

Article

Fish Community Composition and Seasonal Variation in the Coastal Waters of Sanya (Hainan, China) Based on eDNA Metabarcoding

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Abstract

To characterize the fish community composition and seasonal variation in the coastal waters of Sanya, a tropical coastal region on Hainan Island in the northern South China Sea, and to evaluate the applicability of environmental DNA (eDNA) metabarcoding for biodiversity monitoring, water samples were collected from 16 nearshore sampling sites in June 2024 and January 2025. Fish community structure was analyzed using eDNA metabarcoding, combined with diversity indices and multivariate statistical analyses. A total of 488 marine fish species were detected, including 426 species in summer and 205 species in winter, with higher species richness observed in summer. Perciformes was the dominant order in both seasons. Dominant species composition varied seasonally, with a relatively dispersed pattern in summer and a dominance concentration structure in winter. The Chao1 and Shannon indices were significantly higher in summer than in winter, whereas evenness showed no significant seasonal difference. NMDS and ANOSIM analyses revealed significant seasonal differences in community structure, and low Sørensen similarity coefficients indicated substantial species variation between seasons. These results indicate that fish communities in the coastal waters of Sanya exhibit clear seasonal variation. eDNA metabarcoding provides an effective complementary approach for monitoring fish diversity in complex coastal habitats, although its results may be influenced by eDNA transport, degradation, and reference database limitations.

Keywords: eDNA; fish diversity; community structure; seasonal variation; metabarcoding

Key Contribution: This study represents the first cross-seasonal eDNA surveys in the coastal waters of Sanya, providing essential baseline data on regional fish diversity and demonstrating the potential of eDNA for monitoring and conserving coral reef ecosystems.



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1. Introduction

Marine biodiversity is fundamental to maintaining the functions and services of marine ecosystems, among which fish diversity serves as an important indicator of marine ecological health [1]. Sanya (18°09′–18°37′ N, 108°56′–109°48′ E) is located in the tropical zone on the southern coast of Hainan Island, China. The region is bordered by mountains to the north and the sea to the south, forming a narrow terrain that slopes gradually southward. The distinct geographical location and complex hydrological conditions make the coastal

waters of Sanya one of the most important fishing grounds in the South China Sea, as well as critical spawning, feeding, and nursery habitats for marine organisms, supporting rich marine biodiversity. Previous surveys have documented diverse coral reef fish assemblages in the Sanya coastal waters. A recent ecological assessment recorded 36 coral reef fish species belonging to 14 families and 28 genera within the Sanya Coral Reef National Nature Reserve, highlighting the high biodiversity value of these reef ecosystems [2]. At a broader regional scale, recent studies across Hainan Island have reported more than 360 coral reef fish species, indicating that diversity assessments based on localized surveys, such as those from individual reef areas, may substantially underestimate the overall species richness [3]. As a key interface between land and sea, coastal zones are highly susceptible to anthropogenic disturbances (e.g., fishing, coastal development, and pollution discharge) and climate change. In recent years, rapid economic development in Sanya has degraded aquatic environments and altered biological communities. These changes include declining species diversity and intensified soil erosion. The continued expansion of these issues may severely affect regional economic development and environmental protection [4]. Therefore, systematic investigation and assessment of fish species diversity in coastal waters of Sanya are urgently needed to support the effective conservation and management of fishery resources.

A variety of methods have been developed for monitoring fish species diversity, with early studies primarily relying on traditional survey techniques, including net sampling, electrofishing, trapping, market surveys, underwater photography, and sonar detection [5]. However, these approaches often require extensive preparation, exhibit habitat and species selectivity, and may have negative impacts on biodiversity and habitats. In particular, their invasive nature greatly limits their application in studies involving small water bodies and endangered species. Therefore, there is an urgent need for more efficient and non-invasive monitoring approaches to address the limitations of biodiversity assessment and fisheries management.

Environmental DNA (eDNA) refers to genetic material released into the environment by organisms in various forms, such as skin cells, saliva, and other secretions, and is widely distributed in environmental media including water, soil, sediments, and air [6]. eDNA-based approaches involve the collection and enrichment of DNA fragments from environmental samples, followed by species identification through polymerase chain reaction (PCR) amplification and high-throughput sequencing [7]. In recent years, eDNA metabarcoding has attracted considerable attention and has been widely applied in biological and ecological studies [8–10]. Its feasibility for monitoring coral reef fish diversity has been demonstrated in several studies [11]. Moreover, eDNA has been extensively used to investigate fish spatial structure [12], species composition and population abundance [13], as well as biological invasions [14]. The advantages of eDNA include high sensitivity, cost-effectiveness, time efficiency, and non-invasiveness [15,16], as well as reduced dependence on subjective judgment by researchers [17]. It enables efficient species identification and the estimation of relative abundance within biological communities [18].

This study was conducted in the coastal waters of Sanya. As an important tourist city, Sanya is highly influenced by urban development and tourism activities, which may lead to rapid changes in species composition and necessitate timely and accurate biodiversity assessment. In addition, the presence of extensive coral reef protected areas in the coastal region makes traditional survey methods difficult to implement and potentially harmful to reef structures. Currently, studies on coral reef fish diversity in Sanya based on eDNA metabarcoding remain limited. Therefore, it is necessary to conduct a systematic investigation using eDNA approaches. The objectives of this study were to evaluate the feasibility of eDNA for monitoring fish diversity and to provide technical support for long-

term community monitoring, ecological health assessment, and biodiversity conservation in Sanya and further offer reference information for fish ecosystem studies in other coastal regions of China.

2. Materials and Methods

2.1. Sampling Design and eDNA Collection

Based on the distribution of fish fauna and geomorphological characteristics of Sanya, a total of 16 sampling sites were established (Figure 1). Water samples were collected twice, in 13–28 June 2024 (summer) and 8–23 January 2025 (winter). At each site, 20 L of water, including both surface and bottom layers, was collected using a sterile polyethylene container and stored in sterilized polyethylene bottles. Within 24 h of collection, water samples were filtered to concentrate eDNA using mixed cellulose ester membranes with a pore size of 0.45 μm and a vacuum filtration system, allowing DNA to be retained on the filter membranes. Field blanks were included during sampling to monitor potential contamination introduced during field operations. Sterile ddH₂O was transported to the sampling sites, exposed to the same field conditions as water samples, and processed using the same filtration, storage, extraction, and amplification procedures. Depending on water turbidity, two filter membranes were used per sample. Membranes from the same sample were pooled for a single DNA extraction when multiple filters were required due to clogging. After filtration, the membranes were promptly transferred into sterile 2 mL centrifuge tubes and stored at $-80\text{ }^{\circ}\text{C}$ until further analysis. During all procedures, gloves were worn, and membranes were handled using sterile forceps to minimize contamination.

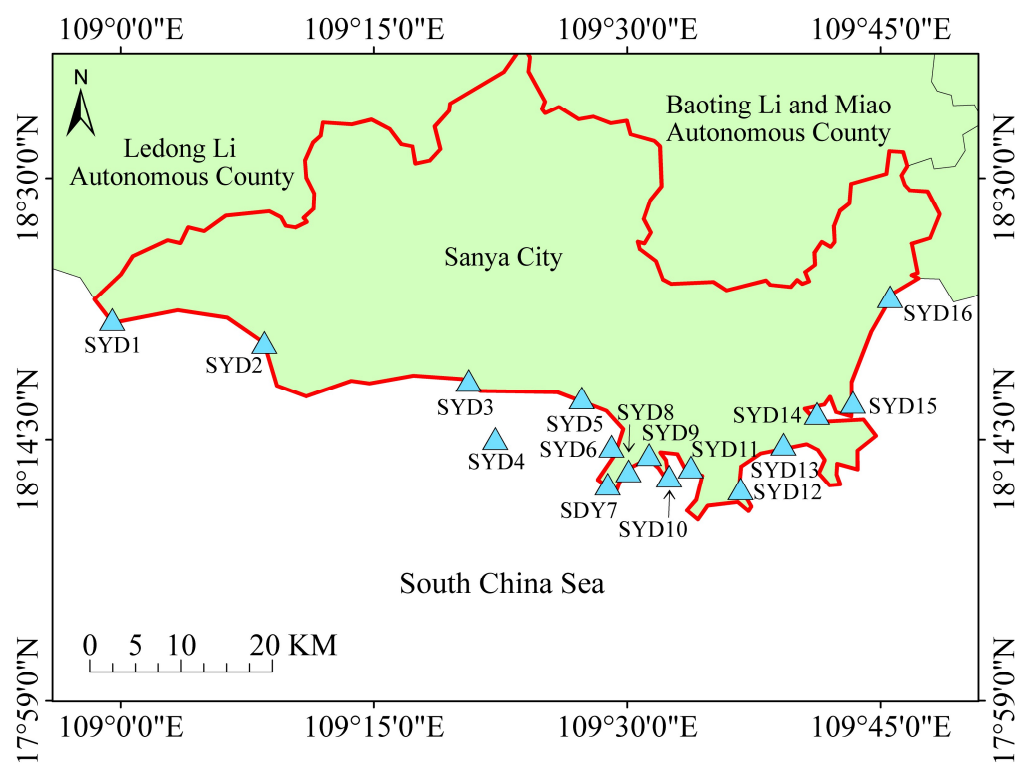


Figure 1. Location of the study area and sampling sites.

