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Molecular diversity of foraminiferal eDNA in sediments and their correlations with environmental factors from the Yellow Sea

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ABSTRACT

Foraminiferal environmental DNA (eDNA) is an efficient and reliable indicator of environmental changes. Here, we investigated the foraminiferal diversity of 25 stations (33–36°N) in the Yellow Sea based on eDNA and presented the first assessment on the correlations between foraminifera diversity and environmental conditions in the region. A total of 71 sediment samples were collected and local environmental parameters were measured. The foraminiferal eDNA was extracted from the samples and part of the small subunit (SSU) rRNA gene was amplified from the samples. After high–throughput sequencing, 1,701,899 amplified reads were detected and clustered into 426 operational taxonomic units (OTUs). The benthic foraminiferal eDNA community diversity and group diversity showed significant positive correlations with water depth and total inorganic carbon (TIC) in sediments, and significant negative correlations with temperature and total organic carbon (TOC) in sediments. However, in terms of the correlation between relative abundance and environmental factors, there were wide variations from species to species. In this work, planktic foraminiferal eDNA was detected in the sediments, and its OTUs number could reflect the changes in ocean currents and upper layer water masses to a certain extent. This is the first study on relationship of foraminiferal eDNA and environmental factors in the Yellow Sea shelf region of the western Pacific Ocean, and it demonstrates that community parameters in foraminiferal eDNA could be a valuable proxy for environmental changes in the shelf sea.

1. Introduction

Foraminifera are widespread and numerous but are sensitive to environmental changes, thus they are a good indicator for environmental monitoring and paleoenvironmental reconstruction (Ganugapenta et al., 2018; Gooday, 2003; ter Kuile et al., 1989). With the development of DNA sequencing technology, next–generation sequencing (NGS) metabarcoding with higher efficiency and lower cost has been gradually applied in foraminifera ecological investigations (Pawlowski et al., 2014b). Foraminiferal environmental DNA (eDNA) and environmental RNA (eRNA) in sediments have been successfully used to monitor the environmental impacts of fish farming (Pochon et al., 2015; He et al., 2019) and oil drilling (Laroche et al., 2016; Frontalini et al., 2020), and adopted in various other surveys in deep–sea environments such as seamounts, deep sea trenches and marine blue holes (Cordier et al., 2019; Li et al., 2020; Shi et al., 2020; Shi et al., 2021). It has been proved that the distribution of planktic foraminifera DNA in surface sediments could reflect the community structure (Morard et al., 2017). After more than two decades of research, foraminifera gene sequence database has become more comprehensive (Pawlowski et al., 2014a). Compared with classical morphological methods, molecular biological methods can be more realistically used to monitor foraminifera diversity in the environment (Cavaliere et al., 2021; Pawlowski et al., 2014a).

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The Yellow Sea, which is located in eastern China, is a semi–enclosed continental shelf shallow sea divided into the northern and southern parts by the tip of the Shandong Peninsula. It is an important channel for the spread of terrigenous materials from East Asia to the Western Pacific and is one of the main areas for the development of marine resources (Wang et al., 1980b; Fan et al., 2011). The Yellow Sea Cold Water Mass, one of the most important hydrological features in the region, is a low–temperature water body located under the seasonal thermocline. The thermocline formed in April, peaked in August, and gradually disappeared until November. In summer, the depth of thermocline is about 20 m, and the uniform cold-water range is below 40 m (Hu and Wang, 2004; Yu et al., 2006). In shelf seas, the abundance and distribution of organisms are easily influenced by factors such as water depth, sedimentary types and nutrient salts. Foraminifera play a key role in the reconstruction of the shelf environment (Langezaal et al., 2006). At the end of the last century, a number of investigations were carried out concerning the distribution of foraminifera in the Yellow Sea based on classical morphology (Cheng et al., 1999; Wang et al., 1980a). In order to reconstruct the past marine environment, the response characteristics of foraminifera to the environment must be clearly defined. However, the benthic foraminiferal eDNA distribution in the Yellow Sea remained unknown.

The aims of this study in the Yellow Sea are: 1) investigate the diversity, community composition and distribution of foraminifera using a molecular approach; and 2) clarify the relationships between the distribution of foraminifera and environmental factors, and explore whether the foraminiferal eDNA in sediments can indicate the local environmental changes.

2. Materials and methods

2.1. Sample collection

In situ sampling was done in the period between 11 and 19 June 2019 in the Yellow Sea (33°00′N–36°00′N, 119°49′E–124°00′E) (Fig. 1, Supplementary Material Table S1). The SBE 911plus CTD (Sea–Bird Scientific, USA) was used to collect seawater samples and record temperature, salinity and water depth at the sampling stations. A range of physical–chemical parameters, namely dissolved oxygen (DO), pH, chlorophyll *a* (Chl–a), and various nutrients (NO_2^-, NO_3^-, NH_4^+, DTN, DTP, DSi), were measured in the seawater samples on–site. A box corer of 0.1 m^2 was used to collect sediment samples at the stations. The undisturbed surface sediment of about 2 cm was scraped with a sampling spoon and put into a sealing bag, which was stored in a –80°C freezer, and brought back to the laboratory for further processing. The sediment particle sizes were measured by a laser diffraction particle size analyzer (Cilas1190l, France), and the particle diameter corresponding to the 50% cumulative particle size distribution in the samples was recorded as the median particle size (D_{50}). Total carbon (TC) and total organic carbon (TOC) in the sediments were measured by the Vario MACRO Cube elemental analyzer (Elementar, Germany) and the difference between TC and TOC yielded total inorganic carbon (TIC).

2.2. Environmental DNA extraction

After the sediment samples were brought back to the laboratory, total environmental DNA was extracted from the samples, and 3 different locations were selected for each sample as replicates to avoid errors caused by spatial heterogeneity. Samples were extracted using the DNeasy PowerSoil Kit (Qiagen, Germany). Each sample was weighed about 0.25 g to obtain 100 μL environmental DNA solution. Except for the cell lysis process that extended the vortex oscillation time to 40 min (Lecroq et al., 2011), the other processes were carried out according to the instructions provided by the kit.

2.3. PCR amplification

The 37F hypervariable region of the foraminiferal SSU rDNA that

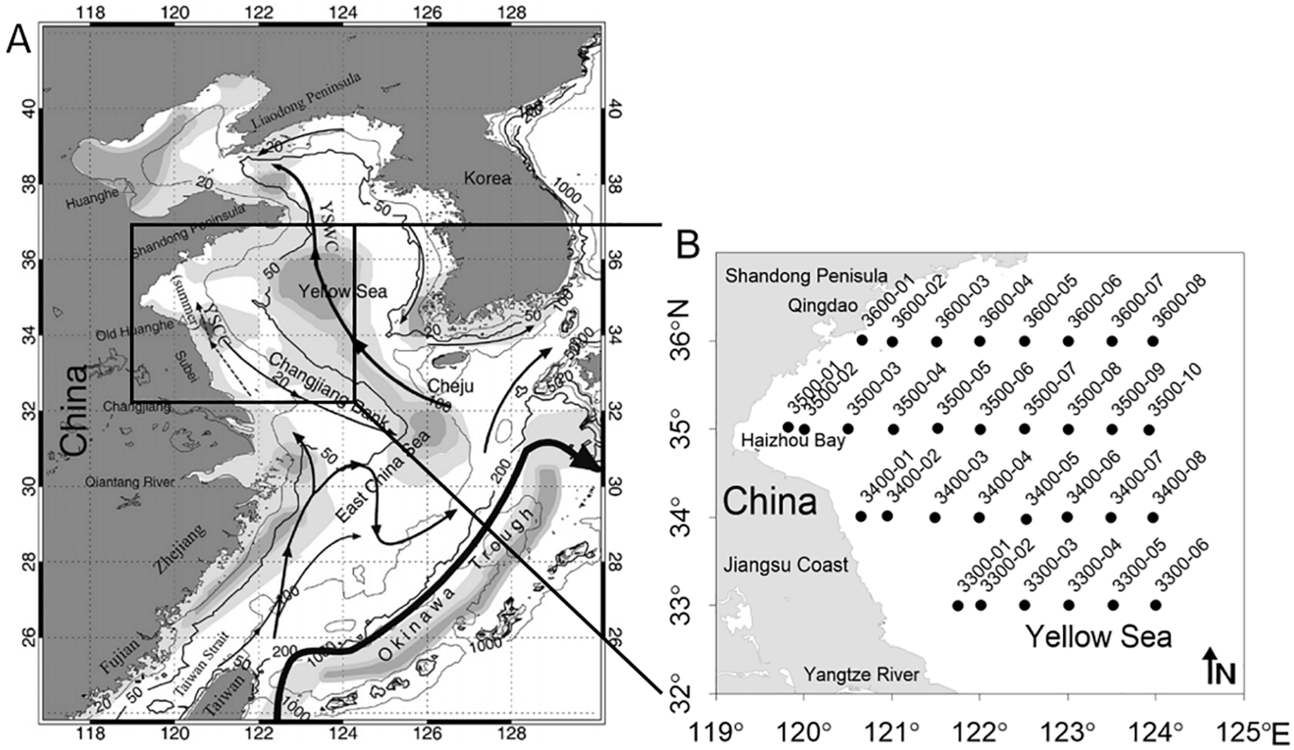

Fig. 1. Study area and sampling stations. (A) Image from Yuan et al. (2008). Topography and currents of the Yellow Sea and East China Sea. The black box indicates the study area. (B) Location of all sampling stations in the study area. Please note that collection of sediment was unsuccessful at Stations 3300–01/02, 3500–01/02/03 and 3600–01, while foraminiferal eDNA extraction failed at Station 3500–05. Environmental parameters were recorded at all stations.

