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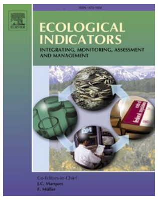

Assessment of macrobenthic communities of rocky intertidal zone from Zhejiang offshore islands with AZTI marine biotic index

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ABSTRACT

The structure of macrobenthic communities in island intertidal zones serves as an important indicator for assessing the health status of ecosystems. In recent years, the unregulated and extensive development and utilization of island resources in China, coupled with the continuous deterioration of the ecological environment in the surrounding marine areas of islands, have significantly impacted the health and stability of island ecosystems. Four investigations during spring (2018, 2021) and autumn (2017, 2020) of the Niushan Island intertidal zones and surrounding waters, located off the coast of Taizhou, Zhejiang, were conducted in this study. The purpose was to explore the external factors influencing the structure of intertidal macrobenthic communities and to assess the ecological status of the island. There was a clear vertical zonation observed in the distribution of the macrobenthic communities. During this survey, the eutrophication index improved, and the temperature, salinity, and suspended particulate matter exhibited seasonal fluctuations. Dissolved inorganic phosphorus and nitrate were the environmental factors that required special attention in coastal ecological monitoring, which significantly affected the distribution of the macrobenthic communities and was strongly correlated with the biotic indices and different ecological group biomasses. Most of the islands were classified as having a moderate status based on the Shannon-Wiener diversity index (H'). The AZTI Biotic Index (AMBI) and Multivariate AMBI (MAMBI) indicated that almost all islands were in good status, and the evaluation results demonstrated that the AMBI and MAMBI tended to overestimate the ecological status of the rocky intertidal zone. There was a significant correlation between the MAMBI and several traditional biological indices, suggesting that the MAMBI could be introduced as an evaluation indicator for the ecological status of rocky reefs. Nevertheless, it is important to consider the boundaries of different assessment levels.

1. Introduction

Due to the geographical isolation of islands, endemic and widespread species often occur, forming biodiversity hotspots that are key points for ecological conservation efforts (Friedlander et al., 2013). Islands near human activity areas are also subject to the unintentional introduction of non-native plants and animals (Hughes and Convey, 2010), which puts these islands at higher ecological risk compared to those located far away from the mainland. The coastal areas of islands are typically surrounded by nutrient-rich marine upwellings (Palumbi, 2003), which provide an abundant food supply, supporting marine organisms such as invertebrates and algae. These biological resources sustain the functioning of island intertidal ecosystems, influence the population dynamics of terrestrial animals through food chains, and constitute one of the nutrient sources between adjacent ecosystems (Roffler et al., 2023; Stapp and Polis, 2003). The rocky intertidal zone, as a transitional area between the marine and terrestrial island domains, forms complex habitats by its dynamic environment (Bertness et al., 2002). Meanwhile, as a concentrated point for the biotic communities in coastal habitats, it supports a wide range of plant and animal species (Ojeda and Muñoz, 1999) and serves as a habitat and foraging ground for birds and other predators (Irons et al., 1986).

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Islands serve as important targets for development and utilization, and endemic species often have difficulty adapting to changes in external conditions due to the difficulty of migration and restricted areas (Huang et al., 2008; Leclerc et al., 2020). In recent years, there has been an increasing trend in the development of uninhabited islands, where habitat fragmentation and vegetation destruction have long-term impacts on macroinvertebrate island communities (Steibl et al., 2021). Meanwhile, aquatic stresses caused by various types of human-induced pressures, including chemical emissions and the emergence of adverse conditions, such as eutrophication and hypoxia (Diaz, 2001), are increasingly evident (Chislock et al., 2013). These external changes contribute to the deterioration of environmental factors, leading to a decline in island biodiversity.

To control the pressures and impacts of external environment changes and human activities, different countries and organizations worldwide have enacted laws and regulations to set monitoring requirements (Vinagre et al., 2017). For instance, the European Union (EU) issued the “Water Framework Directive” (WFD, 2000), which consolidated the existing scattered regulations into a legal management framework. The WFD established a regulatory system that simultaneously protects the inland surface waters, transitional waters, coastal waters, and groundwater. Furthermore, in 2008, the EU issued the “Marine Strategy Framework Directive” (MSFD, 2008), which stipulates environmental monitoring factors for coastal waters, including phytoplankton, macroalgae, macroinvertebrates, and the physicochemical factors supporting biological communities.

Using parameters, such as the macrobenthic community structure, abundance, and biomass, can accurately reflect the status of the water column and the benthic environment, which is the focus of estuarine and coastal ecosystem assessments. Macroalgae respond to changes in biological processes (predation, competition, etc.), climate conditions, and nutrient concentration (Ferreira et al., 2014), thereby altering the dominant species in the community (Portugal et al., 2017). The characteristics of macroinvertebrates are the result of physicochemical factors and interactions of the biological community. Different invertebrate species have different levels of adaptability to environmental conditions and sensitivity to pollution (Patricio et al., 2009).

Ecological status assessment usually involves extracting complex species composition data into biotic indices. Traditional indices include species diversity, species richness, evenness (Boesch, 1973), and other types considering the different responses to environmental changes that categorize organisms into different ecological groups. (Borja et al., 2000) proposed an innovative biotic index for soft substrates, the AZTI Biotic Index (AMBI), based on the sensitivity of the macrobenthic communities to organic matter, and categorized species into five ecological groups (EGI-EGV) based on their sensitivity to pressure gradients. (Muxika et al., 2007) combined the AMBI with habitat richness and diversity to develop a multivariate AMBI (MAMBI) assessment tool. Garaffo et al. (2017a) tested the usability of the AMBI and MAMBI in hard coastal substrates. Hence, researchers developed a curiosity regarding the applicability of these indices in the intertidal island zones of China’s seas.

