1	1	0	1	1
Anabantiformes	0	1	1	0	1	1	0	1	1
Anguilliformes	0	1	1	0	1	1	0	1	1
Atheriniformes	0	1	1	0	1	1	0	1	1
Beloniformes	0	2	2	0	2	2	0	2	2
Blenniiformes	0	1	1	0	1	1	0	1	1
Callionymiformes	0	1	1	0	1	1	0	1	1
Carangaria incertae sedis	0	1	1	0	1	1	0	1	1
Carangiformes	0	1	1	0	1	1	0	2	2
Cichliformes	1	1	2	1	2	2	1	2	2
Clupeiformes	2	2	2	3	6	7	4	7	10
Cypriniformes	2	6	6	2	19	20	2	22	24
Eupercaria incertae sedis	2	4	4	2	4	4	2	4	4
Gadiformes	0	1	1	0	1	1	0	2	2
Gobiiformes	2	3	3	8	12	16	12	17	23
Lophiiformes	0	1	1	0	1	1	0	1	1
Mugiliformes	1	1	1	1	2	2	1	5	5
Osmeriformes	0	1	1	0	1	1	0	1	1
Ovalentaria incertae sedis	1	1	1	1	1	1	1	1	2
Perciformes	5	1	6	5	1	6	5	1	6
Pleuronectiformes	1	2	2	1	2	2	1	2	2
Scombriformes	0	1	1	0	1	1	0	1	1
Siluriformes	1	2	3	1	2	3	1	3	4
Tetraodontiformes	1	1	1	1	1	1	1	1	2
Total	19	39	46	26	68	80	31	83	102

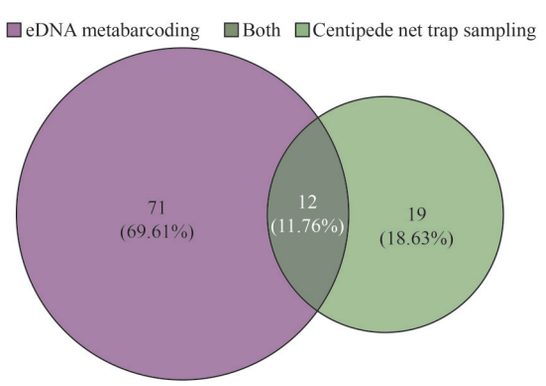

Fig.2 Comparison of fish species among different survey methods

(5 species) and Clupeiformes (4 species) (Supplementary Table S2). The relative abundance of Mugiliformes (*Planiliza macrolepis*) was the highest, accounting for 44.16% of the total orders, followed by Gobiiformes (29.04%). Within Gobiiformes, *Acentrogobius viridipunctatus* (12.89%), *Glossogobius giuris* (4.77%), and *Synechogobius ommaturus* (3.66%) were identified as the dominant species (Fig.3).

The eDNA metabarcoding data revealed the following dominant orders: Mugiliformes (32.55%), Gobiiformes (15.96%), Tetraodontiformes (15.70%), and Cypriniformes (14.07%). The species with the

Table 2 Relative abundance and rank (within method) of shared species based on eDNA read counts and centipede net catch counts

Shared species	Centipede net			eDNA		
	Total read	Relative abundance	Rank (within method)	Total individual	Relative abundance	Rank (within method)
Planiliza macrolepis	329 136	25.13%	1	435	44.16%	1
Acentrogobius caninus	102 436	7.82%	3	4	0.41%	19
Sillago sihama	69 362	5.30%	5	46	4.67%	6
Oreochromis niloticus	9 620	0.73%	20	3	0.30%	22
Butis koilomatodon	7 463	0.57%	23	22	2.23%	9
Acanthopagrus latus	7 070	0.54%	24	53	5.38%	3
Stolephorus commersonnii	6 123	0.47%	26	1	0.10%	31
Periophthalmus modestus	5 104	0.39%	27	16	1.62%	13
Butis butis	5 030	0.38%	29	16	1.62%	12
Acentrogobius chlorostigmatoides	2 059	0.16%	43	8	0.81%	15
Cynoglossus puncticeps	2 015	0.15%	44	2	0.20%	25
Bostrychus sinensis	561	0.000 4	66	1	0.001	27

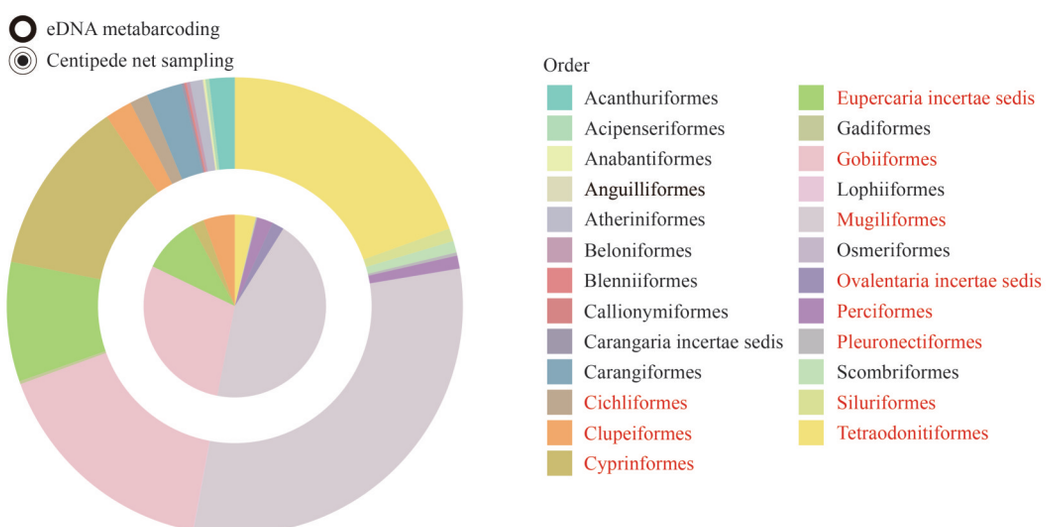

Fig.3 The relative abundance of fish at the order level based on eDNA metabarcoding and centipede net trapping

Legends with red notes were detected by both methods.

highest occurrence frequency were *Planiliza macrolepis* (26.92%), *Takifugu alboplumbeus* (15.70%), *Acentrogobius caninus* (7.32%), and *Cyprinus carpio* (6.64%) (Fig.4a; Supplementary Table S3). Among the 985 centipede net individuals, the four leading orders by occurrence frequency were Gobiiformes (35.53%), Mugiliformes (29.97%), Eupercaria incetae sedis (13.56%), and Clupeiformes (6.26%). *Acentrogobius* (13.31%) was the most representative genus from Gobiiformes, with *A. viridipunctatus* (11.62%) exhibiting a notably high occurrence (Fig.4b; Supplementary Table S4). Upon combining both datasets, the top four orders were identified as Mugiliformes (31.44%), Gobiiformes (24.35%), Tetraodontiformes (10.78%), and Eupercaria incetae sedis (10.68%). Both methods detected eleven orders: Mugiliformes, Gobiiformes, Tetraodontiformes, Eupercaria incertae sedis, Clupeiformes, Cypriniformes, Perciformes, Ovalentaria incertae sedis, Cichliformes, Siluriformes, and Pleuronectiformes (Fig.4c).