encodes the ribosomal subunit is an ideal high–throughput sequencing DNA barcode (Pawlowski et al., 2014b). The region (about 160 bp) was amplified by PCR using foraminifera specific primers s14F3 (5′ ACGCAMGTGTGAAACTTG 3′) and s15ROTEX (5′ GAAAGGACTAGCATATTTAAC 3′) (Lejzerowicz et al., 2013). To label different sources of experimental samples, primers with independent barcode were added to each sample during amplification.

Each PCR reaction volume of 25 μL containing 12.5 μL of 2 × High–Fidelity PCR Master Mix, 0.5 μL of each primer at 10 μM, 2 μL of DNA template and 9.5 μL of ddH_2O. The PCR reactions consisted of a pre–denaturation at 94°C for 5mins, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 60 s and extension at 72°C for 120 s, then immediately followed by additional 10 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 120 s. The annealing temperature was appropriately increased in this experiment to improve the amplification specificity.

PCR amplified samples were detected by 1% agarose gel electrophoresis. The bright positive bands were screened by electrophoresis and purified by DNA Gel Extraction Kit DNA Gel Extraction Kit (Beijing TsingKe Biotech Co., ltd.). After testing the concentration, the samples were sent to Beijing Novogene Co., ltd. TruSeq® DNA PCR Free Library Preparation Kit from Illumina was used to construct the library. After the library was assessed by Qubit, NovaSeq 6000 was used for machine sequencing.

2.4. *Data quality control and processing*

Quality control was carried out on the original data of disembarking to remove low–quality data interference. First, the original data were de–multiplexed to samples according to barcode sequences and primer sequences. After that, the barcode and primer sequences were removed, and FLASH (V1.2.7, https://ccb.jhu.edu/software/FLASH/) (Magoc and Salzberg, 2011) was used for double–end splicing of samples to obtain Raw reads. The Unoise3 (Edgar, 2016) function of Usearch (V11.0.667) was used to de–noise and generate Operational Taxonomic Units (OTUs), and an OTU table was generated with a similarity threshold of 97%. Blast (V2.10.1) was used to compare all OTUs with PR^2 (Protist Ribosomal Reference Database) to obtain species classification and other information.

2.5. *Statistical analysis*

The sample with the least amount of data (403 in this data) was taken as the standard for homogenization of all samples. QIIME (V1.9.1, https://qiime.org/index.html) (Caporaso et al., 2010; Bokulich et al., 2013) was used to calculate the α diversity of the samples, including Observed Otus, Shannon, Chao1, Ace, Simpson, and Margalef, etc. The statistical software R (V4.0.5) and SPSS 22 were used to conduct correlation analysis on the data and generate charts.

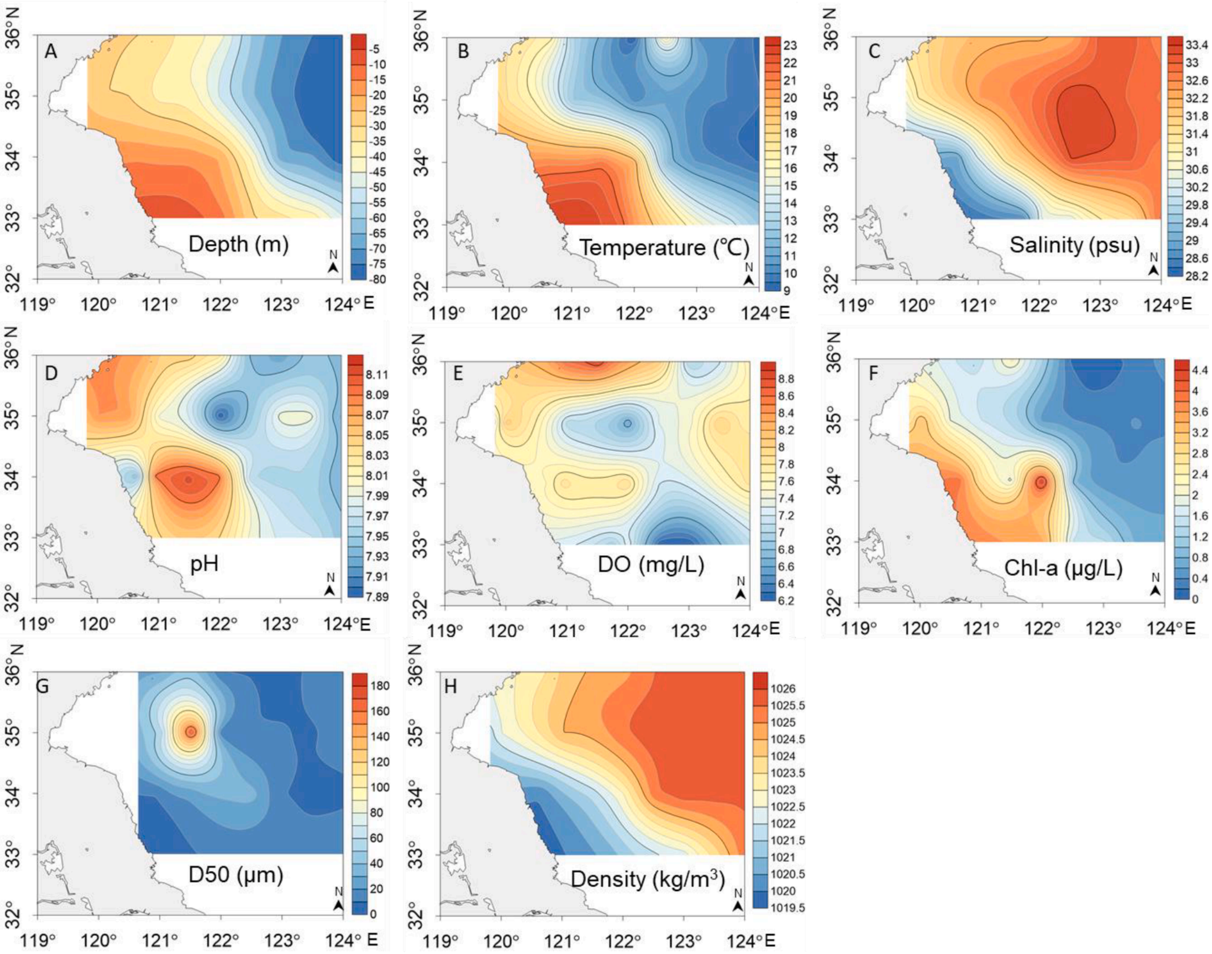

Fig. 2. Distribution of main environmental parameters in the Yellow Sea. (A) Temperature in bottom water; (B) salinity in bottom water; (C) water depth; (D) pH in bottom water; (E) dissolved oxygen (DO) in bottom water; (F) chlorophyll-a (Chl-a) in bottom water; (G) sediment median particle size; (H) bottom water density. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Environmental characterization

The relevant environmental information collected in this study was used to draw contour maps. The water depth in the region, ranging from 10 to 79 m, was shallow in the southwest and deep in the northeast (Fig. 2A). The bottom water was characterized by high coastal water temperature and low offshore water temperature, with a temperature range of 9–22°C (Fig. 2B). Except for the low water temperature of the Yellow Sea Cold Water Mass, the low water temperature of station 3600–04 was 14°C. The salinity of the surveyed area was lower in the southwest coast and higher in the northeast sea (near the Yellow Sea Cold Water Mass), with a salinity range of 31.2–33.1 psu (Fig. 2C). The pH value of the bottom water in the surveyed area was higher inshore and lower offshore, with a pH range of 7.891–8.113 and the lowest pH at station 3500–06 (Fig. 2D). The dissolved oxygen range was 6.20–8.82 mg/L, with two hypoxic differences between stations 3500–06 and 3300–04 (Fig. 2E). The Chl–a content in the bottom water of the Jiangsu coast was higher than that in the offshore water, ranging from 0.1 μg/L to 4.51 μg/L (Fig. 2F). The median particle size (D_{50}) at station 3500–05 was 192 μm, and those at other stations was <50 μm (Fig. 2G). The distribution of bottom water density had a similar pattern to salinity, ranging from 1020.1 kg/m^3 to 1026 kg/m^3.

