

BENTHIC FORAMINIFERAL COMMUNITY STRUCTURE AND DIVERSITY IN INTERTIDAL ZONE NEAR CHINA RESOURCES WENZHOU POWER PLANT

QIAO, L.¹ – REN, C. Z.² – SUN, X. M.¹ – LI, T. J.^{1*} – GUI, F.² – ZHAO, A. R.³

¹Key Laboratory of Sustainable Utilization of Technology Research for Fishery Resource of Zhejiang Province, Zhejiang Marine Fisheries Research Institute, Zhoushan 316012, China

²Marine Science and Technology College, Zhejiang Ocean University, Zhoushan 316004, China

³School of Fishery, Zhejiang Ocean University, Zhoushan 316004, China

*Corresponding author

e-mail: litiejun1982@126.com; phone: +86-580-2299-886

(Received 6th Feb 2023; accepted 27th Apr 2023)

Abstract. Benthic foraminifera are good indicators for monitoring marine environmental pollution and recovery of marine ecosystem. In this study, high-throughput sequencing based on small subunit rDNA and rRNA amplifications was used to assess total and active benthic foraminifera diversity and community composition in intertidal sediments near China Resources Wenzhou Power Plant. The results showed that the closer to the power plant, the lower the diversity and richness of both total and active foraminifera are. The total assemblages had higher values of species diversity and richness than active communities. A total of 33 and 25 foraminifera genera were identified using DNA-based and RNA-based HTS, respectively. The dominant genera included *Vellaria*, *Stainforthia* and *Miliammina* in total foraminiferal communities, and became *Ammonia*, *Miliammina* and *Notorotalia* in active foraminiferal communities at the station slightly away from the power plant; while only one dominant genus, *Haplomyxa*, was detected close to the power plant.

Keywords: high-throughput sequencing, SSU rDNA and rRNA, active foraminifera, intertidal zone, heavy metals

Introduction

Zhejiang Province, located in the southeast coast of China, is an economically developed region in the Yangtze River Delta, which has active economy and strong demand for electricity (Zhang, 2012; Shen et al., 2022). Especially in Wenzhou, the economic development of the region is extremely rapid, and the power shortage has become a bottleneck restricting the economic development. In addition, the grid structure of south Zhejiang power grid is weak, and the power support is insufficient, which cannot ensure the safe, stable and economic operation of the power grid (Yang et al., 2000; Ge, 2006). China Resources Wenzhou Power Plant is located at the southernmost end of Zhejiang power grid, that is, the end of Zhejiang power grid. Its construction greatly optimized the structure of Zhejiang power grid, enhanced the stability and reliability of the power grid, which not only helped to ease the power shortage situation in Wenzhou, but also promoted the sustainable economic development of Wenzhou area. China Resources Wenzhou Power Plant Phase I project has a 2×1000 MW ultra-supercritical coal-fired generating unit (Hu, 2013). In May 2014, both units were put into operation to generate electricity, and the maximum displacement of cooling water is $64.08 \text{ m}^3/\text{s}$. The Phase II expansion project of China Resources Wenzhou Power Plant is located in the west of the Phase I plant. In this project, two 1000 MW ultra-supercritical coal-fired generating units will be expanded

and ultra-low emission facilities will be built simultaneously. The cooling water displacement of Phase II project is basically the same as that of Phase I project.

Intertidal zone is located at the interface between the ocean and the land, which is an important type of marine ecosystem with abundant biological resources. This zone is significantly affected by water temperature, light, wave, tide, salinity and other ecological factors and human interference, which exhibits considerable spatial and temporal habitat heterogeneity at various scales (Meadows and Campbell, 1988; Kon et al., 2020). Benthic community is a representative ecological group in the intertidal zone, which can well reflect the environmental quality changes of the surrounding area (Blasco and Drake, 2008). Benthic foraminifera are unicellular protozoa that widely found on the surface of and within sediments (Tappan and Loeblich, 1988; Tarasova, 2006; Saraswati, 2021). Benthic foraminifera are characterized by small size, short life cycle, high species diversity, wide distribution, good preservation potential in sediments and high sensitivity to various pollutants, which have been regarded as bio-indicators of marine pollution (Murray, 1991; Frontalini and Coccioni, 2011; Gómez-León et al., 2018; Youssef et al., 2021).

