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Spatial and temporal variation of benthic ecological quality evaluation in the Bohai Bay (China) using benthic indices

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Abstract: Bohai Bay, a semi-enclosed bay in northern China, is an important spawning and nursery ground for marine organisms but has experienced long-term anthropogenic pressures associated with land-based pollution, coastal engineering, and aquaculture activities. Assessing the ecological status of benthic habitats is therefore essential for evaluating the effectiveness of recent management and restoration measures. Based on summer surveys conducted from 2019 to 2023, benthic macrofauna and environmental data were used to evaluate benthic ecological quality in Bohai Bay using three commonly applied indices: the Shannon-Wiener index (H'), AZTI's Marine Biotic Index (AMBI), and multivariate AMBI (M-AMBI). The three indices showed generally consistent spatial patterns and interannual trends, although differences in sensitivity were observed. H' and M-AMBI exhibited clearer spatial gradients and higher concordance with habitat conditions, whereas AMBI produced more clustered classifications. Overall, benthic ecological quality in Bohai Bay displayed a significant improving trend during the study period. The proportion of stations classified as slightly disturbed or undisturbed increased from 81% in 2019 to 96% in 2023, while stations in good or high ecological status increased from 56% to 76%. Spatial heterogeneity remained evident, with stations showing moderate disturbance or poor status mainly associated with localized human activities such as aquaculture, anchorage, and marine engineering. Correlation analysis and redundancy analysis further indicated that benthic communities were jointly influenced by water-column and sediment conditions. Active phosphate in the water column was closely associated with broader regional gradients, whereas sediment sulfide played a key role in shaping local benthic ecological quality and community composition. These results indicate that pollution reduction and coastal management efforts have contributed to measurable improvements in benthic ecological quality in Bohai Bay, while localized human pressures continue to influence benthic habitats, and this study provides a multi-index and multivariate framework for assessing benthic ecosystem responses to human activities that supports adaptive management of Bohai Bay and other semi-enclosed coastal systems.

Keywords: benthic macrofauna, Shannon-Wiener index, AMBI, M-AMBI, benthic ecological quality, Bohai Bay

1. Introduction

Gulfs are regions of intense land-sea interaction, characterized by unique physical and chemical properties¹. The mixing of freshwater runoff and seawater driven by tidal currents creates distinct physical, chemical, and biological conditions. China's gulf regions are densely populated, and rapid socio-economic development has increased anthropogenic pressures that have resulted in significant threats to the marine ecosystem².

Bohai Bay, one of the three major bays in the Bohai Sea, is a critical spawning and nursery ground for many marine species³. As a typical shallow (average depth ~10 m) semi-enclosed bay with a silty bottom, its water exchange capacity with the open sea is limited, making it particularly vulnerable to pollutant accumulation and anthropogenic pressures⁴⁻⁶. These pressures have led to severe environmental challenges, including land-based pollution, large-scale coastal engineering, and intensive mariculture operations. Previous ecological assessments revealed degraded benthic conditions in the bay^{3,6,7}. In response, the Chinese government launched the "Comprehensive

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Governance Campaign for the Bohai Sea" in 2018, implementing stringent pollution control and ecological restoration measures⁸. However, no systematic assessment of benthic ecological status has been conducted since these interventions.

Benthic macrofauna, which live at the water-sediment interface, have limited mobility and long lifespans. This makes them slow to escape disturbances and highly sensitive to environmental changes. They can reflect ecological shifts through their population and community responses to both incidental and long-term anthropogenic disturbances. Therefore, they can be considered effective bioindicators of benthic ecological quality in coastal areas⁹⁻¹¹.

In recent decades, benthic indices have been widely applied to evaluate ecological quality in coastal and estuarine ecosystems. The Shannon-Wiener index (H') has long served as a simple yet effective tool for detecting changes in species diversity under anthropogenic stress¹²⁻¹⁴. Building on species sensitivity and tolerance classifications, the AZTI's Marine Biotic Index (AMBI) and its multivariate extension (M-AMBI) offer more detailed diagnostics of community responses to pollution. These indices have been extensively validated in European coastal waters and transitional environments and have been incorporated into monitoring frameworks such as the European Water Framework Directive^{9,15,16}. These indices have since been successfully applied in other regions, including the Mediterranean¹⁷, and North America^{18,19}, demonstrating their broad adaptability. Recent studies have also applied these indices in Chinese marginal seas, such as the Bohai, Laizhou Bay and Yangtze River Estuary, further confirming their diagnostic capacity in regional contexts²⁰⁻²².

Although AMBI and M-AMBI have been applied in Bohai Bay^{3,23}, systematic evaluations of benthic ecological quality after the 2018 governance initiative are still absent. Given the scale of restoration efforts, clarifying their ecological outcomes has become an urgent research need. To address this, our study provides a comprehensive post-implementation assessment based on benthic macrofauna, water quality, and sediment data from 2019 to 2023. Specifically, this study aims to (1) evaluate the current state of benthic ecological quality in Bohai Bay; (2) compare the consistency and efficacy of three benthic indices (H' , AMBI, and M-AMBI) in the regional context; and (3) explore the relationships between ecological status, key environmental factors, and anthropogenic pressures. The findings are intended to deliver scientifically grounded support for ongoing ecological protection and adaptive management strategies in the area.

2. Materials and methods

2.1 Study area and sampling stations

The data analyzed in this study were obtained from the National Marine Ecological Early-warning Monitoring Program (NMEEP), organized by the Ministry of Natural Resources of China. Sampling stations were located to represent the major ecological features and gradients of human stress in the bay. Their placement considered water depth, sediment type, hydrodynamics, and the intensity of nearby human activities. This national monitoring framework provides a standardized and scientifically robust basis for long-term ecological assessment. The number of sampling stations has a slight variation each year (Table 1). However, the locations of the sites remained largely consistent, encompassing a total of 32 sampling stations (Fig. 1). To further document the consistency of spatial coverage across years, Supplementary Table S1 summarizes the sampling years of each station. Notably, 18 stations were sampled in at least four of the five survey years, demonstrating a high degree of spatial stability in the monitoring design and supporting the comparability of annual ecological assessments. Although not all stations were sampled in every year, all surveys followed a consistent sampling protocol and were conducted during the same season. Stations within each year were treated as independent spatial replicates, and interannual analyses focused on overall spatial patterns and index-based classifications rather than strict temporal trend testing at individual stations.

Table 1. Sampling stations in Bohai Bay from 2019 to 2023

Year	2019	2020	2021	2022	2023
sampling sites	16	23	19	29	25

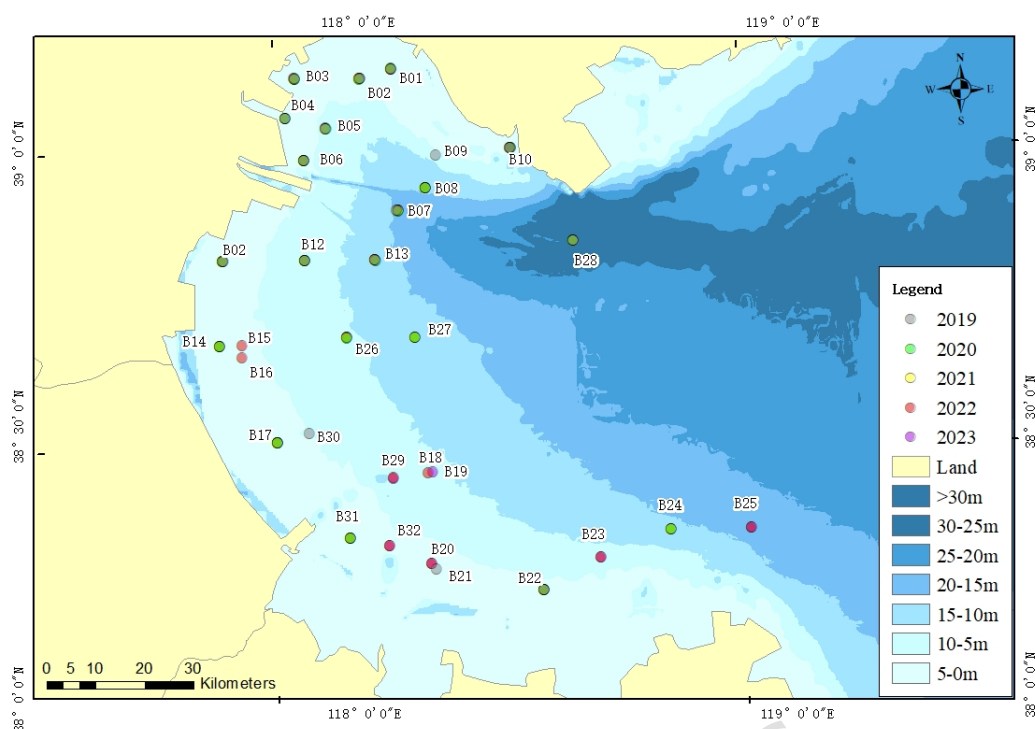


Figure 1. Sampling stations in Bohai Bay.