In the surveyed area, the nitrate ion (NO_3^-) concentration in the bottom water near stations 3300–02 and 3300–03 (33°N,122–123°E) was higher, reaching 7.10–8.27 μmol/L, and 0.28–4.89 μmol/L in the rest of the stations (Fig. 3A). The nitrite ion (NO_2^-) concentration was generally low and the concentration range was 0.03–0.93 μmol/L (Fig. 3B). The concentration of ammonium ion (NH_4^+) content in the bottom water of stations near Haizhou Bay was higher (34–36°N, 120–121°E), such as 3500–01, 02, 03,3600–02, 03, and lowest concentration is at station 3400–06, with the concentration ranged from 0.25 μmol/L to 2.79 μmol/L (Fig. 3C). The distribution of dissolved total nitrogen (DTN) concentration in bottom water become higher near Jiangsu Coast than other area (Fig. 3D). The distribution of dissolved total phosphorus (DTP) in the bottom water tended to be lower in coastal areas and higher in offshore areas, and the concentration of DTP in coastal areas was higher than that in station 3400–01 (Fig. 3E). The concentration range of dissolved silicate (DSi) in the bottom water in the surveyed area was 0.59–21.17 μmol/L, and the lowest concentration was found at station 3400–04 while the highest was at station 3400–01 (Fig. 3F). The distribution of carbon content in sediment samples showed that the TC was relatively high along the northern Jiangsu Coast (such as 3400–01, 02, 03), with a carbon content of 0.32–2.03% (Fig. 3G). The concentration of TOC was lower in coastal waters and

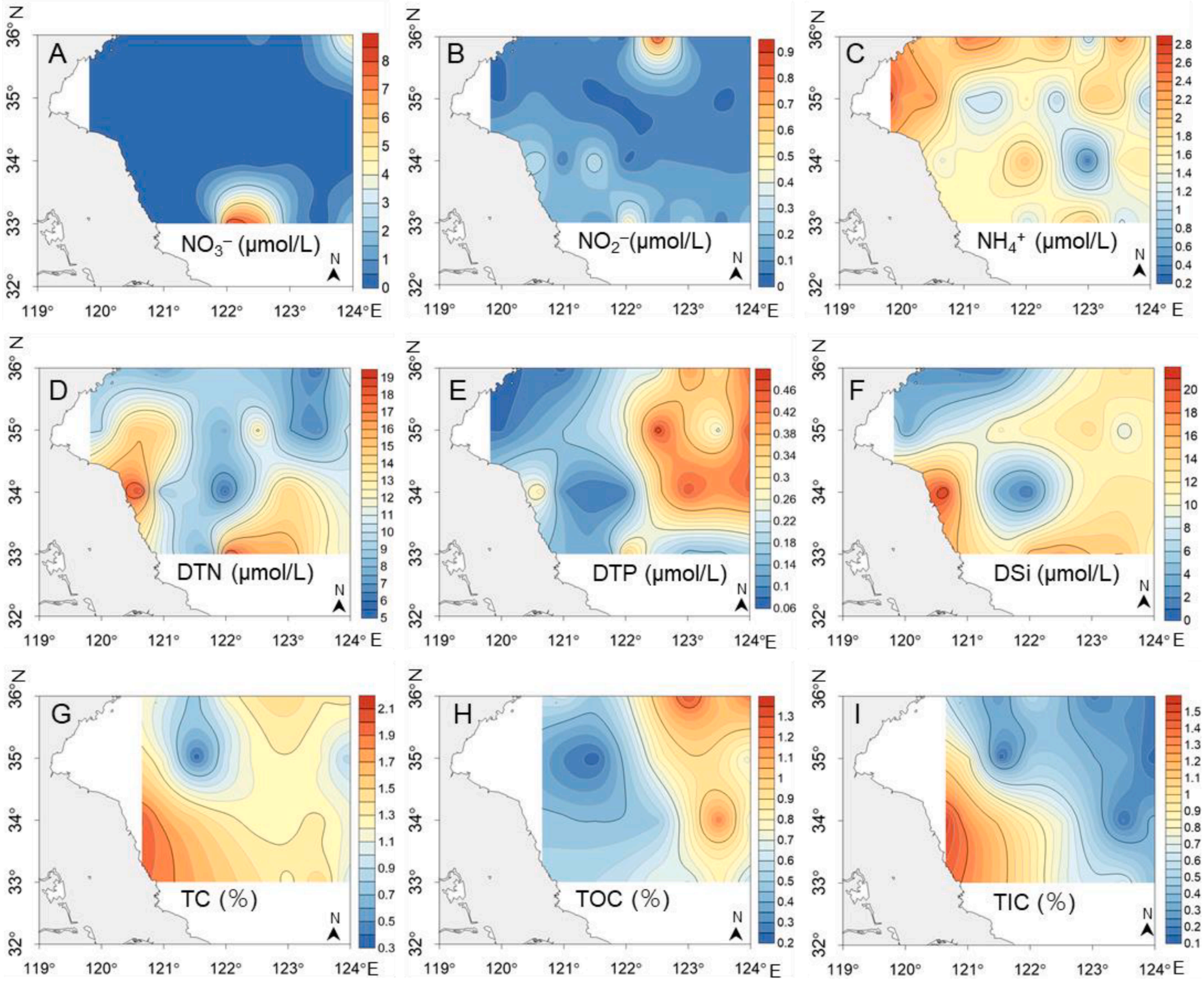

Fig. 3. Nutrient distribution in the Yellow Sea. (A) Nitrate ion (NO_3^-) concentration in bottom water; (B) nitrite ion (NO_2^-) concentration in bottom water; (C) ammonium ion (NH_4^+) concentration in bottom water; (D) dissolved total nitrogen (DTN) concentration in bottom water; (E) dissolved total phosphorus (DTP) concentration in bottom water; (F) dissolved silicate (DSi) concentration in bottom water; (G) total carbon (TC) content in sediments; (H) total organic carbon (TOC) content in sediments; (I) total inorganic carbon (TIC) content in sediments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

higher in offshore waters, with an organic carbon content range of 0.22–1.32% (Fig. 3H). The concentration of inorganic carbon was higher in the Jiangsu Coast (such as 3400–01, 02, 03, 04), and lower in stations of other areas, with the content of inorganic carbon ranged from 0.1 to 1.55% (Fig. 3I).

3.2. Foraminiferal eDNA data overview

A total of 71 samples from 25 stations in the Yellow Sea were sequenced (two samples from stations 3300–03 and 3300–04 failed repeated tests and were not sequenced) (Supplementary Material Table S2). A total of 5,937,127 Raw Paired End reads were obtained, 5,401,478 Raw reads were obtained after splicing, and 5,281,762 Effective reads were left after filtering the low–quality sequences. A total of 426 OTUs and 1,701,899 reads were obtained by generating OTU tables after noise reduction and chimeric removal. Among the 389 OTUs successfully assigned to foraminifers, 41 OTUs were assigned to planktic foraminifers, and 348 OTUs were assigned to benthic foraminifers. The number of OTUs varied from 49 to 247 among the samples (in Supplementary Material). In this study, a rarefaction curve was drawn based on the number of reads and OTUs at each station, as shown in Fig. 4A. It could be seen that with the increase in the number of sequenced reads, the curve of species diversity increased sharply at first and then become flat. The dotted line indicated that the number of species had stabilized even if the number of sequenced samples had continued to increase. Therefore, the sample size of this experiment was sufficient and reasonable.

The 426 OTUs obtained were identified using Blast according to PR^2 (Protist Ribosomal Reference Database). A total of 389 OTUs (1,694,408 Reads) were identified and detailed species annotation information was obtained, while the remaining 37 OTUs (7,491 Reads) were not successfully matched. In the 389 OTUs that had been annotated successfully, the average value of Identity (the similarity between the OTUs representative sequence in the sample and the PR2 database reference sequence) was 97.69%. Among them, OTUs with Identity value greater than 99% accounted for 35.47%, reads with Identity value greater than 99%accounted for 54.73%. and OTUs with Identity value $<$ 90% accounted for only 0.09% (Fig. 4B).