Traditional method for identification and classification of benthic foraminifera was microscopic method, which mainly relies on their morphological characters (Vilela et al., 2011; Parsaian et al., 2018; Bergamin et al., 2019). Since benthic foraminifera are small and highly diverse, morphological identification is not only time-consuming and laborious (Lecroq, 2014), but also might underestimate the diversity of benthic foraminifera (Medinger et al., 2010; Lecroq, 2014; Pawlowski et al., 2014). In addition, in some species, generational alternations or environmental influences lead to the emergence of two or three different morphotypes (Pawlowski, 2000), which cause difficulties in the identification and might result in a large number of inaccurate species descriptions. With the development of DNA sequencing technology, the high-throughput sequencing (HTS) approach provides an effective and convenient method for the identification of foraminiferal species (Lejzerowicz et al., 2013; Pawlowski et al., 2018; Li et al., 2020), which has greatly increased our understanding of benthic foraminiferal community structure and diversity in the sedimentary environment worldwide.

In this study, we performed HTS of small subunit (SSU) rDNA and rRNA to investigate total and living benthic foraminifera in intertidal zone near China Resources Wenzhou Power Plant. The main objectives were to compare total and living benthic foraminiferal community structure and diversity, and explore the response of living foraminiferal assemblages to heavy metals.

Materials and methods

Site and sampling description

This survey was conducted in intertidal zone near China Resources Wenzhou Power Plant (27.50°N, 120.66°E) on May 11, 2021 (*Fig. 1*). Station CN01 (27.60°N, 120.66°E) is 11 km away from the power plant; and station CN02 (27.49°N, 120.69°E) is about 3 km from the power plant. The top ~1 cm of surface sediment was sampled for laboratory analyses. Three duplicate samples were collected at each station. 50 ml of sediment from each sample was separated immediately after collection and stored in centrifuge tubes. One tube was frozen in liquid nitrogen for foraminiferal community analysis, and the other was used for chemical analyses.