Spatial distribution of sampling stations surveyed from 2019 to 2023. Stations sampled in each year are shown in distinct colors to illustrate year-specific coverage. Bathymetry (m) is displayed using a graded blue color scale. The map was created by the authors using ArcGIS 10.8 (Esri, Redlands, CA, USA; <https://www.esri.com>) based on publicly available geographic datasets.

2.2 Sampling methods and procedure

Benthic macrofauna, seawater, and surface sediment samples were collected simultaneously at each station during the summer (late June to early August) from 2019 to 2023, as required by the National Marine Ecological Early-warning Monitoring Program (NMEEP), which mandates a fixed summer sampling period for assessing the baseline ecological status and interannual trends of China's coastal waters.

At each station, three replicate samples of surface sediments were collected using a Van Veen grab and then sieved through a 0.5 mm mesh. All benthic macrofauna on the sieve were removed, fixed with a 4% formalin solution and preserved in 70% ethanol. In the laboratory, all organisms were identified to the lowest possible taxonomic level (typically species) using stereomicroscopy. Taxonomic identification was based on authoritative references, primarily the Checklist of Marine Biota of China Seas²⁴, with subsequent verification against the World Register of Marine Species (WoRMS) to ensure nomenclatural accuracy. A checklist of benthic macrofauna species recorded during the surveys is provided in Supplementary Table S2.

Concurrently, seawater and surface sediment samples were collected for physicochemical analysis. The collection, preservation, and laboratory analysis of all environmental parameters strictly followed the standard methods prescribed by China's national standard "The specification for marine monitoring" (GB 17378-2007). The specific analytical techniques for each parameter were as follows: Water column parameters: Salinity (psu; CTD, in situ); pH (potentiometric method); Dissolved oxygen (DO, mg/L; iodometric titration); nutrients (NH_4^+ , NO_2^- , NO_3^- , PO_4^{3-} ; mg/L; spectrophotometry); COD (mg/L; alkaline potassium permanganate method); Silicate (SiO_4^{2-} ; mg/L; spectrophotometry); Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH_4^+ , NO_2^- and NO_3^- . Suspended solids (SS; mg/L; gravimetric method); Chlorophyll-a (ug/L; fluorometry after acetone extraction). Sediment parameters: Total organic carbon (TOC, % dry weight; potassium dichromate oxidation-diffusion

method); Sulfide (mg/kg dry weight; iodometric titration or methylene blue spectrophotometry). All analyses were performed following strict quality assurance and quality control (QA/QC) protocols.

2.3 Benthic indices and ecological assessment methods

To evaluate benthic ecological quality, we selected three complementary and widely used indices: the Shannon–Wiener index (H'), AMBI, and M-AMBI. These indices represent distinct but synergistic dimensions of benthic community structure and environmental response. H' reflects species richness and evenness, serving as a classical indicator of diversity and community stability. AMBI incorporates species-specific sensitivity and tolerance to organic enrichment and disturbance, thereby linking community composition to pollution gradients. M-AMBI integrates richness, diversity (H'), and sensitivity (AMBI) within a multivariate framework, providing a more balanced assessment that reduces the influence of natural variability. In addition to their broad international use, these indices have been successfully applied in multiple Chinese coastal systems^{20–22}, supporting their suitability for evaluating benthic conditions under the multi-stressor environment of Bohai Bay.

2.3.1 Dominant species identification and H' calculation

H' was employed for each monitoring station, calculated using the following formula:

$$H' = -\sum_{i=1}^S P_i \log_2 P_i$$

Where P_i represents the proportion of individuals of the i -th species relative to the total number of individuals in the sample. The H' incorporates both species richness (the number of species, S) and the evenness of individuals distributed among those species. A higher H' value indicates a more diverse and stable community, which is typically associated with healthier, less disturbed environments. Conversely, a lower H' value suggests a disturbed environment where a few tolerant species may dominate.

When $H' = 0$, the environment is considered severely polluted; $0 < H' \leq 1$ indicates heavy pollution; $1 < H' \leq 2$ indicates moderate pollution; $2 < H' \leq 3$ indicates slight pollution; and $H' > 3$ indicates a clean environment¹⁴.

2.3.2 AMBI Index Calculation

All soft-bottom benthic macrofauna are assigned to five ecological groups according to the sensitivity of benthic communities' response to environmental pollution. The AMBI is calculated based on the species abundance of each ecological group and calculated by the following formula:

$$\text{AMBI} = (0 \times \% \text{EGI} + 1.5 \times \% \text{EGII} + 3 \times \% \text{EGIII} + 4.5 \times \% \text{EGIV} + 6 \times \% \text{EGV}) / 100$$

EGI represents the species sensitive to environmental disturbance, EGII represents the species insensitive to environmental disturbance, EGIII represents the species tolerant to environmental disturbance, EGIV represents the second-order opportunistic species, and EGV represents the first-order opportunistic species¹⁵. %EGI–%EGV is the relative abundance of five ecological groups²⁵.

The fundamental ecological principle of AMBI is that as environmental stress increases, the proportion of sensitive species (EGI) decreases, while the proportion of opportunistic species (EGIV and EGV) increases. Therefore, the AMBI index translates the taxonomic composition of a benthic community into a simple numerical value that reflects its overall sensitivity or tolerance to stress, with a low AMBI value indicating an undisturbed condition and a high value indicating a disturbed one.

The AMBI and M-AMBI were calculated using the AMBI software V6.0, freely available on the AZTI center website (<http://ambi.azti.es>). The scientific names of the benthic macrofauna involved in this study were verified against the Chinese Marine Biological Checklist²⁴ and the WORMS website (<https://marinespecies.org/>). Species were assigned to ecological groups based on the latest classification (11,347 species, updated June 2022). Species present in both this study and the checklist directly adopted their existing group assignments. Species present in this study but not in the checklist were assigned to the ecological group of their congeneric species²⁶. Species not meeting these criteria were categorized as "Do Not Assign." Data from "Do Not Assign" species were excluded from the calculation. The percentage of unassigned individuals exceeding 20% requires cautious interpretation of their AMBI results; if the percentage exceeds 50%, the corresponding AMBI results are considered

unreliable²⁷. This study's percentage of unassigned individuals ranged from 0% to 18.6%. This result means that all samples could be taken into account in the analysis.

The AMBI index assessment categorizes ecological status as follows: $0 < \text{AMBI} \leq 1.2$ indicates an undisturbed condition; $1.2 < \text{AMBI} \leq 3.3$ indicates a slightly disturbed condition; $3.3 < \text{AMBI} \leq 5$ indicates a moderately disturbed condition; $5 < \text{AMBI} \leq 6$ indicates a heavily disturbed condition; and $6 < \text{AMBI} \leq 7$ indicates an extremely disturbed condition²⁷.

2.3.3 M-AMBI Index Calculation

The M-AMBI index builds upon the AMBI index and integrates richness and H' . It is a multivariate approach that combines three complementary aspects of the benthic community: the number of species (Richness), the distribution of individuals among species (H'), and the sensitivity of the species present (AMBI). By integrating these three metrics, M-AMBI provides a more comprehensive and robust assessment of ecological status than any single index alone, as it is less sensitive to natural variability and more responsive to a wider range of anthropogenic pressures.

The application of M-AMBI necessitates the establishment of a reference state. The worst state is an absence of biota (richness and $H' = 0$, AMBI value = 6). The best state is established by evaluating the optimal value of each indicator in the sample.

The M-AMBI index assessment categorizes ecological status as follows: $0 < \text{M-AMBI} \leq 0.2$ indicates a bad status; $0.2 < \text{M-AMBI} \leq 0.38$ indicates a poor status; $0.38 < \text{M-AMBI} \leq 0.53$ indicates a moderate status; $0.53 < \text{M-AMBI} \leq 0.77$ indicates a good status; $0.77 < \text{M-AMBI} \leq 1$ indicates a high status²⁸.