3.3. Taxonomic composition and relative abundance of benthic foraminifera

Blast successfully compared 389 foraminifera OTUs, of which 348 were compared with the benthic foraminifera, a total of 51 species. The OTUs for comparison with benthic foraminifera were assigned to Rotaliida, Monothalamids, Textulariida, Robertinida, and Miliolida at the Order level. The remaining OTUs that were not successfully compared were classified as Others. Rotaliida had the highest proportion with 280 OTUs, accounting for 71.97% of the total and containing 1,255,944 reads. Textulariida was the second highest with 39 OTUs (108,215 reads). Monothalamids only had 19 OTUs, but the number of reads was 196,384, accounting for 11.54%.

Except for stations 3300–03 and 3300–04, the total OTUs of benthic foraminifera varied stably among stations (Fig. 5A). Rotaliida was the dominant group in this area, and was abundant in the coastal waters, especially near the Jiangsu coast and Haizhou Bay (Fig. 5B). Textulariida was more abundant in shallow seas, especially around the north of Jiangsu coast and Haizhou Bay (Fig. 5C). Monothalamids were mainly distributed in 33–34°N, especially along the Jiangsu coast (Fig. 3D). Miliolida and Robertinida have a small amount of data in this study (Fig. 5E–F). Monothalamids were found to be relatively abundant at stations 3500–07 and 3600–03, and as high as 58% at station 3500–07 (Fig. 6).

The heat map based on the relative abundance of benthic foraminifera of different genera at each site (Fig. 7) show that 11 of the 40 genera, such as Monothalamids_XXX, *Leptohyalis*, *Cibicidoides*, *Globobulimina* and *Robertina* were relatively abundant in most stations. These foraminifera were the dominant group in the Yellow Sea. The abundance of seven genera, such as *Cassidulina*, *Elphidiella* and *Miliammina*, was very low at all sites. The remaining 22 genera had higher abundance at some stations and lower abundance at others, and presented patchy distribution in the area.

3.4. Correlation analysis between benthic foraminifera and environmental factors

RDA analysis was performed on α diversity index and environmental factors (Fig. 8A). The results showed that indexes of community abundance, such as Ace index, Chao1 index, Margalef index, and Observed OTUs, were all in the fourth quadrant. Pielou ’evenness index and Simpson diversity index, which represented species evenness, fell in the

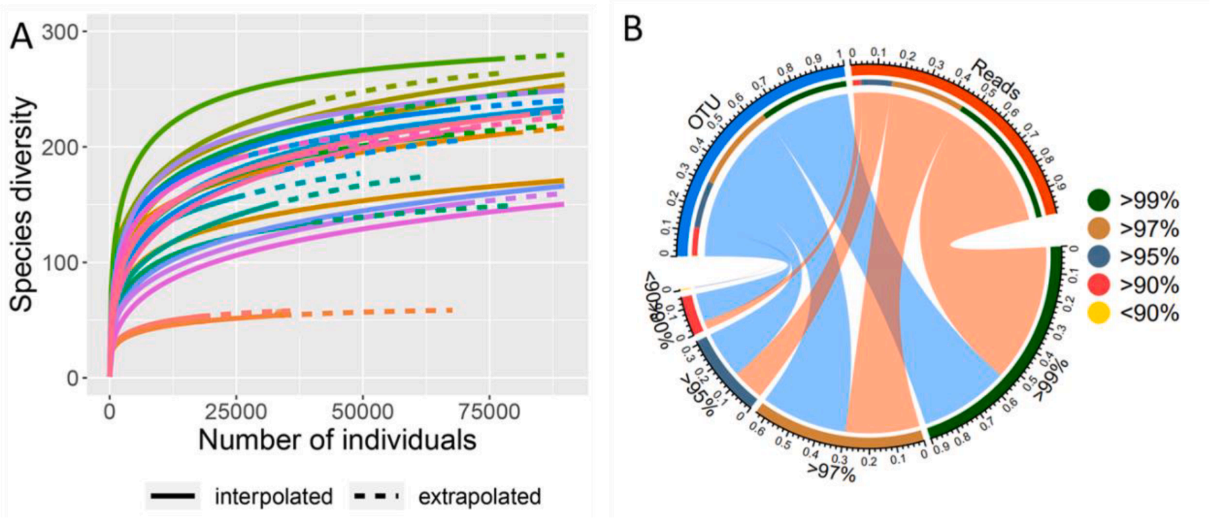

Fig. 4. (A) Rarefaction curves calculated based on OTUs and reads at each station. The solid lines represent the reads abundance plotted based on observed values, and the dotted lines represent the reads abundance extrapolated. (B) The circular graph shows the identity values of OTUs (blue) and reads (orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

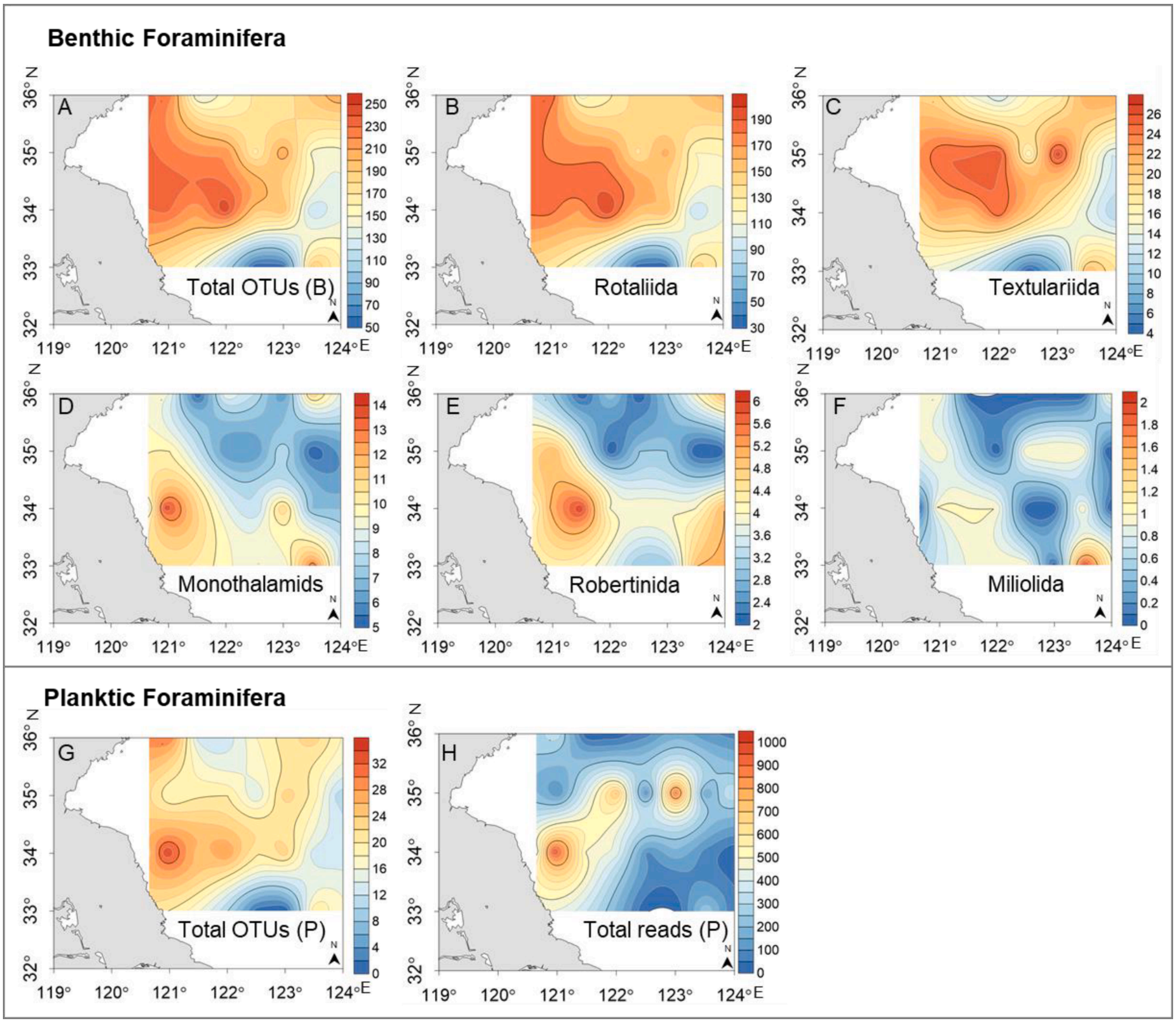

Fig. 5. Distribution of each group in the Yellow Sea. (A) Total number of benthic foraminifera OTUs; (B) OTUs number of Rotaliida; (C) OTUs number of Textulariida; (D) OTUs number of Monothalamids; (E) OTUs number of Robertinida; (F) OTUs number of Miliolida; (G) Total number of planktic foraminifera OTUs; (H) Total number of planktic foraminifera reads. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

first quadrant in the Fig. 8D. They were positively correlated with TC and TIC, but negatively correlated with TOC. Spearman correlation analysis was conducted between α diversity index and environmental factors. The results showed that temperature and TIC were significantly positively correlated with the diversity of benthic foraminifera, while water depth and TOC content was significantly negatively correlated with the diversity of benthic foraminifera (Table 1).

