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Research Paper

Environmental DNA metabarcoding decodes biodiversity restoration via artificial reefs: a case study of Xiaoheishan Island in Bohai Sea, China*

Zhipeng ZHANG[1], Jingjing SONG[2], Feifei QIN[1], Kaikai LIU[2], Yanjie WEI[1], Qirang SHI[1], Mingqi WANG[1, **], Yongshun JIANG[3, **]

[1] *Key Laboratory of Environmental Protection Technology on Water Transport, Tianjin Research Institute for Water Transport Engineering, Ministry of Transport, Tianjin 300456, China*

[2] *Shandong Provincial Key Laboratory for Intelligent Marine Ranching, Marine Science Research Institute of Shandong Province, Qingdao 266104, China*

[3] *Marine Science and Technology College, Qingdao Agricultural University, Qingdao 266000, China*

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Abstract This study surveyed the Xiaoheishan Island artificial reef area in China's Bohai Sea (deployed in 2014) through year-round ecological surveys conducted in 2023. Using environmental DNA metabarcoding technology, we systematically studied the ecological restoration effects and underlying mechanisms of the artificial reef area. The results revealed 91 genetically identified fish species in the artificial reef area, compared to 87 species in the control area, indicating that artificial reefs play a positive role in restoring biodiversity. Principal Component Analysis demonstrated significant seasonal and spatial differences in biological distribution between the artificial reef and control areas (P<0.05). Specifically, the artificial reef area is dominated by small demersal rock-associated fishes such as Gobiidae, Scorpaeniformes, *Lateolabrax japonicus*, and *Hexagrammos otakii*; in contrast, the control area features predominantly pelagic and soft-bottom species including *Liza haematocheila*, *Engraulis japonicus*, *Mugil cephalus*, *Paralichthys olivaceus*, and *Scomberomorus niphonius*. By Canonical Correspondence Analysis, we propose that seasonal migration driven by water temperature and species' life-history strategies promote habitat partitioning between the artificial reef and control areas, which in turn influences the distribution of dissolved oxygen and NH_4^+ in the water. Through the correlation analyses, it is found that although chlorophyll levels were higher in control area, the category and diversity indices of plankton showed marked declines compared to the artificial reef area. We believe that, compared to control areas, artificial reefs can increase the biodiversity of smaller organisms but cannot support large predators and higher biomass.

Keyword: artificial reef; environmental DNA metabarcoding; life-history strategy; water quality survey; Xiaoheishan Island

1 INTRODUCTION

Along with the ongoing increase of population, the supply-demand contradiction on resources including food, energy, and space has become prominent; climate change and pollution also became an issue that challenged the world. The global ecosystem is currently facing unprecedented change and coastal marine habitats are being lost at alarming rates (Hoekstra et al., 2005; Banks-Leite et al., 2020). Particularly, in some Chinese coastal waters, the deteriorating water quality, declining distribution area of typical habitats of seagrass beds and coral reefs, and reduced biodiversity are all

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** Corresponding authors: wangmingqi1991@163.com; jys@qau.edu.cn

threatening the service functions of the ecosystem (Lin et al., 2021). Such degradation trends persist globally despite conservation efforts (Duke et al., 2007; Sala et al., 2012), and the restoration of typical habitats and biological resources has emerged as a critical approach to ensuring marine health and sustainable coastal development (Wu et al., 2022). Therefore, the proposal for marine ranch construction provides a sound research direction for the restoration of typical coastal habitats and biological resources (Yang et al., 2024).

Currently, a primary method of implementing marine ranching is through the creation of artificial habitats (Fowler et al., 2014, 2015). The deployment of artificial reefs has been widely recognized as an effective approach to improving regional marine habitats. Theoretical research on artificial reef construction mainly focuses on the restructuring of community assemblages and the reconstruction of food webs (Zhang et al., 2019; Yu et al., 2020), as well as the evaluation of ecosystem structure, function, and service value (Claisse et al., 2014), collectively enriching the theoretical foundation of artificial reef development. However, the transformation of artificial habitats is a long-term process, and the aforementioned theoretical basis requires validation through long-term investigation. Notably, fish are a key component of habitats (Loreau et al., 2001), yet the mechanisms underlying the adjustment of their community structure remain insufficiently investigated, which partially hinders progress in habitat restoration through effective artificial reef creation and population enhancement.

By implementing large-scale, long-term ecological surveys in artificial reef area to analyze niche characteristics of keystone species and their responses to habitat parameters, as well as integrating the multi-source data on fish life-history strategies, environmental gradients, and community dynamics to carry out comprehensive analysis of interspecific relationship and environment effects (Weiher and Keddy, 1995; Nathan et al., 2000), it will enable quantitative analysis of the restructuring effects of the restoration work on fish communities. This approach provides empirical evidence for scientifically evaluating the influence of habitat restoration on marine ecosystems, thereby increasing scientific evidences for marine ecological restoration practices. Traditional survey methods, including sonar technology, trawl sampling, and microscopic identification (Heino et al., 2011; Fraija-Fernández et al., 2020), require extensive offshore expeditions to obtain ecosystem data from the surveyed areas. However, in practical research, constraints such as economic costs and weather conditions make it difficult for these conventional surveys to comprehensively reflect temporal changes in regional marine biodiversity and abundance (Zhang et al., 2020a). Comparatively, environmental DNA metabarcoding (eDNA), as an emerging marine environmental survey technology, despite the drawbacks of inaccurate quantification and false positives, offers cost-effective, eco-friendly, and time-efficient advantages in reflecting temporal biodiversity distribution characteristics, and has been widely applied in environmental surveys (Taberlet et al., 2012; Thomsen and Willerslev, 2015; Sun et al., 2025).

Xiaoheishan Island is located above the boundary between the Yellow Sea and the Bohai Sea, serving as a crucial maritime region for establishing an ecological security barrier system spanning the Bohai Bay. It belongs to a typical island ecosystem, characterized by simple structure and systemic integrity. Before 2014, pollution in the Bohai Sea was severe (Zhang et al., 2012), and combined with overfishing, the biological resources in the waters around Xiaoheishan Island had declined (Gao et al., 2014). Around 2010, China initiated a series of engineering projects in an attempt to restore coastal resources (Bi et al., 2012). In this context, we established a marine ranching restoration demonstration zone in the coastal areas of Shandong using artificial reefs, hoping to serve as a long-term research and observation sample. The artificial reefs on Xiaoheishan Island are made of reinforced concrete, with external dimensions of 1.4 m×1.4 m×1.2 m. A total of 162 reefs, occupying 220 m^2, were deployed in June 2014. Before and after the deployment, there was no species introduced. In 2023, nearly ten years after the implementation of this project, we conducted an ecological survey of the area and applied eDNA technology during the investigation. We aimed to evaluate the recovery effects on fish diversity, and by studying environmental factors and plankton dynamics, we further investigated the driving mechanisms behind resource restoration. Clarifying the coupling mechanisms between facilities, organisms, and the environment is of great significance for the establishment of marine ranching.