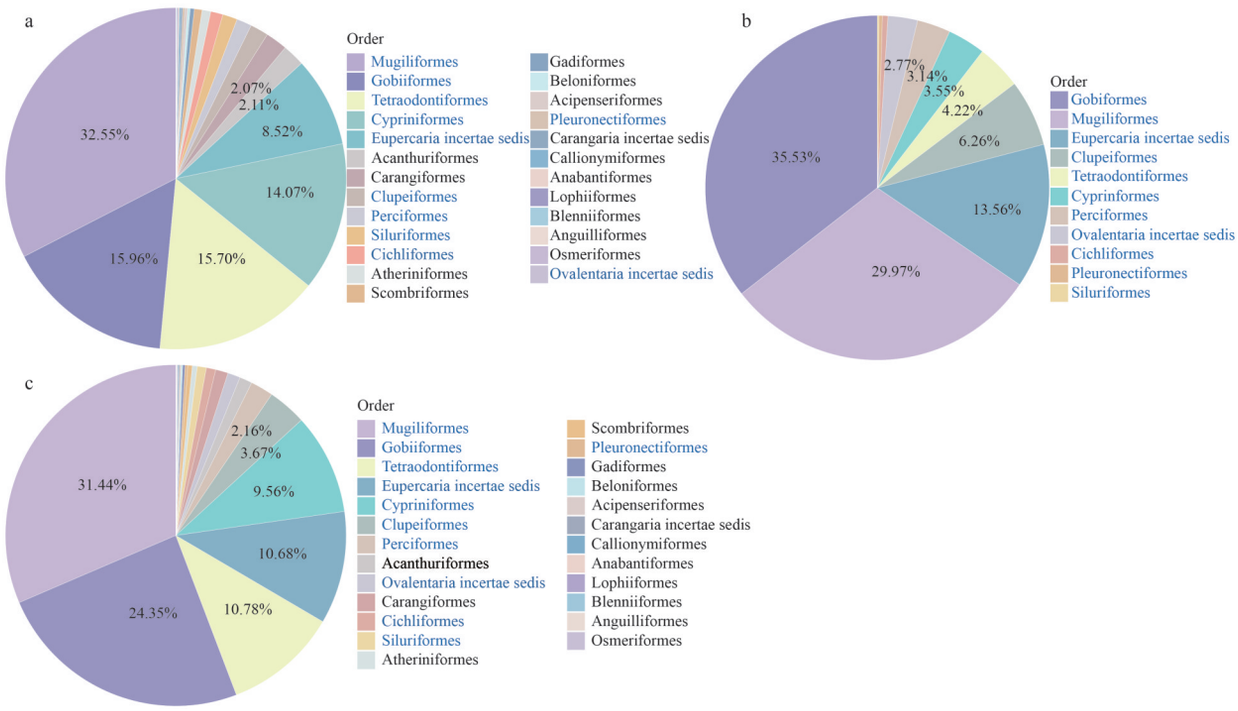

Fig.4 Mean frequency of occurrence at the taxonomic order level across sampling sites

a. centipede net sampling; b. eDNA metabarcoding; c. combined, calculated as a sample-size-weighted average of (a) and (b) using the number of effective samples per method. Legend entries shown in blue denote orders detected by both methods. Sectors with exceeding 2% were labeled with their percentages.

3.2 Evaluation of effectiveness and diversity in two sampling methods

Based on Bray-Curtis dissimilarities, PERMANOVA revealed a significant difference in fish community composition between eDNA metabarcoding and centipede net sampling (F= 17.249, P=0.001). The PCoA ordination showed clear separation between methods along the first two axes, with distinct, non-overlapping group centroids (Fig.5a). PCoA1 explained 33.8% and PCoA2 explained 11.9%, accounting for 45.7% of the total variation. At the overall level, PERMDISP did not detect a significant difference in within-group dispersion between methods (F=1.803, P=0.19; Fig.5b), supporting the view that the PERMANOVA signal primarily reflects centroid shifts rather than differences in multivariate spread. Per-site analyses further indicated significant compositional differences between methods at all four stations (PERMANOVA P<0.05 for each; Supplementary Table S5). Site-level PERMDISP indicated significant dispersion differences at two of the four stations (P<0.05; Supplementary Table S5). Overall, species composition differed significantly between methods at the whole-dataset level (PERMANOVA) and remained significant in per-site contrasts across all four stations. PERMDISP indicated no overall difference in multivariate dispersion between methods; however, site-level PERMDISP was significant at Stations A and D, suggesting heterogeneity in dispersion at these locations.

Detection rates of species were evaluated by comparing eDNA metabarcoding with centipede net sampling. Although the mean detection rate was higher in the eDNA group (62.7%) compared to the centipede net group (53.2%), this difference was not statistically significance (Wilcoxon rank-sum test, P>0.05; Fig.6a). Furthermore, species accumulation curves showed that the number of species detected by both methods approached an asymptote as sampling effort increased, indicating that the sampling intensity was sufficient to capture the predominant components of the assemblage detectable by each method. Throughout the entire range of effort, the eDNA curve remained above that of the centipede net catches, with the final observed richness approximately 2.6 times greater than that of centipede net sampling (Fig.6b).

