



Original Research Article

Integrated multi-method surveys reveal fish diversity in a marine protected area at the northern margin of China's coral reefs

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ABSTRACT

Fish assemblages underpin key ecosystem functions in marine ecosystems, yet baseline information remains limited for many marine protected areas (MPAs), especially those spanning heterogeneous habitats in marginal or biogeographic transition zones. Here, we conducted an integrated fish survey in the National Nature Reserve of Guangdong Nanpeng Islands and adjacent waters (2023–2024), located at the northern margin of China's coral reef distribution, by combining bottom trawling, underwater diving surveys, and environmental DNA (eDNA) metabarcoding. Across all methods, we recorded 201 fish species (15 orders, 70 families, 144 genera), including 119 species not previously documented for the reserve. Species detections were strongly complementary among methods, with limited overlap, substantially broadening taxonomic coverage. Consistent with this pattern, trait-based functional-space analyses showed that integrating approaches markedly expanded multidimensional trait coverage relative to any single method. Comparisons with historical compilations indicate differences in taxonomic and functional structure, with the contemporary inventory showing a higher proportional representation of reef-associated, small-bodied, and low-trophic fishes but lower representation of large predators and previously reported protected species, highlighting potential conservation concerns and underscoring the need for enhanced monitoring and conservation action. Overall, our study updates the regional species inventory for the Nanpeng Islands Reserve and adjacent waters and provides a transferable multi-method framework for evaluating biodiversity and functional structure in habitat-heterogeneous MPAs, thereby supporting long-term monitoring and conservation planning in coastal systems.

1. Introduction

Marine fishes are integral components of ocean ecosystems, playing essential roles in nutrient cycling, energy flow, and

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maintaining ecological stability (Chen et al., 2026; Pratte et al., 2018). In recent decades, however, marine ecosystems worldwide have been subjected to increasing pressures from multiple anthropogenic stressors, including intensified marine heatwaves (Claar et al., 2020; Selig et al., 2012) and overfishing (Robinson et al., 2017). These pressures have resulted in widespread habitat degradation and biodiversity loss (Ainsworth et al., 2020; Hughes et al., 2017), posing substantial risks to fish resources and generating cascading consequences for ecosystem functioning, fisheries sustainability, and coastal livelihoods.

Marine protected areas (MPAs) have been widely implemented as a key management strategy to mitigate biodiversity loss, enhance biomass, and support sustainable fisheries (Benedetti-Cecchi et al., 2024; Fan et al., 2023; Pauly et al., 2002; Sweke et al., 2016). Empirical studies have shown that MPAs often support higher species richness and more complex community structures than adjacent unprotected areas (Sahyoun et al., 2012; Vandeperre et al., 2011). However, the effectiveness of MPAs depends critically on the availability of reliable baseline information on species composition and community structure. Such information remains limited in many regions, particularly in coastal systems characterized by high habitat heterogeneity and rich biodiversity, where comprehensive assessments are challenging.

The National Nature Reserve of Guangdong Nanpeng Islands (hereafter referred to as the Nanpeng Islands Reserve) spans

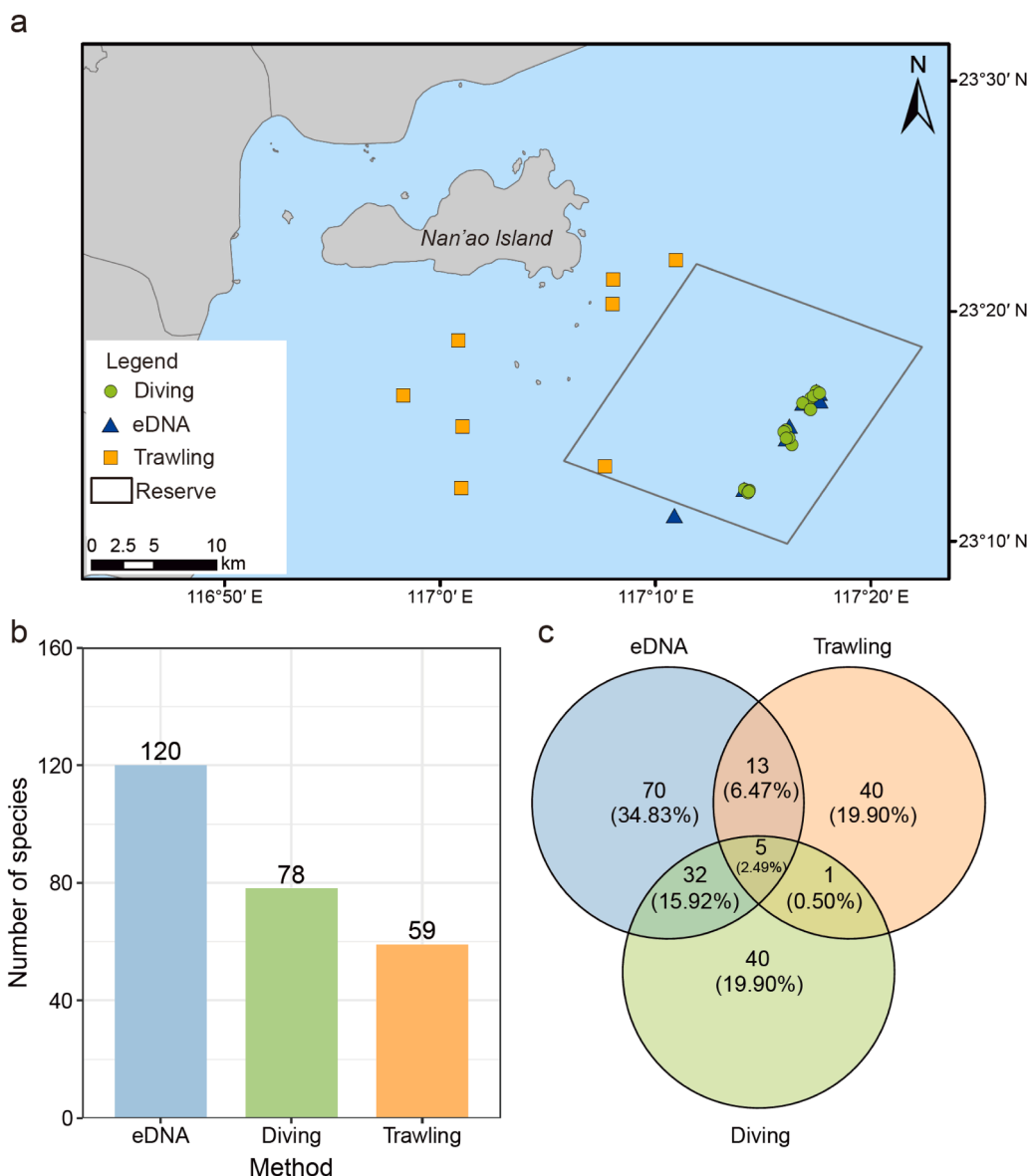


Fig. 1. Study area and fish species composition across survey methods in the Nanpeng Islands Reserve and adjacent waters in the northern South China Sea. Map lines delineate study areas and do not necessarily depict accepted national boundaries. (a) Sampling sites. (b) Number of fish species detected by each of the three survey methods. (c) Venn diagram illustrating the overlap and unique fish species detected by eDNA, trawling, and diving surveys.