2 MATERIAL AND METHOD

2.1 Survey timing, station setup, and sample collection

In March, June, August, and November 2023, four cruises were conducted to the artificial reefs deployed in June 2014 to survey environmental factors of sea water as well as the plankton in Xiaoheishan Island waters. The locations of survey stations are as shown in Fig.1. The seawater sampling method followed the standard GB17378.4-2007, the plankton collection method was in accordance with GB17378.7-2007, and the identification and enumeration were performed using an Olympus CX31 microscope. The sampling depth for the water column was 5 m. The plankton nets had mesh sizes of 64, 77, and 112 μm, respectively. A detailed description of the methodology is provided in Supplementary Material 1. The minimum straight-line distance between the control area and the artificial reef area is 2 km. Since no submarine trenches are present around the island, the differences in water depth and current direction between the control area and the artificial reef area are not significant. Three sampling stations were established in both the artificial reef area and the control area, as shown in Fig.1.

2.2 Biodiversity calculation

Biodiversity was assessed using the Berger-Parker index (d) (Berger and Parker, 1970), Margalef index (d_{Ma}) (Margalef, 1958), Simpson index (λ) (Simpson, 1949), Shannon diversity index (H') (Shannon, 1948), and Pielou evenness index (J') (Pielou, 1966). For the calculation of all indices, we did not use raw read counts (read abundance). Instead, we converted the filtered amplicon sequence variants (ASVs) table into relative abundance data per sample prior to analysis. Specifically, each ASV read count in a sample was divided by the total number of quality-filtered reads for that sample, resulting in a proportional community profile where the sum for each sample equals.

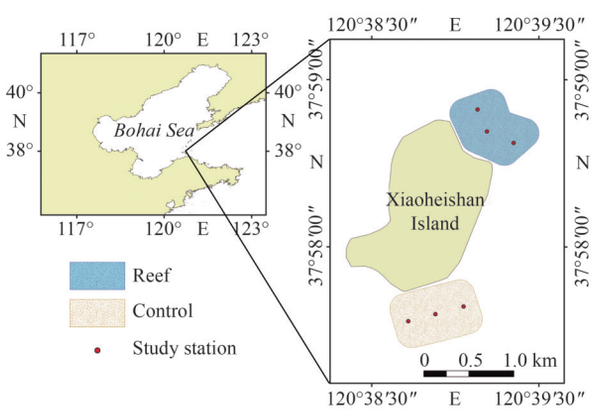

Fig.1 Sampling location map

2.3 eDNA detection method

The eDNA detection and analytical methods followed those described by Stauffer et al. (2021). Two liters of water were collected at each sampling station and transported to the laboratory on ice within 4 h. From both the control and reef areas, six samples were taken at a depth of 5 m. After thorough mixing, each sample was vacuum-filtered through a 0.45-μm mixed cellulose ester membrane. To prevent contamination, the laboratory was sterilized by ultraviolet irradiation, and all filtration equipment was autoclaved prior to use. Following filtration, the membranes were folded with forceps, wrapped in aluminum foil, placed in centrifuge tubes, and stored in liquid nitrogen until eDNA extraction.

Genomic DNA was extracted using a QIAGEN DNA extraction kit, and its concentration and quality were assessed using a Qubit fluorometer. The quantified DNA was then amplified with specific primers, and the amplification products were checked for quality. A two-step PCR approach was employed to construct libraries for high-throughput sequencing. The first PCR was carried out using the MiFish-U primer set (MiFish-U/E-F: 5′-GTYGGTAAAWCTCGTGCCAGC-3′ and MiFish-U/E-R: 5′-CATAGTGGGGTATCTAATCCYAGTTTG-3′) (Miya et al., 2015), which included Illumina adapter overhangs (Miya et al., 2015). Negative controls (no-template water) were included in each PCR run to monitor contamination. The thermal cycling conditions for the first PCR were as follows: initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 98 °C for 20 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; followed by a final extension at 72 °C for 5 min. All samples were amplified in triplicate to account for stochastic amplification bias. A species was only considered “not detected” if it failed to amplify in all replicate reactions, thereby reducing the risk of false negatives resulting from stochastic PCR fluctuations. All samples were amplified in triplicate to mitigate stochastic amplification bias, and the triplicate products were pooled afterward. A second, limited-cycle PCR was then conducted to attach dual-indexed Illumina sequencing adapters and sample-

specific barcodes. The resulting libraries were quantified, pooled in equimolar ratios, and sequenced on an Illumina MiSeq platform using paired-end chemistry. The thermal profile for the indexing PCR was: 95 °C for 3 min; 8 cycles of 98 °C for 20 s, 55 °C for 30 s, and 72 °C for 30 s; with a final extension at 72 °C for 5 min.

Fish gene sequences were taxonomically assigned by referencing the NCBI database. Library preparation from the quantified samples was performed following the standard protocol of the ALFA-SEQ Amplicon Library Prep Kit (FINDROP NDM001E). Library quality was evaluated using a Qubit fluorometer and the Oseq400 High-Throughput Nucleic Acid and Protein Analysis System, and high-throughput sequencing was carried out on an Illumina NovaSeq 6000 platform.

Raw image data from sequencing were converted into raw reads via base calling. These reads were subsequently processed using Yijian Biotechnology's standardized bioinformatics pipeline for eDNA amplicon analysis. Quality control of the raw sequencing data was performed using FastQC. Primer sequences were then trimmed, and paired-end reads were merged with DADA2, which resolves exact ASVs and provides higher resolution than traditional OTU clustering by discriminating single-nucleotide differences. Taxonomic assignment of ASVs was conducted using the RDP classifier (implemented within the DADA2 pipeline) against a curated 12S rRNA reference database (specifically, the MiFish pipeline database), with a minimum bootstrap confidence threshold of 80%. Only assignments meeting or exceeding this confidence level were retained for subsequent ecological analyses. A stringent filtering pipeline was applied to minimize false positives: ASVs with an overall abundance below 0.001% of the total reads were discarded; any ASV detected in negative controls was removed from all samples; the remaining ASVs were compared against the NCBI database using BLAST, retaining only those with ≥97% sequence similarity and ≥99% query coverage to Actinopterygii sequences. The final curated ASV table was used for subsequent ecological and biodiversity analyses.