Consistent with these patterns, sample completeness curves generated using *iNEXT* showed that, once the observed number of individuals

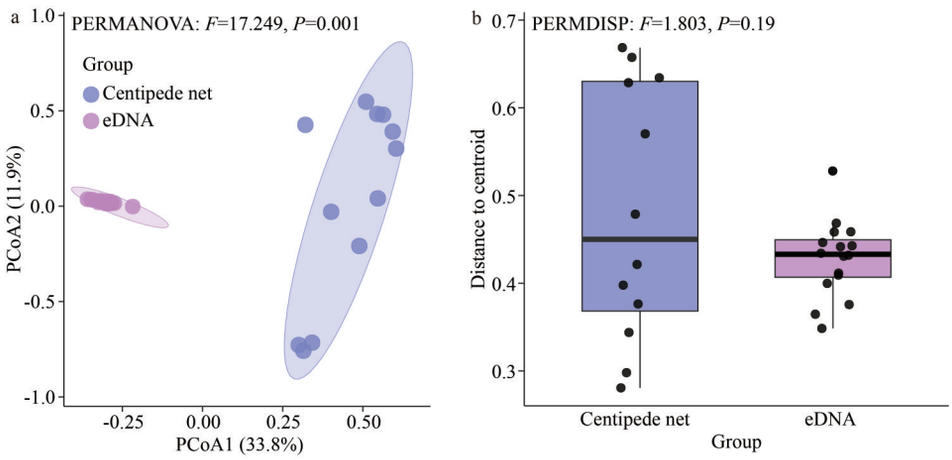

Fig.5 Community differences and within-group dispersion for eDNA and centipede net sampling

a. PCoA ordination based on Bray-Curtis dissimilarities (PERMANOVA); b. PERMDISP results comparing within-group dispersion between methods.

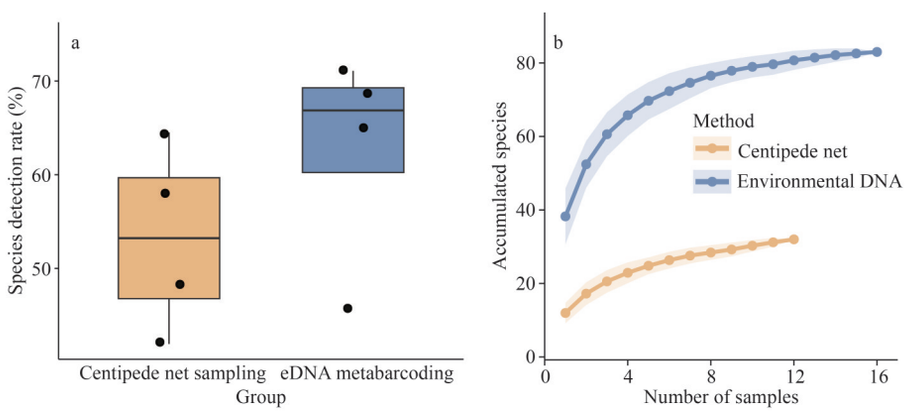

Fig.6 Species detection rates and accumulation curve for the two methods

exceeded approximately 1 000 for centipede net sampling, sample coverage approached saturation, and the extrapolated segment leveled off, indicating limited gains from additional sampling. Conversely, eDNA coverage remained high throughout its observed range, with rarefaction and extrapolation segments converging at a plateau, suggesting that the current sequencing depth provided adequate coverage of the target community (Fig.7a).

Analysis of sample-size-based rarefaction and extrapolation curves further clarified these patterns. For centipede net sampling, the q=0 (species richness) curve continued to increase within the observed range, and its extrapolated confidence interval widened with additional individuals, suggesting under-sampling of rare taxa (Fig.7b). In contrast, the q=1 (exponential of the Shannon index) and q=2 (inverse Simpson index) curves largely reached a plateau, indicating stability in the composition of common taxa. For eDNA, all three diversity orders (q=0, 1, 2) approached a plateau within the observed range, indicating higher sampling sufficiency and greater result stability. Taken together, these patterns indicate that eDNA achieved nearly complete coverage across diversity orders within the sampled range, whereas centipede net sampling still underrepresented rare taxa despite approaching coverage saturation, supporting the complementarity of the two approaches.

3.3 Co-occurrence network and habitat allocation

To explore interspecific associations within fish communities detected by the two survey methods, separate molecular ecological networks were constructed, and significant positive and negative associations were identified using Spearman correlations. A cross-method comparison revealed that, in terms of connectivity, the node with the highest degree was *Glossogobius giuris* in the centipede net network and *Periophthalmus modestus* in the eDNA network. For betweenness and stress centrality, the highest values were

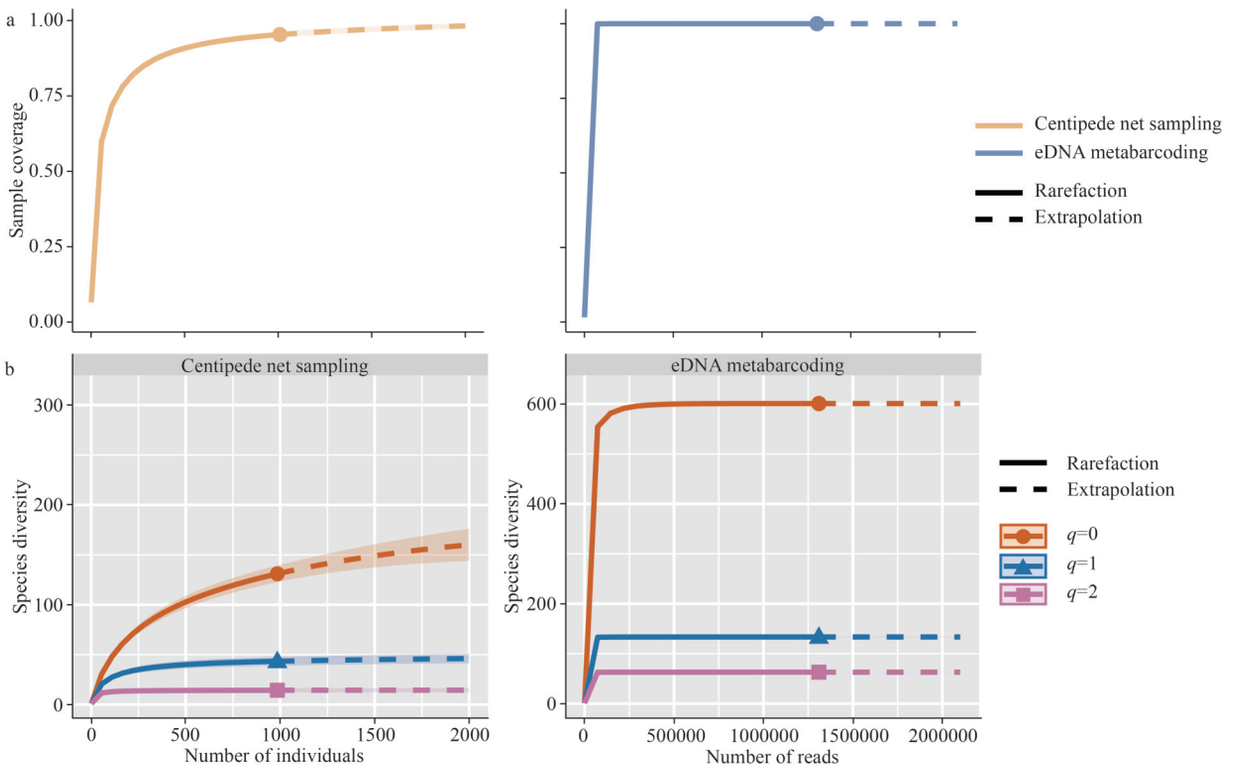

Fig.7 The rarefaction/extrapolation curves for the two methods

a. sample completeness curves; b. sample-size-based rarefaction/extrapolation curves. All rarefaction/extrapolation curves are shown with 95% confidence bands and were extrapolated to twice the observed sampling effort using the *iNEXT* default framework.