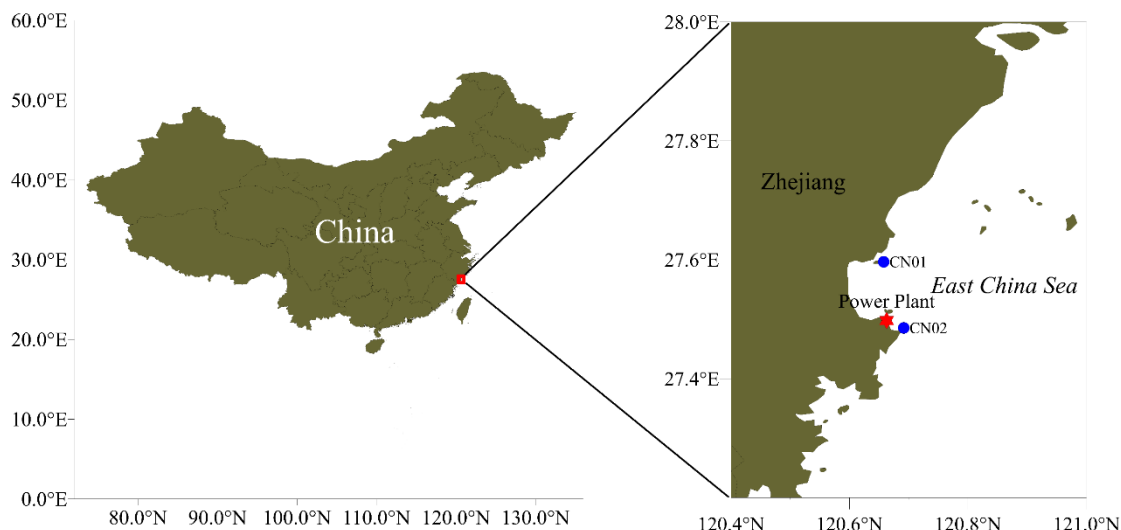


Figure 1. Location map of the study area and sampling stations

Sample measurements

Grain size fractions of sediments ranging from clay to sand with 0.25-phi intervals were detected using a Microtrac S3500 Particle Size Analyzer (Microtrac Ltd., USA). Concentrations of total nitrogen (TN) and total phosphorus (TP) were colorimetrically determined using ultraviolet spectrophotometer (Cary 50, Agilent Technologies Co. Ltd., USA) and visible spectrophotometer (V-1600BPC, Shanghai Mapada Instrument Co., Ltd., China). Organic carbon (OC) contents were measured using potassium dichromate volumetric method. The samples for heavy metal analysis were freeze-dried, homogenized, and reduced to a fine powder. The concentrations of Cu, Zn, Pb, Cr and Cd were analyzed using were determined by atomic absorption spectrometer (AA240FS, Agilent Technologies Co. Ltd., USA). The concentration of As was measured using atomic fluorescence spectrometer (Kylin-S12, Beijing Jitian Instrument Co., Ltd., China). The Hg concentration was analyzed using a Milestone DMA-80 direct mercury analyzer (Milestone, Italy).

Risk assessments of heavy metals in intertidal sediments

The degree of contamination (C_d) and potential ecological risk index (PERI) were important parameters used to evaluate assess heavy metals pollution in sediments (Hakanson, 1980; Zhuang and Zhou, 2021). C_d is calculated using *Equation 1*.

$$C_d = \sum_{i=1}^7 C_f^i \quad (\text{Eq.1})$$

$$C_f^i = \frac{C_i}{C_n^i} \quad (\text{Eq.2})$$

where C_f^i is the contamination factor of heavy metal i , C_i is the measured concentration of heavy metal i , and C_n^i is the background concentration of heavy metal i . The background values of Cu, Zn, Pb, Cd, Cr, Hg and As in sediments from coastal waters of southern Zhejiang Province were 27, 104, 47, 0.124, 86, 0.045 and 10.4 mg/kg, respectively (Zhang et al., 2005).

The calculation formulas for PERI are *Equation 3*.

$$PERI = \sum_{i=1}^7 E_r^i \quad (\text{Eq.3})$$

$$E_r^i = T_r^i \times C_f^i \quad (\text{Eq.4})$$

where E_r^i is the potential ecological risk index of heavy metal i , and T_r^i is the biological toxicity response factor of heavy metal i . The toxicity factors of Cu, Zn, Pb, Cd, Cr, Hg and As were 5, 1, 5, 30, 2, 40 and 10, respectively (Hakanson, 1980; Xu et al., 2008).

The evaluation criteria of the C_f^i , C_d , E_r^i and PERI were shown in *Table 1*.

Table 1. Hakanson evaluation indexes of the contamination degree and potential ecological risk

C_f^i		C_d		E_r^i		PERI	
Range	Degree of contamination	Range	Degree of contamination	Range	Potential ecological risk	Range	Potential ecological risk
< 1	Low	< 8	Low	< 40	Low	< 150	Low
1-3	Moderate	8-16	Moderate	40-80	Moderate	150-300	Moderate
3-6	Considerable	16-32	Considerable	80-160	Considerable	300-600	Considerable
≥ 6	Very high	≥ 32	Very high	160-320	High	≥ 600	Very high
				≥ 320	Very high		

Extraction and sequencing of DNA and RNA

Genomic DNA and RNA of the surficial sediment samples was extracted using a Fast DNA[®] SPIN Kit for Soil (MP Biomedicals, USA) and a Fast RNA[®] Pro Soil Direct Kit (MP Biomedicals, USA) following the manufacturer's instructions, respectively. The concentration and quality of DNA and RNA were examined using a NanoDrop 2000 (Thermo Scientific, USA), followed by agarose (1%) gel electrophoresis. The extracted DNA was temporarily stored at -20°C for subsequent experiments. The purified RNA was reverse-transcribed into cDNA using the SuperScript First-Strand Synthesis System with random hexamers for RT-PCR (Invitrogen, USA). The RNA and cDNA samples were stored at -80°C for subsequent experiments.