2.3.4 Correlation Analysis

This analysis evaluated the relationships between benthic indices (density, H' , AMBI, M-AMBI) and environmental parameters. The assessed parameters included water column and sediment properties, such as Salinity, pH, Suspended Solids (SS), Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Dissolved Inorganic Nitrogen (DIN), Ammonium Nitrogen (NH_4^+), Nitrite Nitrogen (NO_2^-), Nitrate Nitrogen (NO_3^-), Active Phosphate (PO_4^{3-}), Silicate (SiO_4^{2-}), Chlorophyll-a, Sulfide and Total Organic Carbon (TOC).

The goal was to identify potential anthropogenic stressors affecting the benthic community. Data from water and sediment sampling stations within 100 meters of benthic macrofauna stations were used. All samples were collected in the same month. Because macrobenthos, water-column, and sediment samples were collected using different sampling devices, their exact geographic coordinates did not always fully coincide. Therefore, environmental samples located within 100 m of the corresponding macrobenthic stations were regarded as representing the same site for subsequent correlation and RDA analyses.

Spearman correlation analyses were performed. This approach ensured the robustness of the results, as Spearman's method does not require normally distributed data. The analyses were conducted using SPSS software. Correlation significance was determined at $p < 0.05$.

2.3.5 Redundancy analysis (RDA)

Redundancy analysis (RDA) was used to examine the multivariate relationships between benthic ecological indices and environmental variables. H' , AMBI, and M-AMBI were used as response variables. Environmental parameters were used as explanatory variables and were standardized prior to analysis to remove scale effects. Samples containing missing values were excluded, as RDA does not allow incomplete observations. To reduce multicollinearity and improve ecological interpretability, two separate RDA models were constructed. The first model focused on water-column variables, including salinity, pH, SS, DO, COD, DIN, PO_4^{3-} , SiO_4^{2-} , and chlorophyll-a. The second model examined sediment variables, including sulfide and TOC. The significance of the overall RDA models, canonical axes, and individual environmental variables was tested using Monte Carlo permutation tests (999 permutations). All RDA analyses and graphical outputs were conducted using the vegan package in R.

3. Results

3.1 Species, dominant species, biodiversity and biomass

Between 2019 and 2023, the number of benthic macrofauna species ranged from 79 to 118. The

dominant groups were Annelida and Mollusca, with key species including *Ceratia nagashima*, *Musculista senhousia*, *Sternaspis scutata*, and *Nassarius succinctus*. The overall number of benthic macrofauna species has remained relatively stable in recent years. The average benthic macrofauna density was 220 ind/m², and the average biomass was 37.91 g/m² for the same period. Specifically, the average density and biomass figures for each year were as follows: in 2019, there were 197.81 individuals and a biomass of 26.46 g/m²; in 2020, 149.34 individuals and 39.83 g/m²; in 2021, 228 individuals and 74.35 g/m²; in 2022, 209.55 individuals and 37.81 g/m²; and in 2023, 307.8 individuals and 15.90 g/m². Throughout these years, benthic macrofauna density showed a steady increase, while biomass exhibited minor fluctuations.

3.2 The Shannon-Wiener index (H')

The interannual variation of the Shannon-Wiener index (H') is summarized in Table 2. The mean H' value showed a fluctuating upward trend from 2019 to 2023, with the lowest value observed in 2021 (2.27) and the highest in 2023 (2.60). The standard deviation remained high (0.95) across all years, indicating considerable spatial variability in benthic macrofauna diversity within the bay in any given year.

Table 2. H' results in Bohai Bay from 2019 to 2023

Year	H' (Mean $\pm$ SD)
2019	2.33 $\pm$ 0.96
2020	2.37 $\pm$ 0.95
2021	2.27 $\pm$ 1.08
2022	2.58 $\pm$ 1.03
2023	2.60 $\pm$ 1.10

Spatially, the five-year average H' values exhibited a pronounced and consistent gradient across Bohai Bay (Fig. 2). The stations with the lowest diversity, classified as "Moderate polluted" to "Heavy polluted" (predominantly red and orange symbols), were predominantly clustered in the northwestern and southwestern nearshore region. Notably, stations B07, B08, B09, B12, B17, B30 and B31 consistently demonstrated the most impaired ecological conditions throughout the study period. In stark contrast, the central and offshore areas of the bay, along with the southern region near the Yellow River estuary, were characterized by higher H' values, corresponding to "Slightly polluted" or "Clean" status (yellow and green symbols). This pattern shows that benthic diversity and habitat quality were better in the open waters, which have stronger water exchange. In contrast, the most degraded stations were predominantly distributed in nearshore and semi-enclosed areas affected by intense localized human activities, rather than being strictly confined to the immediate coastline. This spatial pattern indicates that benthic degradation in Bohai Bay is driven more by site-specific anthropogenic pressures and hydrodynamic constraints than by simple proximity to the shoreline.

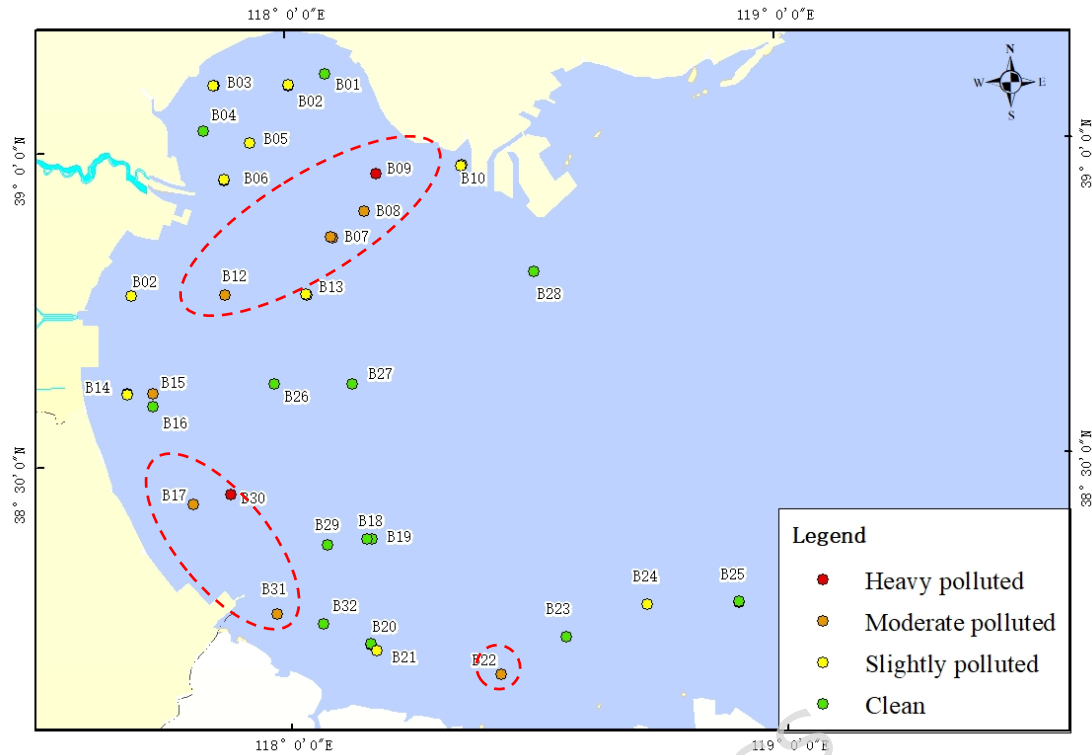


Figure 2. Spatial distribution of the Shannon–Wiener index (H') in Bohai Bay (2019–2023 average). The base map is the same as in Fig. 1.

3.3 AMBI and M-AMBI

The annual mean AMBI values in Bohai Bay ranged from 1.50 to 1.81 between 2019 and 2023 (Table 3), indicating that the area is in a "slightly disturbed" condition. The proportion of stations classified as "slightly disturbed" or "undisturbed" increased from 81% in 2019 to 96% in 2023. A notable improvement was observed after 2019, while one station was categorized as "heavily disturbed" in 2019, no stations fell into this category from 2020 onward (Fig. 3).

Similarly, the annual mean M-AMBI values ranged from 0.54 to 0.64 during the same period (Table 3), reflecting a good ecological status. The percentage of stations classified as being in "high" or "good" status rose from 56% to 76% from 2019 to 2023. Two stations were identified as having "bad" status in 2019, but no "bad" stations were recorded in the subsequent years (Fig. 4). The consistent improvement in ecological status after 2019 is likely related to the implementation of the 'Comprehensive Governance Campaign for the Bohai Sea', which reduced pollutant inputs and strengthened habitat restoration efforts.

