



Article

New Insights into Fish Diversity in the Yellow and Bohai Seas Based on Environmental DNA Technology

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Abstract: Marine biodiversity is increasingly threatened by overfishing and marine pollution. A comprehensive understanding of the biodiversity in the Yellow and Bohai Seas is essential for resource conservation. This study conducted a large-scale survey of fishery resources in the Yellow and Bohai Seas to investigate fish community structure and diversity using eDNA technology. The results showed that 57 and 65 fish species were recorded in spring and summer, respectively, with 46 species identified in both seasons. A total of nine dominant species were identified in spring, mainly belonging to Perciformes, while seven dominant species were identified in summer, primarily from Clupeiformes. In different regions, no significant differences in fish α diversity were observed between spring and summer. However, significant seasonal differences were found in both α and β diversity. At the spatial level, fish species in the Yellow and Bohai Seas occupied similar ecological niches. However, at the temporal level, there are certain differences in the ecological niches of fish communities between seasons. Phylogenetic diversity showed no significant differences at the spatial scale, yet notable seasonal variations were observed at the temporal scale, with spring (661.46) significantly exceeding summer (540.30). The Mantel test indicated that nitrate and salinity were key environmental factors influencing α diversity. RDA analysis revealed that the dominant species in spring were mainly influenced by chlorophyll-a and water temperature, while those in summer were mainly affected by water depth, nitrite, water temperature, and salinity.



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Keywords: environmental DNA; Yellow and Bohai Seas; biodiversity; functional diversity; temporal and spatial patterns

Key Contribution: This study represented the first large-scale investigation of fish diversity in the Yellow and Bohai Seas using eDNA technology. We systematically investigated the spatial and temporal distribution patterns of species diversity among fish communities and explored the influence of environmental factors on diversity indices and dominant species.

1. Introduction

Marine ecosystems cover 70.6% of the Earth's surface and exhibit rich biodiversity [1]. As China's offshore waters, the Yellow and Bohai Seas serve as spawning and nursery grounds for numerous fish species, such as *Penaeus orientalis*, *Larimichthys polyactis*, and *Trichiurus lepturus* [2]. In recent years, the intensification of human disturbance has imposed enormous environmental pressures on the survival of many marine fishes, challenging our ability to formulate sustainable management and conservation plans to maintain ecosystem services. Therefore, developing effective biodiversity management necessitates systematic and large-scale assessments and monitoring [3].

The ability to manage marine ecosystems is often hampered by an inability to efficiently and accurately assess the abundance and distribution of marine species [4]. Species

diversity not only reflects the distribution and composition of species, species richness, and community structure characteristics but also reveals how they adapt to their surroundings [5–7]. Despite the importance of species diversity in ecological research, it alone does not fully capture the state and processes of the ecosystem, as each species possesses its independent evolutionary history and functional traits [8,9]. Phylogenetic diversity (PD) and functional diversity (FD) quantify the evolutionary history and trait values of a given community, respectively. This requires incorporating functional diversity (FD) and phylogenetic diversity (PD) into ecosystem management. Simultaneously, using various biodiversity assessment methods is frequently essential for a more comprehensive understanding of community changes and preservation results [10,11].

Traditional fisheries resource assessments are frequently conducted using trawling or acoustic techniques [12]. These methods monitor a limited number of fish species and can be destructive, hindering fish community conservation efforts [13]. In recent years, the application of environmental DNA (eDNA) in managing marine ecosystems and assessing biodiversity has experienced explosive growth [14]. eDNA metabarcoding technology enables the simultaneous detection of organisms across multiple trophic levels and life domains, characterized by its efficiency, sensitivity, and non-invasiveness [4,15]. The first application of eDNA metabarcoding technology in aquatic ecosystems began in detecting the invasive American bullfrog [16]. Its excellent performance has facilitated the widespread application of this technology. Fraija-Fernández et al. (2020) revealed spatial patterns of fish diversity in a large oceanic area by metabarcoding and sequencing of 12S rRNA genes [17]. Zhou et al. (2022) explored fish biodiversity in Zhoushan waters using eDNA metabarcoding technology [18]. The results showed that the fish community structure varied significantly between summer and winter, with summer exhibiting higher biodiversity. Marques et al. (2021) assessed fish functional and phylogenetic diversity using eDNA. Their results indicated that eDNA analysis provided a better estimate of multiple facets of biodiversity, enhancing our understanding of community structure and function [19].

In this study, we used eDNA metabarcoding technology to estimate the spatio-temporal pattern distribution of multiple biodiversity indicators. Additionally, we explored the relationships between species diversity, functional diversity, and phylogenetic diversity and environmental factors, revealing their responses to these factors. Our results contribute to a deeper understanding of the differences in the Yellow and Bohai Seas, providing critical insights for the conservation and restoration of fishery resources.

2. Materials and Methods

2.1. Sample Sites

In this study, eDNA metabarcoding was conducted in the spring (April 2022) and summer (August 2021) to investigate fish diversity in the Yellow and Bohai Seas (30.00°–39.52° N, 118.78°–123.61° E). Based on geographic location, the surveyed waters can be divided into three regions: the South Yellow Sea (SYS), the North Yellow Sea (NYS), and the Bohai Sea (Figure 1a). During the spring and summer, fish diversity was surveyed at 20 and 28 sites, respectively. However, due to adverse weather conditions, sampling could not be conducted in certain areas of the South Yellow Sea during spring. Our sampling sites covered the migratory routes of fish between the Yellow Sea and Bohai Sea, facilitating the capture of more comprehensive fish data (Figure 1). Based on variations in water depth, we collected surface and bottom water samples from areas with depths of less than 30 m. In areas where the depth exceeded 30 m, we collected surface, mid-depth, and bottom water samples. Water from different depths at the same site was mixed into one sample. The physical properties of the water (water depth, water temperature (T), salinity (S)) were measured at each site using CTD (conductivity, temperature, and depth). Additionally, we measured the concentrations of phosphate (PO_4^{3-}), silicate (SiO_3^{2-}), nitrite (NO_2^-), ammonium (NH_4^+), nitrate (NO_3^-), and chlorophyll-a (Chl-a).