2.2. eDNA Extraction

DNA was extracted from the filter membranes using the D5525 Water DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. Each sample was processed independently, while multiple filter membranes from the same sample were

combined during extraction. The extracted eDNA was immediately stored at -80°C until subsequent PCR amplification.

2.3. PCR Amplification and High-Throughput Sequencing

PCR amplification of the hypervariable region of the mitochondrial 12S rRNA gene was performed using the fish-specific universal MiFish primers: MiFish-F (5'-GTCGGTAAACTCGTGCCAGC-3') and MiFish-R (5'-CATAGTGGGGTATCTAATCC-CAGTTTG-3'). The PCR reaction was conducted in a total volume of 25.0 μL , containing 5.0 μL of 5 $\times$ Reaction Buffer, 5.0 μL of 5 $\times$ GC Buffer, 2.0 μL of dNTPs (2.5 mmol/L), 1.0 μL each of MiFish-F and MiFish-R primers (10 $\mu\text{mol/L}$), 2.0 μL of DNA template (20 ng/ μL), 0.5 μL of DNA polymerase, and 8.5 μL of ddH₂O. Thermal cycling conditions were as follows: initial denaturation at 98°C for 4 min; 30 cycles of denaturation at 98°C for 20 s, annealing at 58°C for 45 s, and extension at 72°C for 50 s; followed by a final extension at 72°C for 8 min. Sterile ddH₂O was used as a negative control to assess potential contamination during PCR amplification. Each DNA sample was amplified in triplicate PCR reactions to reduce stochastic amplification bias. PCR products from technical replicates of the same sample were verified by 2.0% agarose gel electrophoresis, purified using a PCR Purification Kit (Wuhan Canos Technology Co., Ltd., Wuhan, China), pooled and sent to Sangon Biotech (Shanghai, China) for library construction, followed by high-throughput sequencing on an Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA) [19].

To minimize contamination from exogenous DNA, negative controls (ddH₂O) were included throughout the procedures of sample collection, DNA extraction, and PCR amplification, and all consumables used were DNA-free and disposable. Prior to diversity analysis, sequencing data were normalized to reduce the effects of variation in sequencing depth on community analysis results.

2.4. Data Processing and Analysis

Raw sequencing data obtained from the Illumina platform were merged and quality-filtered using PEAR (v0.9.6). Sequences containing ambiguous bases or with quality scores below 20 were removed. Sequences detected in negative controls were removed from the sample dataset before downstream community composition and diversity analyses. The minimum overlap length was set to 10 bp, with a p -value threshold of 0.0001. The merged sequences were further processed using Vsearch (v2.7.1) to remove sequences of non-target lengths, and chimeric sequences were identified and removed using the UCHIME algorithm. The numbers of sequencing reads retained after each quality-control step and the reads assigned to fish taxa are summarized in Table 1. Considering that this study focuses on community structure comparison and ensuring comparability with previous studies, an operational taxonomic unit (OTU)-based approach was employed for sequence clustering. Although amplicon sequence variant (ASV)-based approaches provide higher resolution, we used an OTU-based pipeline because it can reduce the influence of minor sequencing variations and avoid overestimation of species richness caused by redundant sequence variants. OTUs were generated using the UPARSE algorithm implemented in Vsearch (v2.7.1). We chose 99% similarity to minimize over-clustering given the high genetic diversity of tropical reef fishes, and subsequently merged OTUs annotated to the same species. Representative sequences of all OTUs were aligned using the BLAST+(v2.15.0) algorithm against the MitoFish database (<http://mitofish.aori.u-tokyo.ac.jp/>, accessed on 13 July 2026) and the NCBI nucleotide database (<http://ftp.ncbi.nlm.nih.gov/blast/db/>, accessed on 13 July 2026) to obtain taxonomic assignments. Community composition at the class, order, family, genus, and species levels was determined with reference to the Fish10K Genome Project (<https://fish10k.genomics.cn/>, accessed on 13 July 2026), the FishBase

database (<https://www.fishbase.in/search.php>, accessed on 13 July 2026), and relevant ichthyological literatures [20].

Table 1. Summary of sequencing reads at each step of the bioinformatics pipeline.

Seasons	Samples	Rawreads	Cleanreads	Cleanratio	Input	Filtered	Denoised	Non-Chimeric
Summer	SYD1	1,280,204	1,276,120	99.68%	638,060	621,668	617,618	340,937
	SYD2	1,010,166	1,007,014	99.69%	503,507	493,159	489,016	12,345
	SYD3	1,751,848	1,745,368	99.63%	872,684	850,380	845,574	17,970
	SYD4	707,972	705,782	99.69%	352,891	344,513	342,165	286,511
	SYD5	1,969,126	1,962,884	99.68%	981,442	959,292	954,256	56,930
	SYD6	1,396,396	1,391,714	99.66%	695,857	679,314	675,412	107,952
	SDY7	1,208,190	1,204,054	99.66%	602,027	587,163	582,146	75,260
	SDY8	1,326,696	1,322,330	99.67%	661,165	646,809	642,271	113,470
	SYD9	1,076,978	1,073,438	99.67%	536,719	523,800	520,852	249,222
	SYD10	1,379,824	1,375,766	99.71%	687,883	673,402	669,640	363,289
	SYD11	1,310,658	1,306,404	99.68%	653,202	639,163	635,115	165,323
	SYD12	1,222,348	1,218,468	99.68%	609,234	595,136	591,530	284,109
	SYD13	1,538,934	1,534,906	99.74%	767,453	753,197	750,077	650,286
	SYD14	546,598	545,050	99.72%	272,525	267,161	264,949	215,682
	SYD15	1,313,452	1,309,256	99.68%	654,628	640,188	635,467	31,247
	SYD16	832,144	829,562	99.69%	414,781	406,275	398,059	117,278
Winter	SYD1	410,628	409,136	99.64%	204,568	200,173	198,698	139,137
	SYD2	395,816	394,270	99.61%	197,135	192,615	191,707	43,369
	SYD3	365,320	363,972	99.63%	181,986	178,215	177,588	25,615
	SYD4	443,536	441,048	99.44%	220,524	213,134	211,197	51,763
	SYD5	195,056	194,402	99.66%	97,201	95,080	94,353	33,562
	SYD6	257,700	256,602	99.57%	128,301	125,297	124,434	68,482
	SDY7	565,344	562,720	99.54%	281,360	274,420	272,917	200,755
	SDY8	557,128	553,958	99.43%	276,979	269,067	267,276	178,243
	SYD9	598,820	594,040	99.20%	297,020	283,281	281,992	52,533
	SYD10	1,171,262	1,159,294	99.98%	579,647	547,138	546,000	136,384
	SYD11	193,820	192,996	99.57%	96,498	94,223	93,446	53,478
	SYD12	324,414	323,032	99.57%	161,516	157,760	156,122	56,272
	SYD13	493,840	490,820	99.39%	245,410	237,117	236,441	118,097
	SYD14	1,243,310	1,228,238	99.79%	614,119	575,392	574,485	44,555
	SYD15	501,300	497,972	99.34%	248,986	240,903	239,374	66,834
	SYD16	422,520	420,526	99.53%	210,263	204,807	203,179	51,727