The foraminiferal 18S rDNA and cDNA sequences were amplified using foraminiferal-specific primers s14F3 (5'-ACGCAMGTGTGAACTTG-3') and s17 (5'-CGGTCACGTTCGTTGC-3') (Pawlowski, 2000; Lejzerowicz et al., 2013). PCR amplification was performed in 20 µL reactions containing 10 µL of 2× Pro Taq, 0.8 µL of each primer at 5 µM, and 10 ng/µL of DNA template. The PCR reaction consisted of a pre-denaturation at 94°C for 1.5 min, followed by 25 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 55°C and extension at 72°C for 45 s, immediately followed by an additional 10 cycles of denaturation at 94°C for 30 s, annealing for 30 s at 55°C and extension at 72°C for 2 min (Lejzerowicz et al., 2013; Li et al., 2020). The fragments were purified and sequenced on an Illumina MiSeq PE250 platform (Majorbio Bio-Pharm Technology Co., Ltd, Shanghai, China). Illumina sequencing raw DNA and cDNA data were submitted to the Sequence Read Archive (SRA) under the accession numbers SRP347799 and SRP347800, respectively.

Bioinformatic analysis

The paired-end reads from the original DNA/cDNA fragments of each sample were joined using FLASH (version 1.2.11, <https://ccb.jhu.edu/software/FLASH/index.shtml>). Raw reads were quality-controlled using QIIME (version 1.9.1; <http://qiime.org/install/index.html>). OTUs (operational taxonomic units) were generated with 97% similarity cut-off using UPARSE (version 7.0.1090, <http://www.drive5.com/uparse/>). The representative sequences of each OTU were assigned to Protist Ribosomal Reference (PR²) database (<https://github.com/pr2database/pr2database>) using the Basic Local Alignment Search Tool (BLAST) (Guillou et al., 2013). Unannotated OTUs were further assigned to the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>) using BLAST. After taxonomic assignment, OTUs that could not be assigned to foraminifera were removed from the dataset. Finally, to enable statistical analyses and comparisons of data across all samples, standardization among samples was performed by randomly subsampling the OTU table to the number of sequences present in the sample with the lowest amount. Alpha diversity indices such as Shannon and Chao1 were analyzed using Mothur (version 1.30.2, https://www.mothur.org/wiki/Download_mothur).

Data statistical analyses

Statistical analysis of the data was performed using IBM SPSS Statistics 20.0 (IBM, Inc., Armonk, NY, USA), and a Mann-Whitney U Test for independent samples was used to analyze the significance of differences between the two sampling stations. $p < 0.05$ was considered as statistically significant.

Results

Environmental parameters

The average grain sizes of sediments at station CN01 and CN02 were 17.44 μm and 10.58 μm , respectively. The percentages of clay, silt, and sand were 9.25%, 88.00%, and 2.75% at station CN01; and 21.35%, 78.31%, and 0.34% at station CN02, respectively (Table 2). Generally, the sediments at station CN01 were coarser than those at station CN02.

Table 2. The grain size and composition of sediments in intertidal zone near China Resources Wenzhou Power Plant

Station	Grain sizes (μm)	Clay (%)	Silt (%)	Sand (%)
CN01	17.44	9.25	88.00	2.75
CN02	10.58	21.35	78.31	0.34

TN, TP, OC and 7 heavy metals (Cu, Zn, Pb, Cd, Cr, Hg, and As) were measured, and their concentrations are presented in Table 3. The TN, TP and OC concentrations at station CN02 were higher than those at station CN01 ($p > 0.05$). The concentrations of Cu, Zn, Cd, Cr, Hg and As at station CN02 were higher than those at station CN01 ($p > 0.05$), whereas the concentrations of Pb was slightly higher at station CN01 ($p > 0.05$).