2.4 Canonical Correspondence Analysis (CCA)

CCA was employed to quantify the relationships between species composition and environmental variables. We calculated the dominance of fish species throughout the year using a formula and selected 20 fish species as dominant organisms for CCA. The formula for dominance refers to Sun (2001). The specific formula and results are provided in Supplementary Material 2. Prior to analysis, the species relative abundance data were lg(x+1)-transformed to reduce the influence of extreme values. To identify the subset of environmental variables that most significantly explained the variation in species distribution, a preliminary ordination was performed using a partial Monte Carlo permutation test with forward selection (Lepš and Šmilauer, 2003). Only variables with a significant contribution (P<0.05) were retained for the final CCA model. The significance of the overall model and of each canonical axis was tested using Monte Carlo permutations. The analysis was performed using the CANOCO.

2.5 Principal Component Analysis (PCA)

PCA was performed to reduce the dimensionality of the environmental dataset and to visualize the predominant patterns of variation among sampling sites. Prior to analysis, all environmental variables were standardized to eliminate the influence of differing measurement units. The analysis was conducted using a correlation matrix to ensure that all variables contributed equally to the construction of the principal components. Principal components with eigenvalues greater than 1 (Kaiser-Guttman criterion) were retained for interpretation. The relationships between the original variables and the principal axes were examined using the loading scores, which indicate the contribution of each variable to the component. The results are presented in a biplot, which simultaneously displays the distribution of sampling sites and the vectors of environmental variables in the reduced ordination space. Additionally, Analysis of Similarities (ANOSIM) was employed to examine the differences in species distribution between the artificial reef areas and the control areas. All computations and visualizations were carried out using the Omicstudio cloud platform.

2.6 Data analysis

Water quality data was presented as mean±SD and analyzed using SPSS 22.0. First, normality and homogeneity of variance were assessed via Levene's test. Significant differences between artificial reef and control aeras were assessed through one-way analysis of variance (ANOVA). Then, the correlation between biodiversity and water quality parameters was analyzed using Pearson correlation coefficient on the Omicstudio cloud

platform. Furthermore, all figures were generated on the Omicstudio cloud platform (https://www.omicstudio.cn/), in which the distribution heatmaps of fish species were created using standardized data of OTUs.

3 RESULT

3.1 Water quality survey result

The survey results of water quality are summarized in Table 1. It showed no significant difference observed in pH and salinity between the artificial reef and control area. However, other parameters including dissolved oxygen, chemical oxygen demand, phosphate, nitrite, nitrate, ammonia nitrogen, and suspended solids of the two areas showed significant spatial and seasonal variations. Specifically, except in March, dissolved oxygen levels in artificial reef area exceeded that of the control area, and it was significantly higher in August (P<0.05). Chemical oxygen demand showed higher values in control area during June and August but reversed in November and March, with significant seasonal differences in June and March (P<0.05). This trend paralleled those of nitrite, nitrate, and ammonia nitrogen variations. The phosphate concentration exhibited a distinct seasonal pattern: the concentration in the control area surpassed the artificial reef area during August and November, whereas it reversed during June and March. Notably, a statistically significant difference (P<0.05) was observed in June. Furthermore, the values of suspended solids in control area significantly exceeded those in artificial reef area cross all seasons, particularly in November (P< 0.01). Additionally, the level of chlorophyll *a* remained consistently higher in control area.

3.2 eDNA analytical result

The eDNA analysis revealed maximum fish diversity in August (70 species), followed by March (40 species) (Fig.2a). The number of OTUs detected by eDNA was highest in June, followed by August, and March, with the lowest in November (Fig.2b). Overall, *Sebastes schlegelii*, *Hexagrammos otakii*, and *Lycodes ygreknotatus* had the highest total number of OTUs (Fig.2c). The results also present that spatial differentiation emerged between artificial reef area and control area in biological distribution. Specifically, the general distributions of fishes in June showed similarity between the artificial reef area and the control area, with shared

Table 1 Water quality data in control area and artificial reef area of Xiaoheishan Island

Time	Station		pH	Salinity	DO (mg/L)	COD (mg/L)	Phosphate (mg/L)	Silicate (mg/L)	Nitrite-nitrogen (mg/L)	Nitrate-nitrogen (mg/L)	Ammonia-nitrogen (mg/L)	Suspended Solids (mg/L)	Chl *a* (μg/L)	Temperature (℃)
March	Control	Mean	8.21	32.11	9.83	1.42	0.001 8	0.082 3	0.002 6	0.039 3	0.006 6	9.65	0.86	7.7
		Sd	0.03	0.01	0.00	0.00	0.000 0	0.000 2	0.000 0	0.048 1	0.000 0	0.05	0.00	0.1
	Reef	Mean	8.21	32.06	10.00	1.06**	0.002 4**	0.032 2**	0.001 5	0.055 1	0.012 1	9.13	0.51	7.8
		Sd	0.03	0.01	0.00	0.00	0.000 0	0.001 4	0.000 2	0.187 2	0.000 2	0.19	0.00	0.2
June	Control	Mean	8.15	32.18	8.60	0.83	0.004 0	0.216 2	0.000 7	0.017 8	0.012 1	15.30	1.98	15.3
		Sd	0.07	0.00	0.00	0.01	0.000 1	0.000 5	0.002 2	0.000 5	0.002 2	0.70	0.00	0.2
	Reef	Mean	8.16	32.10	9.21*	0.59	0.002 4	0.193 9	0.000 4**	0.006 1	0.006 0	11.80	1.46	15.2
		Sd	0.02	0.02	0.00	0.00	0.000 0	0.000 2	0.000 1	0.364 9	0.000 1	0.36	0.00	0.1
August	Control	Mean	8.24	31.90	7.33	0.48	0.003 8	0.211 3	0.002 6	0.011 5	0.116 8	8.49	0.70	26.5
		Sd	0.03	0.01	0.00	0.00	0.000 2	0.000 2	0.003 2	0.100 2	0.003 2*	0.10	0.00	0.2
	Reef	Mean	8.22	32.05	7.70	0.65	0.002 4	0.197 0	0.005 2	0.014 6	0.131 1	1.33**	1.01	26.3
		Sd	0.01	0.01	0.00	0.00	0.000 1	0.000 3	0.001 5	0.026 0	0.001 5	0.03	0.00	0.1
November	Control	Mean	8.09	31.72	6.66	0.48	0.005 7	0.151 7	0.021 6	0.071 2	0.030 7	9.42	0.22	12.5
		Sd	0.02	0.00	0.00	0.00	0.000 2	0.000 8	0.000 6	0.090 7	0.000 6	0.09	0.00	0.3
	Reef	Mean	8.09	32.12	6.75	0.73*	0.008 0	0.172 7	0.004 0	0.053 4	0.027 9	3.75	0.16	12.8
		Sd	0.05	0.01	0.00	0.00	0.000 1	0.000 8	0.000 7	0.146 9	0.000 7	0.15	0.00	0.1

* means P<0.05; ** means P<0.01; Sd means standard deviation.