RDA analysis results of OTUs of benthic foraminifera at the order level based on different environmental factors were shown in Fig. 8B. The results showed that the data points of the six groups, including the Others, all fell in the second and third quadrants on the left side of the y axis (Fig. 8B). The correlations of Monothalamids, Miliolida and Robertinida with the environmental factors were similarly. In addition, the correlations of Rotaliida, Textulariida and Others with the environmental factors were similarly. Spearman correlation analysis was performed on OTUs of benthic foraminifera at the order level based on different environmental factors. In Spearman correlation analysis (Table 2), the environmental factors that were significantly correlated with foraminifera in each group could be divided into three categories: (1) Temperature and depth: Temperature decreased with the increase of water depth. The diversity of each group was negatively correlated with water depth, but positively correlated with temperature. (2) Chl–a and pH: The diversity of each group increased with the increase of Chl–a and pH. (3) TOC and TIC: The diversity of each group had a significant negative correlation with TOC, and a significant positive correlation with TIC.

In this study, 14 species with ratios of each species to the total reads population greater than 2% were recorded as the dominant species, and the ratio of reads population of these 14 species at each station and environmental factors were analyzed by RDA (Fig. 8C). The species were divided into three different groups, with *Buliminella tenuata*, *Cibicidoides ungerianus*, *Leptohyalis scotti* and *Cibicidoides pachyderma* being positively correlated with NO$_3^-$. The species *Cibicidoides lobatulus* and *Globocassidulina* sp., show positively correlated with temperature, Chl–a, TIC and pH, etc., and negatively correlated with salinity. *Nonionella labradorica*, Monothalamids_XXX_sp., and *Globobulimina turgida* show positively correlated with depth and TOC, etc., and negatively correlated with median grain size (D_{50}). Spearman correlation analysis of relative abundance of dominant species and environmental factors (Table 3) showed that each species had different responses to the environment. Species such as *Bulimina marginata*, *Robertina arctica* and *Globobulimina turgida* had similar environmental responses, were negatively correlated with temperature, Chl–a and TIC, but positively correlated with water depth and TOC. Other species such as *Cibicidoides*

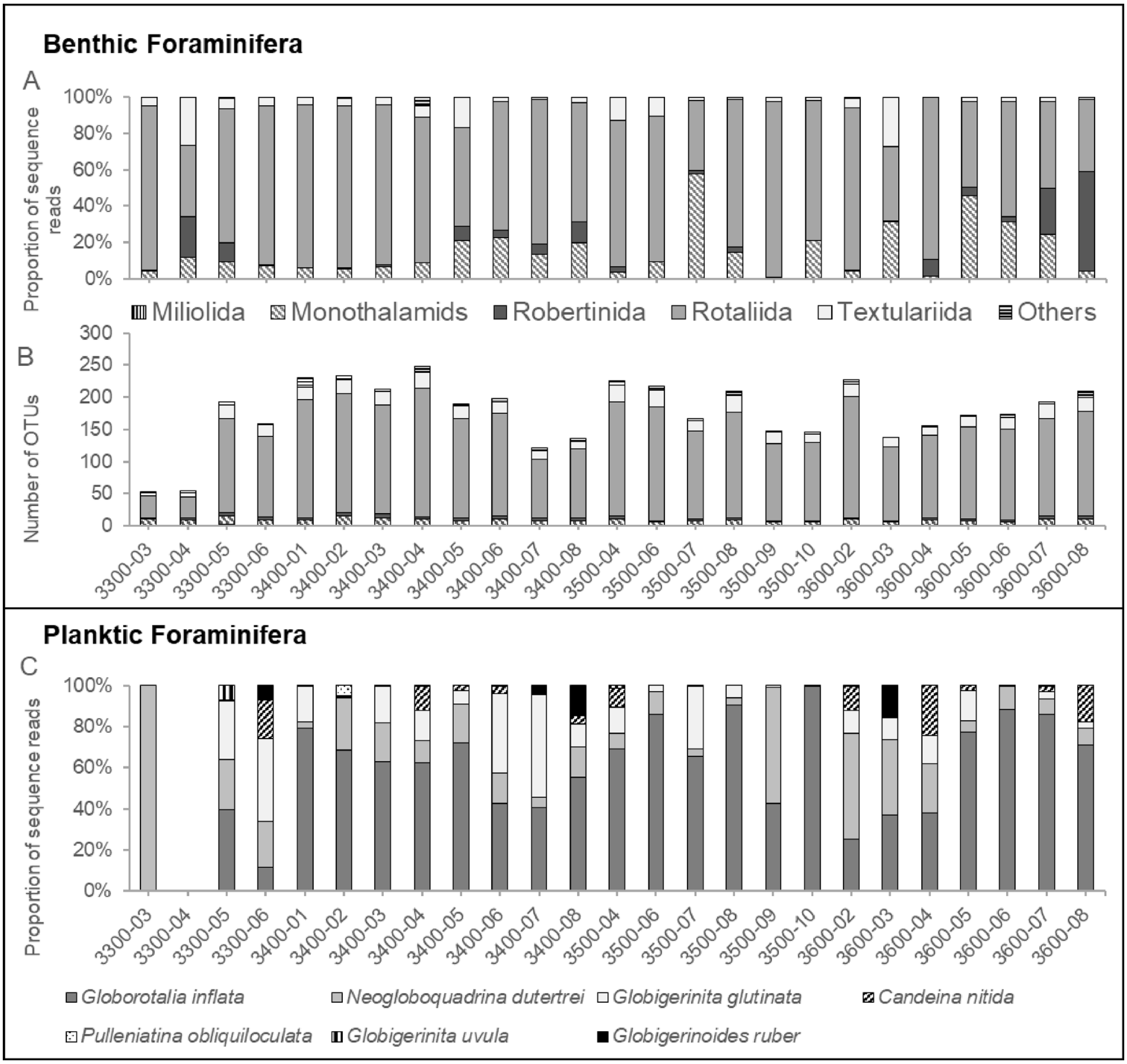

Fig. 6. (A) Proportion of sequence reads in each benthic group at different stations; (B) Numbers of OTUs in each benthic group at different stations; (C) Relative abundance of planktic foraminifera species at different stations.

lobatulus, *Cibicidoides pachyderma*, *Globocassidulina* sp. and *Leptohyalis scotti* had similar environmental responses, were negatively correlated with depth, salinity and TOC, but positively correlated with temperature, Chl–a, D_{50} and TIC.

3.5. Circulation of currents and planktic foraminifera in the Yellow Sea

The ocean currents in the investigated area were drawn according to the literature (Yuan et al., 2008) and shown in Fig. 1. The major flow systems are Yellow Sea Warm Current (YSWC) from southeast to northwest (124–123°E) and Yellow Sea Coastal Current (YSCC) southward from Shandong Peninsula to north of the Jiangsu Coast. YSCC becomes weak and flows northward in the summer (dotted line).

A total of 41 Planktic foraminiferal eDNA were detected in the sediment samples from the Yellow Sea, including 18,340 reads (1.07% of the total number of reads). The obtained planktic foraminifera OTUs were matched to seven species, *Globorotalia inflata*, *Neogloboquadrina dutertrei*, *Globigerinita glutinata*, *Candeina nitida*, *Pulleniatina obliquiloculata*, *Globigerinita uvula* and *Globigerinoides ruber*, from high to low in relative abundance (Fig. 6C). *Globorotalia inflata*, which was the most abundant, contained 13 OTUs and 12,948 reads, accounting for 70.60% of the total number of reads in planktic foraminifera. The distribution of planktic foraminifera in each station was shown in Fig. 5G–H. The number of OTUs was higher along the Jiangsu Coast to the sea area of Qingdao, followed by the number of OTUs near 123°E, which shifted to 124°E in the south. However, there was a low value area of planktic foraminifera to the north of the central part of the surveyed area.

4. Discussion

4.1. Distribution characteristics of benthic foraminifera in the Yellow Sea

This study is the first survey of molecular diversity of foraminifera in the Yellow Sea, and the relationship between the distribution characteristics of benthic foraminiferal eDNA and various environmental factors was explored. The results showed that Rotaliida was the dominant group in the study area, with high abundance in the coastal waters. Monothalamids and Robertinida were detected for the first time. Monothalamids constituted an important part of the community, and the relative abundance of individual stations was more than 50%.