According to the “2017 Statistical Bulletin on Island Surveys” (China, 2018), there are more than 11,000 islands in China, with the East China Sea accounting for 59% of the total. The majority (57%) of these islands are located within 10 km of the mainland, and some surrounding waters of islands have suffered from water quality deterioration, which is particularly concentrated in the Hangzhou Bay and along the coast of Zhejiang Province. The main pollutants are inorganic nitrogen and phosphates, and 95.7% of the surveyed waters surrounding the islands were found to be eutrophic. Meanwhile, ecological restoration is also underway (Deng et al., 2022), with the establishment of special protection zones for aquatic species resources, restoration projects for coastal line improvement, and sludge and garbage clean-up. However, quantifying the restoration effects requires long-term monitoring of the ecological island environment and the expansion of new evaluation systems.

As the province with the most islands in China, Zhejiang emphasized the protection of islands. Our focus in this study was not on the strictly protected islands, but rather on the small islands located a few kilometers from the coastline, which could be easily reached by fishermen with simple boats. We wanted to determine whether their ecological conditions remained good, whether they were affected by pollution due to their proximity to the mainland, and whether it was necessary to include an additional biotic index. Niushan Island was used as an example in our study, while we aimed to explore the temporal and spatial changes of the rocky intertidal macrobenthic community and the surrounding marine environmental factors and attempted to analyze their relationship to identify the environmental factors that influence the community structures. Three types of biological indices, the H’, AMBI, and MAMBI were used to assess the ecological status of the intertidal zone, and. We analyzed the applicability of the indices and discussed the correlations among these indices.

2. Materials and methods

2.1. Study area

Niushan Island lies in Taizhou, Zhejiang, between 28°18′14″N-28°16′20″N latitude and 121°39′29″E-121°41′13″E longitude and is only 1.5 km away from the nearest shoreline. Although close to the shore, this area still experiences active seawater activities, influenced by the freshwater from the Oujiang and Jiaojiang rivers, as well as the Kuroshio tributaries. The coastal tides also have a significant impact, since the coastline in Zhejiang is characterized by long muddy tidal flats, with suspended particulate matter often exceeding 300 mg L^{-1}. Overall, the freshwater from the rivers and the warm currents of the Kuroshio mix in the coastal waters, making the seasonal and interannual variations complex (Zheng et al., 2018).

2.2. Sampling design

Nine sampling locations around Niushan Island, named S1-S9, were selected for this study, as shown in Fig. 1. The straight-line distance from each location to the shoreline can be found in the Supplemental file. Three parallel zones were selected for each sampling location, corresponding to the Upper, Mid, and Lower tidal zones divided by the natural tides. Within each tidal zone, two random sampling sites (25 cm × 25 cm) were designed to collect macrobenthos and algae. Monitoring was conducted in spring and autumn, with the first survey taking place in Autumn 2017 and Spring 2018, while the second survey occurred in Autumn 2020 and Spring 2021. There was a three-year interval between surveys of the same season (recorded as Au1, Au2, Sp1, and Sp2).

All samples (nine sites × three zones × two seasons × two years) were preserved in 5% neutral formaldehyde immediately after sampling. Macrobenthos samples were screened through a 0.5 mm mesh in the laboratory, in which macrobenthic were identified by physical characteristics, then counted and weighed (after removing surface water). Identifications were carried out as far as possible to the species or genus level. The species and genus were corrected and recorded according to the World Register of Marine Species (https://www.marinespecies.org/). Macroalgae samples were cleaned, identified, and weighed, and the recorded algal were corrected according to AlgaeBase (https://www.algaebase.org/). Data were standardized to the record of macrobenthic abundance (ind/m^2) and biomass (g/m^2), and algal biomass (g/m^2).

When the macrobenthos were sampled at each station, the surface seawater (0.5 m depth) was collected in 5 L Niskin bottles. Water temperature (°C) and salinity were measured *in situ* using an RBRconcerto CTD sensor (RBR Inc., Ontario, Canada), and the pH was measured with an Orion 868 pH meter. The suspended particulate matter (SPM) concentration was obtained by filtering 250 mL of water through a cellulose