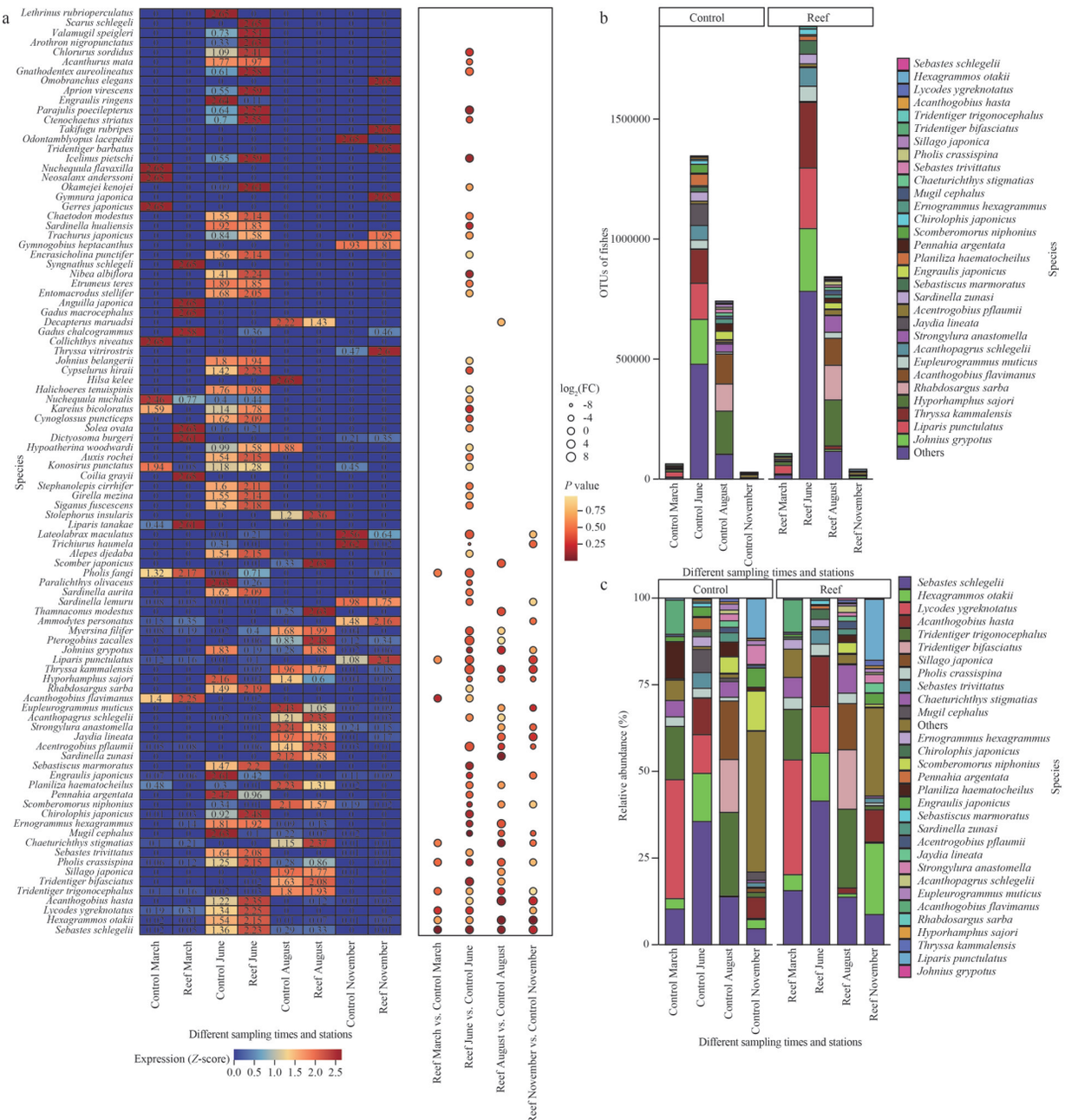

Fig.2 Distribution of relative abundance of fish

a. fish distribution heat map (the values in the heatmap represent the standardized values of OTUs; the bubble size represents the ratio of OTUs in the reef area to those in the control area, and the bubble color represents the statistical significance of the differences.); b. relative abundance percentages (*y*-axis represents the total number of OTUs from all samples within each region); c. relative abundance of fishes.

with high OTU values including *Lycodes albonotatus* and *S. schlegelii*. However, *Ernogrammus hexagrammus*, *Gadus macrocephalus*, *Theragra chalcogramma*, *Anguilla japonica*, and *Syngnathus schlegeli* appeared exclusively in the artificial reef area, while *Liza haematocheila* and *Kareius bicoloratus* were confined to the control area. August surveys showed significantly higher abundances of *Scomberomorus niphonius*, *Hyporhamphus sajori*, *Mugil cephalus*, *Engraulis japonicus*, *Johnius grypotus*, *Paralichthys olivaceus*, and *L. haematocheila* in the control area, whereas artificial reef area exhibited greater prevalence of *Acentrogobius pflaumii* and *Thryssa kammalensis*. *S. schlegelii*, *Acanthogobius hasta*, and *H. otakii* served as higher distribution in both areas, with *T. chalcogramma* unique to the artificial

reef area. The species distribution in November demonstrated general consistency between both areas, though *Hilsa kelee* and *Allanetta woodwardi* appeared exclusively in control area. In March, distinct fish distribution patterns were observed between the artificial reef area and the control area. The high OTUs species in the artificial reef area include *H. otakii*, *Liparis tanakae*, and *Ammodytes personatus*, while the control area was characterized by *S. niphonius*, *Lateolabrax japonicus*, *Trichiurus lepturus*, and *L. tanakae*. Notably, *Konosirus punctatus*, *Myersina filifer*, *L. haematocheila*, and *Sillago japonica* were exclusively found in the control area, whereas *Takifugu rubripes*, *Acanthogobius flavimanus*, *E. hexagrammus*, *Tridentiger barbatus*, and *T. chalcogramma* were unique to the artificial reef area.

3.3 PCA and CCA analysis of fish distribution on Xiaoheishan Island

The result of the PCA analysis depicting the fish distribution around Xiaoheishan Island are presented in Fig.3, which revealed that the first two principal components (PC1 and PC2) collectively explained 47.96% of the total variation in the dataset, accounting for 36.48% and 11.48%, respectively. ANOSIM analysis revealed a statistically significant difference in fish assemblages between the artificial reef and control area (Fig.3a). Furthermore, we found that the species composition was more similar in March and November, whereas it was distinctly different in June and August (Fig.3a). The triplot of the CCA is shown in the Fig.3b. The results indicate that the eigenvalues for SPE AX1 and SPE AX2 were 0.276 and 0.158, respectively, collectively accounting for 80.3% of the total variance.

The CCA results revealed that NH_4^+, water temperature, and DO were highly significantly correlated with eDNA-based species distribution ($P<$ 0.01; Monte Carlo permutation test). During the pre-screening process in the CANOCO software, NH_4^+, water temperature, and DO were selected due to their high explanatory power (Table 2), collectively explaining 91.9% (0.496/0.54) of the total explainable variance.