Based on OTU clustering results, analyses of species composition, dominant species and their variation, diversity indices, and Sørensen similarity coefficients (Cs) were conducted: (1) Species composition analysis. After removing sequences assigned to non-fish taxa (e.g., bacteria, birds, amphibians, and mammals), OTUs assigned to fish with identity $\geq 99\%$ and E-value $\leq 10^{-5}$ were retained. OTUs assigned to the same species were merged. OTUs assigned only to unidentified taxa at the genus or family level were retained as separate taxonomic units only when they represented distinct taxa, but were not counted as confirmed species-level records. These unidentified taxa were reported separately and excluded from species-level richness comparisons to avoid overestimation. The abundance of each fish species was calculated for each sample in Excel, and taxonomic information was annotated and refined. (2) Dominant species and their variation. Based on the filtered fish OTUs dataset after merging, anomalous distribution records were first removed. The McNaughton dominance index (Y) was calculated using the sequence abundance and occurrence frequency of each species. Species with $Y > 0.02$ were defined as dominant species [21], and their distribution patterns across different seasons and sampling sites were compared. (3) Alpha diversity analysis. The Chao1, Shannon, Simpson, and Pielou indices were calculated to reflect community richness, diversity, and evenness, respectively. Differences in alpha diversity indices between summer and winter fish communities were

assessed using Welch's *t*-test, with a significance level of $p < 0.05$. Before performing Welch's *t*-test, the normality of each group was assessed using the Shapiro–Wilk test. Welch's *t*-test was selected because it does not assume equal variances between groups. (4) Beta diversity analysis. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis distances was performed on community data after removing rare and low-abundance species. Analysis of similarities (ANOSIM) was further conducted to test differences in community composition based on 999 permutations. (5) Cs analysis. The Cs of sampling sites was used to quantify the similarity between seasons based on species composition overlap. Data visualization in this study was performed using Origin 2024 software, ArcMap (v10.8.2), the ChiPlot (<https://www.chiplot.online/>, accessed on 13 July 2026) platform, and the Hiplot (<https://hiplot.com.cn/>, accessed on 13 July 2026) platform. The formulas used for the calculations are provided as follows:

$$\text{McNaughton dominance index}(Y) : Y = \frac{n_i}{N} \times f_i \quad (1)$$

$$\text{Chao1 index}(C) : C = S_{obs} + \frac{n_1(n_1 - 1)}{2(n_2 + 1)} \quad (2)$$

$$\text{Shannon index}(H') : H' = - \sum_{i=1}^S \frac{n_i}{N} \ln \frac{n_i}{N} \quad (3)$$

$$\text{Simpson index}(D) : D = \sum_{i=1}^{S_{obs}} \frac{n_i(n_i - 1)}{N(N - 1)} \quad (4)$$

$$\text{Pielou index}(J) : J = \frac{H}{H_{max}} = \frac{H}{\log_x S} \quad (5)$$

$$\text{Sørensen similarity coefficient}(Cs) : Cs = \frac{2c}{a + b} \quad (6)$$

where n_i represents the number of sequences contained in the i -th OTU; N denotes the total number of sequences; and f_i indicates the frequency of occurrence of the i -th OTU across all sampling sites. S represents species richness of the community; n denotes the total number of species with an abundance (number of sequences) greater than zero. H_{max} indicates the maximum possible value of the Shannon index under the condition of equal species richness. S_{obs} represents the observed number of OTUs; n_1 is the number of OTUs represented by a single sequence (singletons); and n_2 is the number of OTUs represented by two sequences (doubletons). a denotes the number of species in summer; b denotes the number of species in winter; and c represents the number of species shared between summer and winter.

3. Results

3.1. Taxonomic Annotation of OTUs

After performing quality control, sequence assembly, chimera removal on the eDNA data obtained from Illumina high-throughput sequencing, and removing fish OTUs detected in the negative controls, a total of 1488 OTUs were obtained from summer samples and 1231 OTUs from winter samples. Among the detected OTUs, 731 showed less than 97% similarity to reference sequences in the databases and could only be assigned to higher taxonomic levels such as order or family (with known historical distributions in the study area). A total of 215 OTUs exhibited similarity between 97% and 99% and were assigned at the genus level. The remaining 1773 OTUs showed greater than 99% similarity. However, among the 1773 OTUs, 14 could be annotated to the species level but were identified as unclassified species in the databases (Table 2). These 14 OTUs were not treated as confirmed

species in subsequent species-level diversity analyses, but were retained as unclassified taxonomic records for reporting taxonomic uncertainty. This limitation may be attributed to morphological similarity among species and uncertainties in the identification process [22]. The species accumulation curves approached a plateau when the sample size reached 15, indicating that the sampling effort was sufficient (Figure 2) (The OTU-based accumulation curves are shown in Figure S1). A summary of sequencing reads, OTUs, and identified fish species for each sampling site is provided in Table 3. In addition, it was observed that multiple fish species corresponded to one OTU, and multiple OTUs were annotated as the same species (Figure 3). This phenomenon is commonly reported in eDNA metabarcoding studies [19] and may result from interspecific sequence similarity, intraspecific sequence variation, as well as nucleotide differences introduced during PCR amplification or high-throughput sequencing [23]. To avoid overestimation of species richness due to redundant OTUs, OTUs assigned to the same species were merged in subsequent analyses, and all downstream analyses of community composition and diversity were conducted at the species level.

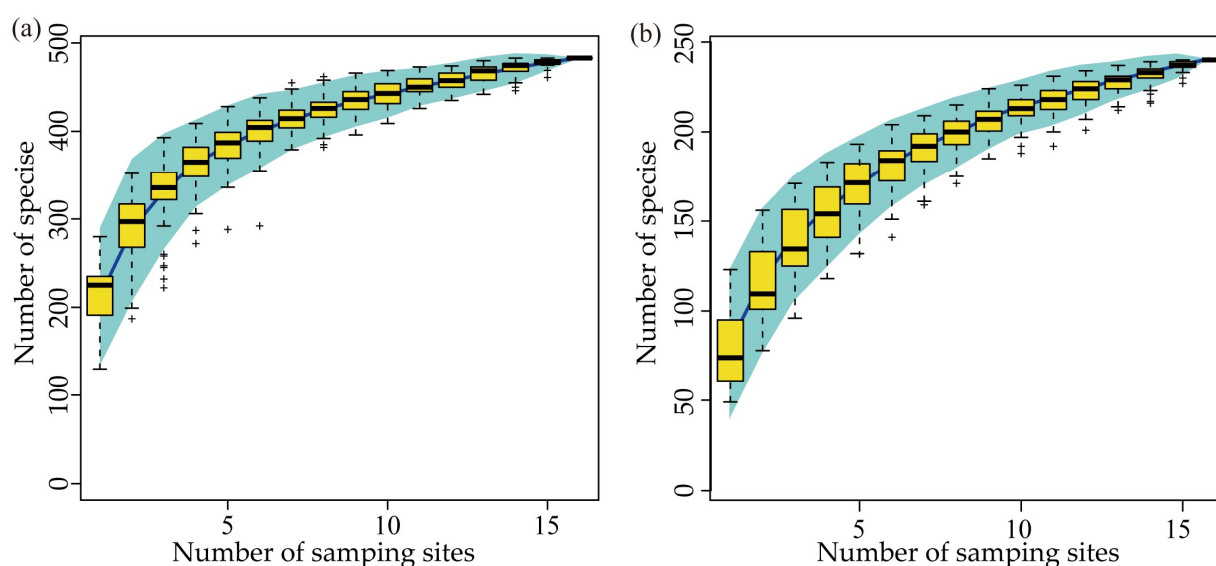


Figure 2. Species accumulation curves for summer samples (a) and winter samples (b).

Table 2. Taxonomic information of 14 OTUs assigned to unclassified species in the databases.