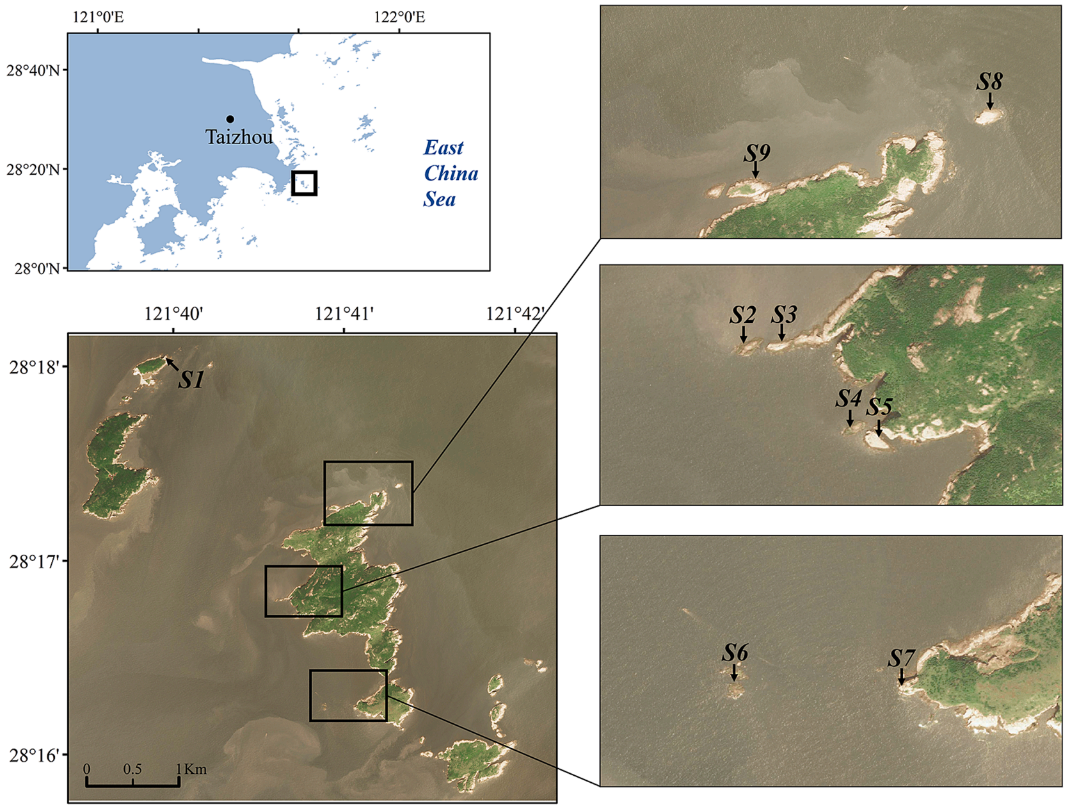

Fig. 1. Distribution of sampling locations S1-S9, around Niushan Island, along the coast of Taizhou, Zhejiang, China (S1: Shitang Sanya island; S2: Xiaochangzui island; S3: Changzui island; S4: Xiaojianbang island; S5: Jianbang island; S6: Niaowo rock; S7: Niaowodong island; S8: Fansuan rock; S9: Niujiaoti rock).

acetate membrane (0.45 mm) and was determined gravimetrically (drying at 70 °C) in triplicate. The dissolved oxygen (DO) concentration was determined using the Winkler method (Knap et al., 1996). The chemical oxygen demand (COD) was determined by potassium permanganate. Water samples for the determinations of dissolved inorganic nitrogen (DIN) included ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and dissolved inorganic phosphorus (DIP) were filtered through a 40 μm nylon pre-filter and then pressure filtered onto a Whatman GF/F glass-fiber filter (precombusted for 4 h at 400 °C). The filtrate was analyzed by segmented flow analysis (SFA) (Koroleff, 1983).

2.3. *Data analysis*

The abundance and biomass of the macrobenthic communities, the biomass of macroalgae, and environmental factors in the waters near each of the nine sampling sites were counted (S1-S9), which were the main results obtained from our survey during Au1, Au2, Sp1, and Sp2. Regarding the environmental factors, the two-way analysis of variance (ANOVA) (Scheirer-Ray-Hare in R) was utilized to analyze the factors that changed over the years and seasons. Furthermore, the Mann-Whitney *U* test was used to determine if there were any differences between the factors from different years or seasons.

Regarding the changes in the macrobenthic community, permutational multivariate analysis of variance (PERMANOVA) (Adonis in R) was utilized to analyze the effects of the factors during years, seasons, and tidal zones on the community structure. The abundance data of the macrobenthic communities was square root-transformed, the Bray-Curtis similarity coefficients were calculated, a similarity matrix for cluster analysis was constructed, and non-metric multidimensional scaling (NMDS) was performed in PRIMER V5.0. The similarity percentage program (SIMPER) was utilized to compare the average contributions of different species to the within-group similarity and between-group dissimilarity (Liu et al., 2023). Environmental factors play an important role in supporting communities, and Canoco V5.0 redundancy analysis (RDA) was used to analyze the explanatory power of factors on the macrobenthic abundance. The angle between the variables of the RDA ordination plot indicates the relationship between them (Peng and Li, 2021).

The method for determining the dominant species of macrobenthic communities were calculated by macroinvertebrates abundance (Zhang et al., 2016). To assess the status of the rocky intertidal ecosystem, PRIMER V5.0 and AMBI V6.0 were used to calculate the Shannon-Wiener diversity index, Margalef's species richness index, and Pielou's evenness index (H'_{Div}, D_{Div}, J_{Div}), and the AMBI of each sampling location based on the macrobenthic abundance. For species that could not be determined for ecological groups through the AMBI software, their ecological groups were replaced by species in the same genus with similar ecological habits. The reference conditions for MAMBI were set by the commonly method in intertidal surveys as follows. High status: AMBI = 0, and increased the highest diversity and richness by 15% during the survey. Bad status: AMBI = 6, diversity and richness = 0 (Liu et al., 2013).

The AMBI was classified according to the count 0–6, with higher value indicating poorer ecological status. The H' and MAMBI also had a different criterion for division; according to this criterion the ecological status was divided into five levels (Table 1).

Table 1
Ecological status by H', AMBI, and MAMBI indicate different criterion levels, reference (Ni et al., 2019).

	H'	AMBI	MAMBI
High	>4	<1.2	>0.77
Good	3–4	1.2–3.3	0.53–0.77
Moderate	2–3	3.3–5.0	0.38–0.53
Poor	1–2	5.0–6.0	0.20–0.38
Bad	0–1	>6.0	<0.20

Diversity indices of macrobenthic and macroalgae biomass were calculated based on the biomasses (H'$_{Bio}$, H'$_{Alg}$). The correlation between these indices was analyzed using the Spearman correlation (Spearman in R) to assess the applicability of the AMBI and MAMBI indices in the rocky habitat.

3. Results

3.1. Environmental parameters

Details of the coastal marine environmental parameters monitored in this survey are shown in Table 2. The two-way ANOVA (year and season) revealed that a single variable significantly influenced the temperature, with a notable increase in spring compared to autumn, and an obvious increase during three years. Both seawater salinity and the SPM were affected by seasonal changes, and it was obvious that the salinity was higher in spring compared to autumn, while the SPM was significantly lower in spring. From the perspective of the environmental factors such as temperature, salinity, and SPM, the waters near Niushan Island exhibited high temperature, high salinity, and low SPM in spring, whereas they showed a relatively low temperature, low salinity, and high SPM in autumn, which were related to the hydrodynamic conditions in the survey area. NO_3^-, NO_2^-, and DIP showed a significant decrease over the past three years.