Spatially, the five-year average AMBI and M-AMBI values revealed a pronounced inshore–offshore gradient (Figs. 5 and 6). The most disturbed stations were consistently concentrated in the northwestern nearshore area, whereas offshore stations and those near the southern bay exhibited better ecological conditions. These spatial patterns correspond well with known environmental pressure gradients, such as higher pollutant inputs and weaker hydrodynamic exchange in nearshore waters.

Table 3. AMBI and M-AMBI results in Bohai Bay from 2019 to 2023

Stations	2019		2020		2021		2022		2023	
	AMB I	M- AMBI	AMB I	M- AMBI	AMB I	M- AMBI	AMB I	M- AMBI	AMB I	M- AMBI
B01	1.35	0.62	1.63	0.83	1.74	0.83	1.60	0.62	1.69	0.72
B02	0.69	0.65	2.62	0.45	2.89	0.72	0.50	0.53	1.40	0.74
B03	0.48	0.85	1.41	0.69	0.78	0.52	2.96	0.32	2.14	0.62

B04	1.39	0.73	0.96	0.73	2.03	0.68			1.59	0.71
B05	2.06	0.39	0.97	0.67	1.59	0.64			2.00	0.55
B06	1.04	0.62	0.59	0.71	1.72	0.70	1.77	0.59	2.50	0.46
B07	3.60	0.34	1.80	0.49	1.80	0.42	0.75	0.56	2.96	0.34
B08			3.67	1.14	0.50	0.52	4.50	0.89	0.75	0.49
B09	4.50	0.19								
B10	5.50	0.11	5.57	0.87			3.50	1.03	5.20	1.11
B11	0.83	0.79	0.98	0.70	2.31	0.58	1.32	0.62	1.67	0.62
B12	2.59	0.40	0.88	0.61	2.95	0.36	2.36	0.54	2.30	0.54
B13	1.77	0.58	3.50	0.38	1.20	0.46	1.00	0.50	0.64	0.68
B14			1.00	0.62	2.58	0.79	0.60	0.61	1.11	0.73
B15							1.09	0.53		
B16							1.79	0.70		
B17			4.00	0.89	1.13	0.57	3.75	0.86	3.00	0.87
B18							1.25	0.87		
B19									0.57	0.90
B20							1.91	0.75	1.21	0.80
B21	0.62	0.64								
B22	0.00	0.48	1.50	0.47	0.25	0.49	0.00	0.47	3.68	0.29
B23							1.04	0.74	1.34	0.83
B24			0.80	0.66	2.02	0.59	1.04	0.66		
B25							1.07	0.59	1.97	0.86
B26			1.02	0.89	1.50	0.71	2.25	0.50	0.86	0.80
B27			1.00	0.97	1.29	0.61	1.77	0.65		
B28	1.73	0.91	2.04	0.82	1.50	0.90	1.50	0.83		
B29							1.50	0.87	1.57	0.83
B30	0.75	0.39								
B31			1.50	0.36	1.51	0.35	1.67	0.54	1.50	0.62
B32							1.13	0.80	1.33	0.91
Average value	1.81	0.54	1.63	0.61	1.65	0.6	1.51	0.59	1.71	0.64

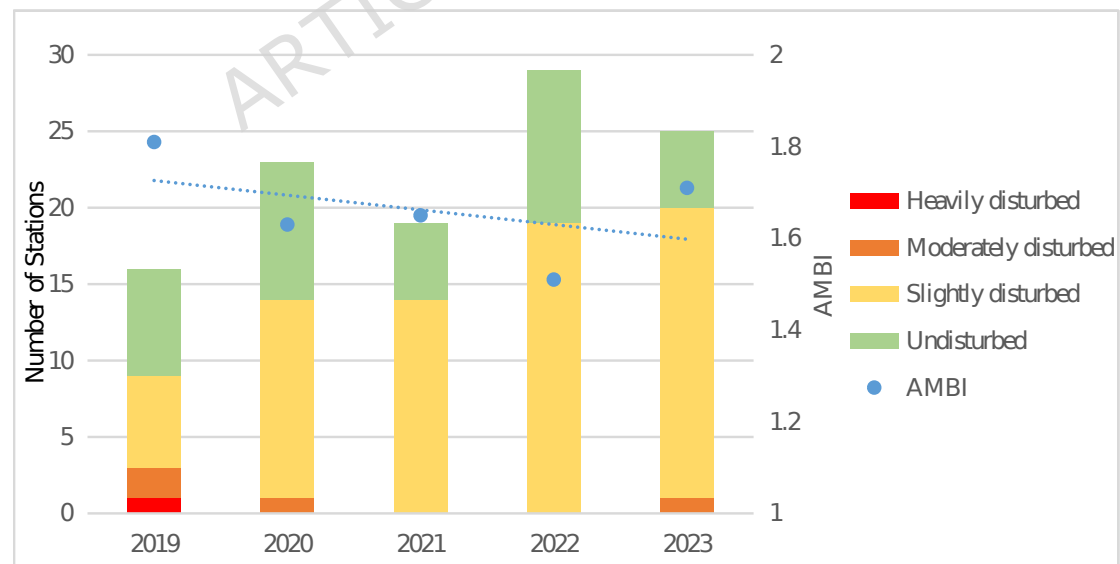


Figure 3. Annual AMBI classification of sampling stations in Bohai Bay from 2019 to 2023.

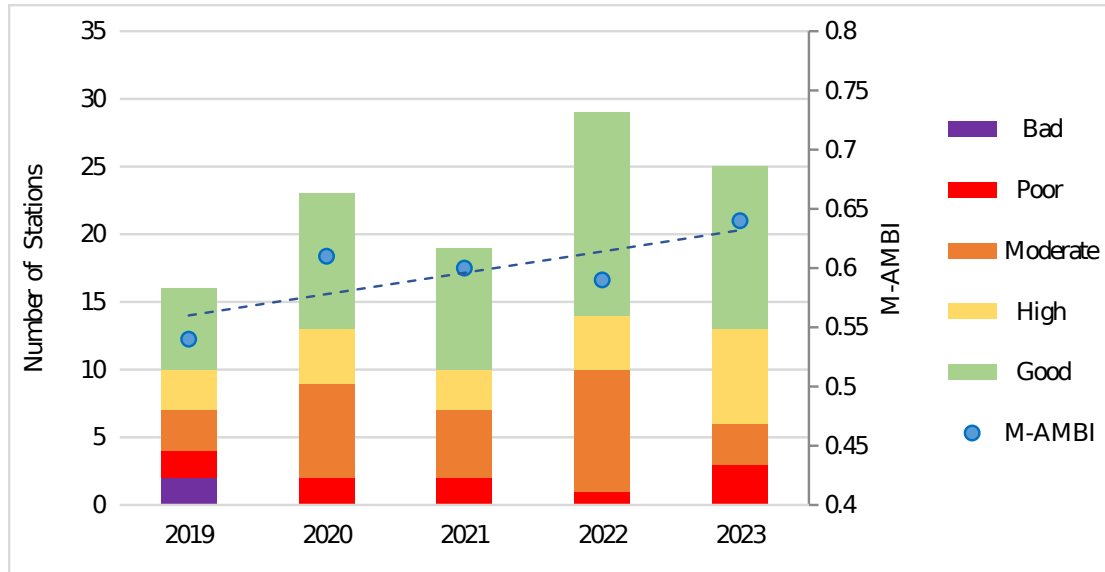


Figure 4. Annual M-AMBI classification of sampling stations in Bohai Bay from 2019 to 2023.