3.4 Diversity indices of phytoplankton, zooplankton, and fish

Biodiversity indices are detailed in Fig.4. The Berger-Parker index (d) inversely correlates with biodiversity, where higher values indicate lower diversity. The Shannon index (H_e') reflects species diversity, and higher values indicate higher

Table 2 Explanatory power and correlations of key environmental factors in the CCA

Environmental factor	Marginal effect	*P*-value	Conditional effect
NH_4^+	0.267	0.002	0.267
WT	0.266	0.004	0.156
DO	0.14	0.004	0.073

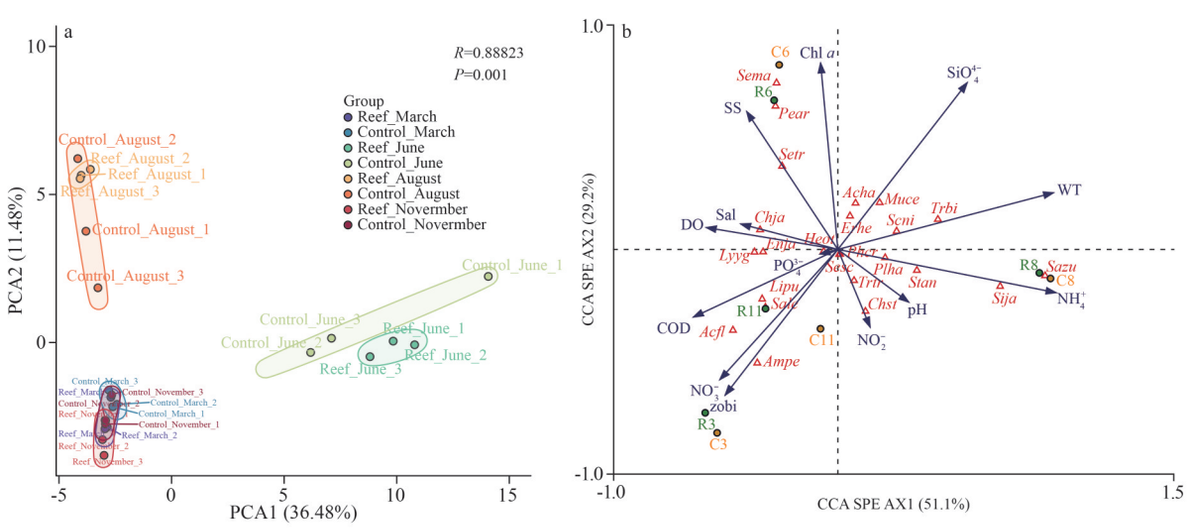

Fig.3 PCA and CCA analyses in Xiaoheishan Island

a. PCA performed on fish distribution across sample period; b. CCA analysis between environmental variables and fish populations and relative abundance. WT: water temperature; Zobi: zooplankton biomass; C3, 6, 8, 11: the relative abundance of fishes in the control area of Mar., Jun., Aug., Nov.; R3, 6, 8, 11: the relative abundance of fishes in the reef area of Mar., Jun., Aug., Nov. Abbreviations for fish species are provided in Supplementary Material 2.

diversity. The Simpson index (λ) measures the degree of dominance, where lower values suggest reduced dominance and higher diversity. The Pielou index (J_e) quantifies evenness, with values approaching 1 indicating more uniform species distribution. This study revealed that phytoplankton diversity peaked in November, while August exhibited greater dominance concentration, indicating that water temperature potentially influences algal distribution. The J_e index demonstrated more balanced distribution of phytoplankton resource in the artificial reef area in June. Contrasting with phytoplankton, the zooplankton in artificial reef area exhibited maximum diversity and evenness in August, while the diversity in March significantly trailed control area level. Furthermore, λ index analysis revealed reduced dominance concentration of fish communities due to the artificial reef. March observations showed synchronized declines in H_e' and J_e values within the artificial reef area, indicating that artificial reef enhanced the stability of fish communities (Magurran, 2021).

3.5 Correlation analysis between environmental factors and the diversity of phytoplankton, zooplankton, and fish

The correlation analyses between environmental factors and the diversity and evenness of phytoplankton, zooplankton, and fish are presented in Fig.5. It is found that, in the control area, the index d of phytoplankton showed direct positive correlations with salinity and nitrate, while other diversity indices