According to traditional morphological studies, the benthic foraminifera in the Yellow Sea area could be classified into three types of

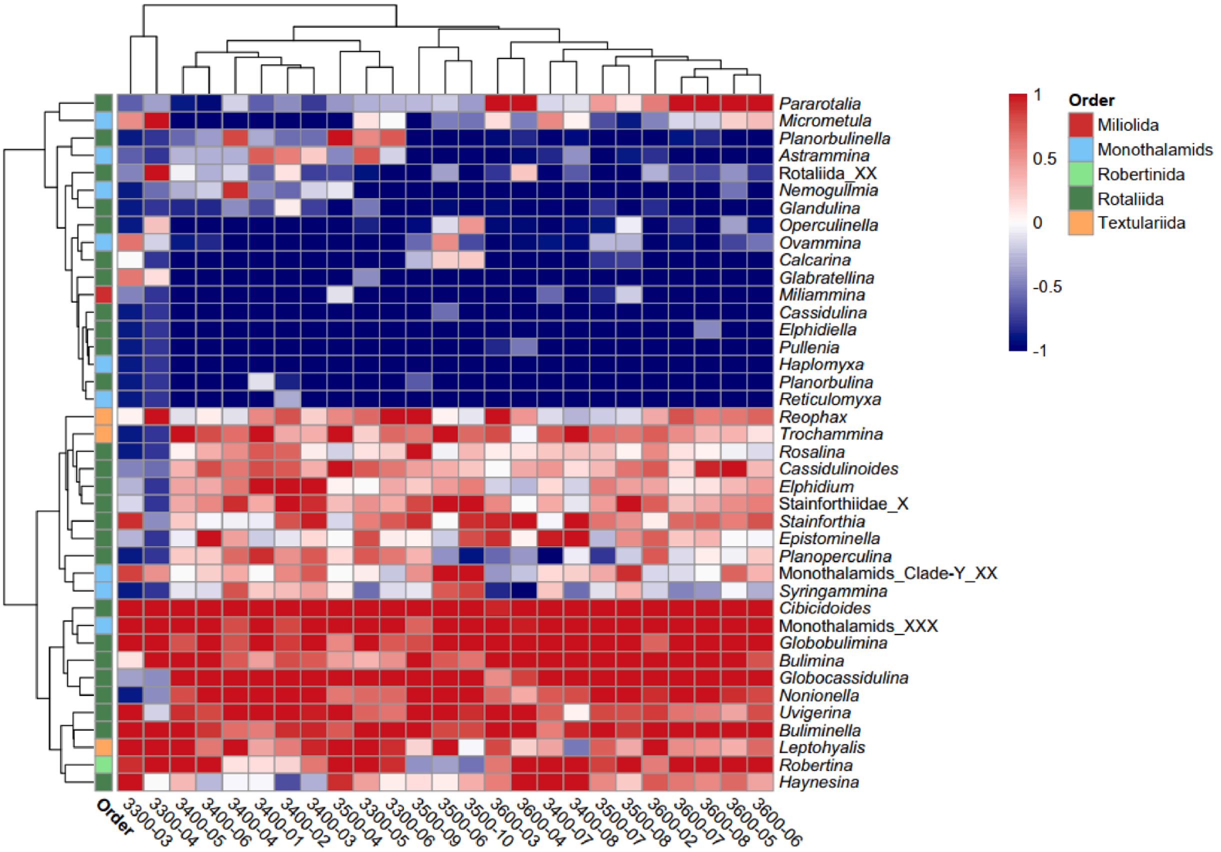

Fig. 7. Genus's level species distribution of benthic foraminifera at each station. The heatmap was generated using the package "pheatmap" in the statistical software R.

chitinous shells, hyaline (Rotaliida), agglutinated (Textulariida) and porcellaneous (Miliolida). Previous studies showed that Rotaliida is the dominant species in this area, which is in agreement with the dominant position of Rotaliida in this study. However, researches showed that the high value of Rotaliida is located in deeper waters (Cheng et al.,1999; Polski, 1959), while in this study, the abundance of Rotaliida was higher in the coastal waters, especially in northern Jiangsu Coast and Haizhou Bay. As for the distribution of Textulariida, morphological studies showed that the abundance of Textulariida was higher in the shallow sea area <50 m (Wang et al., 1980b), and the current study confirmed that premise.

There are several possible reasons to explain the phenomenon that using benthic foraminiferal eDNA showed different taxa distribution pattern with morphology results before. First of all, the morphology needs to obtain a certain quality of surface sediments, so benthic foraminifera abundance may be affected by the deposition rate, such as far away from the coast low deposition rate will get a higher abundance value (Li et al., 2021; Polski, 1959). Secondly, the shells of dead foraminifera may have experienced multiple ocean currents (Dessandier et al., 2018; Horton and Murray, 2006), and the Rose Bengal staining is also a controversial method to determine life or death (Bernhard et al., 2006). Therefore, if the dead individuals cannot be clearly identified, the results will be greatly affected. In addition, the shells of agglutinated groups such as Textulariida may have decomposed; the soft shells of Monothalamids, which are easily destroyed and difficult to preserve, leading to possible biased results. Since it is difficult to preserve the DNA molecules, benthic foraminiferal eDNA obtained by high–throughput method could better reflect the distribution status of living foraminifera, especially for the soft–shell groups and small foraminifera larvae (<0.063 μm), which are difficult to observe morphologically (Pawlowski et al., 2014b).

4.2. Responses of benthic foraminifera to water temperature and depth and their implications

The shallow sea shelf region is a focus area of marine environment research because of its complex and changeable environment and the influence of human activities. The Yellow Sea, an important continental shelf sea in China, is influenced by the inputs of the Yellow River, the Yangtze River, the Han River and the Jinjiang River, etc. (Cheng et al., 1999). In the study area, the water depth increases with distance, and has a range between 10 and 79 m. The temperature gradually decreases with the increase of water depth. As result showed, at the level of community diversity and taxa, both water depth and temperature were important influencing factors. With the increase in water depth and the decrease in temperature, community diversity decreased. The influence of depth on the distribution of foraminifera is usually due to the changes of temperature, salinity, pH and other environmental factors caused by the change of depth (Funnell, 1967). In this study, temperature was the main factor that affected the depth distribution of foraminifera.

Previous studies have shown that temperature is one of the most important environmental parameters for organisms, and planktic foraminifera are often used as thermometer for climate reconstruction studies of ancient oceans (Elderfield and Ganssen, 2000). In laboratory culture studies, it was found that benthic foraminifera communities increased community richness at higher temperatures (Dong et al., 2018; Weinmann and Goldstein, 2016). The Yellow Sea Cold Water Mass is a seasonal hydrological feature. It is a part of water body located below the seasonal thermocline in the central region of the Yellow Sea. It often appears in spring and summer and is characterized by low temperature and high salinity (Yu et al., 2006). The thermocline was formed around April, with a cold-water mass on both sides of the South Yellow Sea. By May, the thermocline strengthened, with a depth of 10–20 m,