approximately 356.79 km² (117°06'26"–117°23'44" E, 23°10'47"–23°23'25" N) and is located at the northeastern margin of Guangdong Province, near the southwestern entrance of the Taiwan Strait. Situated at the intersection of the South China Sea and the East China Sea, the reserve lies within a biogeographic transition zone at the northern margin of China's coral reef distribution (Huang et al., 2015, 2025; Tu et al., 2025). Reef-associated systems at such latitudinal margins are often particularly sensitive to environmental variability and human disturbance (Luo et al., 2026). Despite this vulnerability, baseline biodiversity information for these marginal systems, including the Nanpeng Islands Reserve, remains scarce. The reserve encompasses a diverse array of marine habitats and supports high marine biodiversity, including one first-class nationally protected fish species (*Bahaba taipingensis*) and six second-class protected species (*Cetorhinus maximus*, *Dasyatis bennettii*, *Rhincodon typus*, *Hippocampus kelloggi*, *Hippocampus kuda*, and *Hippocampus trimaculatus*). Nevertheless, comprehensive and up-to-date information on fish species composition and functional trait structure within the reserve and its adjacent waters is still lacking.

A wide range of survey techniques has been developed to assess marine fish assemblages, each characterized by method-specific selectivity, detectability, and habitat accessibility, which can lead to systematic differences in the components of the community sampled. For instance, underwater video surveys are commonly used to document fish distribution and behavior (Boom et al., 2014; Murphy and Jenkins, 2010), roving creel surveys provide insights into community-level patterns (Ferrer et al., 2023; Mallison and Cichra, 2004), and bottom trawling enables large-scale sampling of demersal assemblages (Belcher and Jennings, 2009; DeCelles et al., 2017). More recently, environmental DNA (eDNA) metabarcoding has emerged as a powerful tool for aquatic biodiversity assessment, offering high sensitivity across a broad range of taxa (Andres et al., 2022). When combined with conventional approaches such as diving or trawling, eDNA analysis offers a non-invasive approach that can substantially enhance species detection and improve the completeness of fish community inventories, particularly by revealing cryptic or low-abundance taxa (Deiner et al., 2017; Richards et al., 2022; Thomsen et al., 2012). Consequently, the integration of complementary survey methods is increasingly recognized as an effective strategy for capturing both taxonomic and functional dimensions of fish assemblages in heterogeneous marine environments.

In this study, we conducted an integrated survey of fish assemblages in the Nanpeng Islands Reserve and adjacent waters between 2023 and 2024 by combining underwater diving surveys, bottom trawling, and eDNA metabarcoding. Specifically, we aimed to (1) characterize contemporary fish assemblage composition and functional trait structure across reef and non-reef habitats, and (2) examine broad temporal patterns by contrasting contemporary records with historical inventories. Together, this study provides a robust baseline for long-term ecological monitoring and offers valuable reference information for understanding the structure of fish assemblages in a heterogeneous marine protected seascape.

2. Methods

2.1. Field surveys

From 2023 to 2024, integrated fish surveys were conducted in the National Nature Reserve of Guangdong Nanpeng Islands and adjacent waters using three complementary approaches, with bottom trawling carried out in the summer of 2023 and underwater diving surveys and environmental DNA (eDNA) sampling conducted in the summer of 2024 (Fig. 1a). These approaches were designed to target distinct habitat types and components of the regional fish fauna, thereby improving the completeness of the species inventory, although the surveys were conducted in broadly comparable seasonal periods across different years.

Given that the reserve encompasses both coral reef and non-reef habitats, survey methods were selected according to habitat accessibility and sampling suitability. Bottom trawling was conducted in non-reef habitats at eight sampling stations, with trawling depths ranging from 15 to 30 m and averaging approximately 22 m. At each station, a standardized bottom trawl was towed for approximately one hour at a vessel speed of 3–4 knots. In coral reef habitats, diving surveys were conducted at seven stations to sample reef-associated fishes. Fish specimens collected during both trawling and diving surveys were immediately photographed to record morphological features, and species-level identification was carried out according to *Key to Marine and Estuarial Fishes of China* (Wu and Zhong, 2021) and *Marine Fishes of China* (Chen and Zhang, 2015).

For eDNA analysis, seawater samples were collected from nine stations encompassing both reef-associated and adjacent non-reef habitats. At each station, 10 L of seawater was collected at a consistent depth of approximately 1 m below the surface using a portable 10-L acrylic water sampler, transferred to clean sampling containers, and immediately filtered through 0.2 µm polycarbonate membranes. Field negative control samples were not included during water sampling. To reduce potential contamination, the filtration apparatus and related equipment were thoroughly cleaned and sterilized with ethanol between samples. Filters were placed in 2 mL cryotubes and stored at –80 °C until further processing. All eDNA samples were transported to OneGene Biotech (Suzhou) Co., Ltd. for DNA extraction and sequencing.

2.2. DNA extraction, amplification, sequencing, and bioinformatic processing

DNA was extracted from filter membranes using the TIANamp Marine Animals DNA Kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's protocol. DNA purity and concentration were assessed using a NanoDrop One spectrophotometer (Thermo Fisher Scientific). Environmental DNA was amplified using the fish-specific MiFish universal primer set targeting the mitochondrial 12S rRNA region (MiFish-E/U-F: 5'-GTGCGTAAAWCTCGTGCCAGC-3'; MiFish-E/U-R: 5'-CATA-GTGGGGTATCTAATCCYAGTTTG-3'), following established fish eDNA metabarcoding protocols (Miya et al., 2015). PCR amplification was performed using TaKaRa Premix Taq® Version 2.0 (TaKaRa Biotechnology Co., Dalian, China) under the following conditions: initial denaturation at 98 °C for 30 s, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 58 °C for 30 s, and extension

at 72 °C for 20 s, with a final extension at 72 °C for 5 min. A PCR positive control and a negative (no-template) control were included during PCR amplification to monitor amplification performance and potential contamination. A single PCR reaction was performed for each sample, as the eDNA survey was designed primarily for species inventory. PCR products, including controls, were visualized on 1.5% agarose gels, with no visible amplification observed in the negative control. Only samples showing a clear primary band within the expected size range were retained for downstream processing.