Species	Order	Family	Genus
<i>Ambassis</i> sp.	Perciformes	Ambassidae	<i>Ambassis</i>
<i>Cirripectes</i> sp.	Perciformes	Blenniidae	<i>Cirripectes</i>
<i>Bascanichthys</i> sp.	Ophidiiformes	Bythitidae	<i>Bascanichthys</i>
<i>Symphurus</i> cf. <i>microrhynchus</i>	Pleuronectiformes	Cynoglossidae	<i>Symphurus</i>
<i>Bathygobius</i> sp.	Perciformes	Gobiidae	<i>Bathygobius</i>
<i>Favonigobius</i> sp.	Perciformes	Gobiidae	<i>Favonigobius</i>
<i>Mugilogobius</i> sp.	Perciformes	Gobiidae	<i>Mugilogobius</i>
<i>Oxyurichthys</i> sp.	Perciformes	Gobiidae	<i>Oxyurichthys</i>
<i>Pseudogobius</i> sp.	Perciformes	Gobiidae	<i>Pseudogobius</i>
<i>Minous</i> sp.	Scorpaeniformes	Scorpaenidae	<i>Minous</i>
<i>Sebastapistes</i> sp.	Scorpaeniformes	Scorpaenidae	<i>Sebastapistes</i>
<i>Sillago</i> sp.	Perciformes	Sillaginidae	<i>Sillago</i>
<i>Misgurnus</i> sp.	Cypriniformes	Cobitidae	<i>Misgurnus</i>
<i>Terapontidae</i> sp.	Perciformes	Terapontidae	

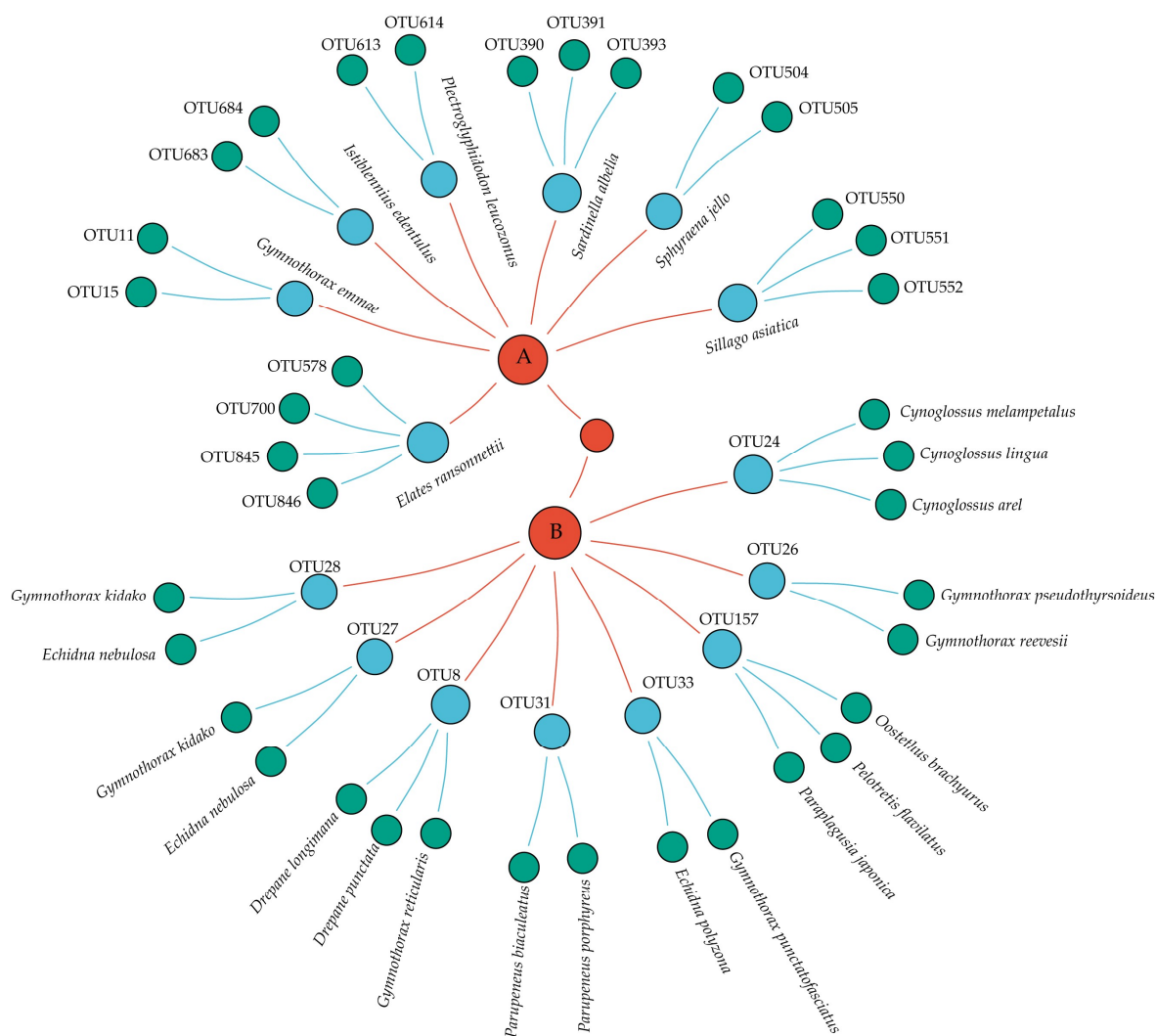


Figure 3. Circle network of multilevel hierarchical relationship between species and corresponding OTUs. A: A species corresponds to multiple OTUs. B: One OTU corresponds to multiple species.

Table 3. Brief introduction to the numbers of fish sequences, OTUs, and identified species.

Seasons	Sampling Sites	Sequences	OTUs	Identified Species
Summer	SYD1	340,937	297	211
	SYD2	12,345	141	118
	SYD3	17,970	159	134
	SYD4	286,511	233	182
	SYD5	56,930	195	158
	SYD6	107,952	204	174
	SDY7	75,260	203	147
	SDY8	113,470	228	175
	SYD9	249,222	257	208
	SYD10	363,289	310	229
	SYD11	165,323	258	203
	SYD12	284,109	326	236
	SYD13	650,286	318	248
	SYD14	215,682	248	197
	SYD15	31,247	204	172
	SYD16	117,278	253	215

Table 3. Cont.

Seasons	Sampling Sites	Sequences	OTUs	Identified Species
Winter	SYD1	139,137	118	90
	SYD2	43,369	60	58
	SYD3	25,615	43	49
	SYD4	51,763	105	99
	SYD5	33,562	88	52
	SYD6	68,482	54	49
	SDY7	200,755	102	62
	SDY8	178,243	105	80
	SYD9	52,533	119	82
	SYD10	136,384	88	62
	SYD11	53,478	76	52
	SYD12	56,272	105	94
	SYD13	118,097	102	41
	SYD14	44,555	81	49
	SYD15	66,834	69	67
	SYD16	51,727	95	81

3.2. Fish Species Composition

Based on taxonomic annotation with a 99% similarity threshold and after merging OTUs assigned to the same species, a total of 539 fish species were identified. Among these, 470 species were detected in summer (belonging to 30 orders, 110 families, and 284 genera), while 239 species were detected in winter (25 orders, 82 families, and 171 genera), indicating that species richness was markedly higher in summer (Figure 4a). In terms of ecological types (Figure 4b), marine fish (488 species) dominated the assemblage in the study area. In summer, 426 marine fish species were identified (25 orders, 96 families, and 247 genera), accounting for 90.6% of the total fish species recorded in summer, whereas in winter, 205 marine species were detected (19 orders, 71 families, and 139 genera), representing 85.8% of the total fish species recorded in winter. In conclusion, only a small number (51 species) of brackish and freshwater species were also detected. Given that marine fish accounted for the overwhelming majority in both seasons and play a key role in structuring coastal ecosystems, subsequent analyses focused primarily on marine fish species, and the 51 brackish and freshwater species were not included.

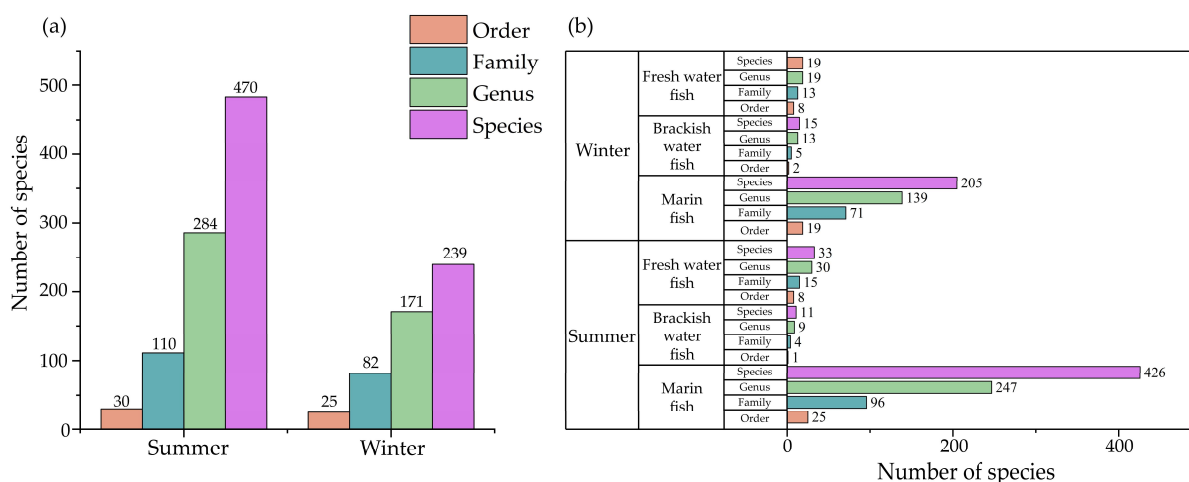


Figure 4. Fish species composition based on eDNA analysis. Numbers of fish orders, families, genera, and species detected in summer and winter (a). Taxonomic composition of marine, brackish-water, and freshwater fishes detected in summer and winter (b).