observed for *Takifugu niphobles* (centipede net) and *Planiliza planiceps* (eDNA). Regarding eigenvector centrality, *G. giuris* ranked highest in the centipede net network, whereas *P. modestus* ranked highest in the eDNA network. For closeness centrality, the top nodes were *G. giuris* (centipede net) and *Cirrhinus molitorella* (eDNA), respectively (Supplementary Table S6).

In the centipede network, 16 significant species pairs were identified (P<0.05), comprising nine positive and seven negative associations. *G. giuris* served as a core node, showing significant links to six species (two positive and four negative). Among the strongest positive associations were *G. giuris-Butis butis* (R=0.89), *Acentrogobius viridipunctatus-T. niphobles* (R=0.77), and *A. viridipunctatus-Planiliza macrolepis* (R=0.73). Among negative associations, the strongest were *B. butis-P. macrolepis* (R=-0.80), *G. giuris-A. viridipunctatus* (R=-0.74), and *G. giuris-P. macrolepis* (R=-0.70) (Fig.8a).

In the eDNA network, interaction patterns were more complex, with 188 significant pairs (P<0.05), including 131 positive and 57 negative associations. The node with the highest degree was *P. modestus*, which was significantly connected to 17 species (12 positive and 5 negative). Other highly connected taxa included *Butis koilomatodon* (15 pairs: 13 positive, 2 negative), *Hilsa kelee* (15 pairs: 11 positive, 4 negative), *Odontamblyopus lacepedii* (15 pairs: 11 positive, 4 negative), and *Rhodeus sinensis* (15 pairs: 11 positive, 4 negative). The three strongest positive associations were *Cynoglossus puncticeps-Lates calcarifer* (R=1.00), *H. kelee-R. sinensis* (R=0.98), and *Acanthopagrus latus-P. modestus* (R=0.98); the three strongest negative associations were *Platycephalus cultellatus-Trachinotus mookalee* (R=-0.89), *A. latus-C. molitorella* (R=-0.87), and *C. molitorella-R. sinensis* (R=-0.87) (Fig.8b).

To examine differences in the water-column (vertical) habitats and distribution environments of key species identified by the two methods, alluvial plots were used for visualization (Fig.9). Overall patterns were broadly similar across methods; however, the relative proportions across vertical and certain environmental categories differed. In both networks, key species were predominantly demersal, resulting in a demersal-dominated vertical habitat profile: demersal taxa accounted for 50% (20 species) in the eDNA network and 75% (9 species)

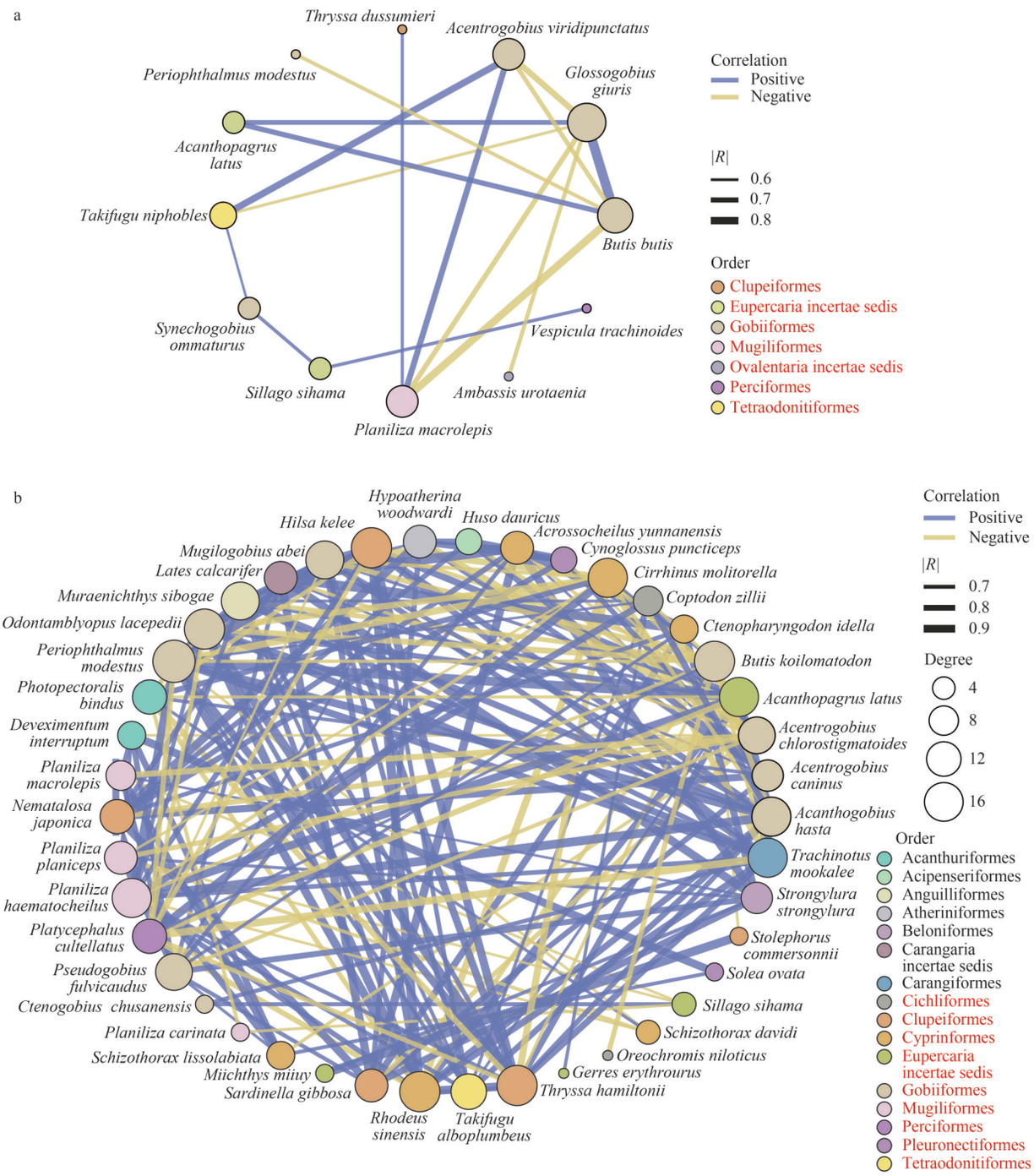

Fig.8 Fish community association (correlation) network in the centipede net (a) and eDNA (b) sampling