The eutrophication index as an indicator of seawater health status revealed a significant interannual and seasonal variation. An obvious decrease was detected over the past three year, and a significant difference was observed between spring and autumn within the same year. Overall, the eutrophication index changed from severe and moderate eutrophication to mild eutrophication in four surveys, and none of the sampling sites showed eutrophication in the final survey.

3.2. Macrobenthos composition

A total of 58 macroinvertebrates species and 15 macroalgae species were recorded in the survey. There were 11 groups of macroinvertebrates found in the benthic habitat, including Nemertea (Phylum); Gastropoda, Bivalvia, Anthozoa, Polychaeta, Polyplacophora, Echinoidea (Class); Cirripedia (Subclass); and Amphipoda, Isopoda, Decapoda (Order). Among these, Gastropoda, Decapoda, Bivalvia, and Anthozoa were the most commonly observed taxa in the survey.

Table 2
Environmental factors along the coastal waters of Niushan Island in each survey.

Parameters	Au1	Au2	Sp1	Sp2
Temperature (°C)	16.16 ± 0.76	19.33 ± 0.16	20.34 ± 0.72	21.28 ± 0.38
Salinity	25.01 ± 0.2	23.42 ± 0.13	28.46 ± 0.07	29.53 ± 0.14
SPM (mg L^{-1})	230.36 ± 122.91	76.57 ± 19.06	14.06 ± 7.08	10.37 ± 1.12
DO (mg L^{-1})	8.48 ± 0.1	7.79 ± 0.05	7.84 ± 0.19	7.71 ± 0.14
COD (mg L^{-1})	1.159 ± 0.374	0.685 ± 0.147	0.816 ± 0.244	0.862 ± 0.245
pH	7.93 ± 0.02	8.11 ± 0.01	8.07 ± 0.02	8.12 ± 0.01
DIP (mg L^{-1})	0.046 ± 0.001	0.036 ± 0.003	0.025 ± 0.004	0.011 ± 0.001
NO_2^- (mg L^{-1})	0.003 ± 0.001	0.004 ± 0.001	0.021 ± 0.002	0.009 ± 0.001
NH_4^+ (mg L^{-1})	0.076 ± 0.012	0.009 ± 0.005	0.039 ± 0.022	0.037 ± 0.006
NO_3^- (mg L^{-1})	0.637 ± 0.016	0.48 ± 0.036	0.401 ± 0.048	0.156 ± 0.007
EI*	8.47 ± 2.99	2.63 ± 0.78	2.10 ± 0.77	0.40 ± 0.12

*: The eutrophication index (EI) of seawater was calculated from chemical oxygen demand (COD), dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP) (Warwick and Somerfield, 2015): $EI = COD \times DIN \times DIP \times 10^6/4500$.

The average abundance of macrobenthic organisms in the intertidal zone of Niushan Island was 353.19 ± 124.78 ind/m^2 with a range of 0–1272 ind/m^2, and the average biomass was 928.52 ± 289.14 g/m^2 with a range of 0–2889.4 g/m^2. Among the taxa, Anthozoa had the highest abundance and biomass, especially *Tetraclita squamosa*. Gastropoda was the second most abundant taxa, followed by Bivalvia. The records of the species, and the abundance biomasses of different macrobenthos taxa in the surveys can be found in the Supplemental file.

Tetraclita squamosa, *Mytilisepta virgata*, *Reishia clavigera*, *Echinolittorina radiata,* and *Siphonaria japonica* were the dominant species in the Au1 and Sp1 surveys, and these species except *S. japonica* were also the dominant species in the Au2 and Sp2 surveys. Over the three years, the number of species increased from 38 to 54, which was mainly observed in Decapoda, Amphipoda, and Anthozoa. During the survey period, there were slightly more species observed in spring compared to autumn, and the rare *Echinoidea* sp. taxa was only observed during the spring survey.

The 15 macroalgae species could be classified into four phyla, with Rhodophyta as the main group in the survey area, while Phaeophyceae and Chlorophyta had fewer species, and Cyanobacteria (*Lyngbya* sp.) appeared only in Au1. The number of macroalgae species decreased over the three years, and the same variation pattern was not seen in the macrobenthic communities between spring and autumn. The highest number of macroalgae was collected during Sp1. This may be due to limitations of the sampling area, which could not cover all algae species within the Niushan Island intertidal zones.

The differences in the structure of the macrobenthic communities, as shown in Table 3, are primarily reflected in the distribution of the different tidal zones, as analyzed through the PERMANOVA test considering the year, season, and tidal zone factors. Besides, no biological samples were collected at the S7 high tide in Sp2. Cluster analysis using Bray-Curtis similarity at 22.72% divided the rocky intertidal zones into two groups: the upper-tide zone and the mid-lower tide zone (Fig. 2). The results of the NMDS ordination plot (2D Stress: 0.009) were the same as the clustering dendrogram. Both statistical and visual analyses corroborated each other in that macrobenthic communities had distinct distribution patterns across tidal zones, with no significant changes observed in terms of year and season.

The SIMPER analysis of different tidal zones revealed that the average similarity between communities in the upper-tide zone was 60.80%, which was higher than the mid-tide zone (58.09%) and the lower-tide zone (40.19%). Among them, *Littorina brevicula* and *Echinolittorina radiata* contributed significantly to the average similarity in the upper-tide zone, with the contribution rate of *E. radiata* exceeding 50%. The top three contributors to the similarity in the mid- and low-tide zones were *Tetraclita squamosa*, *Reishia clavigera*, and *Mytilisepta virgata*, although with different rates of contribution. SIMPER analysis of the macrobenthic community structure in different tidal zones indicated an average dissimilarity of 97.88% between the upper- and the mid-tide zones, 99.01% between the upper- and the lower-tide zones, and 60.33% between the mid- and the lower-tide zones. The contributions of different species to the average dissimilarity between tidal zones are shown in the Supplemental file.