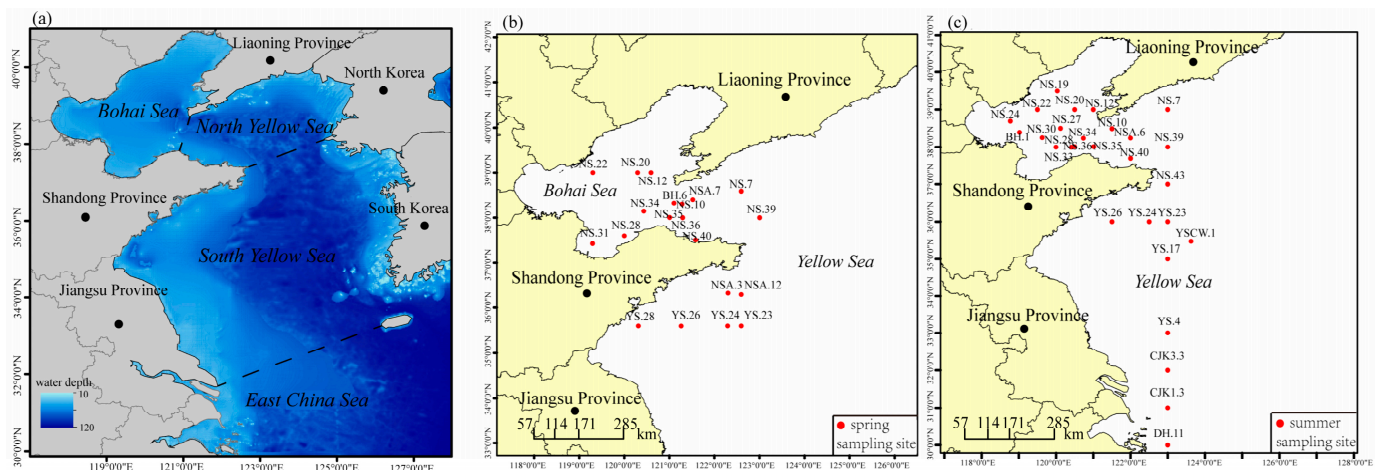


Figure 1. Location of samples collected from the Yellow and Bohai Seas. (a) Details about the marine boundaries. (b) eDNA sampling sites in spring. (c) eDNA sampling sites in summer.

2.2. Sample Collection and DNA Extraction

The mixed water samples were placed in sterile bags and transported to the laboratory at low temperature for filtration. To prevent cross-contamination, sodium hypochlorite was used to sterilize all instruments before filtration. The 2 L water samples were then filtered through a 0.22 µm glass-fiber filter membrane. On each sampling day, 500 mL of ultrapure water was filtered as the negative control, yielding a total of 10 negative controls. The filters were wrapped in sterile tin foil and stored at -20°C until further processing. DNA extraction was conducted according to the protocol outlined in a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), followed by purification with a DNeasy PowerClean Pro Cleanup Kit. The total eDNA obtained was stored at -20°C until PCR amplification.

2.3. Library Construction and Sequencing

For PCR amplification, a universal primer set (MiFish-U/E-F: GTC GGT AAA ACT CGT GCC AGC, MiFish-U/E-R: CAT AGT GGG GTA TCT AAT CCC AGT TTG) was used to amplify the 12S rRNA gene of fish [20]. The PCR assay volume was 25 µL and included 1 µL of DNA template, 17.5 µL of ddH₂O, 2.5 µL of 10× buffer, 2 µL of dNTPs, 1 µL of forward and reverse primers, and 0.15 µL of rTaq polymerase. The PCR protocols used for all samples included an initial denaturation step at 95 °C for 5 min, followed by 40 cycles consisting of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s. The final extension was at 72 °C for 10 min and cooling at 4 °C. The PCR products were detected using a 2% agarose gel to confirm the presence of the target band. Then, samples containing the target bands were used for library construction and sequenced using the 2 × 250 paired-end protocol on the Illumina NovaSeq platform (Santiago, CA, USA).

2.4. Bioinformatics Analysis

The raw data analyses were conducted using QIIME 2 version 2021.4 [21]. The demux plugin was used to demultiplex the raw sequence data. Next, the DADA2 plugin was employed to merge, filter, and remove duplicate sequences [22]. To minimize false positives, ASVs with fewer than 10 sequences were filtered out [23]. Based on the ASV, taxonomy was assigned to ASV using Blastn against the Mitofish database (<http://mitofish.aori.u-tokyo.ac.jp/>) (accessed on 15 May 2024). Note that only ASVs with an identity greater than 98%, a length of at least 160 bp, and an E-value of less than 10^{-5} were retained. Moreover, all samples were resampled using the vegan package (2.6.4), standardizing the sequencing depth to 50,000. The Chao index, the Shannon–Wiener index, the Pielou evenness index, and the Simpson’s diversity index were measured using the vegan package (2.6.4). Non-metric multidimensional scaling (NMDS) was used to compare beta diversity. A stress

value <0.2 indicates that the simulation results are reliable. Functional diversity was calculated using the R package mFD (1.0.6). Functional traits selected for analysis included species characteristics related to environmental factors, life history, morphology, and reproduction, including six continuous variables and four categorical variables. Functional traits were obtained from FishBase (<http://www.fishbase.org>) (accessed on 20 August 2024). Specific traits are provided in Supplementary Material Table S1. The calculation of phylogenetic diversity was performed using the R package taxize (0.9.99). The relationship between environmental factors and diversity indices was examined using the Mantel test, implemented through the R package linkET (0.0.7.4). Additionally, redundancy analysis was conducted with the R package vegan (2.6.4) to explore the influence of environmental factors on dominant species.

Dominant species were identified using the dominance index (Y), with a value of $Y \geq 0.02$ considered as dominant species [24].

$$Y = f_i \times \frac{n_i}{N}$$

In this equation, f_i is the frequency of the i^{th} species occurring in the samples, n_i is the abundance of the i^{th} species, and N is the total abundance of all fish species.

3. Results

3.1. Sequencing Results and Taxonomic Composition

The results of high-throughput sequencing indicated that all samples yielded the target band, with the exception of the blank control groups. In the spring, we obtained a total of 4,410,967 raw reads, from which 3,667,165 (83%) were used for downstream analysis. In the summer, we collected 6,926,030 raw reads, with 5,844,574 (84%) used for downstream analysis. The results indicated that we obtained a substantial number of high-quality sequences through sequencing.