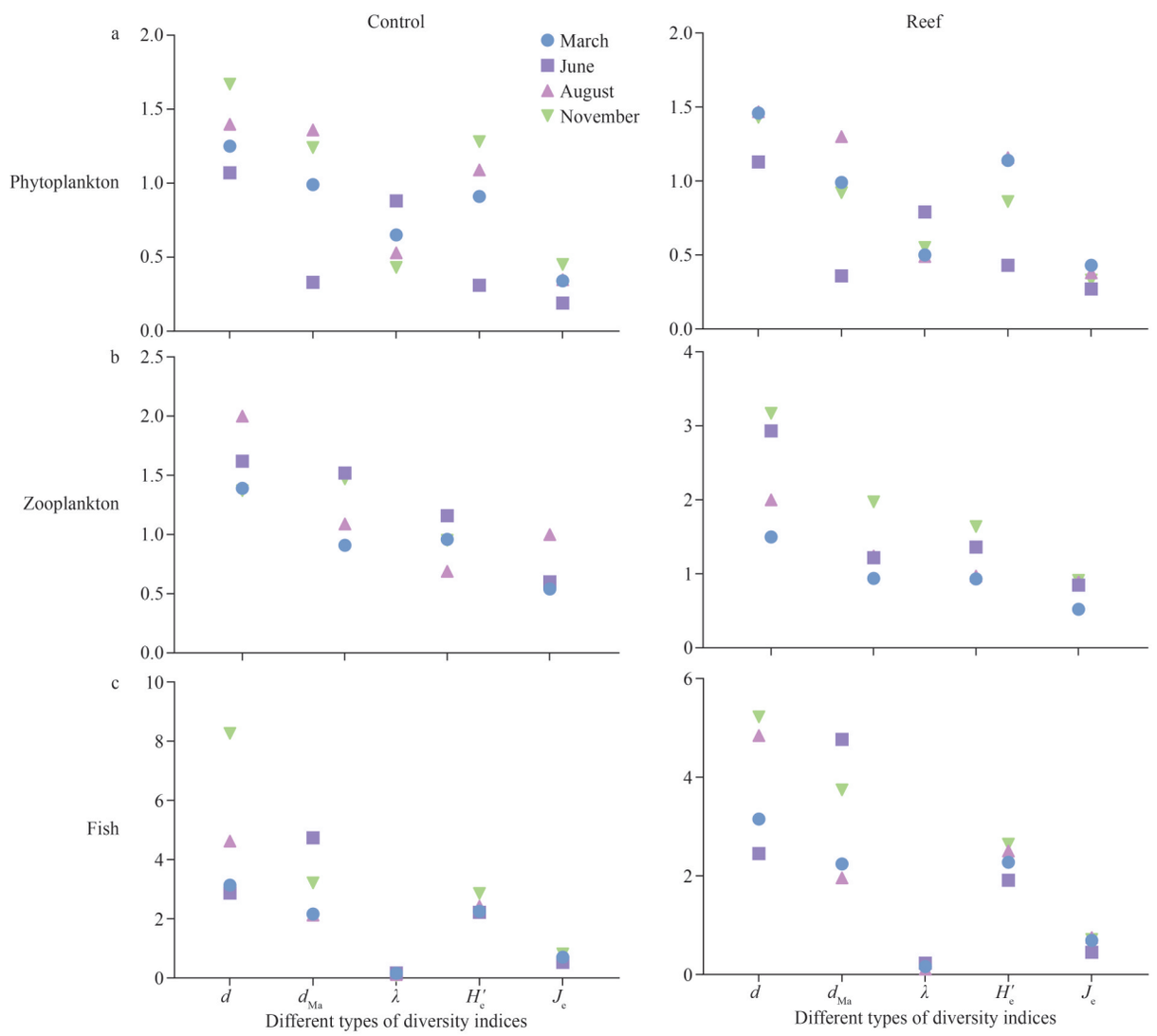

Fig.4 Seasonal diversity indices of phytoplankton, zooplankton, and fish in control area and artificial reef area of Xiaoheishan Island

exhibited no significant correlations with environmental factors, in which temperature and ammonia nitrogen displayed negative correlations with phytoplankton diversity, suggesting their potential role as limiting factors. Zooplankton communities demonstrated direct positive correlations only between ammonia nitrogen and H_e, and between temperature and J_e. Fish communities exhibited direct positive correlations between index d with salinity and nitrate, and between d_{MA} and suspended solids, while ammonia nitrogen, temperature, and silicate showed indirect negative correlations with fish diversity indices. In the artificial reef area, diversity indices of phytoplankton showed no direct correlations with environmental factors, contrasting with control area where pH, dissolved oxygen, phosphate, ammonia nitrogen, and temperature exerted negative influences on diversity indices of phytoplankton. For zooplankton, the d_{MA} index positively correlated with dissolved oxygen, while nitrate, nitrite, ammonia nitrogen, and temperature predominantly exhibited indirect negative correlations, and pH and salinity predominantly presented no significant correlations with diversity indices of zooplankton. The index J_e of fish in artificial reef area directly correlated with suspended particle density, which also showed indirect positive correlations with other indices. Most environmental factors including temperature, pH, COD, silicate, and phosphate demonstrated indirect negative correlations with diversity indices of fish.

4 DISCUSSION

Artificial reefs function as engineered artificial marine habitats that modify seabed substrates to provide spawning grounds, predator refuge, etc., for marine creatures, thereby enhancing the biodiversity and facilitating marine resource restoration (Baine, 2001). This study took the artificial reef area in Xiaoheishan Island (Bohai Sea, China) as the case. Using eDNA technology, we investigated the restoration effects of the artificial reef area. Based on results including water quality monitoring, we explained the restoration mechanisms of the artificial reef area from both biological and environmental perspectives.

4.1 Influence of artificial reef on fish distribution

In 2013, prior to the deployment of artificial reefs at Xiaoheishan, fishery surveys using A-frame trawls indicated that fish assemblages throughout the sea area were predominantly composed of 21 species collectively, including *K. punctatus*, *H.*

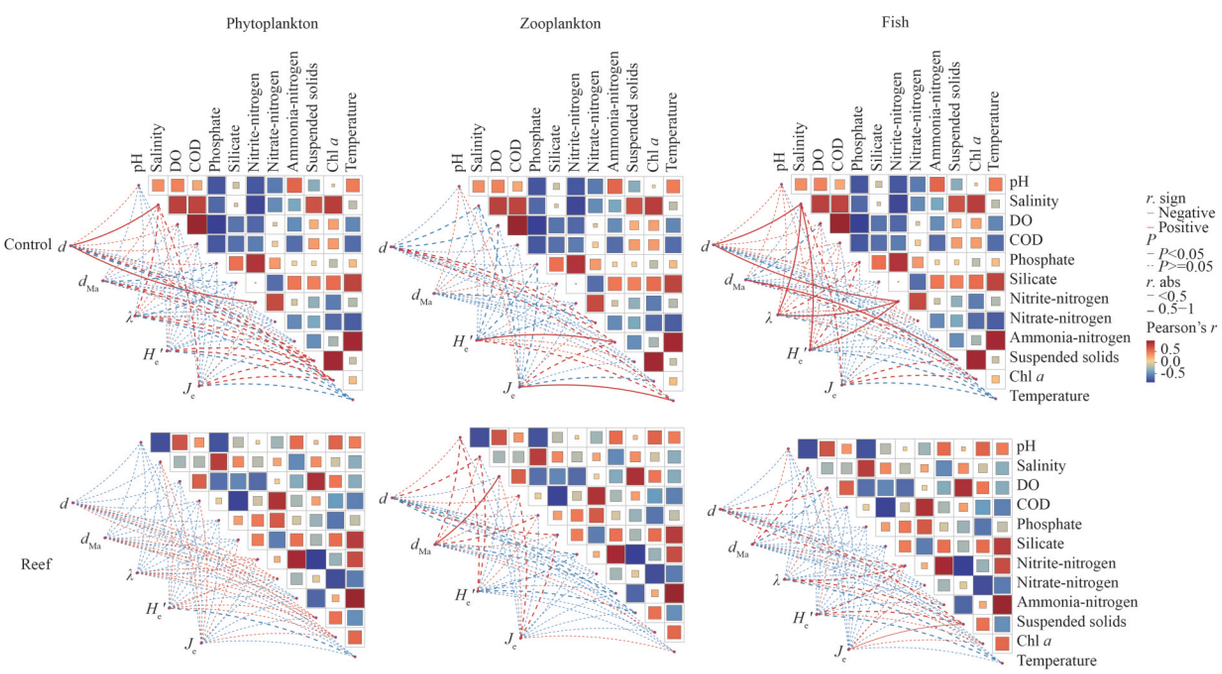

Fig.5 Correlation analysis between environmental factors and diversity of phytoplankton, zooplankton, and fish

The small squares represent the results of Pearson correlation analysis among water quality parameters, red indicate positive correlations, blue indicate negative correlations. The size of the colored area represents the strength of significance, larger area denotes stronger significance; the solid blue lines represent a significant negative correlation (P<0.05), the blue dotted lines represent a non-significant negative correlation (P>0.05), the solid red lines represent a significant positive correlation (P<0.05), and the red dotted lines represent a non-significant positive correlation (P>0.05).