The composition at the order and family levels is shown in Figure 5. In summer, Perciformes was the most species-rich order, with 292 species detected, accounting for 68.5% of marine fish species. This was followed by Clupeiformes (21 species, 4.9%) and Anguilliformes (20 species, 4.7%). In winter, Perciformes remained the dominant order, accounting for 67.3% of marine fish species, indicating its consistent dominance across seasons, although its relative proportion decreased compared with summer. In contrast, Anguilliformes accounted for 6.3% in winter, showing an increased proportion relative to summer and suggesting seasonal variation at the order level. Overall, the marine fish community in the coastal waters of Sanya was characterized by the dominance of Perciformes at higher taxonomic levels, together with the co-occurrence of multiple orders and families, reflecting high species richness and a complex community structure.

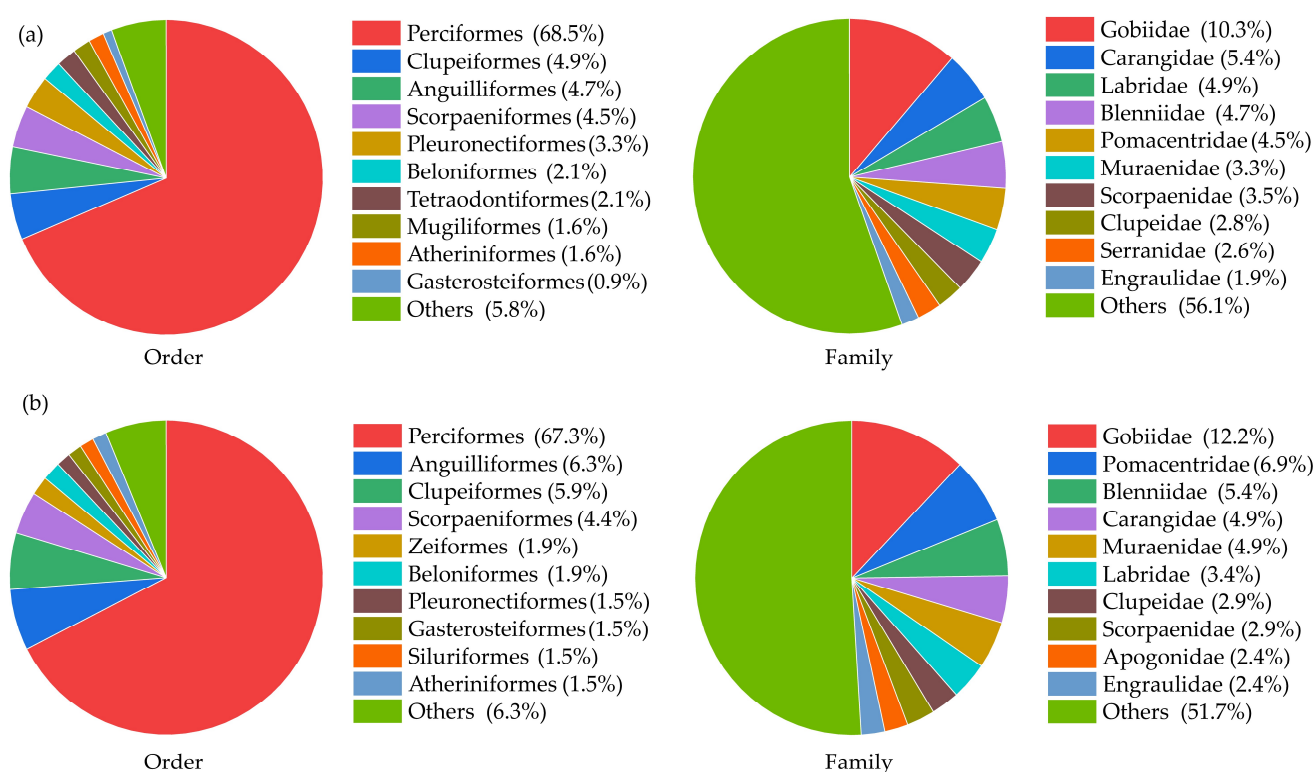


Figure 5. Taxonomic composition of fish communities at the order and family levels in summer (a) and winter (b) based on eDNA.

3.3. Dominant Fish Species and Their Seasonal Variation

The results showed that nine dominant species ($Y > 0.02$) were identified in both seasons; however, differences were observed in species composition and dominance patterns between summer and winter (Table 4). In summer, *Abudefduf septemfasciatus* and *Atherinomorus lacunosus* shared the highest dominance index ($Y = 0.122$), followed by *Atherinomorus pinguis* ($Y = 0.061$), while the remaining dominant species showed Y values ranging from 0.022 to 0.043. The sum and coefficient of variation (CV) of Y for nine dominant species were 0.49 and 73.66%, respectively, in summer. Overall, the summer community exhibited a pattern of co-dominance among multiple species. In contrast, the dominance structure in winter showed a dominance concentration pattern. *Siganus guttatus* exhibited the highest dominance index ($Y = 0.217$), markedly higher than that of other dominant species. *Enneapterygius philippinus* and *Istigobius ornatus* had dominance indices of 0.114 and 0.082, respectively, while the remaining dominant species had Y values ranging from 0.021 to 0.042. The sum and CV of Y for nine dominant species were 0.606 and 94.69%, respectively, in winter.

Table 4. Dominant fish species and their Y values in summer and winter.

Dominant Species in Summer	Y	Dominant Species in Winter	Y
<i>Abudefduf septemfasciatus</i>	0.122	<i>Siganus guttatus</i>	0.217
<i>Atherinomorus lacunosus</i>	0.122	<i>Enneapterygius philippinus</i>	0.114
<i>Atherinomorus pinguis</i>	0.061	<i>Istigobius ornatus</i>	0.082
<i>Encrasicholina heteroloba</i>	0.043	<i>Chrysiptera biocellata</i>	0.042
<i>Herklotsichthys quadrimaculatus</i>	0.039	<i>Plesiops coeruleolineatus</i>	0.037
<i>Plotosus canius</i>	0.030	<i>Blenniella bilitonensis</i>	0.035
<i>Enneapterygius philippinus</i>	0.026	<i>Gymnothorax miliaris</i>	0.033
<i>Gerres filamentosus</i>	0.025	<i>Chromis fumea</i>	0.025
<i>Trachinotus ovatus</i>	0.022	<i>Callogobius okinawae</i>	0.021

Because eDNA sequence abundance may be affected by biological and technical factors, it was interpreted as a proxy for relative detection intensity rather than a direct measure of actual species abundance in this study. From the perspective of spatial distribution, the abundance of dominant species across sampling sites also exhibited clear seasonal differences. In summer, dominant species were detected at most sampling sites and were distributed relatively evenly (Figure 6a). In contrast, the abundance of dominant species was obviously high at some sampling sites in winter, such as SYD1, SYD7, SYD8 and SYD10 (Figure 6b). Overall, the spatial distribution of fish abundance of dominant species shifted from a relatively dispersed pattern in summer to an abundance concentration pattern in winter.