Table 3. Basic statistics of heavy metals in intertidal sediments near China Resources Wenzhou Power Plant

Station	TN (mg/kg)	TP (mg/kg)	OC (%)	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)	Cd (mg/kg)	Cr (mg/kg)	Hg (mg/kg)	As (mg/kg)
CN01	138	322	0.079	10	56	25	0.052	32	0.039	9.2
CN02	435	451	0.43	14	68	24	0.070	43	0.047	9.3

TN: total nitrogen; TP: total phosphorus; OC: Organic carbon (OC)

At station CN01, the order of the C_f^i values for the seven heavy metals was As (0.88) > Hg (0.87) > Zn (0.54) > Pb (0.53) > Cd (0.42) > Cr (0.37) = Cu (0.37). The C_d value was 3.98 (Fig. 2a). Thus, station CN01 was lowly contaminated with heavy metals. The E_r^i values at station CN01 were in the following order: Hg (34.67) > Cd (12.58) > As (8.85) > Pb (2.66) > Cu (1.85) > Cr (0.74) > Zn (0.54). The PERI value was 61.89 (Fig. 2b), indicating that station CN01 was in a low ecological risk level. The order of the values of C_f^i at station CN02 was Hg (1.04) > As (0.89) > Zn (0.65) > Cd (0.56) > Cu (0.52) > Pb (0.51) > Cr (0.50) (Fig. 2a). The results showed that station CN02 was lowly contaminated by heavy metals, except for Hg (moderate contamination). The C_d value was 4.69 (Fig. 2a), indicating a low degree of contamination at station CN02. The values of E_r^i at station CN02 were as follows: Hg (41.78) > Cd (16.78) > As (8.94) > Cu (2.59) > Pb (2.55) > Cr (1.00) > Zn (0.65) (Fig. 2b), which indicated that Hg was at moderate potential ecological risks, while the other six heavy metals were at low potential ecological risks. The PERI value was 74.46 (Fig. 2b), suggesting that station CN02 was in a low ecological risk level.

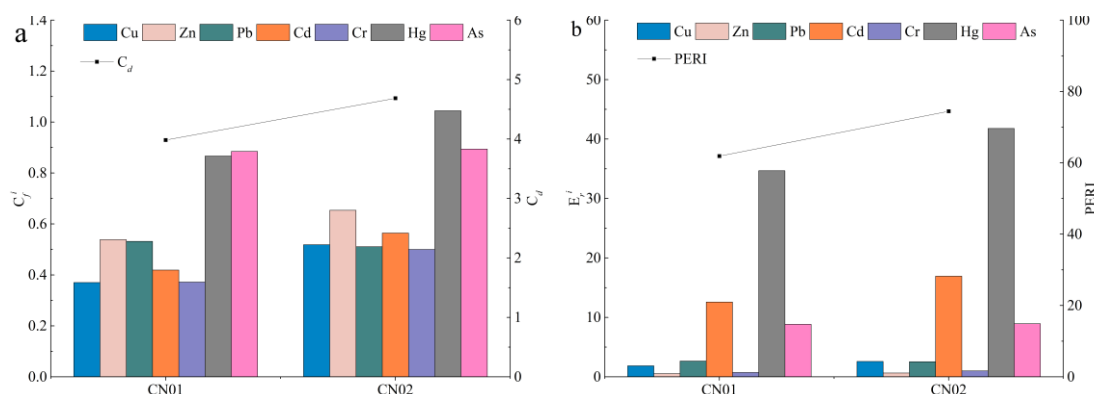


Figure 2. Degree of contamination (a) and potential ecological risk index (b) of heavy metals in intertidal sediments near China Resources Wenzhou Power Plant

High-throughput sequencing data

A total of 780,944 raw reads were obtained using the high-throughput sequencing based on SSU rDNA and rRNA amplifications of all samples. After quality control of the raw reads, a total of 661,549 clean reads and 252 OTUs were obtained (Table 4). On the basis of the taxonomic information, 384,485 sequences were annotated to foraminifera, accounting for 3.39%–99.84% of total sequences (Table 4; Fig. 3).