Through sequence annotation, a total of 76 fish species were identified during spring and summer, belonging to 13 orders, 41 families, and 69 genera (Supplementary Material Table S2). The number of species identified in both spring and summer was 46 (Figure 2a). In spring, a total of 57 fish species were identified, classified into 11 orders, 33 families, and 51 genera. Among these, six taxa were annotated only at the genus and family levels, such as *Lepidotrigla*, *Sebastes*, *Macroramphosus*, Tetraodontidae, Callionymidae, and Pleuronectidae. In summer, a total of 65 fish species were identified, classified into 13 orders, 38 families, and 59 genera. Among these, eight taxa were annotated only at the genus and family levels: *Hexagrammos*, *Chelidonichthys*, *Saurida*, *Takifugu*, *Macrorhamphosus*, *Siganus*, and Callionymidae. Perciformes were the majority during the two surveys, followed by Pleuronectiformes. Spatially, the number of species identified in the Bohai Sea, South Yellow Sea, and North Yellow Sea during spring was similar (Figure 2b). Additionally, the summer survey revealed that the South Yellow Sea had the highest number of species (Figure 2c). In spring, a total of nine dominant species were detected. Apart from *Scophthalmus maximus* and *Konosirus punctatus*, the remaining species were observed at each sampling site. Notably, the non-native species, *Scophthalmus maximus* was observed at 95% of the sampling sites (Table 1). Among the seven dominant species identified in summer, only *Engraulis japonicus* was found at each sampling site (Table 2).

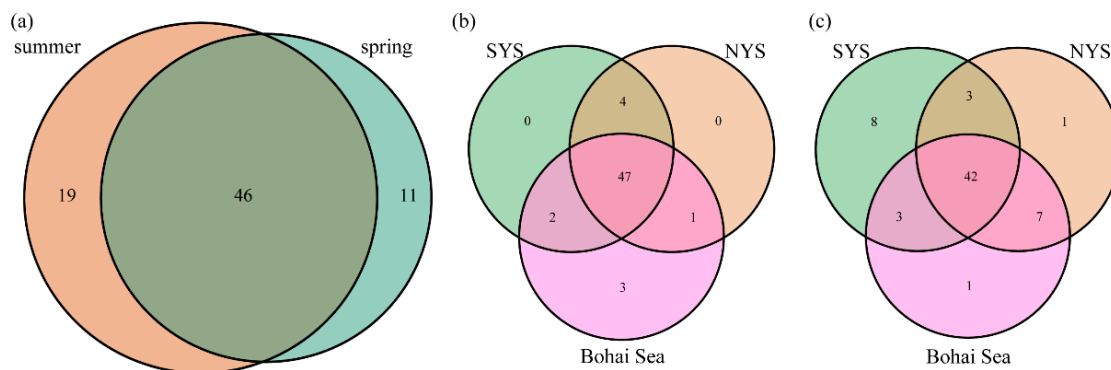


Figure 2. Venn plot showing the number of fish species detected through eDNA metabarcoding in the Yellow and Bohai Seas. (a) Fish species detected in both spring and summer. (b) Fish species detected in different seas in spring. (c) Fish species were detected in different seas in summer.

Table 1. Degree of dominance of dominant species and frequency of occurrence in spring.

Dominant Species	Order	Frequency of Occurrence (%)	Y
<i>Engraulis japonicus</i>	Clupeiformes	100	0.372
<i>Ammodytes personatus</i>	Perciformes	100	0.200
<i>Pholis fangi</i>	Perciformes	100	0.078
<i>Setipinna tenuifilis</i>	Clupeiformes	100	0.066
<i>Amblychaeturicht hexanema</i>	Perciformes	100	0.030
<i>Scophthalmus maximus</i>	Pleuronectiformes	95	0.029
<i>Johnius grypotus</i>	Perciformes	100	0.027
<i>Liparis tanakae</i>	Scorpaeniformes	100	0.025
<i>Konosirus punctatus</i>	Clupeiformes	90	0.021

Table 2. Degree of dominance of dominant species and frequency of occurrence in summer.

Dominant Species	Order	Frequency of Occurrence (%)	Y
<i>Engraulis japonicus</i>	Clupeiformes	100	0.382
<i>Hexagrammos</i>	Scorpaeniformes	67.9	0.116
<i>Benthoosema pterotum</i>	Myctophiformes	89.3	0.030
<i>Eupleurogrammus muticus</i>	Perciformes	71.4	0.026
<i>Liparis tanakae</i>	Scorpaeniformes	64.3	0.024
<i>Konosirus punctatus</i>	Clupeiformes	67.9	0.021
<i>Thryssa kammalensis</i>	Clupeiformes	71.4	0.020

3.2. Alpha and Beta Diversity of Fish Communities from Various Perspectives

Based on eDNA survey data, we calculated the alpha diversity of fish communities in the Yellow and Bohai Seas. Overall, the Chao index, Shannon's entropy index, Simpson's diversity index, and Pielou's evenness index in spring were significantly higher than that in summer (Figure 3). Based on spatial position, we compared the alpha diversity indices of the South Yellow Sea, North Yellow Sea, and Bohai Sea during spring and summer. The results revealed that the Chao index in spring ranged from 24 to 44, while the Shannon's entropy index ranged from 2.990 to 3.721. The Simpson's diversity index varied from 0.943 to 0.972, and the Pielou's evenness index ranged from 0.650 to 0.680. Furthermore, the Kruskal–Wallis test indicated no significant differences in alpha diversity among these regions. The alpha diversity indices observed in summer were similar to those observed in spring, with the Chao index ranging from 11 to 41, the Shannon's entropy index ranging from 2.320 to 3.674, the Simpson's diversity index varying between 0.903 and 0.972, and the Pielou's evenness index ranging from 0.651 to 0.690. There were no

significant differences among the three regions. The diversity index values of each site are provided in Supplementary Material Tables S3 and S4.