Nodes represented species, with the radius proportional to their degree. Node color indicated the taxonomic order. Orders labeled in red font were detected in both networks. Edges connecting nodes denoted significant associations. Blue lines indicated positive correlations, while yellow lines indicated negative correlations. Edge width was scaled according to the absolute correlation coefficient ($|R|$). All associations were derived from Spearman rank correlations with Benjamini-Hochberg adjustment (adjusted $P<0.05$) and an RMT-based similarity threshold ($|R|>0.16$). Only species present in at least 40% of samples were retained for analysis.

in the centipede net network. In the eDNA network, pelagic-neritic species were the least represented (22.5%, 9 species), whereas in the centipede net network, benthopelagic species comprised the smallest share (8.3%, 1 species).

For distribution environments, flows associated with estuary/brackish habitats were the largest (eDNA: 13.95; centipede net: 4.67). Secondary

contributions in the eDNA network came from coastal/nearshore areas (10.20) and freshwater (rivers/lakes) (6.37), whereas the centipede net network showed secondary contributions from sandy/muddy bottoms (2.17), coastal/nearshore areas (1.33), and mangroves (1.33) (Fig.9). Other habitat types, such as lagoon, reef/hard-bottom, intertidal/tidal flats, and seagrass/vegetated, exhibited relatively small flows (each axis weight less than 2), indicating limited contributions to the composition of key species.

3.4 The network stability, vulnerability, and complexity of different fish communities

The eDNA network exhibited significantly higher robustness than the centipede net network (Wilcoxon test, $P<0.001$), indicating a stronger ability to preserve overall connectivity under random node loss (Fig.10a). Vulnerability was quantified as the relative decrease in global efficiency following the removal of each node, compared to the original network (Fig.10b). The eDNA network exhibited lower vulnerability than the centipede net network, indicating that the average impact of node failure on network efficiency was smaller in the eDNA network.

To quantify the degree of association and segregation among species within fish communities, network-level cohesion indices were calculated. Positive cohesion sums the contributions of all positive edges and reflects the overall tendency toward coordinated change or niche convergence (Fig.10c), while negative cohesion sums the contributions of all negative edges and represents the overall strength of mutual exclusion or niche differentiation (Fig.10d). Both positive and negative cohesion were significantly higher in the eDNA network compared to the centipede net network (Wilcoxon test, $P<0.001$). These results indicate that the eDNA network exhibited stronger signals of both co-occurrence and mutual exclusion, consistent with more pronounced aggregation and segregation. Overall, the eDNA network exhibited stronger signals of both co-occurrence and mutual exclusion,

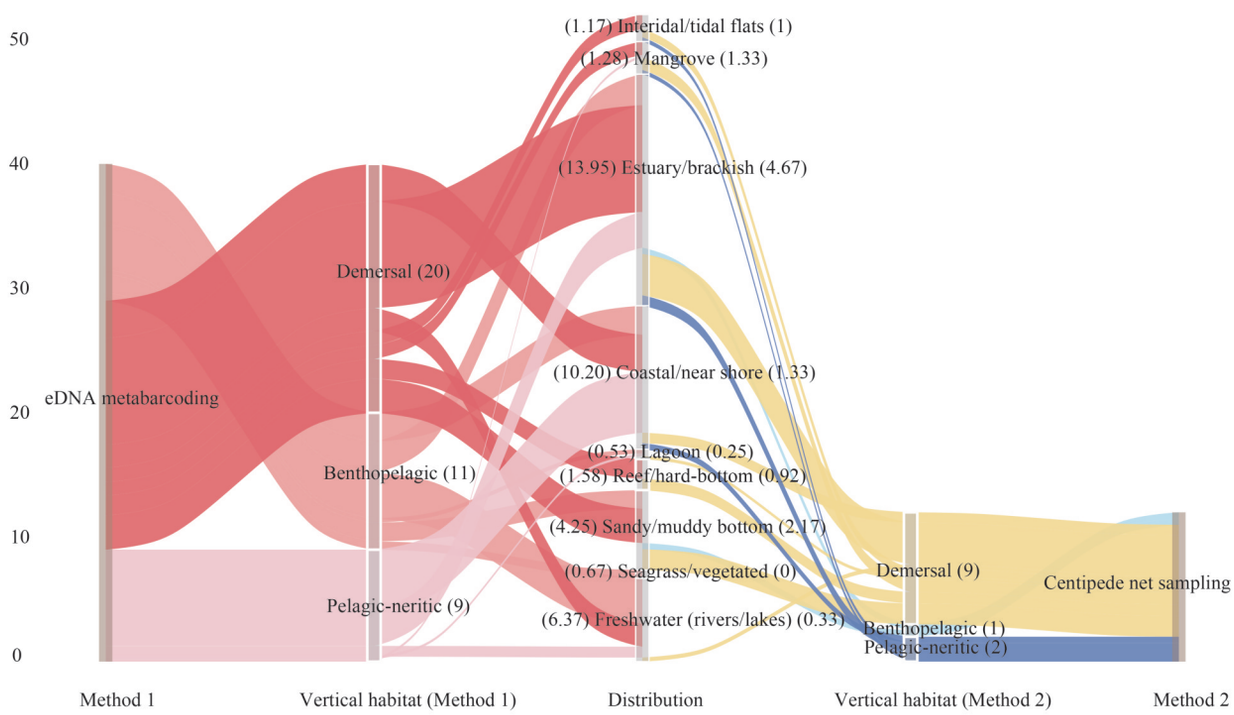

Fig.9 Alluvial plot of key species by method, vertical habitat, and habitat class

This figure employs a five-axis alluvial layout. Vertical habitat denotes the common water-layer guild of key species identified by each method (demersal, benthopelagic, pelagic-neritic). The generalized environment where key species commonly occur, categorized into nine classes (e.g., intertidal/tidal flats, mangrove, estuary/brackish, coastal/nearshore, etc.). For species annotated with multiple attributes, weights were equally apportioned across tags and aggregated by method×vertical habitat×distribution. Band widths and stratum heights are proportional to weighted species counts. Labels on Axis 2 and Axis 4 report method-specific totals by vertical habitat. Labels on Axis 3 are formatted as "(eDNA) distribution (centipede)", showing the method-specific weighted totals for each distribution class.

characterized by more pronounced aggregation and segregation. Furthermore, the sensitive analysis revealed that these stability patterns (robustness, vulnerability, and cohesion) remained consistent under moderate alternative cutoffs ($|R_{ij}|$≥0.4–0.6). Whereas $|R_{ij}|$≥0.7–0.8 produced highly sparse, fragmented networks (especially for eDNA) that limited structural comparability.