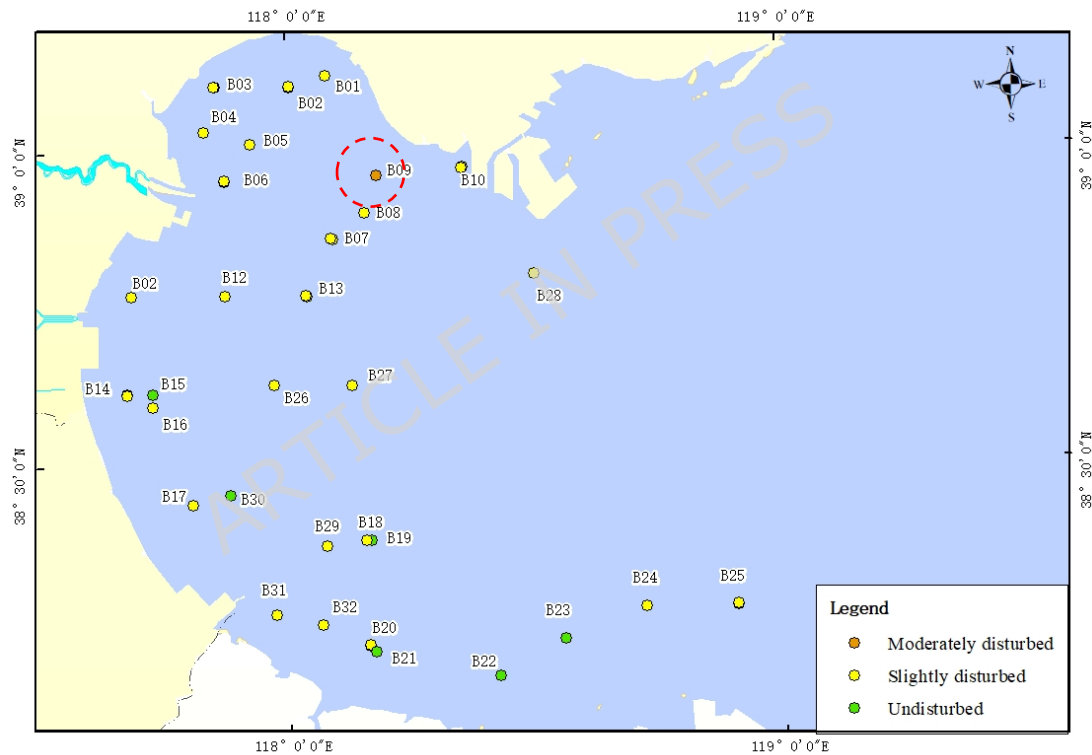


Figure 5. Spatial distribution of five-year average AMBI values in Bohai Bay.

The base map is the same as in Fig. 1.

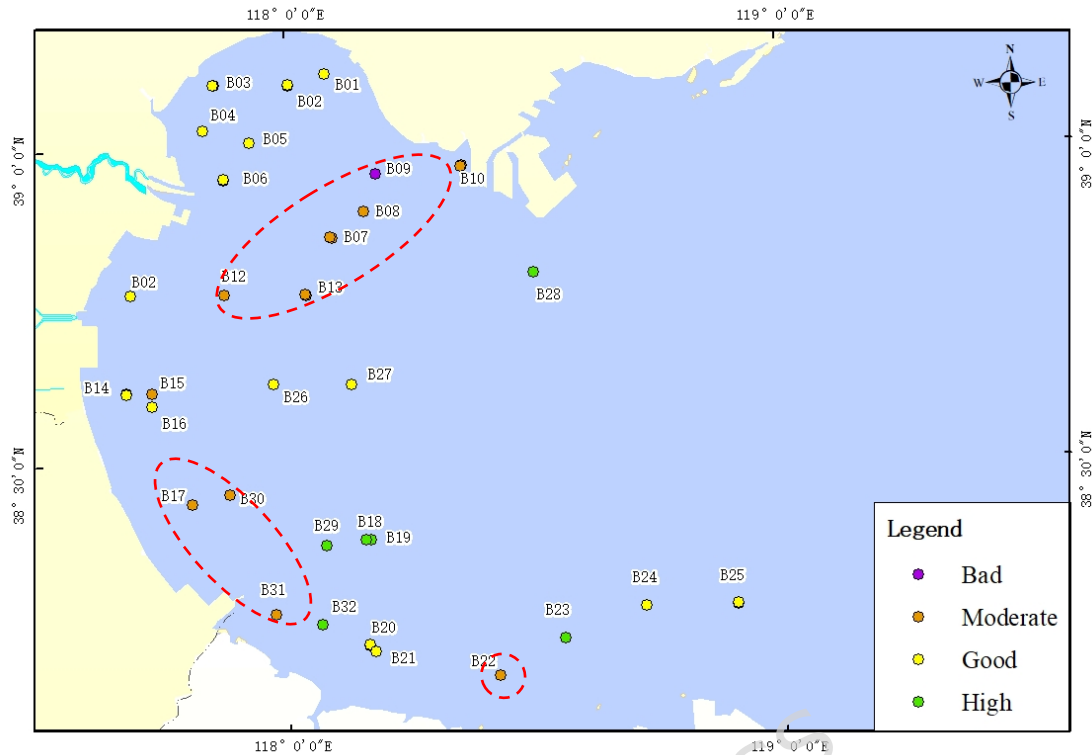


Figure 6. Spatial distribution of five-year average M-AMBI values in Bohai Bay.

The base map is the same as in Fig. 1.

3.4 Consistency and differences among the three benthic indices

Despite their different conceptual foundations, H' , AMBI, and M-AMBI revealed broadly consistent spatial and temporal patterns in Bohai Bay. All three indices showed gradual improvement after 2019 and identified the same nearshore northwestern area as the most degraded region, while consistently classifying offshore stations as being in better ecological condition. This agreement is clearly reflected in the spatial distributions (Fig. 2, Fig. 5, Fig. 6), where all indices delineate an inshore–offshore gradient shaped by hydrodynamic conditions and proximity to anthropogenic pressures. Such consistency indicates that the indices are responding to the same underlying ecological gradients.

However, the indices differed markedly in how they represented the magnitude of ecological variation across stations. H' exhibited a wide dynamic range, with strong contrast between degraded nearshore sites and more diverse offshore communities, as shown by the broad span of color values in Fig. 2. M-AMBI displayed a similarly wide spread (Fig. 6), effectively separating clearly impaired stations—characterized by low diversity and dominance of tolerant taxa—from offshore stations achieving high ecological status. These two indices therefore retained strong discriminatory power across the full disturbance gradient.

In contrast, AMBI showed a narrower spatial distribution of values, with most stations clustered within the "undisturbed" to "slightly disturbed" classes (Fig. 5). This compressed gradient reduces its ability to distinguish among stations experiencing moderate but ecologically meaningful changes in community composition. The reduced spatial contrast in AMBI is particularly evident at the chronic hotspot stations (e.g., B07, B08, B10, B12, B13, B17, B30, B31, B22), where H' and M-AMBI consistently indicate pronounced degradation, while AMBI often categorizes the same stations in milder disturbance classes.

Taken together, these results demonstrate that while the three indices identify the same spatial patterns and long-term trajectory of ecological improvement, they differ in diagnostic sensitivity. H' and M-AMBI resolve finer differences across the disturbance gradient, especially in impacted nearshore areas, whereas AMBI provides a more conservative assessment under the current multi-

stressor regime. M-AMBI emerges as the most balanced single indicator because it integrates structural and sensitivity-based information, while the combined interpretation of all three indices enhances confidence in diagnosing benthic ecological status in Bohai Bay.

3.5 Results of correlation analysis

To examine the relationships between benthic indices and environmental parameters, Spearman correlation analyses were applied to the five-year summer dataset (Table 4, Fig. 7). This non-parametric approach was selected because several environmental variables did not meet normality assumptions and exhibited skewed distributions.

The results showed that the diversity index (H') and the multivariate assessment index (M-AMBI) showed significant positive correlations with active phosphate concentrations. In contrast, benthic macrofauna density, H' , and M-AMBI were significantly negatively correlated with sulfide, whereas AMBI exhibited a significant positive correlation. These results indicate that sulfide is a major environmental stressor promoting pollution-tolerant taxa while reducing overall abundance, diversity, and ecological quality.

A significant negative correlation between M-AMBI and total organic carbon (TOC) was also observed, emphasizing the detrimental effect of organic enrichment on benthic condition. Suspended solids (SS) showed significant monotonic relationships with all benthic indices, suggesting that elevated SS levels may influence benthic communities through indirect or threshold-related mechanisms rather than simple linear responses.

Other environmental variables, including Salinity, pH, COD, DIN, NH_4^+ , NO_2^- , NO_3^- , and chlorophyll-a, exhibited no significant correlation with any benthic index. These results suggest that these factors were not the primary drivers shaping benthic macrofauna community patterns in Bohai Bay during the study period.