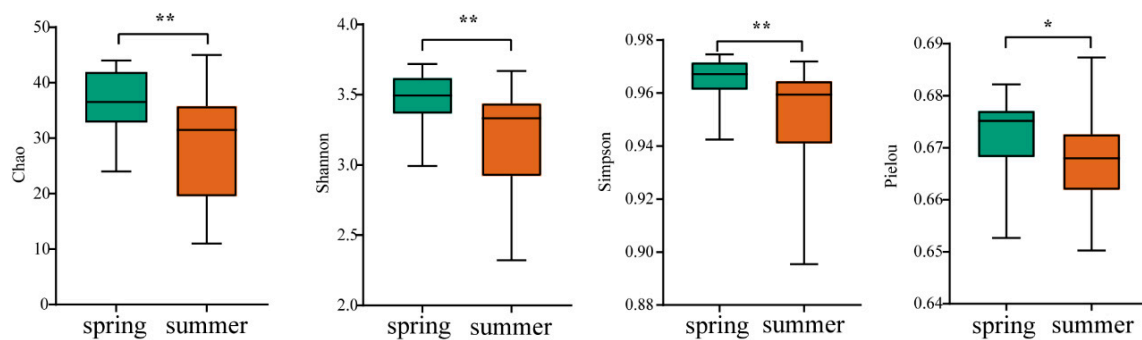


Figure 3. Seasonal variation in fish alpha diversity in the Yellow and Bohai Seas. “*” represents $p \leq 0.05$; “**” represents $p \leq 0.01$. The p -value was obtained through a t -test.

As illustrated in Figure 4a, the structure of fish species communities exhibited significant seasonal changes ($p = 0.001$), with a closer relationship observed among the fish community structures at different sites during spring. A permutational multivariate analysis of variance (PERMANOVA) in spring revealed that significant differences in fish community structures were observed only between the South Yellow Sea and the Bohai Sea ($p < 0.05$). In summer, significant differences in fish community structures were observed between the South Yellow Sea and the Bohai Sea ($p < 0.01$) as well as the North Yellow Sea ($p < 0.05$). Detailed information regarding the permutational multivariate analysis of variance can be found in Supplementary Material Tables S5–S7.

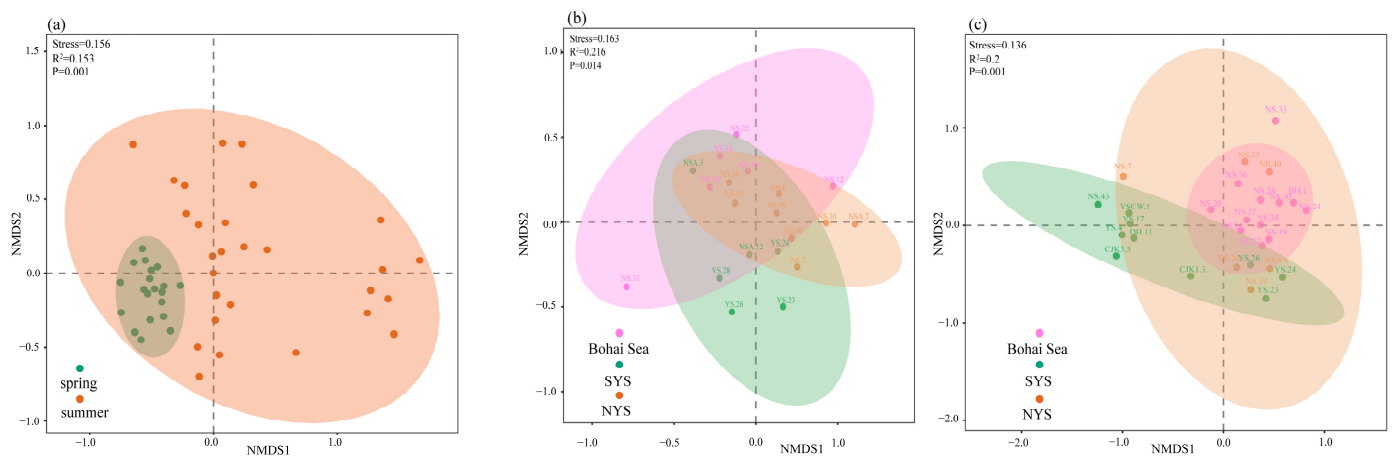


Figure 4. NMDS analysis of fish species in the Yellow and Bohai Seas in spring and summer. Ellipses represent a 95% confidence interval. (a) A comparison of fish community structures between spring and summer. (b) A comparison of fish community structures among different seas in spring. (c) A comparison of fish community structures among different seas in summer. SYS indicates the South Yellow Sea. NYS indicates the North Yellow Sea.

3.3. Functional and Phylogenetic Diversity of Fish Communities from Various Perspectives

Using the ecological and biological traits of the detected fish, we calculated the indices of functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv). Figure 5 illustrates the seasonal differences in functional diversity indices among marine areas. FRic ranged from 0.04 to 0.24 in spring and from 0.0003 to 0.39 in summer, indicating no significant differences in the functional space occupied by fish communities between seasons ($p > 0.05$). FEve refers to the distribution uniformity and relative abundance of a

particular group within a functional space. The FEve ranged from 0.85 to 0.89 in spring and from 0.84 to 0.94 in summer. Although the mean FEve in spring was higher, the seasonal differences in FEve were not significant. FDiv represents the distribution pattern of species abundance within functional space. FDiv exhibited significant differences between seasons, with higher values observed in summer. Additionally, we compared functional diversity across various marine regions (Supplementary Material Figures S1 and S2). In spring, there were no significant differences in functional diversity among marine regions (Figure S1). The differences in the FRic among these regions were minimal. The trends of FEve and FDiv in the South Yellow Sea, North Yellow Sea, and Bohai Sea showed a pattern of initially decreasing followed by increasing. In summer, a significant difference in FDiv was observed only between the South Yellow Sea and the Bohai Sea, exhibiting a continuously increasing trend from the South Yellow Sea northward to the Bohai Sea (Figure S2).