4 DISSCUSSION

Global attention has increasingly focused on the use of non-invasive techniques to monitor and characterize fish biodiversity and assess the health of aquatic ecosystems, particularly in vulnerable coastal and estuarine wetlands. eDNA metabarcoding has become a mainstream approach for monitoring global biodiversity and assessing ecosystem health. It has shown rapid and sensitive performance in mudflat environments, where habitat structure is complex, and hydrodynamics are strong. The Gaoqiao mangrove forest, located at the mouth of Yingluo Bay in northern Zhanjiang Province, is recognized as a typical system where mangrove, mudflat, and estuarine environments converge (Zhao et al., 2022b; Wu et al., 2025). Research on fish biodiversity in this area has remained limited. In the present study, parallel surveys were conducted to provide a systematic account of fish diversity and community structure within the mudflat zone and to further analyze network associations and stability, thereby informing future monitoring and management efforts.

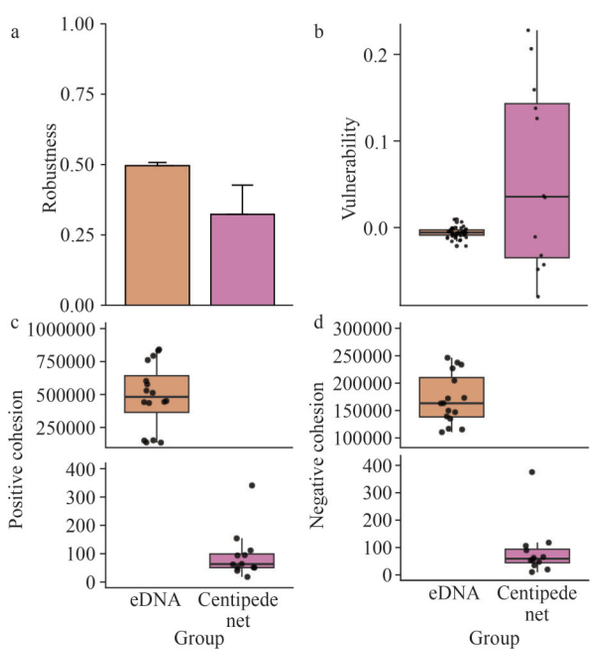

Fig.10 Stability of fish community networks (eDNA vs. centipede net)

4.1 Fish diversity of Gaoqiao mudflat in the Yingluo Bay esturay

This study identified a total of 102 fish species across 26 orders, 50 families, and 85 genera. The eDNA method detected a greater number of species than centipede net sampling (Supplementary Tables S1–S2). This pattern aligns with findings from studies in mangrove and mudflat ecosystems, where eDNA achieved higher detection rates and broader taxonomic coverage compared to a single gear type (Wang et al., 2024a). Comparisons of relative abundance and within-method rank for the 12 species detected by both methods (Table 2) showed that several dominant taxa were consistently represented across approaches, whereas others exhibited clear rank discordance. These patterns suggest that eDNA read proportions and net-caught individual counts may reflect distinct aspects of community structure, likely driven by variations in sampling integration and gear selectivity, as well as methodological factors related to primer performance, vertical integration of eDNA signals, and spatial coverage of passive net sampling. Within this context, the higher species richness detected by eDNA is consistent with its capacity to recover signals from taxa that are infrequently captured by passive nets, including low-abundance, spatially dispersed, or transient species, as reported in previous studies (Deng et al., 2024). However, the rank-abundance comparisons presented here indicate that this enhanced detection does not imply a direct correspondence between read abundance and local biomass or catch abundance. Rather, eDNA provides a complementary perspective on community composition that integrates across microhabitats and temporal windows, while centipede nets retain advantages in resolving locally abundant benthic taxa and providing information at the individual level.

The eDNA metabarcoding method can detect higher taxonomic diversity, whereas traditional gillnets more effectively differentiate community composition among habitats, such as between mangrove and seagrass assemblages (Qiu et al., 2023). In the present study, Mugiliformes and Gobiiformes exhibited high relative abundance in both methods, with values of 30.53% and 16.50% for eDNA and 44.16% and 29.04% for centipede net sampling, respectively. These results are consistent

with established patterns of fish diversity in the nearby coastal waters of the Beibu Gulf (Yang et al., 2025). Reviews by Wang et al. (2023) reported Mugiliformes, Gobiiformes, and Perciformes as the most species-rich orders in the Beibu Gulf, and a DNA barcoding-based reference library developed by Jiang et al. (2023) further confirmed the high prevalence of Mugiliformes and Gobiiformes in this region. Collectively, these studies support the conclusion that the taxonomic features observed here are representative of the region.

4.2 Community composition and sampling sufficiency

PERMANOVA on Bray-Curtis dissimilarities showed significant differences between methods, while PERMDISP indicated similar within-group dispersion. Therefore, the effect reflected a centroid shift rather than dispersion. The first two PCoA axes explained 45.7% of the variance, consistent with a clear separation between methods. This difference was likely driven by the distinct hydrological and temporal scales captured by each method. Previous studies have demonstrated that hydrological spatiotemporal dynamics are primary drivers of changes in fish community structure and that fluctuations in discharge can lead to marked shifts in species composition (Railsback et al., 2021). Periodic changes in water level have been reported to alter fish abundance and redistribute communities across space and time (Ngor et al., 2018). River discharge has also been identified as a key hydrological variable underlying among-reach differences in fish communities (Judes et al., 2021). The eDNA method relied on single water collections followed by high-throughput sequencing, thereby yielding an integrated DNA signal from the mixed water column. This integrated signal encompassed DNA from multiple water layers, transported by tidal processes, thereby increasing the likelihood of capturing signals from transient or spatially dispersed taxa, including flood-pulse migrants and pelagic species in the upper water column (Thomsen et al., 2012; Andruszkiewicz et al., 2017). Centipede nets functioned as passive gear and primarily captured fish from bottom or near-bottom waters during alternating flood and ebb tides. Consequently, centipede net sampling tended to record low-velocity and benthic taxa, and its catch was directly influenced by tidal water-level changes (Marley et al., 2020; Mehdi et al., 2021; Philpott et al., 2025).