Table 4. Spearman correlation coefficients between benthic indices and key environmental parameters

environmental parameters	density	H'	AMBI	M-AMBI
Salinity	-0.163*	-0.190*	-0.009	-0.149
pH	0.091	0.162*	-0.113	0.202*
SS	0.296***	0.235**	-0.329***	0.274**
DO	-0.259**	-0.219**	0.147	-0.208*
COD	-0.082	-0.128	0.041	-0.114
DIN	0.024	0.033	0.003	-0.005
NH_4^+	-0.073	-0.136	0.112	-0.154
NO_2^-	-0.002	0.063	0.022	0.062
NO_3^-	0.023	0.03	-0.016	-0.011
PO_4^{3-}	0.293***	0.306***	0.027	0.299***
SiO_4^{2-}	-0.179*	-0.171*	0.135	-0.161
Chlorophyll-a	-0.138	0.036	0.03	0.06
Sulfide	-0.447***	-0.401***	0.420***	-0.498***
TOC	-0.212*	-0.155	0.228*	-0.253*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

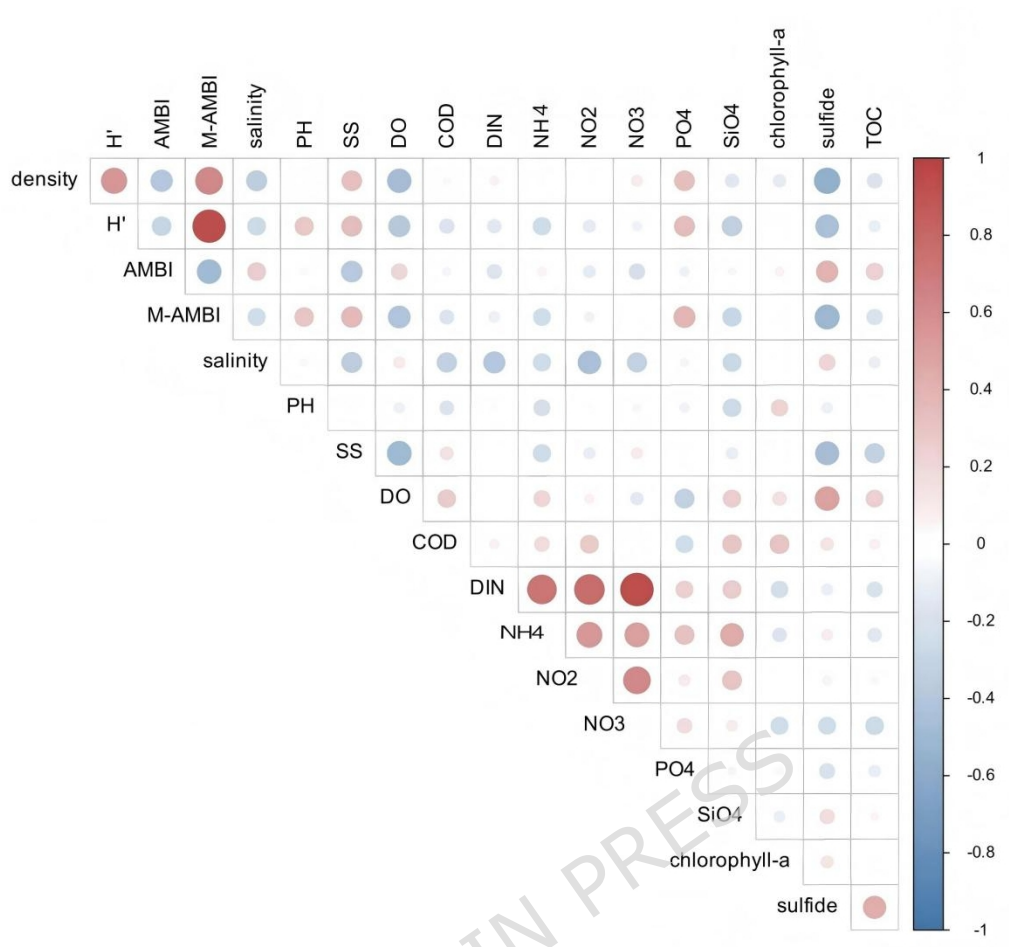


Figure 7. Results of Spearman correlation analyses between the benthic indices with environmental parameters in Bohai Bay.

Environmental variables shown in the figures are abbreviated for clarity (e.g., $PO_4 = PO_4^{3-}$, $SiO_4 = SiO_4^{2-}$, $NO_3 = NO_3^-$, $NO_2 = NO_2^-$, $NH_4 = NH_4^+$), as defined in the Methods.

3.6 Redundancy analysis of benthic indices and environmental variables

To further explore the multivariate relationships between benthic ecological indices and environmental variables, redundancy analysis (RDA) was applied to the standardized dataset. When water-column variables were considered, the overall RDA model was significant (permutation test, $p < 0.05$), indicating that water quality exerted a detectable influence on benthic ecological condition. The first two RDA axes accounted for 29.13% (RDA1) and 0.12% (RDA2) of the constrained variation, respectively, and thus represent the dominant water-column gradients structuring benthic ecological patterns (Fig. 8a). To improve interpretability and reduce multicollinearity, environmental variables were screened using variance inflation factor analysis and forward selection. As a result, DO , PO_4^{3-} , SiO_4^{2-} , and NO_3^- were retained in the final ordination, while other water quality parameters showed limited explanatory power. In the RDA ordination, H' , AMBI, and M-AMBI exhibited differentiated responses along the primary environmental gradient, with AMBI and M-AMBI generally aligned with higher nutrient and oxygen-related conditions, whereas H' was oriented in the opposite direction. In contrast, the sediment-based RDA model was highly significant ($p = 0.001$). The first two sediment RDA axes accounted for 17.69% (RDA1) and 0.03% (RDA2) of the constrained variation, respectively (Fig. 8b). Given the limited number of measured sedimentary variables, sulfide and total organic carbon (TOC) were both included in the final sediment-based RDA model. Sulfide emerged as the dominant explanatory factor, showing a strong positive association with AMBI and a clear negative association with H' , whereas TOC reflected a secondary gradient related to organic enrichment. Overall, the RDA results are consistent with the correlation analysis, indicating that water-column variables describe a

broad environmental gradient, whereas sedimentary factors—particularly sulfide—show a stronger and more direct association with benthic ecological indices in Bohai Bay.

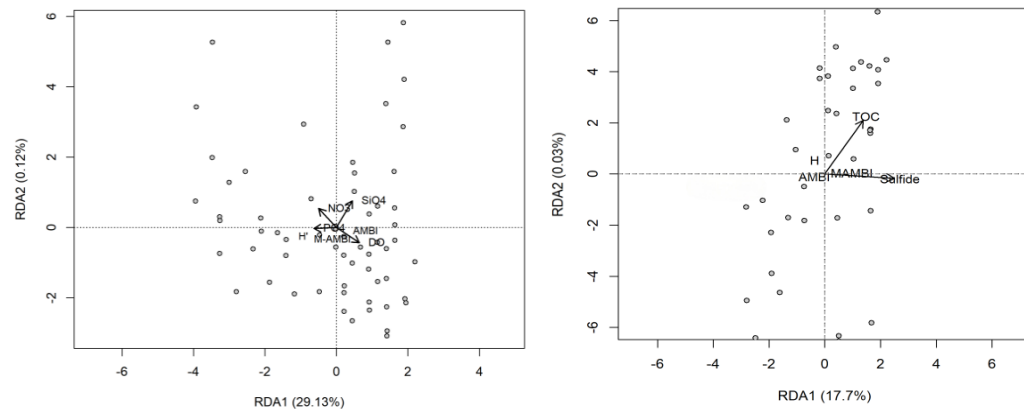


Figure 8. Redundancy analysis (RDA) of benthic ecological indices and environmental variables in Bohai Bay.

(a) Relationships between benthic indices and selected key water-column variables (DO, PO_4^{3-} , SiO_4^{2-} , and NO_3^-). (b) Relationships between benthic indices and selected sediment variables (sulfide and TOC). Arrows indicate environmental gradients, and points represent benthic ecological indices. For clarity, environmental variables are abbreviated in the ordination plots (e.g., $\text{PO}_4 = \text{PO}_4^{3-}$, $\text{SiO}_4 = \text{SiO}_4^{2-}$, $\text{NO}_3 = \text{NO}_3^-$), as defined in the Methods.