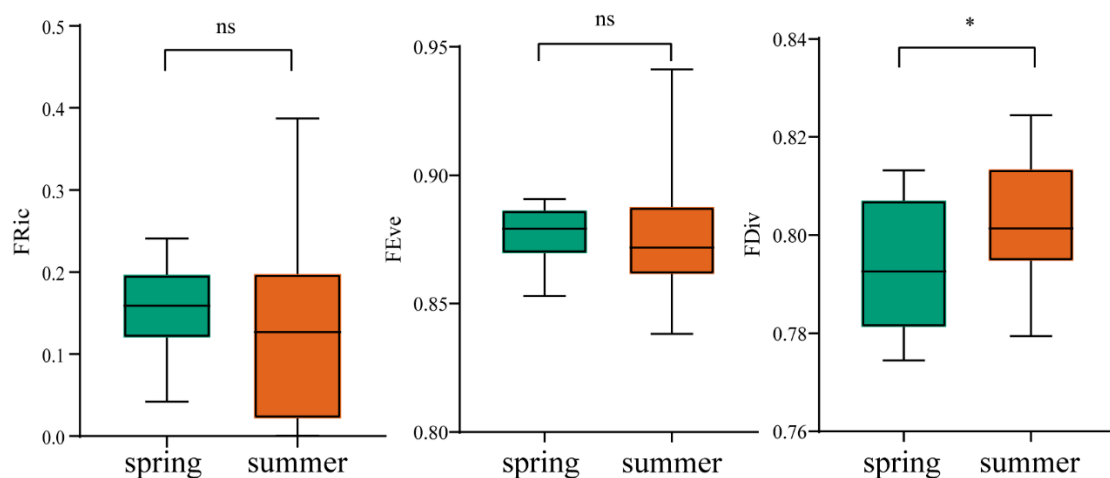


Figure 5. Seasonal variation in fish functional diversity in the Yellow and Bohai Seas. “*” represents $p \leq 0.05$; “ns” represents $p > 0.05$.

The results of phylogenetic diversity differed between seasonal and spatial levels (Figure 6). The phylogenetic diversity index of fish species in spring ranged from 484.96 to 758.50, significantly higher than that of summer (287.87–714.69). However, at the spatial level, the South Yellow Sea, North Yellow Sea, and Bohai Sea exhibited similar phylogenetic diversity in both spring and summer.

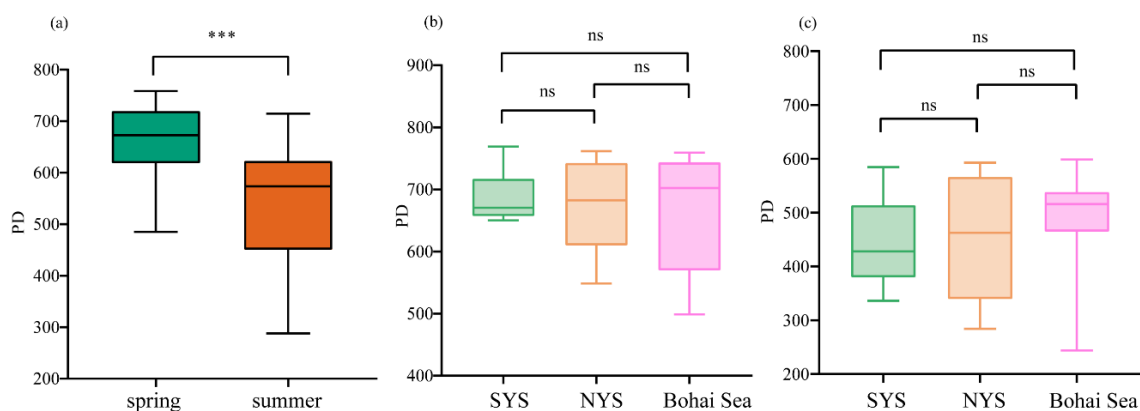


Figure 6. Phylogenetic diversity of fish species in the Yellow and Bohai Seas. (a) Seasonal variations in fish phylogenetic diversity. (b) Spatial variations in fish phylogenetic diversity during the spring. (c) Spatial variations in fish phylogenetic diversity during the summer. SYS indicates the South Yellow Sea. NYS indicates the North Yellow Sea. “****” represents $p \leq 0.001$; “ns” represents $p > 0.05$.

3.4. Relationship between Biodiversity and Environmental Factors

Biodiversity is inextricably linked to the environment. As shown in Figure 7a, the Chao index in spring was mainly influenced by NH_4^+ ($p = 0.05$), NO_3^- ($p = 0.027$), T ($p = 0.04$), and S ($p = 0.034$). The Shannon–Wiener index showed a significant positive correlation with NH_4^+ ($p = 0.046$), NO_3^- ($p = 0.021$), and S ($p = 0.032$). The Simpson’s diversity index was primarily influenced by NH_4^+ ($p = 0.049$), NO_3^- ($p = 0.022$), T ($p = 0.039$), and S ($p = 0.028$). Additionally, the Pielou evenness index was mainly influenced by NO_3^- ($p = 0.012$) and S ($p = 0.02$). However, the influence of environmental factors on diversity in summer was relatively homogeneous. The Shannon–Wiener index, Simpson’s diversity index, and Chao index were influenced by NO_3^- ($p < 0.05$), while the Pielou evenness index was primarily influenced by S ($p = 0.018$). The Mantel test revealed the relationship between functional diversity and environmental factors (Figure 8). In spring, FRic showed a significant positive correlation with NO_3^- ($p = 0.029$), T ($p = 0.022$), and S ($p = 0.012$). In summer, FDiv showed a significant correlation with water depth ($p = 0.04$), while FRic and FEve exhibited no significant correlations with environmental factors.