otakii, *Pholis fangi*, *Zoarces elongatus*, *S. schlegelii*, and *M. filifer* (Zhang et al., 2017, 2020a). In 2014, a total of 220 artificial reef units were deployed at Xiaoheishan Island to reverse this desertification trend by introducing complex hard structures. Alter the physical substrate from a homogeneous, silt-covered bottom to a complex, three-dimensional habitat, providing shelter, spawning, and nursery grounds for fish and other organisms. The control sites in our study represent the current, degraded baseline condition of the area. After nearly a decade of seabed habitat modification, we observed that small reef-associated species including *S. schlegelii*, *H. otakii*, *L. pallidus*, and *Chaeturichthys stigmatias* exhibited significant prevalence, a finding consistent with our preliminary investigations (Zhang et al., 2017, 2021). The significantly higher species richness and abundance observed at the artificial reef sites demonstrate their effectiveness in catalyzing biodiversity recovery from this degraded state. This result was similar with Liu et al. (2015a), whose found that the deployment of artificial reefs positively influences the aggregation and growth of *S. schlegelii*. In contrast, the adjacent natural control area is predominantly characterized by nektonic fishes, sand-associated demersal species and small pelagic fish species such as *K. punctatus* are exclusively distributed in non-reef zones. This indicates a geospatial divergence in fish assemblages around Xiaoheishan Island, resulting from the deployment of artificial reefs. Using PCA, we found that the presence or absence of reefs has a significant effect on the distribution of fish populations (Supplementary Material 3). Jiang et al. (2016) similarly found that while artificial reef deployment enhanced local biodiversity, it also led to spatial differences in the distribution of swimming fish compared to control areas. This substrate-driven distribution theory aligns with findings from U. S. reef restoration projects, where the topographic modifications by different types of artificial reefs induced distinct biological distributions (Lemoine et al., 2019). The increase in fish species detected by eDNA at the artificial reef sites indicates that these structures promote biodiversity recovery, likely by providing new habitats, which was similar with Komyakova et al. (2019).

Beyond topographic factors, our eDNA dataset reflected patterns consistent with the known migratory behaviors of several fish species. For instance, *Planiliza hematocheilus*, a small estuarine species that migrates inshore in March and returns offshore after September (Liu et al., 2015a), was detected in the control area in early March but was largely absent in November. Similarly, higher read counts of *Pennahia argentata* were observed in the control area in June, coinciding with its reported spawning migration period toward sandy-mud substrates. The increased prevalence of the demersal fish *L. japonicus* in the control area during the same period may also correspond to its documented migratory characteristics (Kasai et al., 2018). These detection patterns align with established behavioral ecology, suggesting that migratory behavior likely influenced the temporal and spatial distribution of eDNA signals in our study. The CCA results strongly supported this inference, revealing a profound influence of water temperature on fish distribution (Fig.3b). This finding provides evidence for the impact of fish migration on spatial distribution. As a result, from June to August, the increase of water temperature and expanding of fish mobility range drove the detection of more eDNA from a wider variety of fish at Xiaoheishan Island. However, the number of fish species detected via eDNA declined in November and March. This result was consistent with previous research, which confirmed higher fish abundance in summer in Bohai Sea areas due to migratory activities (Shan et al., 2016). Furthermore, the surveys revealed amphipod larvae presence in the artificial reef area in August (Supplementary Material 4). This presents the influence of artificial reef on zooplankton diversity restoration, and we suggested artificial reefs interfering with the activity of water column may be the reason for the increase in richness species detected of planktonic animal (Supplementary Material 4). Similarly, Yang et al. (2019) demonstrated that artificial reefs can alter seafloor topography. Such topographic modifications attenuate wave energy, enhance the retention of organic matter within local water masses, and are considered one of the mechanisms through which artificial reefs promote regional biodiversity. From above, we consider that fish distribution patterns are primarily governed by species-specific living habits, and the artificial reefs induced disparities of fish distribution in different areas by changing the substrate environment.

4.2 Influence of artificial reefs on fish distribution by mediating material exchange

Artificial reefs can modify the patterns of material exchange in marine environments (Falcão

et al., 2007, 2009), while concentrations of marine nutrients, temperature, and salinity also influence fish distribution (Cheng, 2000; Castillo-Rivera et al., 2002). These factors may further affect behaviors such as fish spawning through their impact on primary productivity (Říha et al., 2022). Therefore, beyond accounting for fish behavioral ecology, we conducted water quality analyses in both artificial reef deployment areas and control marine zones. We also explored the mechanisms through which environmental changes affect fish community composition by establishing linkages between environmental factors and the distribution of lower trophic organisms (phytoplankton and zooplankton) within these areas.

To elucidate the intrinsic regulatory mechanisms, comparative analyses of water quality, phytoplankton composition, and zooplankton composition and biomass were conducted between the control area and artificial reef area. Previous studies have shown that artificial reefs contribute to the restoration of biological resources, and by doing so, further induce changes in nutrient levels within the artificial reef area. (Falcão et al., 2007, 2009). Although the composition of plankton species at Xiaoheishan Island showed no difference from previous studies (Sun et al., 2013), this study identified differences in water quality, primary productivity, as well as the species composition and biomass of primary consumers between the control and the artificial reef area (Table 1; Supplementary Material 4), and revealed a correlation between water quality and biodiversity indices (Fig.3).

In this study, the overall water quality of Xiaoheishan Island maintained pronounced seasonal variations within the area (Zhang et al., 2020c). Different from previous studies, this study found higher nutrient levels in natural area compared to artificial reef area, with significant higher diversity of both organic matter, suspended solids, and plankton, which is inconsistent with the findings of Wan et al. (2024) who found that artificial reefs were beneficial to the improvement of nutrients in reef area. Our previous studies revealed declining trophic levels in reef-associated fish communities during the initial 2 years after the deployment of artificial reefs, indicating artificial reefs' influence on food chain and primary productivity within the area (Zhang et al., 2020c), so we attempted to explain this phenomenon by analyzing the composition of fish communities and the potential for changes in the food chain. In control areas, small planktivorous fish like Japanese anchovy (*E. japonicus*) that feed on diatoms and microzooplankton exhibit significantly higher reads compared to artificial reef zones. Since blue mackerel (*S. niphonius*) prey on these anchovies, their distribution is correspondingly greater in natural waters. *S. niphonius* belongs to large fish, so its feces can provide higher nutrient supplements to the sea area (Rempel et al., 2022), which further exacerbates the gap in nutrient and suspended matter concentrations between the natural sea area and artificial reef area (Table 1). Furthermore, the CCA analysis pointed to a strong NH_4^+-biological distribution correlation (Fig.3b; Table 3). Although the CCA models the influence of environmental factors on the community, the strong association between fish assemblages and nutrient gradients may also reflect a potential feedback mechanism, whereby increased fish biomass and aggregation at reef sites could enhance local nutrient regeneration.