The tidal zones were not only different in species composition. The

Table 3
PERMANOVA analysis for three factors (year, season, and tidal) based on macrobenthic abundance.

Factors	DF	Sum Of Squares	R^2	F	p
Year	1	0.473	0.01357	3.1043	0.0221
Season	1	0.348	0.00999	2.2850	0.0601
Tital	2	18.357	0.52624	60.1988	0.0001
Residual	103	15.704	0.45020		
Total	107	34.883	1.0000		

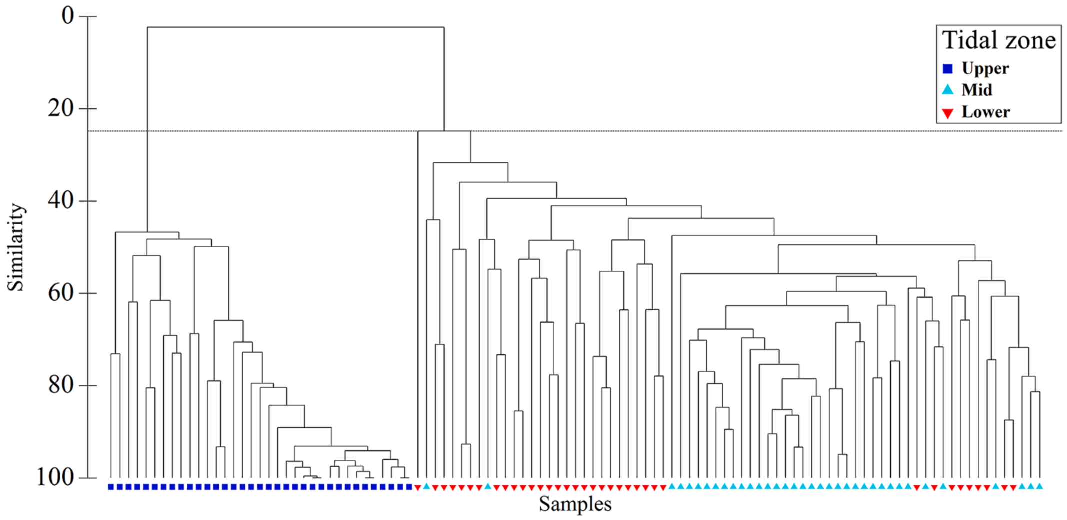

Fig. 2. Clustering analysis of macrobenthic communities in different tidal zones of Niushan Island.

Kruskal-Wallis test was used to determine whether there was a significant difference in macrobenthic abundance, biomass, and algal biomass between different tidal zones. The pairwise comparison results indicated significant differences in the macrobenthic abundance and biomass between each tidal zone, in the order of mid- > lower- > upper-tide zone. There was no significant difference in the algal biomass between the upper- and the mid-tide zone, but that of the lower-tide zone was significantly higher than those of the others (Fig. 3).

3.3. *Ecological indicator*

Another focus of this survey was to examine whether the distance to the islands and human activities influenced ecological health. In fact, there was no significant correlation (Spearman, $p > 0.05$) between the macrobenthic biotic indices (H'_{Div}, J_{Div}, and D_{Div}) and the distance from the shore at each sampling site. There was a significant difference in the richness index between the two autumn surveys. There were no significant changes in the diversity index and evenness index across the seasons and years (Fig. 4).

During sample processing, it was noted that algae were not collected in every tidal zone, and the distribution of algal biomass varied among the different tidal zones (Fig. 3c). As a result, the indices calculated from the algal biomass, such as the evenness index and richness index, could not be tested for correlation. Only the diversity index that was calculated based on both macrobenthic biomass and algal biomass (H'_{Bio} and H'_{Alg}) could be analyzed with other indices. The correlation analysis was conducted using the coefficient and p-values (Spearman in R), and only significant correlations ($p < 0.05$) are shown in the Fig. 5.

Among them, the biotic indices calculated from the abundance showed a relatively high correlation with the MAMBI, particularly in the richness index and diversity index; however, these correlations were not reflected in the AMBI. In general, there was a significant positive correlation between the MAMBI and biotic indices calculated from the abundance and biomass of the macrobenthic communities, with several indices showing correlations greater than 0.8.

It is worth noting that different biotic indices can be used to classify the ecological status into different levels. For better representation, a heatmap of the ecological status for each sampling site based on the biotic index was shown in Fig. 6. Although there was a high correlation between the MAMBI and H', the heatmap did not reflect consistent change. Overall, the results of the AMBI and MAMBI provided a better assessment of the quality of the reef habitat compared to the H'.

3.4. *Relationships with environmental factors*

In this study, there was a significant negative correlation between NO_3^- and the H' and MAMBI ($p < 0.05$). The RDA ordination plot of all macrobenthic composition and environmental factor variables during the survey period and the first two axes explained 53.94% of the species variation and 23.08% of the species-environment correlation. Monte Carlo significance tests (9999 iterations) indicated that environmental factors had different impacts, among which NO_3^- ($F = 2.5$, $p = 0.002$) and DIP ($F = 2.0$, $p = 0.02$) reached significance levels and were identified as the primary environmental factors affecting the distribution characteristics of the macrobenthic communities (Fig. 7A).