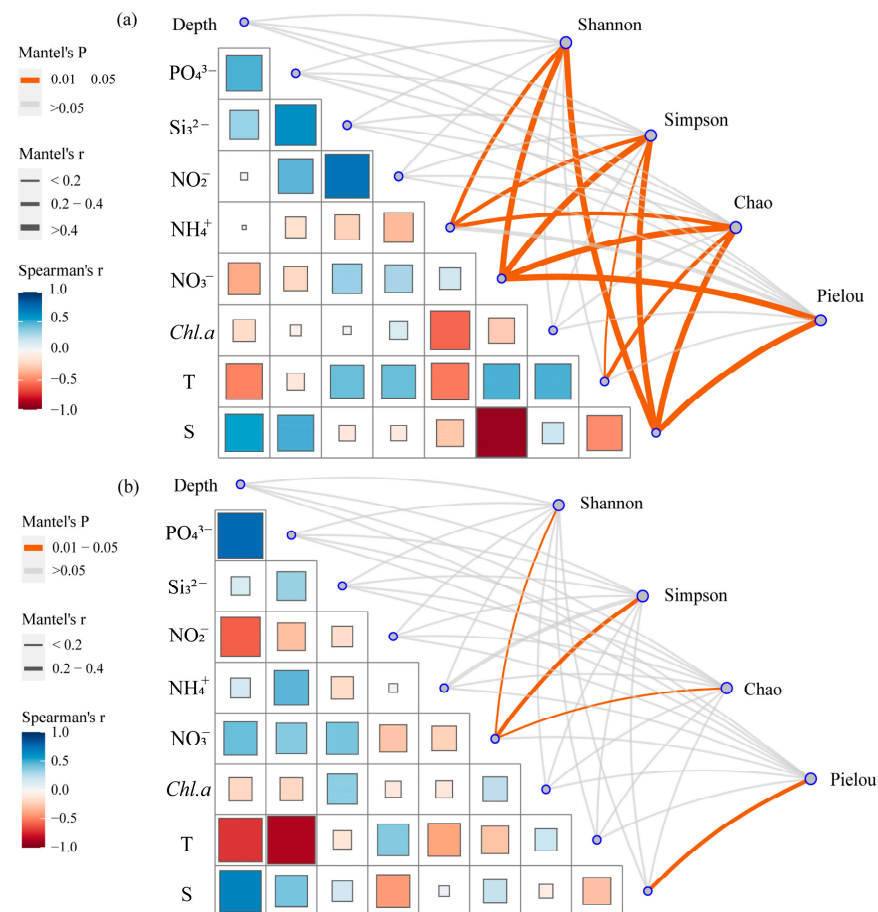


Figure 7. Mantel test of alpha diversity index and environmental factors. (a) Correlation between alpha diversity and environmental factors in the Yellow and Bohai Seas in spring. (b) Correlation between alpha diversity and environmental factors in the Yellow and Bohai Seas in summer.

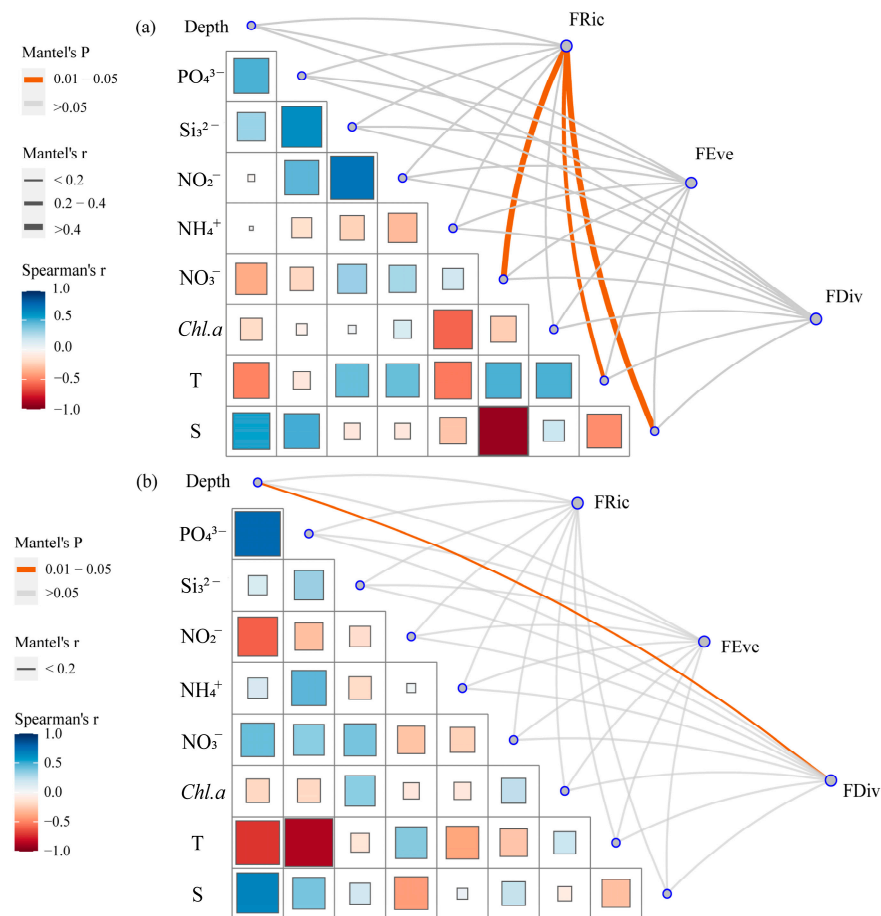


Figure 8. Mantel test of functional diversity index and environmental factors. (a) Mantel test of environmental factors and functional diversity in the Yellow and Bohai Seas in spring. (b) Mantel test of environmental factors and functional diversity in the Yellow and Bohai Seas in summer.

To understand the reasons for the differences in fish community diversity among various marine regions, we conducted an RDA analysis (Figure 9). The Envif tests are provided in Supplementary Material Tables S8 and S9. The RDA ranking model was significant ($p < 0.05$), effectively illustrating the relationship between species and environmental factors (Figure 9a). The first axis was strongly correlated with T ($p < 0.001$) and Chl-a ($p \leq 0.01$), which were considered key environmental factors influencing fish communities. The second axis exhibited a strong correlation with S and SiO_3^{2-} . Furthermore, T and Chl-a were positively correlated with the distribution of fish communities in the South Yellow Sea. Water depth, S, and NH_4^+ exhibited positive correlations with the distribution of fish communities in the North Yellow Sea and the Bohai Sea. As shown in Figure 9b, the RDA model reliably illustrated the relationship between dominant species and environmental factors ($p < 0.05$). NO_2^- and T exhibited a significant positive correlation with the distribution of *Eupleurogrammus muticus*, *Hexagrammos*, *Konosirus punctatus*, and *Thryssa kammalensis*. Water depth and S showed a significant positive correlation with the distribution of *Benthosema pterotum*, *Engraulis japonicus*, and *Liparis tanakae*.