In terms of sampling sufficiency, both methods converged toward saturation, but eDNA consistently yielded higher richness and completeness. Rarefaction patterns suggested that centipede net sampling may under-represent less frequently captured taxa, whereas eDNA reached stable estimates across diversity orders within the sampled range. These findings support the use of eDNA as a high-coverage tool in tidal mudflats, while centipede nets remain valuable for providing complementary trait-level information, underscoring the benefits of a combined approach. Additionally, these results align with the consensus that eDNA achieves high coverage efficiency in tidal and estuarine systems (Shaw et al., 2016; Banchi et al., 2024). The mean detection rate quantified in this study also supported this view (Fig.6a), with eDNA at 62.7% and centipede net at 53.2%, which corroborated the conclusion in Section 4.1 while reflecting evidence at a different level, namely, sample-based rates versus total species richness. Therefore, the sequencing depth employed here was sufficient to capture most community members, which supports the feasibility of eDNA as an efficient and comprehensive tool for mudflat monitoring. However, beyond sampling effort, primer choice can influence amplification coverage and taxonomic representation in fish eDNA metabarcoding, potentially affecting the relative detectability of different groups.

To evaluate the potential coverage of the MiFish 12S primers for the regional species pool, we conducted an in-silico PCR analysis based on the species recorded in this study. The results showed clear differences in amplification coverage among taxonomic orders (Supplementary Table S7). This pattern is consistent with previous findings from studies applying MiFish primers in estuarine and tidal systems (Zhang et al., 2020a; Polanco et al., 2021). Overall, primer performance, together with factors such as water sampling volume, can influence eDNA detection outcomes (Shu et al., 2021; How et al., 2024). Therefore, primer-specific effects should be considered when interpreting differences in community structure between eDNA-based and net-based surveys. By comparing these two approaches, the present study provides a baseline reference for fish community assessment using different survey methods. At the same time, centipede-net sampling provides irreplaceable complementary information on functional and biological traits, including body size, biomass, and individual characteristics. For more detailed ecological interpretation, future studies are recommended to

incorporate multi-primer strategies, sampling across different water layers and tidal phases, and multi-gear, multi-site designs, in combination with local reference databases, to improve cross-method comparability and strengthen inference in this dynamic mudflat–estuary system.

4.3 Construction of fish community co-occurrence networks and topological features of key species

Two co-occurrence networks were constructed using Spearman correlations, retaining only species or OTUs with an occurrence frequency of 40% or higher (Fig.8). To ensure reliability, the thresholding followed protocols consistent with previous studies (Ma et al., 2021; Gu et al., 2023). The eDNA network contained more nodes and links, with 42 nodes and 188 links, compared to 12 nodes and 16 links in the centipede net network. The average degree increased from 2.67 to 8.95, and higher transitivity was observed, with values of 0.417 for the centipede net and 0.74 for the eDNA network. The average clustering coefficient was also higher for eDNA, measuring 0.641, compared to 0.404 for the centipede net. Modularity was stronger in the eDNA network, with a value of 0.448, compared to 0.264 for the centipede network (Supplementary Table S6). These results indicate a higher frequency of triadic closures and a more compact structure in the eDNA network (Li et al., 2025). In addition, modules in the eDNA network were densely connected internally and sparsely connected between modules, which indicated clearer compartmentalization (Mruzek et al., 2025). This feature was consistent with the framework that links environmental heterogeneity to modularity in fish co-occurrence networks. Related studies have reported that spatial heterogeneity in salinity, depth, and tidal gradients was captured more completely by eDNA, which contributed to stronger modular differentiation at the network level (Mruzek et al., 2025).

In contrast, degree centralization, betweenness centralization, and closeness centralization were higher in the centipede network, indicating a stronger dependence on a few key hubs. At the species level, the most connected node was *Glossogobius giuris* in the centipede net network and *Periophthalmus modestus* in the eDNA network. Species with the highest betweenness were *Takifugu niphobles* for the centipede net network and *Planiliza planiceps* for the eDNA network, which suggested a bridging role that linked subgroups and enhanced overall connectivity (Watts and Strogatz, 1998). Differences between the two networks reflected complementary methodological biases and ecological information. Centipede net sampling was more effective at resolving benthic and substrate-associated local hubs, whereas eDNA was better at revealing complex cross-water-column co-occurrence patterns.

4.4 Alluvial analysis of vertical habitat and distributional environments for key species

Alluvial visualizations revealed that, although the overall patterns were similar, the two methods differed in the relative weighting of vertical habitats and distributional environments. Both networks were dominated by demersal species. The proportion of demersal taxa was 50% for eDNA with 20 species and 75% for the centipede net with 9 species. eDNA exhibited higher coverage of benthopelagic and pelagic-neritic guilds, whereas the centipede net had the lowest coverage of benthopelagic taxa. The coverage of benthopelagic taxa may be linked to their cruising behavior and gear entry dynamics (Krueger et al., 1998). The lower relative share of pelagic-neritic taxa under eDNA may be related to water-column dilution, DNA residence time, and primer or reference library performance for offshore water-column groups (Jackman et al., 2025; Silva et al., 2025). These patterns were consistent with gear placement and behavioral selectivity. Centipede nets more readily intercept bottom-dwelling or slow-moving fishes but were less efficient for mid- and lower-water column migrants or highly mobile species (Wang et al., 2019c). In contrast, eDNA integrated genetic signals across water layers and tidal-salinity gradients, which increased sensitivity to off-bottom transition-zone taxa (Zhang et al., 2022). However, only 1.5 L of surface water was collected per site in this study. Given the documented tidal pumping and vertical stratification in Yingluo Bay, the present “whole-water-column” interpretation should be treated as a mixed-layer signal under the sampled tidal conditions. To better capture vertical heterogeneity and strengthen this inference, future surveys should incorporate parallel multi-depth sampling across tidal phases.

Along the distributional axis, eDNA showed higher weights in estuary/brackish, coastal/nearshore, and freshwater categories, with values of 13.95, 10.20, and 6.37, corresponding to 34.88%, 25.50%, and 15.93%, respectively. This indicates a stronger sensitivity to mixing zones, nearshore waters, and

freshwater inputs (Wang et al., 2024a). Centipede net sampling exhibited higher weights for sandy/muddy bottoms and mangroves with values of 2.17 and 1.33, corresponding to 18.08% and 11.08%, which reflected a sampling advantage at soft-bottom and root-fringe microhabitats (Chen et al., 2022). Lagoon, reef/hard-bottom, intertidal/tidal flats, and seagrass/vegetated habitats carried small weights in both methods, each below two on the relevant axis. This likely reflects the dominance of mangrove–mudflat habitats and the scarcity of target species in the study area. It may also result from constraints on access and representativeness, as nets are difficult to standardize in reef or seagrass areas, and eDNA signals are more easily diluted in high-energy environments (Rey et al., 2023; Silva et al., 2025). Taken together, the two methods are complementary across vertical layers and habitat gradients, thereby improving recognition of ecological allocation and coupling for key species (Qiu et al., 2023; Trebitz et al., 2025).