Following the exclusion of Metazoa, Cryptophyta, and unclassified sequences, the remaining foraminifera sequences were randomly rarefied to 9692, which was used in further analyses of community composition and diversity.

Table 4. The numbers of sequences in quality control analysis

Samples	Raw reads	Clean reads	Total OTUs	Foraminiferal reads
CN01 (DNA)	161,037	129,630	172	129,418
CN01 (RNA)	244,027	187,045	102	186,941
CN02 (DNA)	86,317	59,277	27	58,434
CN02 (RNA)	289,563	285,597	22	9,692
Sum	780,944	661,549	252	384,485

Note: OTUs: operational taxonomic units

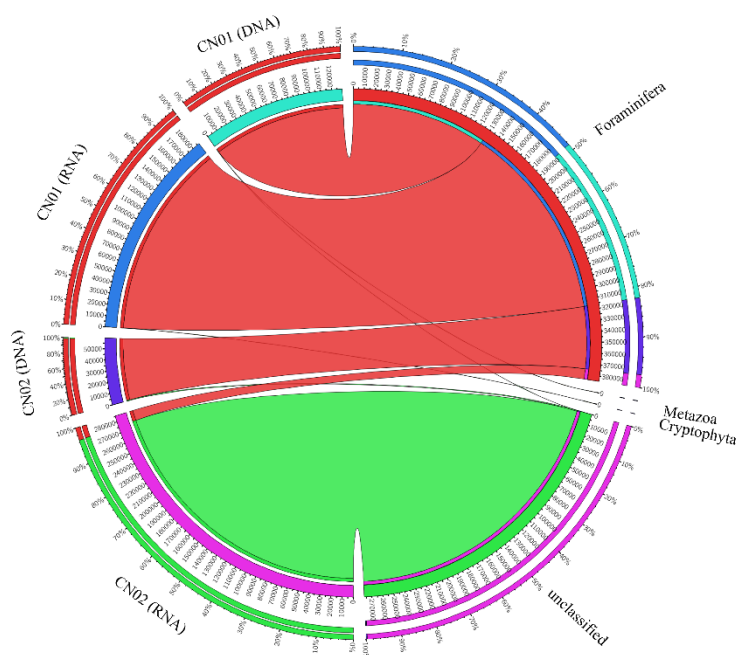


Figure 3. Circular representation of sequences assigned to foraminifera and unclassified groups

Foraminiferal diversity and community composition

Foraminifera diversity and richness were estimated using the Shannon (*Fig. 4a*) Chao1 indices (*Fig. 4b*), respectively. The results showed that the total assemblages had higher values of species diversity and richness than those of active communities. In both total and active assemblages, the mean values of the Shannon and Chao1 indices of station CN01 were the higher than CN02.

The total and active foraminiferal community composition in intertidal zone near China Resources Wenzhou Power Plant revealed by HTS is shown in *Figure 5*. At the class level, four classes, Globothalamea, Monothalamea, Nodosariata and Tubothalamea, were identified (*Fig. 5a*). At station CN01, the total foraminiferal communities were dominated by Monothalamea (42.75%), Globothalamea (36.19%)