PCR products were quantified using GeneTools Analysis Software (Version 4.03.05.0, SynGene). Based on concentration estimates and an equal-mass principle, PCR amplicons from all retained samples were pooled and purified using the E.Z.N.A.® Gel Extraction Kit (Omega, USA), with the target DNA fragments eluted in TE buffer. Library preparation followed the standard protocol of the ALFA-SEQ Amplicon Library Prep Kit. The quality of the library was assessed using Qubit and the QSEP400 High-Throughput Nucleic Acid and Protein Analysis System. Sequencing was performed on an Illumina NovaSeq 6000 platform with paired-end 150 bp reads (PE150). The target sequencing depth was approximately 150,000 raw reads per sample, and the observed read depth across the nine eDNA samples ranged from 157,121 to 342,441 raw reads per sample. All nine eDNA samples passed sequencing quality control.

Raw sequencing reads were processed using QIIME2 (Bolyen et al., 2019). Adapter and primer sequences were removed using Cutadapt, followed by denoising with DADA2 to infer amplicon sequence variants (ASVs). ASVs were taxonomically assigned in QIIME2 using the *classify-sklearn* classifier trained on a curated reference database integrating 12S sequences and complete mitogenomes from NCBI, BOLD, and MitoFish, supplemented with unpublished mitogenomes generated from additional fish specimens collected during previous surveys to improve taxonomic coverage and assignment accuracy. Primer-trimmed reference sequences were extracted and used for taxonomic assignment within QIIME2. To reduce potential false positives, preliminary species-level annotations from the eDNA dataset were further screened using historical records and distributional information. Specifically, each annotated fish species was cross-referenced against occurrence records from FishBase, GBIF, and iNaturalist, together with historical records from the Nanpeng Islands Reserve and ichthyological references documenting species distributions in the South China Sea, southern East China Sea, and Taiwan Strait. In total, 422 ASVs were initially inferred from the nine eDNA samples, of which 259 were taxonomically assigned and 163 remained unassigned. The assigned ASVs corresponded to 163 preliminary species-level annotations, which were further screened using historical records and distributional information. Only species considered biogeographically plausible for the study region were retained in the final species list. During this screening step, 43 preliminary species-level annotations, corresponding to 75 ASVs, were removed, including freshwater or biogeographically implausible taxa, unverified annotations, and ambiguous species-level assignments. The final eDNA species list included 120 species corresponding to 184 ASVs. Although this screening step cannot fully eliminate uncertainty inherent in eDNA-based detection, it improves confidence in the retained species records.

2.3. Historical data compilation

To provide context for contemporary observations, we compiled historical records of fish species occurrence in the Nanpeng Islands Reserve and adjacent waters from available sources. Two primary datasets were used: (1) the species list reported in *Marine Ecology and Biodiversity of Nanpeng Islands* (Li et al., 2009), which documented marine biota (including fishes) in the reserve; and (2) an unpublished dataset from a 2021 *baseline survey of the National Nature Reserve of Guangdong Nanpeng Islands* (internal report). To ensure taxonomic consistency, all species names were standardized and updated according to the World Register of Marine Species (WoRMS) and FishBase.

2.4. Community diversity analysis

Alpha diversity metrics, including species richness, Shannon diversity, Simpson diversity, and Pielou's evenness, were calculated for each sampling site using the *vegan* package in R. Differences among sampling methods were evaluated using pairwise Wilcoxon rank-sum tests. Community compositional differences among methods were examined using Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarities. Statistical significance of group differences was assessed using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. In addition to the overall comparison among the three methods, a subset PERMANOVA was conducted between the eDNA and diving datasets, given their greater overlap in reef-associated sampling coverage. To assess whether PERMANOVA results might be influenced by differences in within-group dispersion, we tested for homogeneity of multivariate dispersion (PERMDISP) using the *betadisper* and *permutest* functions in the *vegan* package in R, with significance assessed using 999 permutations. To assess sampling completeness within each survey method, we generated site-based species accumulation curves using occurrence (presence/absence) data across sampling sites. Mean accumulation curves were estimated from random permutations using the *vegan* package in R.

2.5. Fish functional traits

Fish functional trait data for all detected species were primarily obtained from the FishBase database using the *rfishbase* package in R (Boettiger et al., 2012), including maximum total length, trophic level, life span, growth rate (K), and water column position. Diet information of fish was supplemented from *Marine Fishes of China and Adjacent Waters* (Liu et al., 2024). Maximum total length was further categorized into ordered size classes for descriptive summaries, following published thresholds (Zhao et al., 2024). Trophic level was also summarized into 0.5-unit classes for descriptive summaries, following previous coral reef fish studies (Graham et al., 2017). In contrast, both maximum total length and trophic level were retained as continuous variables for constructing the multidimensional functional space and calculating functional richness (FRic). For species with missing trait information, values were

supplemented from relevant literature sources to ensure completeness of the trait dataset. Functional traits were classified as numerical or categorical variables (Table 1).

2.6. Functional space and functional diversity

A multidimensional functional space was constructed using the *mFD* package in R (Magneville et al., 2021). Pairwise trait distances between species were calculated using Gower's distance, which accommodates mixed numerical and categorical traits. The resulting distance matrix was subjected to a Principal Coordinates Analysis (PCoA) to project species into a continuous functional space. The quality and optimal dimensionality of the functional space were evaluated using Mean Absolute Deviation (MAD). A four-dimensional functional space (MAD = 0.032) was selected as the optimal representation (Fig. S2). Convex hulls were delineated within this functional space for fish communities detected by different survey methods. Functional diversity was quantified using Functional Richness (FRic), defined as the volume of multidimensional functional space occupied by all species within a community (Cornwell et al., 2006; Mouillot et al., 2013; Villeger et al., 2008).