However, in artificial reef aera, a positive correlation between reef zones and small reef-affiliated fish species such as gobies and *S. schlegelii* revealed declining trophic levels in reef-associated fish communities like our previous study showed in 2020 (Zhang et al., 2020b). Zhang et al. (2015) also demonstrated that the deployment of artificial reefs can alter the food sources for marine organisms, thereby influencing their distribution and growth. Based on the aforementioned findings, we propose a hypothesis: in control areas, the food web structure appears to be predominantly based on the classic trophic chain, involving the sequential transfer of matter and energy along the pathway: phytoplankton→zooplankton→small fish→medium-sized fish→large fish. Furthermore, fish assemblages in these areas are largely composed of free-swimming species, as the absence of reef structures may facilitate their foraging movements. In contrast, within artificial reef zones, the presence of reef structures seems to promote the development of food webs that rely more on benthic and detrital pathways. These areas tend to be dominated by demersal fishes and small reef-associated species, while large pelagic predators are relatively scarce. Moreover, by providing shelter and attracting organisms for spawning and predator avoidance, artificial reefs likely contribute to enhanced species diversity among smaller organisms. This hypothesis is consistent with eDNA data and is further supported by differences in dissolved oxygen (DO) concentrations. We observed that DO levels in

natural marine areas are generally lower than those in artificial reef zones, suggesting a higher biomass in natural waters. Additionally, CCA analysis indicates that dissolved oxygen is significantly correlated with species distribution. This hypothetical pattern was shown in Fig.6.

The correlation analysis seemed to further proved this speculation (Fig.3). We have calculated the correlation between the diversity and functional evenness of plankton and the water quality, and found positive regulation of plankton diversity by nitrate, ammonia nitrogen, temperature, and salinity in the natural sea area. Furthermore, the suspended particulate matter and nitrate concentrations exert direct positive regulatory effects on both fish diversity and functional evenness in natural waters, indicating that food particle availability in the water column actively promotes fish distribution. We infer that the abundance of planktonic organisms (especially phytoplankton) in natural areas attracts small fish and promotes the composition of regional fish communities by attracting large swimming predators. Consequently, the aggregation of high trophic level fish in reef areas may enhance local nutrient regeneration through biological processes (e.g., through excretion, egestion, and bioturbation), creating a feedback loop that potentially influences nutrient availability (Liang et al., 2022).

Distinct from natural sea areas, the artificial reef area showed predominant positive regulation by dissolved oxygen. The reason may be that, despite the high diversity of plankton and fish in the reef area, most of them are small with low biomass and oxygen consumption, making the dissolved oxygen conditions more conducive to the growth of plankton, hence with higher plankton capacity. Notably, the artificial reef area exhibited significant reduction of silicate in autumn, coinciding with the bloom of Chaetoceros in the same period, indicating certain effect of the artificial reefs on the distribution of nutrients. Furthermore, the observed biological assemblage around Xiaoheishan Island, characterized by key groups such as microzooplankton, *S. schlegelii*, and Gobiidae species (Supplementary Materials 2 & 4), suggests that a short food chain mode—centered on these components—serves as a primary driving mechanism for fish community assembly in this artificial reef ecosystem.

5 CONCLUSION

In conclusion, we found significant differences in both community composition and environmental regimes between control and artificial reef deployment areas. The living habits of fish also function as a key role in their spatial distribution. We propose that the biogeographical differentiation induced by artificial reefs could, to some extent, promote population

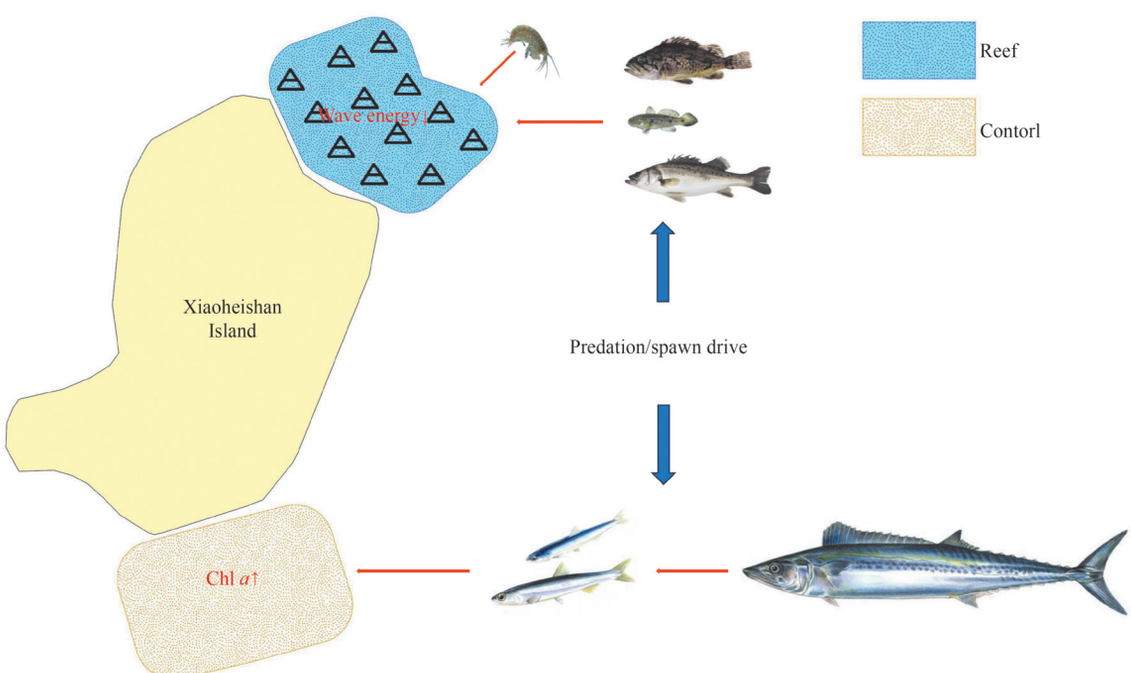

Fig.6 Schematic diagram of the food web at Xiaoheishan Island

growth of specific species and positively contribute to the restoration of small-organism diversity. This study provided theoretical foundations for subsequent stock enhancement programs; however, the regional segregation effect of artificial reefs on marine organism distribution requires further validation through traditional sampling methods. Moreover, food web analysis based on stomach content or stable isotope techniques will be crucial for verifying the hypotheses proposed in this study.

6 DATA AVAILABILITY STATEMENT

All study data are included in the article and supplementary files.

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Electronic supplementary material

Supplementary material (Supplementary Materials 1–4) is available in the online version of this article at https://doi.org/10.1007/s00343-026-5327-x.