4. Discussion

4.1 Temporal Trends and the Effectiveness of Ecological Governance in Bohai Bay

The benthic ecological status of Bohai Bay showed a clear improvement from 2019 to 2023, as reflected consistently across all three benthic indices (H' , AMBI, and M-AMBI). The gradual disappearance of "bad" or "heavily disturbed" classifications and the increasing proportion of stations attaining higher ecological status indicate that benthic habitats are recovering from the historically degraded conditions documented in earlier decades. Although long-term raw monitoring datasets prior to 2019 were not available within the standardized NMEEP framework used in this study, multiple peer-reviewed assessments conducted between 2008 and 2012 consistently reported that most areas of Bohai Bay were in "Moderate" to "Bad" condition^{3,23,29}. These published studies therefore provide a reliable pre-restoration baseline against which the post-2019 recovery can be interpreted.

A key factor underpinning the observed recovery is the large-scale environmental governance undertaken in the region. The Comprehensive Governance Campaign for the Bohai Sea was implemented from 2018 to 2020 as a three-year intensive restoration initiative. This campaign comprised four major categories of actions: land-based pollution control, marine pollution control, ecological protection and restoration, and environmental risk prevention. These categories encompassed integrated measures such as substantial reductions in land-based pollutant discharges, stricter regulation of coastal development, remediation of riverine inputs, and improved management of aquaculture activities. These actions have been shown to significantly improve water quality in the Bohai region^{30,31}. The correspondence between the timing of these governance efforts and the subsequent improvement in benthic indices strongly suggests that the policy intervention played a central role in facilitating ecological recovery.

Although macrobenthic communities often respond more slowly than water or sediment quality, many coastal recovery studies have documented clear improvements within 3-5 years following marked reductions in anthropogenic pressures. The five consecutive years of NMEEP monitoring in this study already capture such a response window. The consistent positive trends across H' , AMBI, and M-AMBI therefore represent a plausible and timely ecological reaction to the large-scale governance measures initiated in 2018.

While all three benthic indices detected the same overall improvement trend and localized degradation hotspots, their diagnostic behaviors differed due to their underlying ecological foundations. H' captured strong spatial contrasts because it is sensitive to changes in richness and evenness associated with habitat stress. M-AMBI showed similar discriminatory ability because it integrates both structural (diversity-based) and functional (sensitivity-group) information, enabling a nuanced differentiation of community states along the disturbance gradient. In contrast, AMBI tended to classify many stations within a narrower disturbance range, reflecting its primary sensitivity to organic enrichment. The relatively compressed disturbance gradient produced by AMBI can be further explained by the sedimentary conditions of Bohai Bay. The five-year mean TOC content was generally low, ranging from 0.45% to 0.58%, indicating that organic enrichment is not the dominant stressor across most stations. Because AMBI is fundamentally designed to respond to shifts in ecological groups along organic enrichment gradients, it may overestimate disturbance or reduce spatial discrimination when organic loading is limited. This diagnostic behavior aligns with the theoretical sensitivity framework described by Wu et al.³², reaffirming that AMBI alone may not fully capture the multi-stressor environmental context of Bohai Bay. Consequently, the broader and more integrative structure of M-AMBI offers a more balanced representation of benthic condition under such circumstances. Overall, the coherence among the three indices provides strong evidence of ecological improvement following the 2018 governance intervention, while the differences in diagnostic sensitivity highlight the advantages of multimetric approaches. M-AMBI offers the most balanced and robust single indicator for routine ecological assessment, whereas the combined interpretation of H' , AMBI, and M-AMBI strengthens confidence in detecting ecological trajectories and identifying persistent hotspots of disturbance.

4.2 Spatial Heterogeneity and Impacts of Localized Anthropogenic Pressures

A clear inshore-offshore gradient in ecological quality was observed. Offshore stations consistently showed better conditions, while over 70% of the most degraded stations were within 10 km of the coastline. This pattern reflects the cumulative effects of land-based pollution and limited water exchange in nearshore waters, contrasting with the greater dilution capacity offshore.

Spatial analysis directly linked these degradation hotspots to intense human activities, primarily aquaculture zones and anchorages (Fig. 9). Specifically, stations B09 and B10 are adjacent to the Nanpu intertidal aquaculture area, while stations B15, B17, and B30 are adjacent to the Huanghua aquaculture area; additionally, stations B07, B08, and B13 are located near the Dagukou anchorage.

The mechanisms through which aquaculture impacts benthic macrofauna composition and distribution^{33,34} are well-understood. It enriches sediments with organic waste, driving hypoxia and toxic sulfide production, which is a key factor negatively correlated with benthic macrofauna health in our study³⁵. This process strongly selects for pollution-tolerant species and can lead to the dominance of smaller-bodied organisms. Similarly, anchorage activities cause chronic physical disturbance that destabilizes sediments and further stresses benthic communities.

The biological response was unambiguous. Degraded stations were dominated by polychaetes such as *Linopherus ambigua*, *Capitella capitata*, and *Sigambra bassi*, indicating that organic enrichment and disturbance cause particular polychaete species to increase while the number of echinoderms decreases^{36,37}. This shift toward AMBI Ecological Groups IV and V, and the concomitant loss of sensitive taxa, is a globally recognized signature of anthropogenic stress³⁸. Furthermore, such engineering activities have been shown to promote the miniaturization of benthic species³⁹, aligning with our findings.

In conclusion, the persistent degradation in northwestern Bohai Bay is a direct result of localized pressures from aquaculture and shipping. These activities override broader improvements and create a legacy of impact that requires targeted management, affirming the findings of previous regional studies that identified these areas as chronic pollution hotspots.

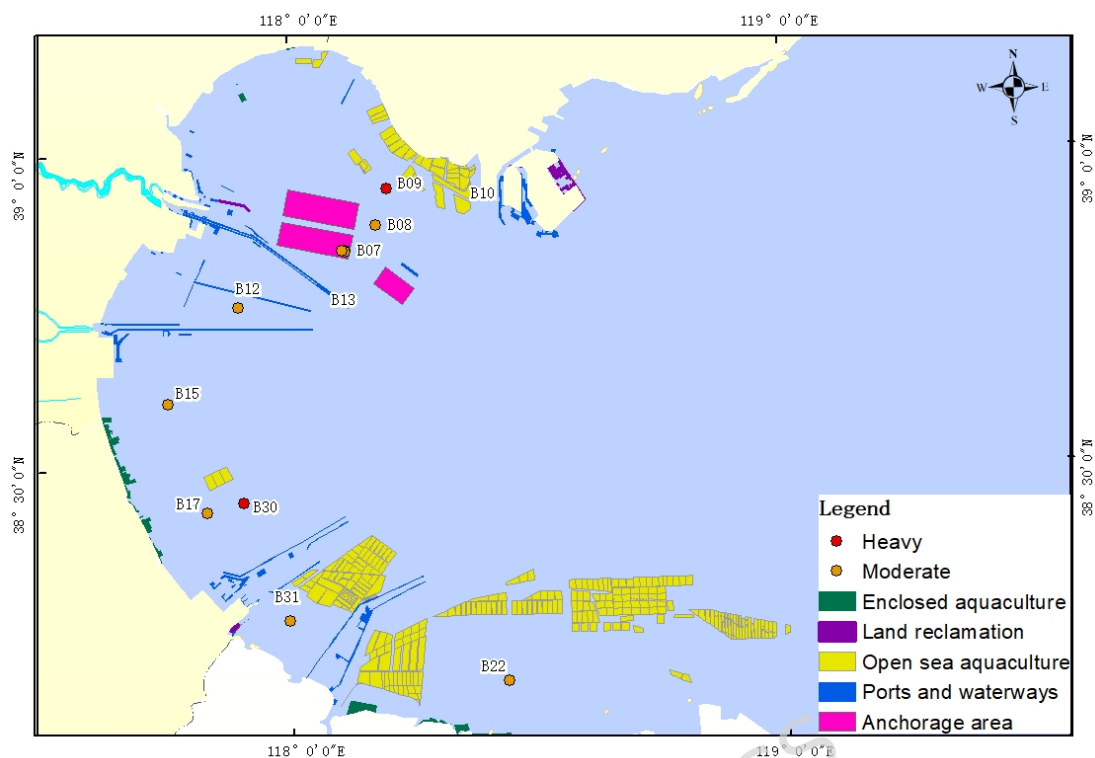


Figure 9. Comparison of moderate or bad stations with marine engineering construction in Bohai Bay.

The base map is the same as in Fig. 1.

4.3 Key Environmental Drivers and Their Interactive Effects

The correlation analysis identified sulfide in the sediment and active phosphate in the water as the two most significant environmental factors correlated with benthic ecological quality indices in Bohai Bay. The mechanisms behind these correlations are rooted in fundamental biogeochemical processes and are well-supported by existing literature.