3. Results

3.1. Species composition and diversity

Across all survey methods, a total of 201 fish species were recorded from the National Nature Reserve of Guangdong Nanpeng Islands and its adjacent waters, representing 15 orders, 70 families, and 144 genera. Among these, eDNA metabarcoding detected 120 species, underwater diving surveys recorded 78 species, and bottom trawling identified 59 species (Fig. 1b). Only five species (*Stephanolepis cirrhifer*, *Parupeneus biaculeatus*, *Sebastiscus marmoratus*, *Pelates quadrilineatus*, and *Siganus fuscescens*) were detected consistently across all three survey methods. Notably, 70 species were detected only by eDNA metabarcoding, 40 only by diving surveys, and 40 only by trawling surveys (Fig. 1c), likely reflecting method-specific detection as well as differences in sampling location, habitat coverage, and survey year.

At the family level (top 20), the taxonomic composition of detected fishes differed among survey methods (Fig. 2a). Within the eDNA dataset, Serranidae (16.61%), Lethrinidae (12.88%), Pomacentridae (12.58%) and Monacanthidae (11.68%) accounted for the largest proportions of detected records. In contrast, diving surveys were primarily characterized by Pomacentridae (19.63%), Labridae (18.22%), and Serranidae (10.45%), whereas trawling surveys were dominated by Sciaenidae (16.53%), Sparidae (11.88%), and Mullidae (11.08%).

At the species level (top 20), the relative composition of detected taxa also varied across survey methods (Fig. 2b). Within the eDNA dataset, *Lethrinus haematopterus* (12.71%), *Monacanthus chinensis* (11.63%), and *Abudefduf bengalensis* (10.29%) represented relatively large proportions of detected records. Diving surveys showed higher proportions of *Sebastiscus marmoratus* (8.76%), *Lethrinus nebulosus* (7.06%), and *Chaetodon modestus* (5.37%), whereas trawling surveys most frequently recorded *Evynnis cardinalis* (11.40%), *Pennahia argentata* (10.27%), and *Upeneus japonicus* (10.11%). *Siganus fuscescens* was the only species that appeared in the top 20 lists across all three survey methods.

Species accumulation curves showed signs of approaching saturation across the three survey methods, most clearly for eDNA, although complete asymptotes were not reached for diving and trawling (Fig. S1). These patterns suggest that each method captured a substantial portion of its detectable species pool, although differences in sampling effort and survey year should be considered when comparing methods. Alpha diversity analysis showed that eDNA metabarcoding recovered greater detected species richness than

Table 1
Summary of functional traits for fish species in the Nanpeng Islands Reserve.

Trait	Type	Categories / Units	Data source
Maximum total length	Numeric (grouped for descriptive summaries)	Small: <35 cm Medium: 35 cm–65 cm Large: ≥65 cm	FishBase; (Zhao et al., 2024)
Trophic level	Numeric (grouped for descriptive summaries)	2.0–2.5 2.5–3.0 3.0–3.5 3.5–4.0 ≥4.0	FishBase; (Graham et al., 2017)
Life span	Numeric	years	FishBase
Growth rate (K)	Numeric	year ⁻¹	FishBase
Diet	Factor	Herbivorous (HE) Omnivorous (OM) Carnivorous (CA)	(Liu et al., 2024)
Water column position	Factor	Pelagic-oceanic (PO) Pelagic-neritic (PN) Demersal (DE) Reef-associated (RA) Benthopelagic (BE)	FishBase

Note: Maximum total length and trophic level were grouped only for descriptive summaries.