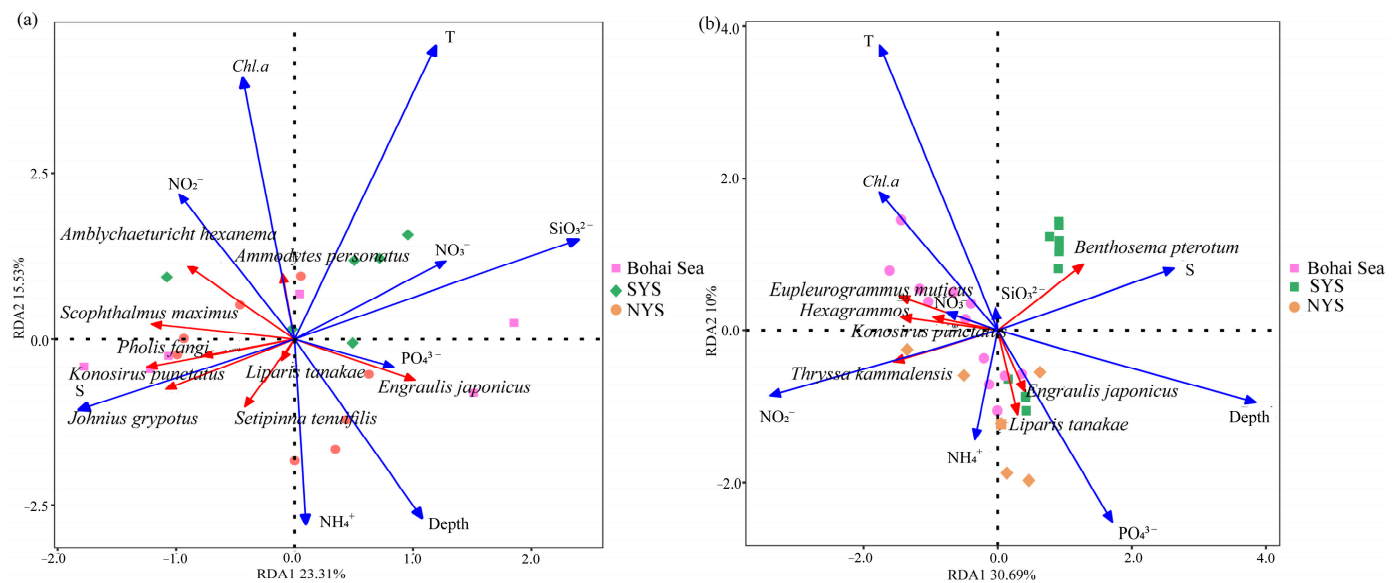


Figure 9. RDA analysis reveals the relationship between dominant species and environmental factors. (a) Relationships between 9 dominant species and environmental factors in spring. (b) Relationships between 7 dominant species and environmental factors in summer.

4. Discussion

Biodiversity conservation is essential for the sustainable development of human societies and is closely intertwined with human progress [25]. However, human production activities and environmental pollution have resulted in the degradation of marine ecosystem functions and a significant reduction in biodiversity [26,27]. Therefore, it is essential to investigate fishery resources in the Yellow and Bohai Seas and their response mechanisms to environmental factors.

4.1. Composition of Fish Communities

In this study, a total of 57 and 65 species were identified in spring and summer, respectively. A related study indicated that the diversity of fish species in the South Yellow Sea decreased from south to north [28]. The higher number of fish species identified in summer may be attributed to the greater number of sampling sites in the South Yellow Sea during this season. Shan et al. (2013) conducted a fishery resources survey in the South Yellow Sea through four trawlers and caught 109 fish species. Perciformes (45) was the most diverse, followed by the Scorpaeniformes and Clupeiformes. Their results indicated that, apart from *Larimichthys polyactis*, *Eupleurogrammus muticus*, and *Pampus argenteus*, smaller-sized pelagic and bottom-dwelling fish of lower economic value, such as *Engraulis japonicus*, *Setipinna tenuifilis*, *Lophius litulon*, and *Liparis tanakae*, were predominant [28]. This finding is consistent with our results. Dai et al. (2022) investigated the fish populations in Laizhou Bay during spring, summer, and autumn using eDNA, identifying a total of 47 fish species [29]. Wang et al. (2024) conducted a fisheries resource study in the Bohai Sea using eDNA and trawl surveys. The eDNA and trawl surveys identified 55 and 38 fish species, respectively, with the dominant species mainly consisting of smaller-sized pelagic fish species. And their results indicated that species such as *Engraulis japonicus*, *Setipinna tenuifilis*, *Hexagrammos otakii*, *Cynoglossus joyneri*, *Acanthogobius hasta*, *Cynoglossus semilaevis*, and *Liparis tanakae* were detected [30]. These fish species were observed in our surveys, indicating the reliability of the findings. A turnover of dominant species was observed compared to previous studies. A previous study indicated that the dominant species in 1995 were *Trichiurus lepturus*, *Larimichthys polyactis*, *Cynoglossus semilaevis*, and *Platycephalus indicus* [31]. However, in our study, the dominant species primarily consisted of pelagic fishes from the Perciformes, Clupeiformes, and Scorpaeniformes. This succession

may be related to years of overfishing and environmental degradation [32]. In general, smaller-sized, short-lived pelagic fish presently dominate the Yellow and Bohai Seas' fish communities [31]. In our study, several taxa were not identified at the species level in both spring and summer. This limitation may be attributed to the specificity of the primers used when eDNA is derived from closely related species [33].

4.2. Fish Diversity in the Yellow and Bohai Seas

Based on eDNA metabarcoding, we compared the diversity indices at various spatial and temporal levels in the Yellow and Bohai Seas. At the seasonal level, both alpha and beta diversity differed significantly between spring and summer. The differences in fish diversity indices among seasons may be associated with seasonal spawning, feeding behaviors, and overwinter migration [34]. In spring, as water temperatures rise, fish in the South Yellow Sea and North East China Sea migrate northward to spawn in the Yellow Sea and Bohai Sea [35]. Generally, fishes in the Yellow Sea reproduce between May and August [36]. The significant release of eDNA during spring spawning may account for the higher alpha diversity index compared to that of summer. However, there were no significant regional differences in alpha diversity in the Yellow and Bohai Seas. The results indicated comparable community richness and evenness in the Yellow and Bohai Seas. Fishery resources in the Bohai Sea are primarily derived from the Yellow Sea, indicating a strong interdependence in the development of fishery resources between the two regions [37]. From a spatial perspective, we believe that compared to the South Yellow Sea, the North Yellow Sea was closer to the Bohai Sea, leading to a higher similarity in species between the North Yellow and the Bohai Seas. In a study of the Yellow Sea's central and southern sections, Shan et al. (2014) demonstrated that the diversity indices of fish communities among various sections showed minimal variation, thus corroborating our findings [38].