Furthermore, the correlations between the abundances and biomasses of macrobenthic communities in different ecological groups (EG) of the AMBI and the environmental factors were analyzed. A significant

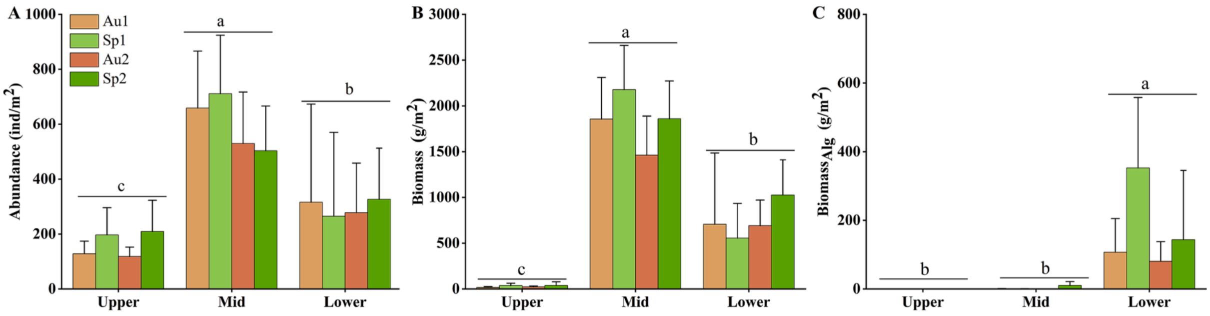

Fig. 3. Macrobenthic abundance (A), biomass (B), and algal biomass (C) in different tidal zones. Letters represent significant differences between treatments ($p < 0.05$).

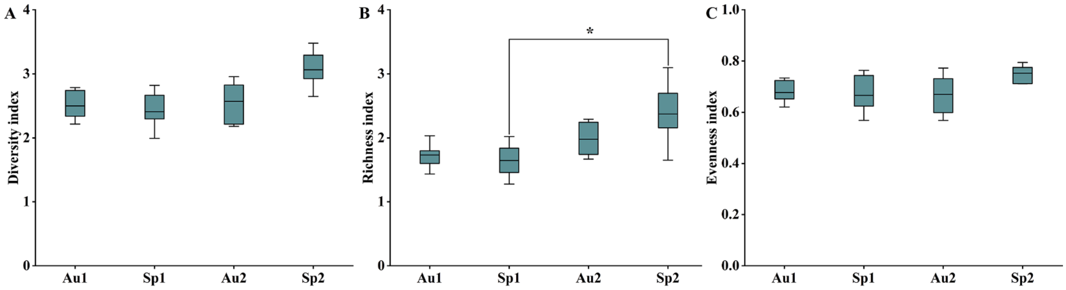

Fig. 4. Intertidal Shannon-Wiener diversity index (A), Margalef's richness index (B), and Pielou's evenness index (C) of Niushan Island.

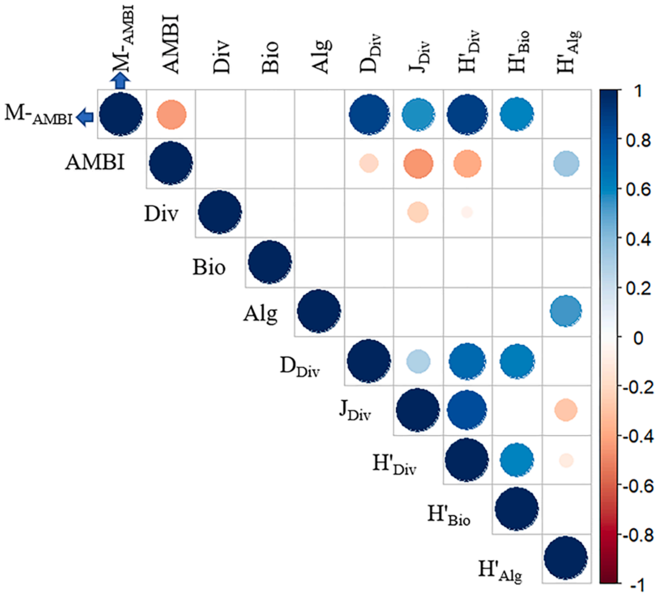

Fig. 5. Correlation between the biotic indices of Niushan Island intertidal zone (Block color represents correlation positive and negative; block size represents correlation size).

correlation was observed between DIP (F = 5.5, p = 0.008) and the biomass (Fig. 7B), a negative correlation was seen with the EGI and EGII biomasses, and a positive correlation was shown with the EGIII biomass.

4. Discussion

4.1. Impacts of environments on macrobenthic communities

A three-year survey was conducted on the intertidal macrobenthic communities in the island intertidal zone of the nearshore waters of Zhejiang, China. Undoubtedly, this study acquired data on the abundance, biomass, and traditional biotic indices of macrobenthic communities. The biomass and abundance of *T. squamosa*, which is a common species in the rocky reef habitat (Grabowski et al., 2005), were the highest in the intertidal zone among them. Rocky reef habitats are widely distributed along the elongated coastlines, where the macrobenthic community structure varies temporally and spatially (Beuchel et al., 2006; Chelchowski et al., 2022). The discovery of temporal differences requires long-term observations over the years, as changes in environmental parameters drive interannual and seasonal variations in the community structure of macrobenthos. Meanwhile, researchers could invariably observe differences between the intertidal zones, with clear vertical zonation, and our findings confirmed this general result. Significant differences in the macrobenthic community structure between different intertidal zones were observed through PERMANOVA, which was related to natural tidal-induced differences in the physicochemical environments (Degraer et al., 1999). These differences are the