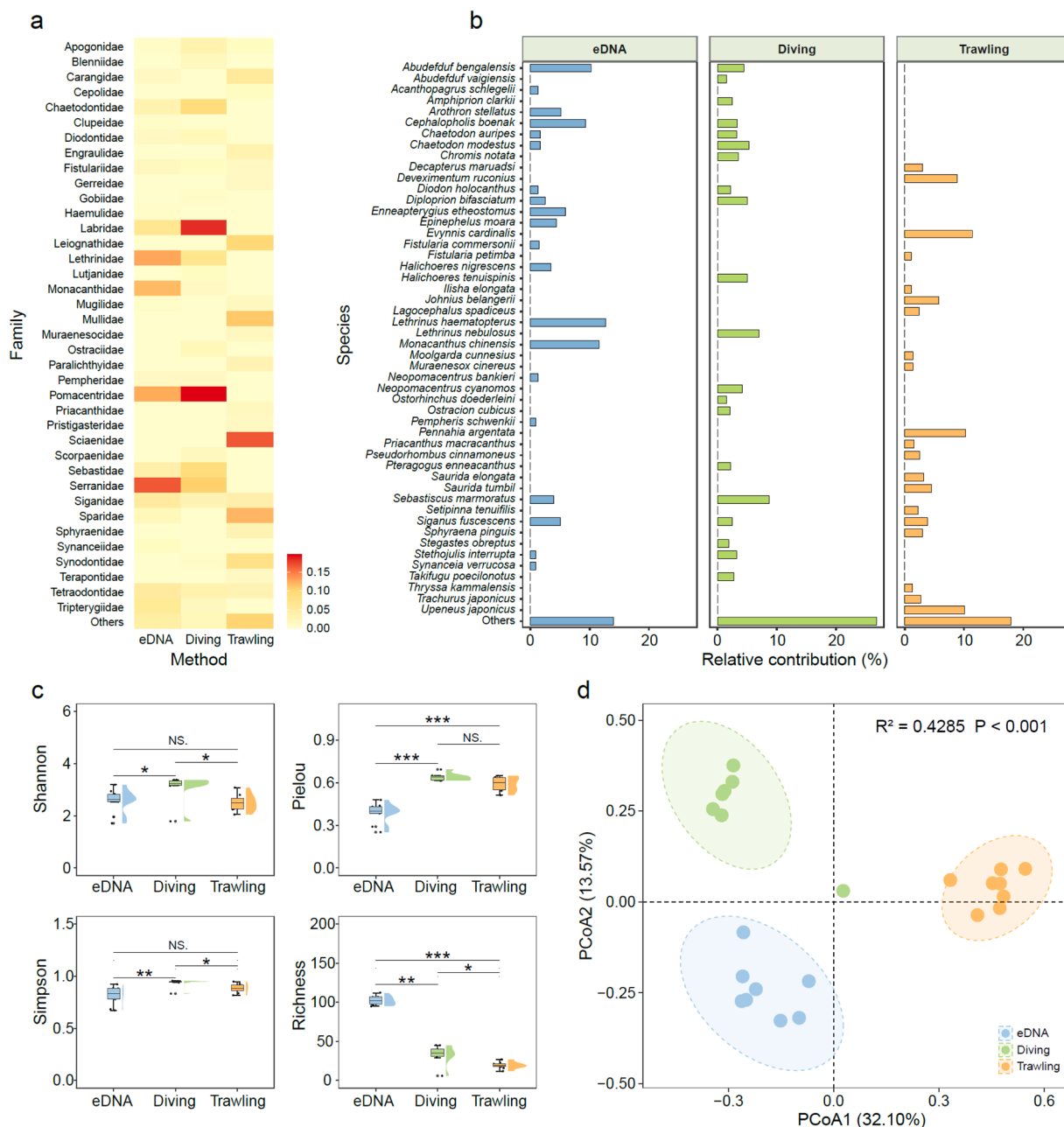


Fig. 2. Taxonomic composition and diversity of fish assemblages across three survey methods. (a) Relative taxonomic composition at the family level (top 20 families). (b) Relative taxonomic composition at the species level (top 20 species). (c) Alpha diversity metrics, including species richness, Shannon diversity, Simpson diversity, and Pielou's evenness. Significance levels for panel (c) are indicated: $p > 0.05$ (NS); $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***). (d) Beta diversity patterns among methods, visualized using principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarities. In panel (d), ellipses indicate 95% confidence intervals around group centroids, and differences among methods were tested using PERMANOVA. All analyses were based on ASV-level relative read abundance for eDNA and species-level relative abundance for diving and trawling surveys.

diving and trawling surveys (Fig. 2c). Species evenness was lower in the eDNA dataset than in the other two methods, whereas Shannon diversity was highest in diving surveys. Principal Coordinates Analysis (PCoA) showed that the first two axes explained 32.10% and 13.57% of the variation, respectively. PERMANOVA results indicated significant differences in the detected assemblage composition among the three survey methods (Fig. 2d; $R^2 = 0.4285$, $p < 0.001$). PERMDISP showed no significant difference in multivariate dispersion among methods ($F = 0.295$, $p = 0.809$), suggesting that the significant PERMANOVA result primarily reflected differences in assemblage composition rather than unequal within-group dispersion. A subset analysis comparing the eDNA and diving

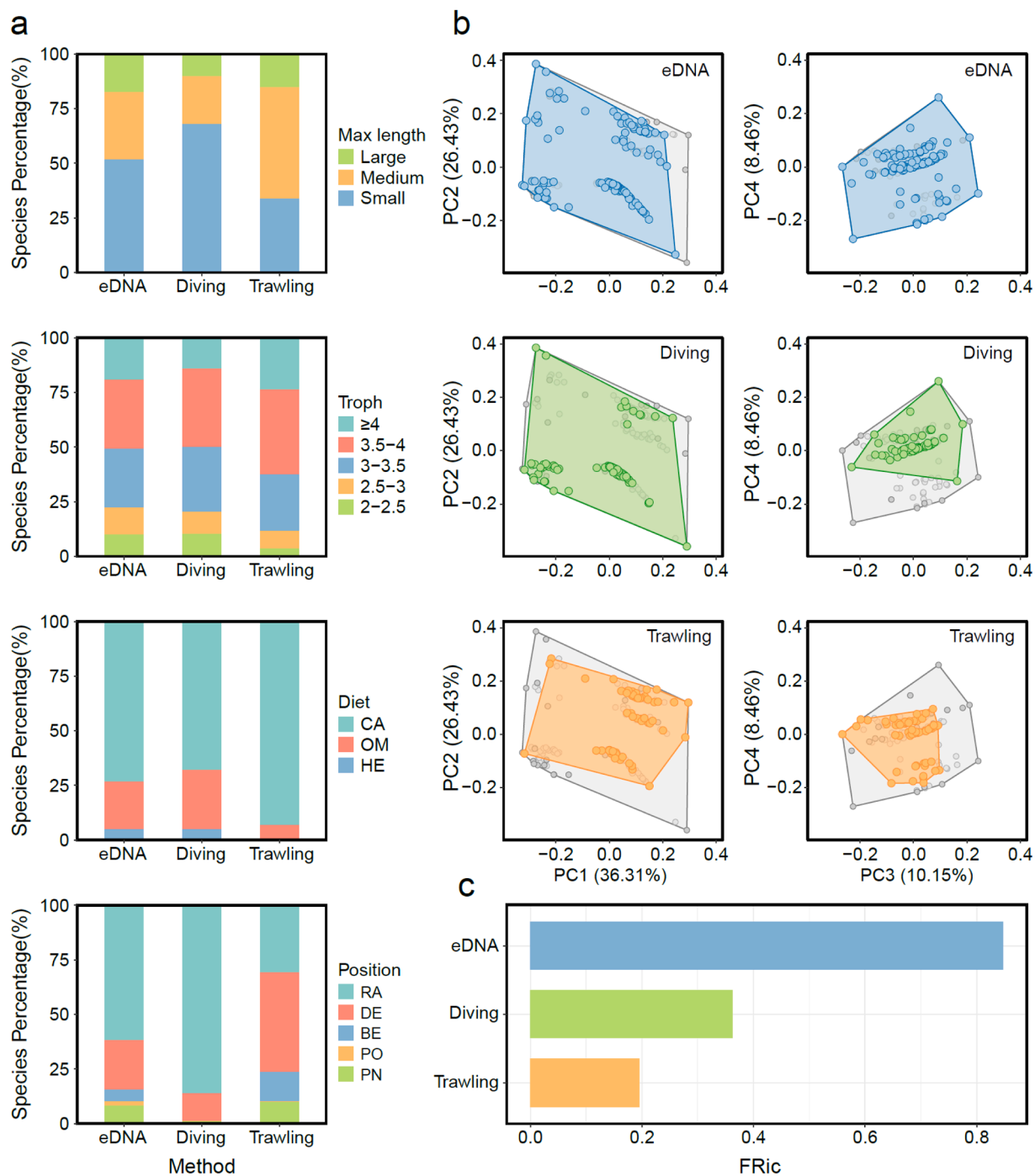


Fig. 3. Fish functional trait composition and functional diversity captured by each survey method. (a) Distribution of key functional trait categories (body size, trophic level, diet, and water column position) among fish species detected by eDNA, trawling, and diving. Max Length: Large (≥ 65 cm), Medium (35 cm–65 cm), Small (< 35 cm). Diet: Carnivorous (CA), Omnivorous (OM), Herbivorous (HE). Water column position: Reef-associated (RA), Demersal (DE), Benthopelagic (BE), Pelagic-oceanic (PO), Pelagic-neritic (PN). (b) Functional space of fish detected by the three methods in Nanpeng Islands Reserve. (c) Functional richness (FRic; convex-hull volume) compared among methods.

datasets showed the same pattern, with significant differences in detected assemblage composition (PERMANOVA: $R^2 = 0.2487$, $F = 4.6344$, $p = 0.001$) and no significant difference in multivariate dispersion (PERMDISP: $F = 0.399$, $p = 0.602$).