4.5 Comparison of the network structure of stability

To our knowledge, studies assessing fish community network stability using eDNA monitoring data remain limited. Given the strong hydrodynamics and mosaic habitats present in mangrove-mudflat-estuary transitions, differences in robustness and interaction structure were expected between networks constructed by different methods. Ecological networks provide an effective means to characterize species associations and to evaluate ecological stability (Yuan et al., 2021). Accordingly, we quantified stability using three metrics: robustness (the proportion of nodes in the largest connected component after random node removal), vulnerability (the decline in global efficiency following node failure), and cohesion (the aggregate strength of positive and negative correlations presenting coordination and mutual exclusion). Results indicated that the eDNA network exhibited higher robustness, lower vulnerability, and stronger positive and negative cohesion than the centipede net network. These features indicated the co-occurrence of coordinated coupling and compensatory segregation. This pattern aligns with the view that higher network complexity can enhance system robustness, as well as with propositions that the ratio of negative to positive cohesion can serve as warning of instability and that moderate negative correlations can dampen synchronous fluctuations (Hernandez et al., 2021; Artime et al., 2024; Srednick and Swearer, 2024). The relatively high stability observed in the eDNA network may be related to its higher network complexity. Previous studies of microbial and fish co-occurrence networks in estuarine and nearshore systems have shown that increases in node richness and edge density are often associated with greater network robustness and stronger overall correlation structure, particularly in systems characterized by strong hydrodynamic forcing and high environmental variability (Seymour et al., 2020; Sun et al., 2025). In highly disturbed tidal systems, networks are also reported to maintain overall connectivity by increasing connection redundancy (Fu et al., 2025; O'Brien and Clements, 2025), which is consistent with the stability patterns observed in the eDNA-based network in this study as a structural response to a highly dynamic environment. In this study, the eDNA network was constructed using unstandardized cumulative read data, and its node number, edge number, and weight density were substantially higher than those of the centipede net-based network. Under these conditions, the absolute values of stability metrics should be interpreted within the specific context of the study system and network construction framework. When target systems, hydrodynamic settings, data scaling, and thresholding criteria differ, direct numerical comparisons across studies may be misleading. Therefore, the interpretation of network stability in this study focuses on the relative differences between survey methods within the same system, rather than on absolute comparisons across different studies.

Based on the mapping between structure and function, two key guidelines for monitoring and management were proposed. First, a layered and parallel monitoring design is recommended. eDNA is well-suited for rapid cross-layer and cross-module scanning and for early detection of anomalies. Centipede net sampling is appropriate for calibrating local benthic networks and providing individual-level data such as body size and biomass. Repeated sampling across seasons and tidal phases, combined with multi-marker assays, improved reference databases, standardized net deployment, and enhanced time-series analyses, is likely to increase sensitivity to network reorganization and changes in stability. Second, differential protection of key species is recommended. In soft-bottom and mangrove-edge zones, demersal "backbone" species such as *Glossogobius giuris* and their associated assemblages should be maintained to stabilize

benthic structure. Along water-column and estuarine transition corridors, bridging species such as *Periophthalmus modestus* and *Planiliza planiceps* should be safeguarded to preserve vertical coupling and overall connectivity. It is important to note that co-occurrence networks represent coordinated fluctuations rather than direct causal interactions. Clarifying the mechanisms behind key links and nodes will require time-series data, functional trait information, and experimental validation. Overall, targeted monitoring and protection focused on hubs, bridges, and module cores are expected to enhance both local resistance and system-wide connectivity in mangrove–mudflat complexes, thereby increasing the resilience and recovery potential of fish communities.

4.6 Synthesis and outlook

This study showed that eDNA and centipede net sampling formed a complementary chain of evidence across vertical strata and habitat gradients. eDNA better revealed transition-zone dynamics in the water column and mixing areas, whereas centipede net sampling resolved benthic hubs at soft-bottom and root-fringe boundaries. Embedding both methods in a layered and parallel framework, coupled with local reference-library development and multi-marker strategies, can preserve spatiotemporal comparability and improve detection of migratory or cryptic taxa as well as interpretation of key species-habitat coupling. By linking biodiversity indicators to carbon-rich mangrove-mudflat mosaics and by analyzing changes along ecotones, this integrated approach is expected to support actionable and interpretable network evidence for restoration, priority setting, and blue-carbon stewardship in mangrove systems. Further research could optimize water column layout and sampling depth settings during eDNA sampling to obtain more comprehensive community information and enhance the characterization of fish community structure in the mangroves of Yingluo Bay.

5 CONCLUSION

A baseline of fish diversity was established for the Zhanjiang mangrove mudflat using eDNA metabarcoding and centipede net sampling. Differences and complementarities between the methods were compared in terms of community composition, sampling sufficiency, and network characterization. Across both methods, 103 species were recorded, with significant differences observed in relative abundance, frequency of occurrence, and taxonomic composition. Rarefaction-extrapolation and sample-coverage analyses indicated that sampling using both methods had essentially reached saturation. Co-occurrence networks analysis revealed that the eDNA network was larger, more clustered, and more modular, exhibiting higher robustness and stronger cohesion.

The distribution of hub and bridge species varied depending on the method used. Centipede net sampling was more sensitive to benthic key nodes, whereas eDNA was more effective at detecting cross-layer and transition-zone associations. Alluvial analyses indicated that eDNA was more responsive to changes in estuarine mixing, nearshore waters, and freshwater inputs, while centipede net sampling performed better along mudflats and mangrove edges. These results support a layered and parallel monitoring framework that combines eDNA and centipede net sampling to achieve spatial and scale complementarity. Future research should include repeated sampling across seasons and tidal phases, the use of multiple primer sets and improved reference databases to reduce bias, standardized gear deployment with time-series analyses, and investigation of the ecological mechanisms underlying key species. Taken together, the integrated evidence provides a practical foundation for monitoring fish diversity and assessing network stability in the Zhanjiang Mangrove Nature Reserve. It also informs habitat management and prioritization efforts that safeguard biodiversity, sustain sedimentary carbon stocks, and support ecosystem functions in blue-carbon mangrove landscapes.

6 DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Electronic supplementary material

Supplementary material (Supplementary Tables S1–S7 and Figs.S1–S2) is available in the online version of this article at https://doi.org/10.1007/s00343-026-5439-3.