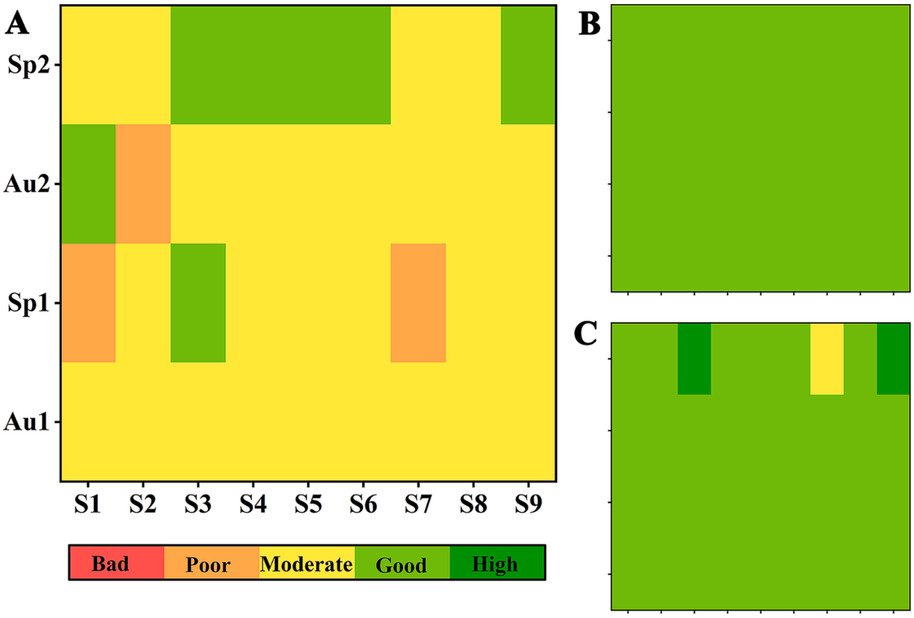

Fig. 6. Heatmap of the ecological environment status assessment by H' (A), AMBI (B), and MAMBI (C).

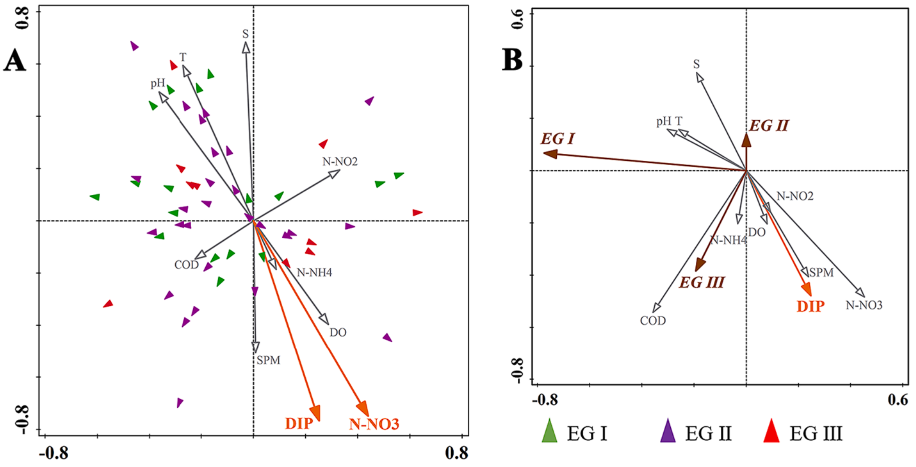

Fig. 7. Relationship between the macrobenthic abundance (A) and biomasses of different ecological groups (B) with environmental factors.

results of surveys on the macrobenthic community, where algae are also important constituents on rocky shores. As shown in the statistical graph of the algal biomass in the surveyed intertidal zones, macroalgae were concentrated mainly in the low-tidal zone, which was determined by its growth characteristics (Mann, 1973).

In addition to the vertical distribution of the intertidal zones, external environmental factor changes are also important factors influencing community variations (Carcedo et al., 2015). According to long-term monitoring in the Yellow Sea and East China Sea (Li et al., 2016), in spring, the coastal waters are influenced by warm currents formed by the Kuroshio branch, resulting in high temperature, high salinity, and low suspended sediment. Historical monitoring of the warm current's influence extends northward to the Yangtze River Estuary. In summer, the freshwater of the Yangtze River can extend to the Taiwan Strait (Liu et al., 2007). In autumn and winter, the Kuroshio moves northward, invading the Yellow Sea. Meanwhile, the water beneath the Kuroshio rises, forming the coastal flow and transporting more sediment (related to the existence of muddy tidal flats along the Zhejiang), which becomes the main source of suspended sediments in the East China Sea. The coastal waters exhibit low temperature, low salinity, and high suspended sediment.

The environmental factors monitored in this survey are consistent with the patterns summarized by Li et al. (2016). The influence of the sea currents in the Niushan Island area (where the coastal water depth is 10–15 m) is straightforward, and is primarily influenced by the coastal current, especially in autumn and winter when the coastal current intensifies. Although the environmental factors in coastal waters are heavily influenced by external factors such as thermal discharge and river runoff, the temperature, salinity, and suspended sediment changes observed in the Niushan Island waters correspond to the overall trends in the East China Sea caused by the Kuroshio warm current and the coastal current (Wang et al., 2020).

The summary of these changing patterns is of great significance for predicting interannual and seasonal variations in certain species sensitive to salinity, temperature, and suspended sediment changes. At present, this is especially true with the increasing occurrence of abnormal weather phenomena such as El Nino and heatwaves (Meehl et al., 2000). The changes in the macroinvertebrate communities have lag and persistence characteristics (Neto et al., 2010). Therefore, monitoring the changes in sensitive organisms can assist in understanding the climate and environmental factors, and predict the trends in community changes after abnormal weather, thus avoiding the aggregation and outbreak of harmful organisms (Epstein, 1999). The development of such monitoring techniques still requires long-term, extensive surveys of macrobenthic communities in the field and corresponding environmental factor monitoring.

In this study, the Pearson's test did not find any correlation between the abundance, macrobenthic biomass, and environmental factors, which differs from surveys in marine areas, where the abundance and biomass of organisms are often influenced by factors such as water depth and chlorophyll (Karakassis and Eleftheriou, 1997). The factors influencing the organisms of the intertidal zone are complex, and different wave impacts lead to different community structures (McQuaid and Branch, 1985). Regarding the relationship between environmental factors and the macrobenthic composition, RDA indicated that factors such as temperature, salinity, and suspended sediment, which varied with seasonal patterns, had no significant impact on species composition. However, NO_3^- and DIP showed a significant correlation with the macrobenthic community distribution. Nitrogen and phosphorus nutrients play an important role in the community structure of invertebrates (Ortega Cisneros et al., 2011).