3.2. Functional trait composition and functional diversity

Fish species detected by the three survey methods exhibited distinct distributions across functional trait categories (Fig. 3a). In terms of body size, species detected by eDNA and diving surveys were predominantly small-sized, accounting for 62 species (51.67%)

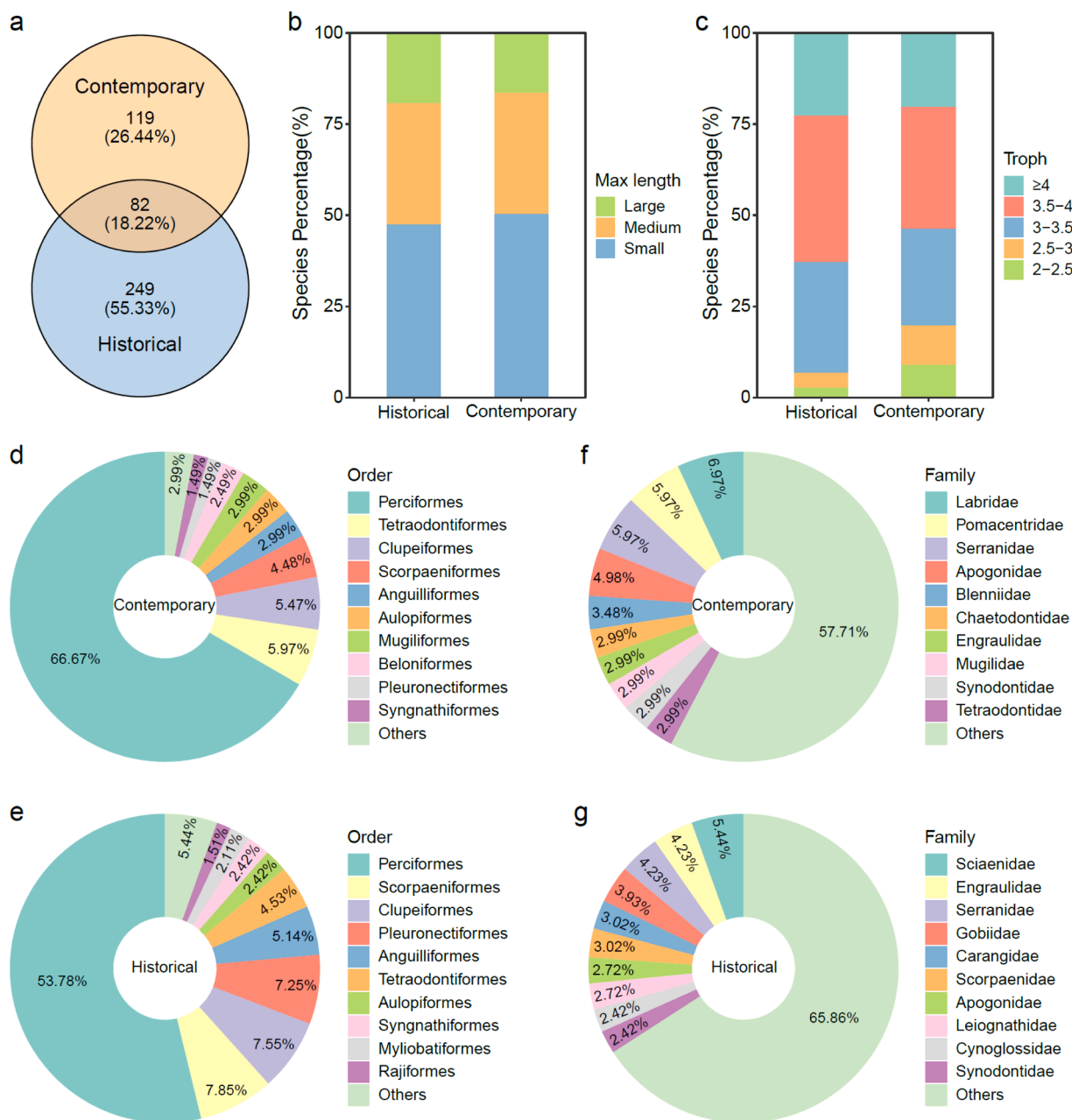


Fig. 4. Taxonomic and trait composition of fish assemblages in historical versus contemporary surveys. (a) Venn diagram showing the overlap and uniqueness of fish species recorded in historical and contemporary surveys. (b) Comparison of body size composition between historical and contemporary species, categorized by maximum total length (small, medium, and large). (c) Comparison of trophic-level composition between historical and contemporary species, with species grouped into five trophic-level classes (2.0–2.5, 2.5–3.0, 3.0–3.5, 3.5–4.0, and ≥4.0). (d–e) Taxonomic composition of fish assemblages at the order level in contemporary (d) and historical (e) surveys. (f–g) Taxonomic composition of fish assemblages at the family level in contemporary (f) and historical (g) surveys.

and 53 species (67.95%), respectively, whereas medium-sized species constituted the largest proportion of species detected by trawling surveys (30 species; 50.85%).

Across all methods, most detected species were distributed within trophic levels ranging from 3.0–3.5 to 3.5–4.0. Carnivorous species dominated all datasets, representing 73.33% of species detected by eDNA surveys, 67.95% by diving surveys, and 93.22% by trawling surveys. With respect to water column position, eDNA surveys detected species occupying all five categories, diving surveys recorded three categories, and trawling surveys recorded four categories. Reef-associated species dominated the eDNA and diving datasets, whereas trawling surveys were primarily composed of demersal species.

The functional trait space constructed from selected traits showed that the first two principal coordinate axes (PC1 and PC2) explained 36.31% and 26.43% of the total variation, respectively, while PC3 and PC4 explained 10.15% and 8.46% (Fig. 3b). PC1 was associated with increasing body size, life span, and trophic level (Fig. S3c), whereas PC3 was associated with decreasing body size and life span and with increasing growth rate (Fig. S3d).