In our study, FRic and FEve exhibited no significant differences between spring and summer. These results indicated that the functional characteristics of spring and summer were comparable, with comparable species occupying similar functional niches and an even distribution of functions, reflecting analogous efficiency in resource use. Additionally, the presence of a large number of identical species (46) accounted for the lack of significant differences in FRic between seasons. The FDiv in summer was significantly higher than that in spring, indicating a stronger degree of niche complementarity and weaker competition in the Yellow and Bohai Seas during the summer [39]. The dominant species differed between spring and summer, occupying different niches in the ecosystem. This variation may be one of the factors contributing to differences in FDiv. The FRic in spring and summer showed no significant differences among the three marine areas, suggesting that the fish communities in these regions have comparable functional characteristics. A positive correlation is commonly observed between species richness and FRic, suggesting that functional richness tends to increase with an increase in species number [40]. However, the South Yellow Sea had the highest species richness in summer, yet its functional richness was the lowest. This can be explained by the phenomenon of mutual replacement among species with similar niches, which results in functional redundancy [41]. In a study of fish phylogenetic diversity in the Yellow and Bohai Seas, the phylogenetic diversity index was significantly higher in spring than in summer. This finding indicated that the evolutionary differences between species in spring were greater than those in summer. Phylogenetically related species occupy similar niches in the ecosystem, while more distantly related populations may exhibit greater evolution.

4.3. Relationships between Fish Communities and Environmental Factors

The development and growth of fish are generally influenced by environmental factors [42]. Hickox et al. (2000) used remotely sensed data to investigate climate and seasonal variations in the East China Sea, Yellow Sea, and Bohai Sea. Their results indicated that most fronts occurred during autumn and winter, disappeared in spring, and nearly disappeared in summer [43]. The seasonal variations of fronts influence sea surface temperatures, which

subsequently affect fish feeding and migration. Therefore, we speculated that the seasonal variation of fronts may contribute to the lack of a significant correlation between biodiversity and environmental factors in summer. Moreover, Jiang et al. (2024) explored the impact of fronts on fish community structure using eDNA. The results indicated that fronts separated water masses with different environmental characteristics, thus attracting fish to their most suitable habitats [44]. In a study of the diversity of demersal fish communities in the Yellow and East China Seas during winter, Cheng et al. (2000) found that the Chao index, Shannon–Wiener index, and Pielou evenness index of fish populations exhibited significant positive correlations with water depth, water temperature, and salinity at the bottom [45]. However, in a winter survey of demersal fish in the Yellow and East China Seas, Liu et al. (2000) found that among the diversity indices, only the Chao index showed significant positive correlations with depth, surface water temperature, and surface salinity [46]. Moreover, in temperate regions with pronounced seasonal changes, salinity often serves as a main environmental factor influencing fish community structure and diversity [47]. Functional diversity plays a crucial role in maintaining ecosystem functioning [48]. Sagouis et al. (2017) investigated the functional diversity of 112 reservoirs in France and found that temperature and salinity were key environmental factors influencing this diversity [49]. In addition, Yan investigated the functional diversity of fish communities in Leizhou Bay, demonstrating that temperature affected functional richness, water depth influenced functional evenness, and both water depth and pH impacted functional divergence [50].

Variation in the spatial and temporal distribution of fish populations typically correlates with changes in dominant species [51]. The differences among dominant species were associated with seasonal migrations, including wintering and spawning [52]. Zhang et al. (2022) conducted a study in the Bohai Sea to evaluate fishery resources and the suitability of spawning habitats. The research indicated that water temperature, salinity, and water depth were significant environmental factors affecting fish spawning from April to September [53]. In our study, 73% of the dominant species were consistent with their findings, indicating that temperature was a key environmental factor influencing fish spawning. Generally, Chlorophyll-a is regarded as a proxy for food availability [54]. In regions of high primary productivity, food is more available, ensuring adequate growth conditions for juvenile fish [55]. Furthermore, water depth, NO_2^- , water temperature, and salinity are important environmental factors that influence the distribution of dominant species during the summer. The reaction of fish to varying water depths may be influenced by associated environmental changes, as well as their morphological adaptations and behavioral patterns [56]. For example, *Engraulis japonicus* is generally found in the pelagic zone, while *Eupleurogrammus muticus* exhibits a benthopelagic preference [57,58]. The effects of water depth on fish may be limited by dissolved oxygen and water temperature. As ectothermic animals, fish body temperature and gonad development are directly influenced by water temperature [59,60]. Salinity is considered an important factor influencing the spatial and temporal distribution of fish [61]. The incubation of eggs, yolk sac absorption, swimbladder expansion, larval growth, and osmoregulation in most species are dependent on salinity [42].

5. Conclusions

We explored the seasonal and spatial variations in fish diversity and their relationship to environmental variables in the Yellow and Bohai Seas by using eDNA metabarcoding technology. Our study identified a total of 13 orders and 76 fish species, with Perciformes being the dominant taxa. There were significant differences in the composition and structure of fish communities between seasons. Spatially, structural differences in fish communities were significant in all groups except for those between the North Yellow Sea and the Bohai Sea. Between seasons, only FDiv exhibited significant differences in spring and summer. Additionally, at the spatial level, there were no significant differences in functional diversity during spring. However, significant differences in FDiv were observed between the South Yellow Sea and the Bohai Sea in summer. Phylogenetic diversity analysis indicated that

phylogenetic diversity was higher in spring than in summer. By exploring the relationship between environmental factors and diversity indices, NH_4^+ , NO_3^- , T, and S were identified as key factors influencing the diversity indices in both seasons. Our findings provided important data for a deeper understanding of the ecological characteristics of the Yellow and Bohai Seas, as well as for biodiversity conservation efforts.

In our future research, eDNA will be used to monitor endangered and invasive species in the Yellow Bohai Sea. Traditional methods are often unreliable for monitoring endangered species and invasive species. It is important to investigate the distribution and abundance of these species through long-term monitoring and implement timely measures to protect ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes9110435/s1>, Figure S1: Spatial change in fish functional diversity index in the Yellow Sea and Bohai Sea in spring; Figure S2: Spatial change in fish functional diversity index in the Yellow Sea and Bohai Sea in summer; Table S1: Selection of functional traits; Table S2: Detection results of the eDNA and trawling surveys; Tables S3–S5: Replacement Multiple Variance Analysis between groups in the Yellow Sea and Bohai Sea in spring; Table S6: Replacement Multiple Variance Analysis between groups in the Yellow Sea and Bohai Sea in summer; Table S7: Replacement Multiple Variance Analysis between groups in the Yellow Sea and Bohai Sea in spring and summer; Table S8: Envif test of environmental factors and species in the Yellow Sea and Bohai Sea in spring; Table S9: Envif test of environmental factors and species in the Yellow Sea and Bohai Sea in summer.

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