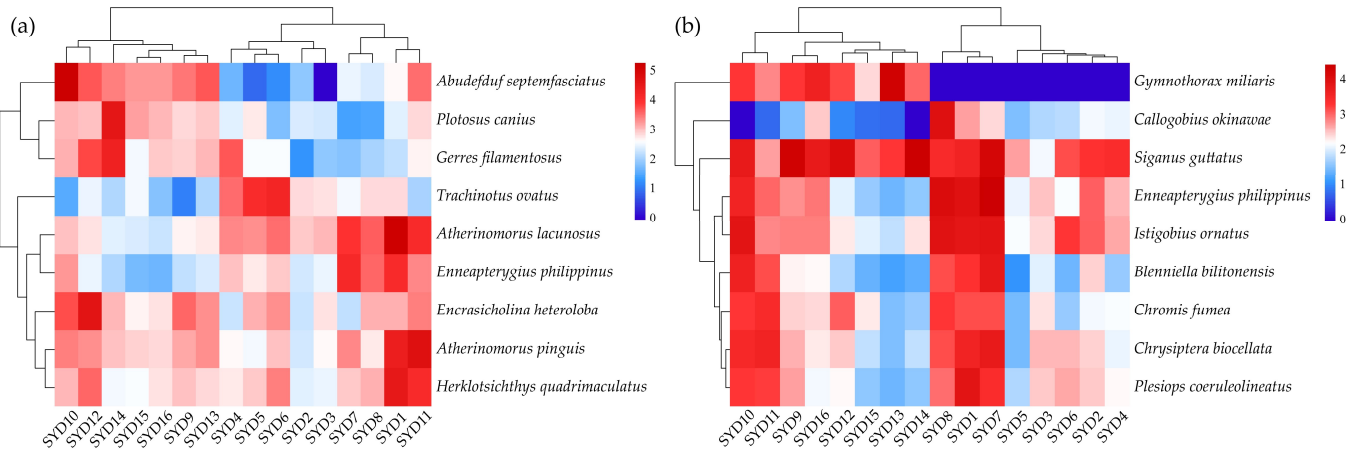


Figure 6. Heatmap of abundance of dominant fish species at each sampling site in summer (a) and winter (b). Note: The data were transformed using the base-10 logarithm (log₁₀) prior to analysis.

In addition to dominant species comparison, shared species were also evaluated using Welch’s *t*-test to analyze seasonal differences (Figure 7), the mean dominance index of shared species in summer and winter is approximately 6.97×10^{-3} and 6.66×10^{-3} , respectively, indicating a very small difference. Welch’s *t*-test revealed no significant seasonal difference between seasons ($p = 0.70$), with a negligible effect size (Hedges’ $g = 0.03$) and a 95% confidence interval overlapping zero (CI₉₅%: -0.13 to 0.20). In addition, Bayesian analysis yielded $\log_e(BF_{01}) = 2.28$, providing further support for the null hypothesis. Taken together, no statistically significant differences were detected between summer and winter for shared species, indicating that seasonal variation in the fish community was relatively limited at this metric.

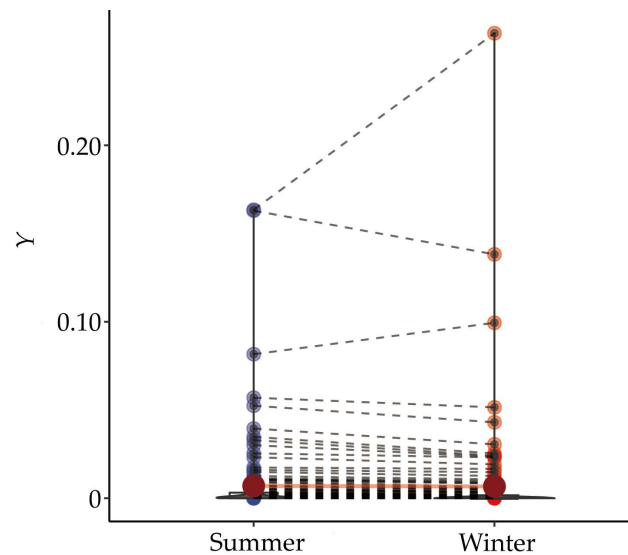


Figure 7. Relative abundance comparison of shared fish species. Note: $\mu_{\text{mean}}(\text{Summer}) = 6.97 \times 10^{-3}$, $\mu_{\text{mean}}(\text{Winter}) = 6.66 \times 10^{-3}$, $n = 137$, Dashed lines indicate paired observations.

3.4. Seasonal Differences in Alpha Diversity of Fish Communities

Based on eDNA data, the Chao1, Shannon, Simpson, and Pielou's index were calculated to compare alpha diversity of fish communities between summer and winter (Figure 8). The results showed that all four indices were higher in summer than in winter, with Chao1 (186.75 ± 36.711 vs. 55.13 ± 14.997), Shannon (3.33 ± 0.651 vs. 2.32 ± 0.812), Simpson (0.89 ± 0.112 vs. 0.76 ± 0.243), and Pielou (0.64 ± 0.130 vs. 0.58 ± 0.203). Welch's *t*-test indicated that the Chao1 and Shannon indices were significantly higher in summer than in winter ($p < 0.001$), with large effect sizes (Hedges' $g = 4.58$, 95% CI: 3.23 to 5.93; Hedges' $g = 1.34$, 95% CI: 0.57 to 2.11, respectively). The Simpson index showed no significant difference between seasons ($p = 0.08$), although it exhibited a higher trend in summer, with a moderate effect size (Hedges' $g = 0.63$, 95% CI: -0.08 to 1.35). Similarly, Pielou's index did not differ significantly between seasons ($p = 0.33$), showing a small effect size (Hedges' $g = 0.34$, 95% CI: -0.35 to 1.04). These results indicate that seasonal variation mainly affected species richness and diversity, whereas community evenness showed relatively limited changes.

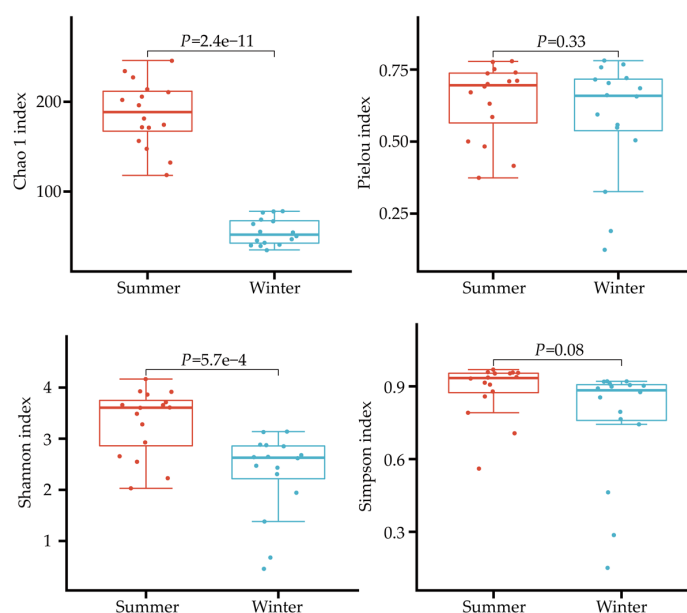


Figure 8. Comparison of alpha diversity indices of fish communities between summer and winter.

NMDS based on Bray–Curtis distance was performed on community data after removing rare and low-abundance species (Figure 9). The stress value was 0.170, indicating a moderate but acceptable representation of the community dissimilarity structure. Therefore, the ordination results should be interpreted with caution. Further analysis using ANOSIM revealed a significant difference in community structure between seasons ($R = 0.813$, $p = 0.005$, based on 999 permutations), indicating that inter-seasonal variation was substantially greater than intra-seasonal variation. These results are consistent with the NMDS ordination pattern and indicate that fish community composition differed significantly between the two seasons. However, the absence of concurrent environmental measurements prevents further assessment of the ecological mechanisms responsible for these differences.

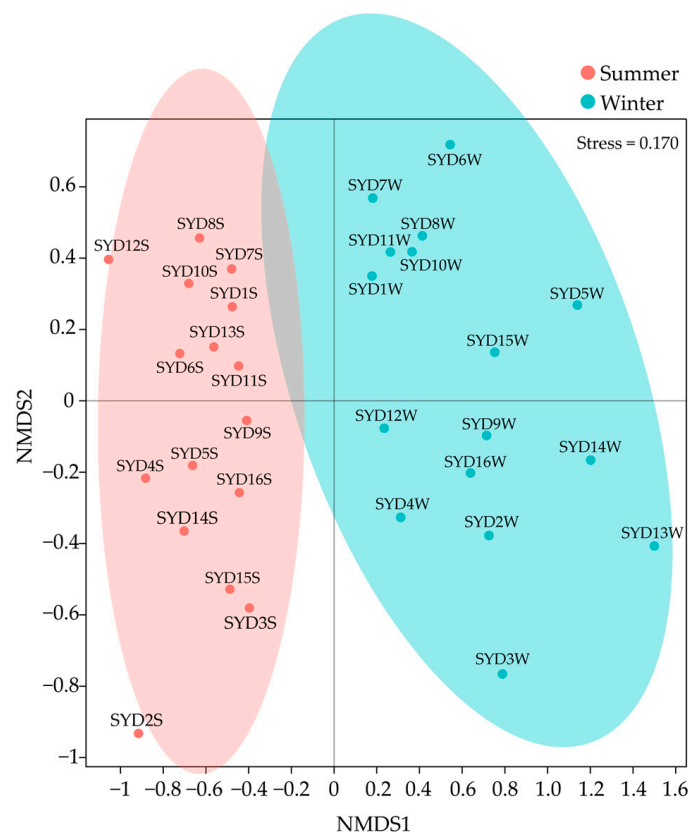


Figure 9. Seasonal variation in fish community structure revealed by NMDS. Note: SYDiS represents the i -th sampling site in summer, whereas SYDiW represents the i -th sampling site in winter.