Differences in the extent of functional trait space occupied by species detected by each survey method were observed (Fig. 3b, c). Functional richness (FRic), calculated from the species detected by each survey method, was highest for eDNA surveys (0.8473), intermediate for diving surveys (0.3621), and lowest for trawling surveys (0.1952) (Fig. 3c).

3.3. Comparison of historical and contemporary fish assemblages

Comparisons between historical and contemporary fish inventories revealed differences in species occurrence and trait composition in the Nanpeng Islands Reserve and adjacent waters. Historical compilations documented 331 fish species, representing 19 orders, 97 families, and 224 genera, whereas contemporary surveys recorded 201 species belonging to 15 orders, 70 families, and 144 genera. A total of 82 species (18.22%) were shared between historical and contemporary datasets, while 119 species detected in the present study were not recorded in historical sources, resulting in a substantially updated regional species inventory (Fig. 4a). Of the 119 newly recorded species, 46 were detected exclusively by eDNA metabarcoding, 38 were recorded only through underwater diving surveys, and 12 were detected solely by trawling surveys, while the remaining 23 species were detected by more than one method (Fig. S4). In contrast, 249 species listed in historical sources were not detected in the contemporary surveys. Among the seven nationally protected fish species reported in historical records, only *Hippocampus trimaculatus* was detected in the contemporary surveys.

Taxonomic composition differed between historical and contemporary datasets at both the order and family levels (Fig. 4d–g). At the order level (Fig. 4d–e), Perciformes accounted for the largest proportion of species in both periods (53.78%, 178 species historically; 66.67%, 134 species contemporarily). In the historical records, Scorpaeniformes (7.85%) and Clupeiformes (7.55%) were the next most species-rich orders, followed by Pleuronectiformes (7.25%), Anguilliformes (5.14%), and Tetraodontiformes (4.53%). In the contemporary records, Tetraodontiformes (5.97%) and Clupeiformes (5.47%) accounted for the highest proportions after Perciformes, followed by Scorpaeniformes (4.48%), with all remaining orders each contributing less than 3% of species. At the family level (Fig. 4f–g), Labridae accounted for 6.97% (14 species) of the contemporary dataset, followed by Pomacentridae and Serranidae (both 5.97%, 12 species each), whereas Sciaenidae accounted for 5.44% (18 species) of the historical dataset, with Engraulidae and Serranidae each accounting for 4.23% (14 species).

Comparison of trait composition based on species inventories indicated differences between historical and contemporary datasets. The proportion of small-sized species was higher in the contemporary inventory, whereas large-sized species were less represented, although the overall size composition pattern remained broadly similar (Fig. 4b). Trophic composition also differed, with greater representation of low-trophic-level species (2.0–2.5 and 2.5–3.0) and lower representation of higher-trophic-level species (3.0–3.5, 3.5–4.0, and ≥ 4.0) in the contemporary inventory (Fig. 4c).

4. Discussion

4.1. Patterns of fish assemblage composition and functional traits

Comparison with historical inventories revealed clear differences in species occurrence, taxonomic composition, and functional trait structure in the Nanpeng Islands Reserve and adjacent waters. At the family level, the contemporary inventory showed a higher proportional representation of reef-associated families, notably Labridae (6.97%) and Pomacentridae (5.97%), whereas historical records were characterized by greater contributions from Sciaenidae (5.44%) and Engraulidae (4.23%) (Fig. 4). This contrast is consistent with the many coral-reef-associated taxa newly recorded in the present surveys, and may partly reflect differences in survey methods and reef-habitat coverage between contemporary sampling and historical compilations. Notably, the contemporary surveys detected fewer nationally protected species than reported historically. Of the seven fish species previously documented as nationally protected within the reserve, only the three-spot seahorse (*Hippocampus trimaculatus*) was recorded in our surveys. Similarly, many species listed in historical sources were not detected in the contemporary surveys, which may reflect differences in sampling effort, spatial and temporal coverage, survey methods, and detectability, as well as possible ecological changes. Given these uncertainties, strong inference about community-level temporal change remains limited. Nevertheless, the limited detection of historically recorded species and protected taxa is noteworthy and highlights the need for targeted monitoring and strengthened conservation actions focused on vulnerable species.

Beyond taxonomic contrasts, the contemporary inventory also differed from historical records in size structure and trophic composition. Small-bodied and lower-trophic-level fishes were proportionally more represented, whereas large-bodied, top-trophic-level species were less represented. Similar patterns in size structure and trophic composition have been frequently reported in reef fish

communities under fishing pressure, particularly in the reduced representation of large-bodied and high-trophic-level species (Pauly et al., 1998; Robinson et al., 2017). Given the increasing anthropogenic pressures on coastal waters in the region, including fisheries and tourism-related activities, these factors may provide one possible context for the lower representation of large and carnivorous fishes in the contemporary inventory. More broadly, coral reef fish assemblages can also be influenced by climate-related stressors and habitat degradation, which may reduce habitat quality and food availability for fishes (Claar et al., 2020). Similar patterns toward smaller fish sizes have also been reported from other coral reef systems in the South China Sea, including the Xisha and Nansha Islands (Capitani et al., 2021; Huang et al., 2023; Zhang et al., 2022), suggesting that the patterns observed here may be aligned with broader regional signals, while also being influenced by local conditions.

Differences in trophic structure may additionally be associated with variation in basal resource availability and local environmental conditions. The greater representation of lower-trophic-level fishes in the contemporary inventory may be consistent with changes in food resources, potentially including greater availability of algal or detrital resources. In and around the Nanpeng Islands Reserve, previous studies have reported declining water quality in some coastal areas, particularly in association with intensified human activities and the expansion of nearby aquaculture zones (Cai et al., 2020; Zhou et al., 2011). Elevated nutrient concentrations in nearshore waters may affect benthic conditions and food resources, potentially contributing to the trophic patterns observed here. Although coastal ecosystems can exhibit resilience (Standish and Parkhurst, 2024), the taxonomic and trait differences observed between historical and contemporary inventories, together with the limited detection of historically reported protected species, highlight potential conservation concerns for the reserve. However, differences in sampling effort, spatial and temporal coverage, survey methods, and detectability limit strong inference about the drivers of these patterns. Future monitoring would benefit from standardized, long-term multi-method surveys with broader temporal and habitat coverage to improve comparability with historical records.