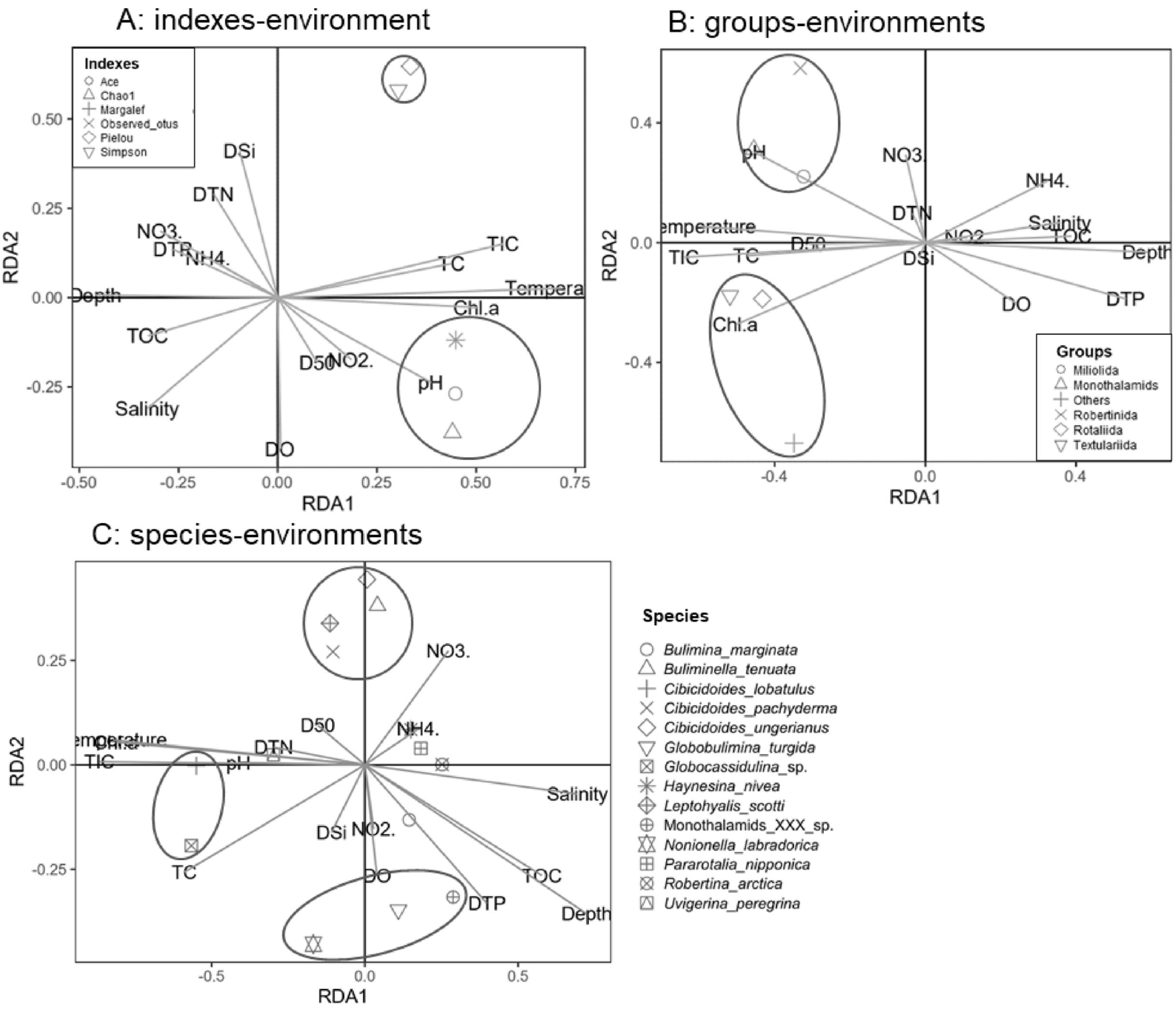

Fig. 8. RDA correlations with environmental factors were demonstrated at three levels: community indexes (A), group composition (B) and dominant species (C).

Table 1
Spearman correlation R value between community diversity and environmental factors at each station. The two–tailed test is used, and those with significant R values are highlighted in bold. $*p < 0.05$ $^{**}p < 0.01$.

Indexes	Chao1	OTUs	Ace	Simpson	Margalef	Pielou
Depth	**–0.296***	–0.137	**–0.270***	–0.152	–0.137	–0.172
DO	0.058	–0.031	–0.086	0.037	–0.031	–0.008
T	**0.403****	**0.310****	**0.385****	0.225	**0.310****	0.228
Salinity	–0.081	0.004	–0.032	–0.214	0.004	**–0.246***
pH	0.181	0.052	0.111	0.091	0.052	0.102
Chl–a	0.144	0.015	0.157	0.200	0.015	0.197
D$_{50}$	0.166	0.138	0.200	0.026	0.138	0.035
NO$_2^-$	–0.014	–0.069	0.006	–0.089	–0.069	–0.140
NH$_4^+$	–0.224	–0.137	–0.186	–0.138	–0.137	–0.109
NO$_3^-$	–0.230	–0.229	**–0.253***	–0.202	–0.229	–0.181
DTP	–0.122	–0.017	–0.046	–0.085	–0.017	–0.128
DTN	–0.096	–0.138	–0.010	–0.092	–0.138	–0.090
DSi	–0.092	–0.031	–0.049	0.119	–0.031	0.125
TC	**0.271***	**0.291***	**0.258***	**0.247***	**0.291***	0.221
TOC	–0.215	–0.140	**–0.257***	**–0.258***	–0.140	**–0.253***
TIC	**0.292***	**0.238***	**0.362****	**0.304***	**0.238***	**0.314****

and the sea water presented a three-layer structure vertically. In August, the thermocline was at its peak, which was also the peak of the cold-water mass. In autumn, the thermocline gradually weakened. In November, the thermocline only existed below 30 m, and its intensity also decreased significantly. By December, both the cold–water mass and the thermocline disappeared (Hu and Wang, 2004; Yu et al., 2006).

Yellow Sea Cold Water Mass is an important hydrological feature in the Yellow Sea. In this study, samples were collected in June 2019, when the Yellow Sea Cold Water Mass was forming. The regional community richness of benthic foraminifera decreased in the Yellow Sea Cold Water Mass. Studies showed that the Yellow Sea cold water mass had a significant impact on the community composition of microbenthic

Table 2

Spearman correlation R value between groups and environmental factors at each station. The two–tailed test is used, and those with significant R values are highlighted in bold. $*p < 0.05$ ** $p < 0.01$.

Groups	Rotaliida	Monothalamids	Textulariida	Robertinida	Miliolida
Depth	**–0.327****	**–0.420****	**–0.373****	–0.211	–0.229
DO	–0.022	–0.177	–0.054	**–0.285***	0.015
T	**0.320****	**0.479****	**0.354****	0.211	0.227
Salinity	–0.093	–0.270*	–0.064	–0.125	–0.094
pH	0.187	**0.294***	**0.324****	**0.257***	**0.403****
Chl–a	**0.235***	**0.294***	**0.293***	0.211	**0.238***
D_{50}	**0.332****	0.167	**0.407****	0.184	**0.306****
NO_2^-	–0.197	0.057	–0.152	0.141	–0.189
NO_3^-	–0.188	0.005	**–0.259***	0.139	–0.041
NH_4^+	**–0.247***	–0.210	–0.201	–0.085	–0.158
DTN	–0.227	0.073	**–0.283***	**0.247***	–0.048
DTP	**–0.261***	**–0.300***	**–0.376****	–0.202	**–0.327****
DSi	–0.097	0.176	–0.071	0.216	–0.093
TC	0.179	**0.418****	0.204	**0.323****	0.050
TOC	**–0.307****	**–0.272***	**–0.345****	–0.107	–0.195
TIC	**0.384****	**0.506****	**0.388****	**0.281***	0.196

Table 3

Spearman correlation between reads ratio of dominant foraminifera and environmental factors. Foraminifera species with more than 2 % reads are selected and classified at the order level. Two–tail test is used for correlation analysis, and marked with significant R value are highlighted in bold. $*p < 0.05$, ** $p < 0.01$.

Species	Depth	DO	T	Salinity	pH	Chl–a	D_{50}
Rotaliida							
Bulimina marginata	0.353**	0.387**	–0.400**	0.105	0.024	–0.277*	–0.068
Buliminella tenuata	–0.213	0.034	0.229	–0.168	0.240*	0.147	0.285*
Cibicidoides lobatulus	–0.414**	0.063	0.475**	–0.283*	0.165	0.361**	0.04
Cibicidoides pachyderma	–0.108	–0.360**	0.349**	–0.026	–0.114	0.01	0.009
Cibicidoides ungerianus	–0.06	–0.016	0.088	0.097	–0.079	–0.029	0.241*
Globobulimina turgida	0.251*	0.011	–0.139	0.148	–0.111	–0.329**	–0.409**
Globocassidulina sp.	–0.249*	0.011	0.342**	–0.139	0.116	0.254*	–0.008
Haynesina nivea	0.200	0.206	–0.282*	–0.035	–0.059	–0.157	–0.164
Nonionella labradorica	0.023	0.075	0.165	0.115	0.141	–0.047	0.106
Pararotalia nipponica	0.287*	0.482**	–0.356**	0.051	0.06	–0.286*	–0.205
Uvigerina peregrina	–0.393**	–0.002	0.345**	–0.109	0.285*	0.315**	0.244*
Monothalamids							
Monothalamids_XXX_sp	0.314**	–0.122	–0.102	0.366**	–0.118	–0.370**	–0.173
Textulariida							
Leptohyalis scotti	–0.474**	–0.270*	0.532**	–0.061	0.280*	0.270*	0.434**
Robertinida							
Robertina arctica	0.354**	–0.137	–0.342**	0.127	–0.257*	–0.320**	–0.358**