3.5. Seasonal Similarity of Fish Communities at Each Sampling Site

To quantitatively assess the compositional stability of fish communities across seasons at the site scale and to further reveal their spatial distribution patterns, the C_s was calculated based on fish species detected in summer and winter at each sampling site (Figure 10). Most sites exhibited C_s values between 0.20 and 0.40, indicating that although many shared species were detected in both seasons, a large proportion of marine fish showed clear seasonal occurrence patterns.

Among all sampling sites, SYD1 exhibited the highest seasonal similarity ($C_s = 0.439$). At this site, 211 and 90 fish species were detected in summer and winter, respectively, with 66 species shared between seasons, indicating relatively high compositional similarity compared with other sites. In contrast, sites such as SYD13 ($C_s = 0.194$) and SYD14 ($C_s = 0.252$) showed lower seasonal similarity. Although these sites had relatively high species richness in summer, the proportion of shared species with winter was low.

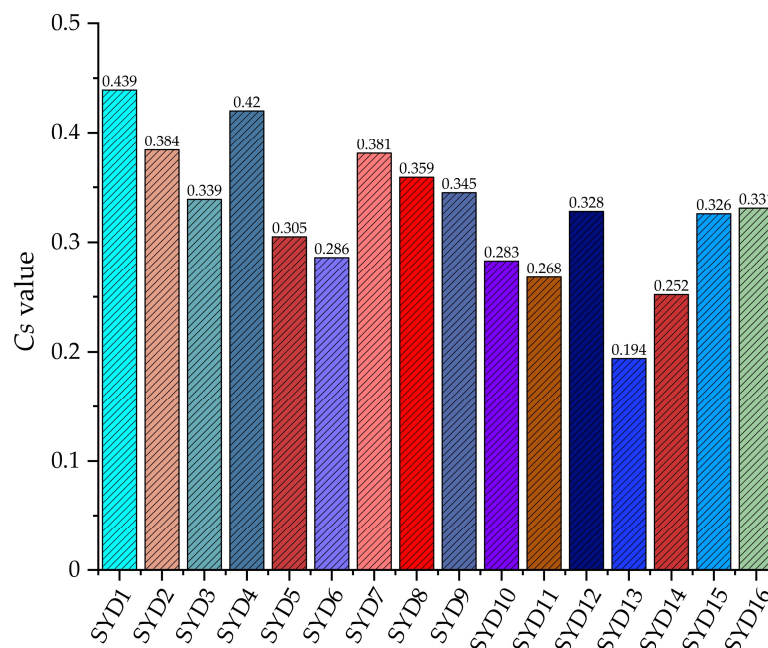


Figure 10. Cs values of fish communities between summer and winter at each sampling site.

The spatial distribution of Cs exhibited clear heterogeneity (Figure 11). Overall, SYD1, SYD4, SYD7, SYD8, and SYD9 are mainly located in nearshore waters far from human disturbance as well as within coral reef protected areas. These sites exhibit relatively high community similarity, indicating that the ecological environment is relatively stable, the biological community structure is well maintained, and the influence of anthropogenic disturbance is minimal. In contrast, sampling sites with smaller bubble sizes are primarily concentrated in sandy beaches with intensive human activities. Due to continuous disturbances from tourism, coastal development, and other anthropogenic factors, these areas show lower community similarity, reflecting instability in community structure and a certain degree of degradation. Generally speaking, community similarity declined from protected areas to heavily disturbed sites, indicating that human activity may be a key driver of coastal community structure.

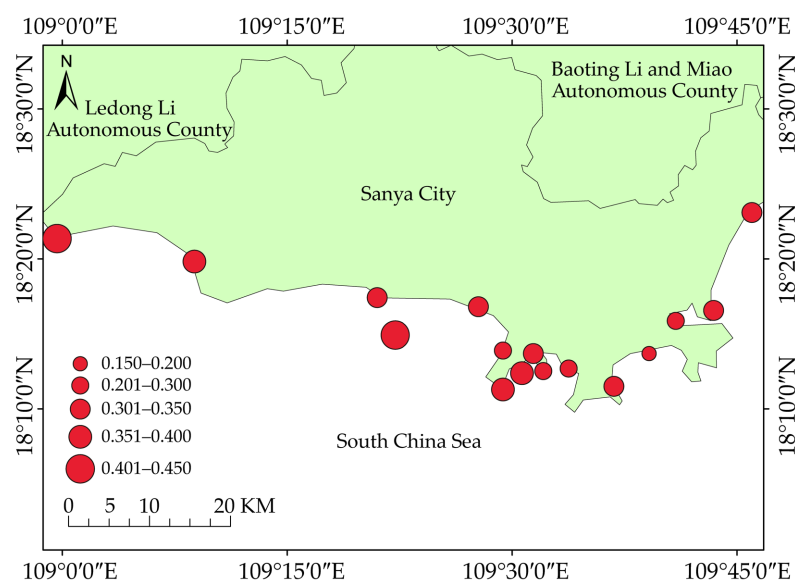


Figure 11. Comparison of Cs among western, central, and eastern regions. Note: Bubble size is proportional to the Cs value.

4. Discussion

4.1. Comparison of Community Structure and Species Number with Previous Studies

In previous eDNA surveys conducted around West Island, Sanya (based on a single-season survey), Perciformes was identified as the dominant taxon, comprising as high as 70.73% of the marine fish species [24]. In this study, SYD4 was situated near West Island. Perciformes was also the most species-rich group at this site, accounting for 66.67% of the total marine fish species. The primary order is consistent with previous eDNA findings. From the perspective of dominant taxonomic composition, eDNA metabarcoding demonstrated the capability in species detection and community structure characterization.

In terms of traditional survey methods, Luo et al. [25] conducted fishery resource surveys using bottom trawl nets around Wuzhizhou Island in Sanya (located between SYD15 and SYD16) in August and November 2020, and in January and April 2021, capturing a total of 174 fish species (2 classes, 12 orders, 65 families, and 123 genera). Similarly, Qu et al. [26] performed two bottom trawl surveys around Hainan Island in August and November 2024, detecting 228 fish species (15 orders, 69 families, and 147 genera) at Sanya sites. However, a total of 488 marine fish species were identified from two seasonal eDNA surveys in this study, which is substantially higher than the number of species recorded by recent traditional methods. This discrepancy underscores the advantages of eDNA metabarcoding in detecting hidden, benthic, burrowing, low abundance, or rare taxa [27,28]. For instance, two nationally protected seahorses (*Hippocampus spinosissimus* and *Hippocampus trimaculatus*) were successfully detected, demonstrating its potential for early detection of rare species and identification of critical habitats [29]. Moreover, numerous Anguilliformes (eels) and high richness of Gobiidae were found. Many Anguilliformes and gobies are typically small, benthic, or cryptic, inhabiting coral crevices or soft substrates [30–32], making them difficult to capture by traditional gear. eDNA approaches in this study address the limitations of traditional methods, show higher sensitivity in species detection, and contribute to a more complete understanding of fish diversity.

4.2. Seasonal Variation of Fish Communities

This study detected 426 marine fish species in summer and 205 in winter, indicating markedly higher species richness during the warmer season. Several factors may contribute to this seasonal difference. Previous studies have suggested that elevated summer seawater temperatures can accelerate fish metabolic rates and increase activity, resulting in greater release of DNA-containing materials (e.g., skin cells, mucus, and excreta), which may enhance eDNA detectability [33]. In addition, summer phytoplankton blooms boost primary productivity and food availability, and many species also enter their reproductive period in summer, temporarily increasing local abundance and overall fish diversity [34]. Seasonal environmental disturbances, including increased rainfall, wave action, typhoons, terrestrial runoff, and water exchange, may also influence eDNA transport, resuspension, and persistence [35]. Conversely, lower temperatures in winter may reduce fish activity or lead to seasonal migration of many species, thereby contributing to the lower number of detected species. These mechanisms are consistent with the observed seasonal pattern but were not directly evaluated in the present study.