4.2. Integrating survey methods in heterogeneous MPAs

The Nanpeng Islands Reserve exemplifies an MPA encompassing a mosaic of habitat types, from coral reefs and rocky outcrops to soft-sediment bottoms. This habitat heterogeneity supports high fish diversity but also poses a challenge for monitoring, since different species and functional groups occupy different niches and differ in detectability. Our results show that no single method captured the full breadth of taxonomic and functional diversity in this complex seascape. Instead, each survey approach sampled a partially overlapping subset of the community, and integration across methods provided a substantially more complete representation of assemblage structure. In addition, most species recorded by diving and trawling surveys were represented in our 12S reference database, further highlighting the complementarity among the survey methods, although part of the observed among-method variation may also be associated with temporal variation and differences in data structure.

Differences in detected assemblages among methods likely reflect both method-specific detectability and the distinct habitat contexts sampled by each approach. Trawl surveys, due to their invasive nature and requirement for relatively uniform seafloor conditions, are best suited to non-reef areas with simple topography (Danielsen et al., 2005) and therefore primarily sampled demersal and soft-bottom-associated taxa. Diving surveys, by contrast, can be implemented on complex substrates and thus offer distinct advantages in coral reef habitats, where trawling is impractical or destructive (Bobsien and Brendelberger, 2006). Consistent with these constraints, diving surveys in reef zones recorded fishes spanning three water-column position categories, whereas trawl surveys in non-reef zones recorded four categories. Herbivorous fishes were not detected in trawl samples (Fig. 3), likely reflecting the predominantly sandy and muddy substrates in trawled areas, which provide limited suitable habitat and food resources for herbivorous fishes but may be more favorable for omnivorous and carnivorous taxa. Together, these results underscore strong habitat associations in the distribution of species and functional groups (Hitchman et al., 2021). In addition, diving and trawl surveys rely on specialist taxonomic expertise for accurate species identification, which can limit efficiency and consistency across large or long-term monitoring programs (Baird and Hajibabaei, 2012; Deiner et al., 2017).

eDNA metabarcoding provided an important complementary perspective that partially mitigates some limitations of conventional surveys. Because eDNA is collected from water, it is non-invasive and can detect taxa regardless of behavior and microhabitat use, as long as their genetic material disperses to the sampled water (Dejean et al., 2012; Goldberg et al., 2011). In our study, eDNA detected the greatest number of species overall and was the only method that detected species spanning all five water-column position categories. High sensitivity and broad taxonomic coverage of eDNA have also been reported in other aquatic surveys (Darling and Mahon, 2011; Jerde et al., 2011; Olson et al., 2012). Nonetheless, eDNA metabarcoding can yield false positives and remains sensitive to both ecological processes and methodological decisions, which can influence taxon detection and assignment reliability. For example, DNA transport, reference-database completeness, sampling design, and bioinformatic processing may all contribute to differences between eDNA-based detections and conventional survey results. These limitations further emphasize the value of cross-validation using conventional approaches (Fujii et al., 2019). Moreover, eDNA is generally more reliable for presence/absence inference than for abundance or demographic structure. Accordingly, eDNA should be viewed as complementary rather than a replacement: it enhances detection of cryptic or low-abundance taxa, while visual and capture-based methods remain essential for quantifying biomass, size structure and providing ecological context.

Overall, our multi-method approach demonstrates that each technique captures a distinct subset of the fish assemblage and that integration is needed to approximate overall community composition. Consistently, functional-space analyses show that the integrated dataset captures a substantially larger trait volume than any single method alone. Similar method complementarity has been reported in other multi-method monitoring efforts, where different conventional surveys yield method-dependent community estimates and where integrating eDNA with underwater visual surveys improves biodiversity detection and conservation relevance in species-rich

coral reef systems (Acre et al., 2020; Muenzel et al., 2024). Accordingly, in habitat-heterogeneous MPAs, integrative monitoring is essential for robust biodiversity assessment and for detecting management-relevant changes across multiple components of fish communities.

5. Conclusion

This study demonstrates the value of an integrated, multi-method survey strategy for characterizing fish assemblages in a habitat-heterogeneous marine protected area. By combining diving surveys, bottom trawling, and eDNA metabarcoding in the Nanpeng Islands Reserve and adjacent waters, we documented 201 fish species (15 orders, 70 families, 144 genera), including 119 species not previously recorded for the reserve. This multi-method approach not only substantially updated the species inventory but also captured a wide array of functional traits present in the community. Each method sampled a distinct subset of taxa associated with different ecological attributes (e.g., body size, trophic position, and habitat use), and no single approach captured the full breadth of functional trait space observed across habitats. Integrating methods was therefore necessary to more fully characterize assemblage-level biodiversity across habitats. Comparisons with historical compilations indicated differences in community composition and trait structure between historical and contemporary inventories, including a greater proportional representation of smaller, lower-trophic-level taxa and lower representation of large predators and historically reported protected species in the contemporary inventory. Although differences in survey design, sampling effort, and detectability limit strong inference about temporal change, these contrasts provide an updated baseline for future monitoring and highlight potential conservation concerns that warrant sustained and targeted monitoring efforts. Overall, our results underscore that combining complementary survey techniques is essential to obtain a robust and functionally informative understanding of fish biodiversity in complex coastal seascapes. The approach and baseline established for the Nanpeng Islands Reserve offer a transferable framework for biodiversity assessment and long-term monitoring in other heterogeneous MPAs and strengthen the evidence base for conservation planning and adaptive management.

Author contributions

Wenliang Zhou conceived and supervised the project. Wenliang Zhou, Han Lai, Kuo Gao, Lan Qiu, Wenbin Huang, Yiting Chen, Shichao Wei, Shaotong Tu, Qian Li, Tao Luo, Zhongwen Ding, Zhiwei Zhang, Wen Yu, and Zhaojie Peng conducted the field surveys and collected samples. Han Lai identified the fish species. Han Lai and Kuo Gao compiled the historical survey data, processed the sequencing data, and carried out the bioinformatic and statistical analyses. Han Lai and Kuo Gao drafted the manuscript. Wenliang Zhou critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Fish sampling was carried out following approval from the Animal Experiment Ethics Committee in the Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), China.

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Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.gecco.2026.e04265](https://doi.org/10.1016/j.gecco.2026.e04265).

Data availability

Data will be made available on request.

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