	NO_2^-	NO_3^-	NH_4^+	DTN	DTP	DSi	TC	TOC	TIC
Rotaliida									
Bulimina marginata	–0.110	0.061	0.204	–0.309**	0.102	–0.217	–0.175	0.255*	–0.406**
Buliminella tenuata	0.050	–0.032	0.338**	–0.04	–0.232	–0.198	0.051	–0.15	0.173
Cibicidoides lobatulus	0.173	–0.194	–0.286*	–0.051	–0.280*	–0.007	0.421**	–0.287*	0.420**
Cibicidoides pachyderma	0.096	–0.029	–0.179	0.103	–0.072	0.267*	0.301*	–0.116	0.274*
Cibicidoides ungerianus	–0.208	0.050	–0.159	–0.437**	–0.184	–0.318**	–0.095	–0.062	0.020
Globobulimina turgida	0.137	–0.041	0.081	0.172	0.324**	0.227	0.326**	0.358**	–0.110
Globocassidulina sp.	–0.074	–0.320**	–0.223	0.031	–0.038	0.071	0.387**	–0.283*	0.385**
Haynesina nivea	–0.066	0.207	0.030	–0.173	–0.046	–0.118	–0.167	0.239*	–0.228
Nonionella labradorica	–0.118	–0.237*	–0.085	–0.147	0.053	0.034	0.273*	–0.083	0.128
Pararotalia nipponica	–0.251*	0.035	0.238*	–0.469**	–0.040	–0.237*	–0.077	0.346**	–0.422**
Uvigerina peregrina	–0.123	–0.384**	–0.008	0.111	–0.133	–0.029	0.188	–0.368**	0.435**
Monothalamids									
Monothalamids_XXX_sp	0.393**	0.120	–0.073	–0.091	0.180	0.025	–0.077	0.316**	–0.285*
Textulariida									
Leptohyalis scotti	0.067	–0.133	–0.074	–0.029	–0.339**	–0.073	0.175	–0.274*	0.427**
Robertinida									
Robertina arctica	0.115	0.392**	0.153	–0.143	0.115	0.133	0.111	0.477**	–0.249*

organisms, such as nematodes and bacteria (Li et al., 2006; Xu et al., 2016). We believe that the effect of the Yellow Sea Cold Water Mass on benthic foraminifera community should not be ignored, but it may be seasonal. Such seasonal ecological effects are often not reflected in traditional morphological studies because of information buried in sediments over a long period of time. In our study, some of the 14 dominant species with an average relative abundance greater than 2%, such as *Cibicidoides ungerianus*, *Uvigerina peregrina*, *Leptohyalis scotti* and *Cibicidoides lobatulus*, were severely affected by the Yellow Sea Cold Water Mass and rapidly decreased due to temperature reduction.

However, not all foraminifera are adversely affected by low temperatures and we found that the relative abundance of dominant species such as *Bulimina marginata*, *Robertina Arctica* and *Pararotalia nipponica* in our samples presented an extremely significant negative correlation with temperature ($p < 0.01$). *Bulimina marginata* is adapted to a salinity range of 29–36 psu, and a temperature range of 7–20°C, has been shown to be an indicator of the temperature–salinity gradient between cold–-fresh Subantarctic Shelf Water (SASW) and warm–salty Subtropical Shelf Water (STSW) (Eichler et al., 2014). *Robertina Arctica*, a cold–-water foraminifera, has been reported in the waters off northern Norway and the northern Gulf of Russia near the Arctic (Eichler et al., 2014; Kniazeva and Korsun, 2019; Korsun et al., 1995). Studies on temperature adaptation of *Pararotalia nipponica* are still blank. However, studies have shown that *Pararotalia nipponica* is a salt–tolerant species, and 33‰ (33 psu) is its best salinity condition. *Pararotalia nipponica* can survive in salinity as high as 100‰ (100 psu), but is intolerant to low salinity (Nigam et al., 2006). The Yellow Sea Cold Water Mass is characterized by low temperature and high salinity. *Pararotalia Nipponica* may be more tolerant to the low–temperature sea area because of its preference for high–salinity environment.

4.3. Response of benthic foraminifera to carbon in sediments and its implications

As one of the most important calcifying organisms in the ocean, foraminifera participate in the geochemical carbon cycle (Barker and Elderfield, 2002; Langer et al., 1997; Pettit et al., 2013). Calcareous foraminifera synthesize calcite shells by calcifying calcium ions and carbonate from seawater (de Nooijer et al., 2014). Studies have shown a linear relationship between inorganic carbon content, calcium ion concentration, carbonate concentration, and foraminifera calcification (ter Kuile et al., 1989). Foraminifera contribute significantly to the inorganic carbon content in the oceans (Fujita and Fujimura, 2008; Pawłowska et al., 2017). In this study, the α diversity index of foraminiferal stations was significantly and positively correlated with TIC content, while Rotaliida, Textulariida and Monothalamids were significantly and positively correlated with TIC content.

Organic carbon content in the environment is closely related to foraminifera growth, and different foraminifera species have specific range of organic carbon adaptation (Bouchet et al., 2021; Dessandier et al., 2015). As an important continental shelf region in China, the Yellow Sea is rich in organic matters in sediments under the influence of input from the Yellow River, Yangtze River, Han River, Jinjiang and other terrestrial sources. The range of organic carbon content in the study area is 0.22–1.32%. In particular, the organic carbon content in the middle and eastern part of the study area is higher than the world average level of amazon and Mississippi shelf (average 0.75%) (Berner, 1982; Kang et al., 2014). In this study, the α diversity index of benthic foraminiferal sites was negatively correlated with TOC content, and OTUs of Rotaliida Textulariida Monothalamids was negatively correlated with TOC content. The negative effects of organic carbon on benthic foraminifera communities might have significantly reduced some species, such as *Globocassidulina* sp., *Uvigerina peregrina*, *Leptohyalis scotti* and *Cibicidoides Lobatulus*. RDA analysis results were consistent with this.

The decrease in evenness means that opportunistic species, such as *Bulimina marginata*, Monothalamids_XXX sp., *Robertina arctica*, *Globobulimina turgida* and *Pararotalia nipponica*, whose relative abundance is significantly positively correlated with TOC content, may gain growth advantages in this region. And studies also proved that *Bulimina marginata* is the main group in the high organic matter area (Loubere et al., 1999; Martins et al., 2016).

4.4. Distribution of planktic foraminifera in relation to ocean currents

Planktic foraminifera, which floats on the surface of the ocean and are passively transported by ocean currents, are highly sensitive to changes in the marine environment. They are therefore called the mirror of ocean currents (Wang et al., 1988). Globigerina ooze is an important component of seafloor sediments and is widely used in biostratigraphy analysis of paleoceanography (Kaiho and Lamolda, 1999; Crundwell et al., 2008). In addition, studies have shown that DNA preserved in marine sediments can preserve the integrity of the ecological structure and diversity of marine surface plankton (Capo et al., 2015; Morard et al., 2017). In this study, 1.07% of the eDNA distribution of foraminifera obtained by high–throughput sequencing was noted as planktic foraminifera. The distribution of planktic foraminifera was basically consistent with the direction of ocean currents. Since planktic foraminifera mostly live in tropical open oceans, there have been no reports of planktic foraminifera in the North Yellow Sea and Bohai Sea, but there have been many studies in the East China Sea and South China Sea (Kubota et al.,2010; Schiebel and Hemleben, 2005). Samples of this experiment were collected in June in summer. In combination with Yuan et al., (2008), we speculated that the planktic foraminifera in the surveyed area were transported from south to north by the Yellow Sea Warm Current (YSWC) and the Yellow Sea Coastal Current (YSCC) prevalent in spring and summer, and encountered the southward Yellow Sea Coastal Current (YSCC) in the Yellow Sea, so 122–123°E had a low abundance gap from north to south. Cheng et al. (1999) also found planktic foraminifera in the surface sediments of the Yellow Sea by using traditional morphological methods, and the distribution was also concentrated in the southeast and southwest areas of the surveyed area, with an abundance of about 1%. Although the content of planktic foraminiferal eDNA extracted from the marine surface sediments is low, it is an important indicator of the current conditions in this area.

5. Conclusions

In this study, we analyzed the benthic foraminiferal eDNA from the surface sediments in the Yellow Sea shelf of the Western Pacific Ocean for the first time using high–throughput sequencing technology. Rotaliida was the dominant group of the benthic foraminifera, while Monothalamids and Robertinida were found for the first time in this area. Monothalamids were an important part of the community, and its relative abundance of eDNA in individual stations was more than 50%. Correlation analysis showed that temperature, sea depth and sediment carbon content were the main environmental factors affecting the eDNA diversity of the benthic foraminifera community. The benthic foraminiferal eDNA community diversity and group diversity showed a significant positive correlation with sea depth and total inorganic carbon (TIC) in sediments, and showed a significant negative correlation with temperature and total organic carbon (TOC) in sediments. The distribution of eDNA community parameters in benthic foraminifera is indicative and has great potential for environmental monitoring.

CRediT authorship contribution statement

Yifei Cao: Conceptualization, Data curation, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Yanli Lei:** Conceptualization, Data curation, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing. **James Kar–Hei Fang:** Conceptualization, Supervision, Visualization, Writing – review & editing. **Tiegang Li:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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.2022.109294.

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