Sulfide showed a strong negative correlation with all biological indices (density, H' , M-AMBI). It is a potent toxin produced by anaerobic bacteria in organically enriched, oxygen-depleted sediments. Our findings confirm its role as a primary stressor, consistent with studies from aquaculture zones where sulfide accumulation creates biological dead zones^{40,41}.

Conversely, phosphate correlated positively with H' and M-AMBI. As a key nutrient, it fuels phytoplankton growth^{42,43}. The subsequent sedimentation of this organic matter provides a vital food source for detritivorous macrofauna, supporting higher productivity and diversity⁴⁴.

Critically, these factors interact. Excess phosphate can initiate a negative feedback loop: it promotes algal blooms, increasing organic matter sinking to the seabed. This material is decomposed by microbes, which consume oxygen and create ideal conditions for sulfate-reducing bacteria to produce toxic sulfide. This synergy explains the severe degradation in areas receiving combined nutrient and organic waste inputs like aquaculture areas.

Overall, the benthic ecological condition in Bohai Bay reflects a balance between nutrient-driven food supply and sulfide-induced habitat stress, providing a mechanistic basis for the statistical patterns observed in both correlation and multivariate analyses.

4.4 Anthropogenic pressures as drivers of benthic ecological responses

The spatial patterns of benthic ecological quality in Bohai Bay reflect the imprint of long-term anthropogenic pressures acting through environmental modification. Although key environmental variables explain the immediate responses of macrobenthic communities, their spatial distribution and intensity are largely shaped by human activities.

Nearshore zones of Bohai Bay are characterized by intensive aquaculture, port operations, and anchorage activities. These pressures increase organic matter inputs and modify local hydrodynamic and sediment conditions, leading to reduced habitat stability. The persistence of relatively poor ecological status at several coastal stations suggests that localized anthropogenic pressures may constrain benthic recovery, even under broader regional improvements in environmental conditions. At the bay-wide scale, improvements in water-column conditions provide a favorable environmental background for general benthic recovery. These large-scale environmental gradients are closely associated with reductions in land-based pollution and regional management efforts. However, multivariate analysis shows that sedimentary stressors, particularly sulfide accumulation, impose a stronger and more immediate constraint on benthic ecological quality at the local scale. This difference helps explain why benthic degradation remains pronounced in high-pressure coastal zones, even where overall water quality has improved.

Together, these results indicate that benthic ecological responses in Bohai Bay are governed by a dual mechanism: regional-scale enhancement of water-column conditions that facilitates widespread recovery, and localized sedimentary degradation driven by persistent anthropogenic pressures that limits recovery at specific sites. Effective management therefore requires both continued bay-wide pollution control and targeted mitigation of sediment degradation in nearshore and high-impact areas. Nevertheless, several data-related limitations should be considered when interpreting these results. The present analysis was based on summer-only surveys, which may not fully capture seasonal variability in benthic macrofauna communities and environmental conditions. In addition, sampling stations were not completely consistent across all five years, which limits the ability to conduct strict long-term temporal trend analyses at individual sites. These data gaps may introduce uncertainty in resolving fine-scale temporal dynamics. However, the consistent sampling season, standardized field and laboratory protocols, and the use of multiple complementary benthic indices support the robustness of the observed spatial patterns and overall improvement trends at the bay-wide scale.

4.5 Implications for Management and Future Research

The results of this study provide practical implications for the management of benthic ecosystems in Bohai Bay and highlight priorities for future research. The overall improvement in benthic ecological quality confirms the effectiveness of large-scale governance measures in reducing diffuse pollution and improving general habitat conditions.

However, the persistence of relatively poor ecological status at several nearshore stations indicates that bay-wide pollution control alone is insufficient. Localized anthropogenic pressures, particularly from aquaculture and coastal engineering activities, continue to constrain benthic recovery in specific areas. These findings underscore the need for targeted management actions, including stricter control of organic waste discharges in aquaculture zones and integrated measures to reduce nutrient inputs that contribute to sediment deterioration.

From a methodological perspective, the present assessment relied on three benthic indices (H' , AMBI, and M-AMBI) derived from related community attributes. While these indices consistently captured major ecological patterns, they may not fully reflect all aspects of benthic ecosystem change. Future monitoring programs in Bohai Bay would benefit from incorporating indices based on different ecological principles, such as the ABC curve, the Benthic Opportunistic Polychaetes Amphipods index (BOPA), and the Feeding Evenness Index, to provide a more comprehensive evaluation of benthic habitat condition^{17,22,45}.

Looking forward, climate change pressures threaten to undermine recovery efforts. Projected increases in sea surface temperature will reduce oxygen solubility, exacerbating hypoxia, while more intense rainfall events will amplify nutrient runoff, thus intensifying the eutrophication-sulfide cycle⁴⁶. Ocean acidification may also impair the calcification processes of foundational mollusk species, potentially altering base community structure⁴⁷. Therefore, sustaining long-term ecological recovery demands that future research and adaptive management strategies integrate these climate drivers with local anthropogenic pressures through predictive modeling and enhanced monitoring to ensure

the long-term effectiveness of management strategies in a rapidly changing climate.

5. Conclusion

This study evaluated the benthic ecological quality of Bohai Bay using summer benthic macrofauna data collected from 2019 to 2023 and three widely applied indices (H' , AMBI, and M-AMBI). The assessment results showed that the three indices exhibited largely consistent spatial patterns and interannual trends, with H' and M-AMBI displaying stronger spatial discrimination and higher concordance with observed habitat conditions. Overall, the benthic ecological quality of Bohai Bay showed a clear improving trend during the five-year period, reflected by an increasing proportion of stations classified as slightly disturbed or undisturbed.

Spatially, offshore stations generally exhibited better ecological conditions than nearshore stations, while stations with moderate disturbance or poor ecological status were mainly associated with localized human activities, particularly aquaculture zones, anchorage areas, and marine engineering sites. Changes in community composition, characterized by the increased dominance of opportunistic polychaete species, further indicated the influence of sedimentary stress and physical disturbance on benthic ecological structure.

Correlation analysis and redundancy analysis revealed that benthic ecological quality in Bohai Bay is regulated by environmental drivers operating at different spatial scales. Active phosphate in the water column and sulfide content in sediments were identified as key factors associated with benthic ecological indices, with sedimentary stressors exerting a stronger and more immediate influence on local benthic community structure. These results highlight the importance of jointly considering water-column and sedimentary processes when assessing benthic ecological quality in semi-enclosed coastal systems.

Taken together, our findings suggest that the recent ecological improvements observed in Bohai Bay are broadly consistent with enhanced pollution control and integrated coastal management efforts, while also emphasizing the persistence of localized stressors that can constrain benthic recovery. In this context, the results highlight the need for continued reduction of land-based nutrient inputs, particularly phosphate, through improved wastewater treatment and watershed management; adaptive and site-specific regulation of aquaculture and anchorage activities to mitigate localized disturbance; enhanced monitoring and management of sediment quality with special attention to sulfide accumulation as a key ecological stressor; and targeted ecological restoration efforts in degraded nearshore and semi-enclosed areas.

Several limitations of this study should be acknowledged. The assessment was based exclusively on summer surveys, which capture periods of high biological activity but do not fully represent seasonal variability in benthic communities. In addition, interannual comparisons were constrained by partially non-identical sampling stations across years, which may introduce uncertainty in spatial-temporal interpretations. The absence of standardized pre-2019 benthic monitoring data further limited the ability to quantitatively compare pre- and post-restoration conditions, necessitating reliance on published studies as a reference baseline. These limitations do not invalidate the observed recovery trends but should be considered when interpreting the magnitude and drivers of ecological improvement.

Despite these constraints, this study provides a robust baseline for future benthic monitoring and underscores the importance of sustained, long-term observations to accurately track ecological trajectories and support ecosystem-based management in Bohai Bay and other semi-enclosed coastal systems.

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Declaration of Interest Statement

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

Author contributions

All authors contributed to the study's conception and design. Conceptualization, material preparation, data collection and methodology were performed by [Z.R.],[X.Y.] and [L.J.]. Data curation and methodology were also performed by [Z.R.],[X.Y.],[A.Y.],[Z.Y.] and [L.Z.]. The manuscript was revised by [Z.R.], [X.Y.]. The formal analysis and first draft of the manuscript were written by [Z.R.]. Reviewing and editing, validation, resources, project administration, and funding acquisition were performed by [L.W.]. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

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