In terms of dominance structure, summer communities exhibited a relatively even distribution of dominance among multiple species (co-dominance), whereas winter communities showed a concentration of dominance, with *Siganus guttatus* markedly outranking others. This shift from a dispersed to a concentrated pattern reflects the same environmental drivers: summer's warmth, productivity, and reproductive activity allow multiple species to share coastal resources evenly, while winter's harsher conditions favor tolerant species, leading to dominance concentration. Furthermore, summer communities were

characterized mainly by pelagic or highly mobile species, whereas winter communities were dominated by benthic or demersal species, indicating that different ecological groups respond differentially to seasonal changes. These patterns suggest that fish communities adapt via niche adjustments across seasons, evident in both species composition and dominance structure, consistent with previous observations [36,37].

Alpha diversity indices also revealed clear seasonal differences, though with varying responses [38]. Chao1 and Shannon indices were significantly higher in summer, indicating increased species richness and total diversity, likely due to the environmental and biological factors mentioned above [39]. However, Simpson and Pielou indices showed no significant seasonal differences, suggesting that evenness remained relatively stable [40]. Beta diversity analyses (NMDS and ANOSIM) confirmed distinct community compositions between seasons, reflecting stable seasonal structural changes rather than random site-level variation. Higher summer temperatures and biological activity likely facilitated more fish entering nearshore waters, releasing richer eDNA signals and producing more complex community structures, whereas reduced activity or migration in winter simplified the community [41,42].

Taken together, the seasonal dynamics in the coastal waters of Sanya are characterized by increased species richness and total diversity in summer, shifts in dominant species composition (with higher dominance of certain taxa in winter), and significant compositional turnover, while community evenness remains relatively stable. Different diversity indices capture distinct aspects: alpha diversity reflects changes in richness and total diversity, dominant-species analysis highlights adjustments in locally dominant taxa, and beta diversity reveals species variation and compositional reassembly across seasons.

4.3. Spatial Variation of Fish Communities

Notably, *Gymnothorax miliaris* was identified as one of the dominant species in winter ($Y = 0.033$); however, its eDNA signal was not detected at several sampling sites (e.g., SYD1–SYD8), exhibiting a clear spatially discontinuous distribution. *Gymnothorax miliaris* belongs to the order Anguilliformes and is typically a benthic, burrow-dwelling species that inhabits coral reef crevices or rocky substrates, showing strong dependence on microhabitat structure. Considering the characteristics of the study area, coral reefs in the coastal waters of Sanya are mainly distributed in the central and eastern regions, whereas they are relatively scarce in the western region. This spatial distribution of habitats explains the observed variation in the occurrence of *Gymnothorax miliaris* among sampling sites. Apart from *Gymnothorax miliaris*, the Sørensen similarity analysis also revealed the spatial heterogeneity among sampling site. Previous studies have shown that eDNA signals in aquatic environments are influenced not only by the actual distribution of target organisms but also by processes such as transport, retention, resuspension, and degradation. Therefore, the observed spatial variation in community similarity among sites is likely the result of the combined effects of local hydrodynamic conditions and habitat heterogeneity [43,44].

4.4. Limitations of eDNA Metabarcoding in Monitoring Fish Diversity

The results of this study demonstrate that eDNA metabarcoding has high applicability in tropical coastal areas, particularly in coral reef environments where traditional survey methods are constrained, and can effectively complement or even extend the scope of conventional biodiversity assessments. However, fish communities in the study area may also be influenced by multiple environmental factors, including water temperature, ocean currents, and coral reef habitat structure. Therefore, the interpretation of eDNA-based results should be approached with caution, taking into account the ecological characteristics and environmental dynamics influencing eDNA signals [8,45]. One important limitation

of this study is the lack of concurrent environmental measurements during sampling, including water temperature, salinity, dissolved oxygen, chlorophyll-a, and turbidity. These environmental factors may influence both fish community distribution and eDNA detection by affecting DNA shedding, degradation, and transport processes. Therefore, although significant seasonal differences in fish community composition were observed, the present study cannot determine the specific environmental drivers responsible for these patterns. The results should thus be interpreted as a descriptive characterization of seasonal variation rather than a mechanistic explanation. Future studies integrating environmental monitoring with eDNA metabarcoding will be necessary to elucidate the ecological processes underlying fish community dynamics.

A total of 51 freshwater and brackish fish species were detected in this study. However, the detection of freshwater fish DNA does not necessarily indicate the presence of established freshwater fish populations in coastal waters. Several possible explanations may account for these records. First, terrestrial runoff may transport water containing DNA from freshwater and brackish species into coastal areas [46], and eDNA can persist in aquatic environments for days to weeks, allowing it to be detected during sampling. Second, anthropogenic activities may contribute to the introduction of non-marine DNA signals into coastal waters, including domestic wastewater discharge, aquaculture escapees, vessel discharge, and fish release practices. Third, limitations associated with short amplicon lengths and incomplete reference databases may lead to potential misassignments during taxonomic annotation. Another possible explanation of brackish species is that some species with broad salinity tolerance may occasionally enter estuarine and nearshore environments. Therefore, these detections should be interpreted as freshwater-associated DNA signals rather than direct evidence of established freshwater fish communities in coastal waters. Similarly, although the detection of rare taxa such as *Hippocampus* spp. demonstrates the high sensitivity of eDNA metabarcoding, these records should still be interpreted cautiously because low-abundance detections may be influenced by trace contamination or DNA transported from nearby or distant habitats through water movement [47]. Therefore, eDNA-based results are more suitable for comparative analyses of community composition across seasons or sampling sites, rather than for directly inferring the instantaneous presence or population size of species at a given site [8,45]. Specifically, the ecological traits of species and regional environmental context should be considered to avoid overinterpretation of DNA signal sources.

eDNA-based analyses are influenced by multiple methodological factors, including sampling strategies, DNA preservation conditions, PCR amplification bias, and the completeness of reference databases [48]. Some OTUs could not be reliably assigned to the species level, which may be attributed to the short length of amplified fragments or the absence of reference sequences for certain taxa. These limitations constrain the taxonomic resolution of species identification to some extent [49,50]. Therefore, eDNA approaches still rely on baseline species inventories and morphological identification derived from conventional survey methods. The integration of eDNA techniques with traditional approaches can improve the accuracy and reliability of fish biodiversity assessments [8,45].

The use of a single primer pair and a single genetic marker should also be considered when interpreting the present results. Although the MiFish primers targeting the mitochondrial 12S rRNA gene are widely used for fish eDNA metabarcoding, primer mismatches may occur in some taxa, leading to preferential amplification of certain fish groups and reduced detection probability for others [51]. Therefore, false negatives cannot be completely excluded, and the fish diversity detected in this study may still underestimate the true community composition. Moreover, the taxonomic resolution of the 12S rRNA marker varies among fish lineages. This limitation, together with incomplete representation of regional

fish taxa in reference databases, may partly explain why 731 OTUs in this study showed less than 97% similarity to available reference sequences. Future studies could combine multiple molecular markers, such as 12S rRNA, COI, and 16S rRNA, because different markers and primer sets may recover complementary components of fish communities [52]. Such multi-marker approaches, together with improved regional reference databases and conventional survey methods, could reduce primer-specific bias, increase species detection, and improve the reliability of species-level taxonomic assignments.

5. Conclusions

This study demonstrated clear seasonal variation in fish communities in the coastal waters of Sanya using eDNA metabarcoding. Higher species richness and diversity were observed in summer than in winter, and community composition differed significantly between seasons, highlighting the importance of seasonal sampling for biodiversity assessment. These findings provide important baseline information for the conservation and management of coastal and coral reef ecosystems in Sanya and support the use of eDNA metabarcoding as an effective complementary tool for long-term fish biodiversity monitoring. Future monitoring programs should integrate eDNA with traditional survey methods, environmental variables, and hydrodynamic information to improve the accuracy and ecological interpretation of biodiversity assessments.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes11070419/s1>, Figure S1: OTU-based accumulation curves for summer samples (a) and winter samples (b).

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