4.2. *Availability of biotic indices*

According to the species lists from the AMBI, 58 macroinvertebrates species were divided into three ecological groups in this study. EGI consists of 23 species, which are mostly rare species, such as Polyplacophora, most of Isopoda, Echinoidea, and Anthozoa, carnivorous Decapoda, and rare species in Gastropoda and Bivalvia, or species that only appear in unpolluted environments, such as *Actinia equina* and *Diadumene lineate* (Rueda et al., 2021). EGII includes 26 species, including several Polychaeta, like *Polynoidae* sp., which are low-density distributed, and the common species that gather in the high-tidal zone, such as *Littoraria articulata* and *Littorina brevicula*. Additionally, *Tetraclita* sp., which has a planktonic feeding mode, can experience population outbreaks under slight disturbances due to their short life cycle, strong reproductive ability, and rapid colonization in vacant habitats (Farrell, 1991). EGIII consists of nine species that appear in large numbers when the environment becomes unbalanced, such as *Pseudonereis variegata* and *Nemertites sp*. These organisms mainly feed on surface sediments (Hutchings, 1998), and the high suspended matter content in the surveyed area provides food source; therefore, they are mostly found in the mid- and lower-tidal zones. This can provide a reference for rocky reef organisms to be classified by the AMBI as ecological groups for other surveys.

In general, the classification catalog developed for soft sediment habitats is also applicable to certain rocky reef invertebrates, including macroinvertebrates with a wide distribution and survivability in various substrate environments, such as Amphipoda (e.g., *Apohyale grandicornis*), Polychaeta (e.g., *Aglaophamus sinensis*), Bivalvia (e.g., *Barbatia obliquata* and *Placuna placenta*), and Gastropoda (e.g., *Mitrella albuginosa*). These organisms are also widely distributed in mudflats, and the taxonomic grouping results are reliable. However, it is worth noting that most Gastropoda and Bivalvia species in this study were still attached to rocky reefs, such as the family *Reishia*, *Cellana toreuma*, *Nipponacmea schrenckii*, *Patelloida pygmaea*, and Cirripedia (family *Balanidae*). These sessile animals are not included in the AMBI biological catalog as they require specific substrate environments and are less likely to survive in soft sediment habitats (Liao et al., 2007). These organisms often have calcareous opercula and hard shells, demonstrating strong adaptability to environmental changes (Gazeau et al., 2013). However, from the correlation between the biomass of different ecological groups and environmental factors, EGI and EGII showed a negative correlation with DIP, EGIII showed positive correlation with DIP, and the delineation of the AMBI groups retained a certain level of credibility (Wu et al., 2022). In subsequent studies, it is important to pay attention to the organic pollution status and sediment quality indices to better understand the applicability of biological indices (Sun et al., 2018).

It was surprising that no EGIV or EGV were found in the sampling at Niushan Island, since the AMBI was originally developed to assess the community structure of soft sediment habitats. EGIV mainly consists of small Polychaeta that feed on subsurface sediments, while EGV is mostly a detrivorous group that prefers reducing environments. These feeding habits and specific environmental preferences could not provide by common rocky reef habitats (Hutchings, 1998). Additionally, the AMBI assesses the impact of organic pollution on the macrobenthic community structure, which is hard to carry out in rocky substrate environments that are difficult to attach muddy sediments. Since rocky reef organisms are mostly carnivores or planktophagous belonging to EGI and EGII, the AMBI calculation for rocky reef habitats tends to indicate a good and high ecological status (Warwick and Somerfield, 2015). Although the MAMBI incorporates H' to consider the community structure, the definition of a "High" status level in the MAMBI determines the assessment results. High status level referred to Liu et al. (2013) in this study; however, this value requires adjustment according to different ecological environments.

In other studies, Garaffo et al. (2017b) found that the AMBI is more suitable for assessing the quality of bivalve bed habitats and serves as a powerful management tool for hard substrate monitoring projects. Additionally, the MAMBI overestimates the ecological status of rocky reefs, which is consistent with our assessment results at Niushan Island. Santibañez-Aguascalientes et al. (2023) demonstrated through historical data from a wide range of areas that both the AMBI and MAMBI showed corresponding changes to human and natural disturbances, and these tools are suitable for the large-scale and long-term assessment of ecological environmental status that can be applied to coastal habitats.

5. Conclusion

In this study, the focus was on the macrobenthic community structure in the intertidal zone of Niushan Island and the environmental factors within the marine area. This was the first investigation of the biological community and the water conditions on this island, and the first analysis of the applicability of the AMBI in the rocky reef intertidal zone. During the survey, the seasonal variations of the environmental factors were consistent with the long-term monitoring data from the East China Sea. This was characterized by high temperature, high salinity, and low suspended solids in spring, and low temperature, low salinity, and low suspended solids in autumn. There was a gradual decrease in the eutrophication levels, transitioning from highly eutrophic to non-eutrophic, which indicated an improving water quality. Additionally, the RDA ordination analysis showed that NO_3^- and DIP were the main environmental factors influencing the distribution of the macrobenthic community, which need to emphasize monitoring in subsequent offshore ecological assessments.

Compared to H', both the AMBI and MAMBI generally overestimated the ecological status of the rocky intertidal zone. Although richness and diversity indices calculated from abundance showed a significant positive correlation with the MAMBI, the introduction of soft-bottom sediment biological indices in the ecological assessment of the island's rocky intertidal zone requires a reconsideration of the boundary value setting of the different ecological statuses.

CRediT authorship contribution statement

Sujie Tian: Formal analysis, Writing – original draft, Writing – review & editing. **Yibo Liao:** Conceptualization, Writing – review & editing, Funding acquisition. **Yanbin Tang:** Methodology. **Qinghe Liu:** Methodology. **Rongliang Zhang:** Formal analysis. **Lu Shou:** Funding acquisition. **Jiangning Zeng:** Project administration.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110923.

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