and Tubothalamea (20.69%). However, Globothalamea was the most abundant class (56.13%), followed by Tubothalamea (31.86%) and Monothalamea (11.89%) in active foraminiferal communities. At station CN02, both total and active foraminiferal communities were dominated by Monothalamea, with relative abundance of 99.74% and 100.00%, respectively. At the order level, 7 foraminifera orders were identified (Fig. 5b). At station CN01, Allogromiida was the most abundant order in total foraminiferal communities, with relative abundance of 39.33%, followed by Rotaliida (27.09%) and Miliolida (20.69%). While, in active foraminiferal communities, the most abundant order was Rotaliida (55.98%), followed by Miliolida (31.86%). At station CN02, the relative abundances of unclassified Monothalamea were highest, accounting for 95.44% and 100.00% of the total and active foraminifera abundance, respectively. At the family level, 33 foraminifera families were identified (Fig. 5c). At station CN01, the total foraminiferal communities were dominated by Allogromiidae (37.60%), Stainforthiidae (17.07%) and Miliamminidae (16.39%). However, Ammoniididae was the most abundant family (41.76%), followed by Miliamminidae (31.20%) and Notorotaliidae (10.94%) in active foraminiferal communities. At station CN02, only one family, Reticulomyxidae, was dominated, with relative abundance of 95.44% and 100.00%, respectively. At the genus level, 33 genera for total foraminifera and 25 genera for active foraminifera were identified (Fig. 5d). At station CN01, the dominant genera included *Vellaria* (37.60%), *Stainforthia* (17.07%) and *Miliammina* (16.39%) in total foraminiferal communities, and became *Ammonia* (41.76%), *Miliammina* (31.20%) and *Notorotalia* (10.94%) in active foraminiferal communities. At station CN02, only one dominant genus, *Haplomyxa*, was detected, which contributed 95.44% and 100.00% of the total and active foraminifera abundance, respectively.

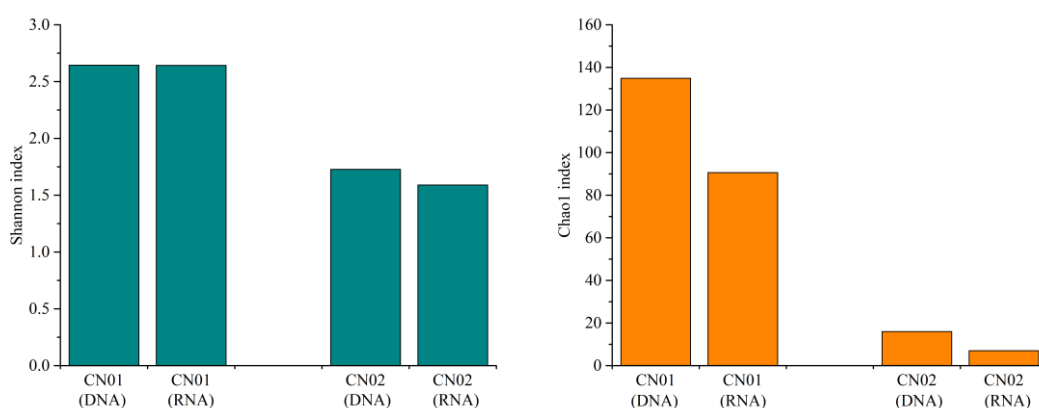


Figure 4. OTU-level Shannon index (a) and Chao1 index (b) of total and active foraminiferal community

Discussion

Benthic foraminifera are good indicators for monitoring marine environmental pollution and recovery of marine ecosystem (Frontalini and Coccioni, 2008; Suokhrie et al., 2017; Klootwijk et al., 2021). During the past 20 years, molecular techniques based on analysis of DNA sequences offered new tools for the identification of foraminiferal species and studies of their diversity and distribution (Pawlowski, 2000; Pawlowski and Holzmann, 2002, 2008; Pawlowski et al., 2011, 2014; Moss et al., 2016; Li et al., 2020).

Unfortunately, DNA sequences could not distinguish between active and dead individuals (Qiao et al., 2022). In morphological methods, sediment samples are usually stained with Rose Bengal to recognize living individuals, but the effectiveness of this method is quite disputed (Murray and Bowser, 2000; Pawlowski and Holzmann, 2008; Schönfeld et al., 2012). Ribosomal RNA (rRNA) is normally present in active cells and degrades rapidly in deceased cells (Segev et al., 2012). Therefore, RNA-based HTS might provide a new and efficient method for detecting active foraminifera (Pawlowski et al., 2014; Pochon et al., 2015). In present study, HTS based on SSU rDNA and rRNA amplification was used to evaluate total and active benthic foraminiferal community structure and diversity in intertidal sediments near China Resources Wenzhou Power Plant.

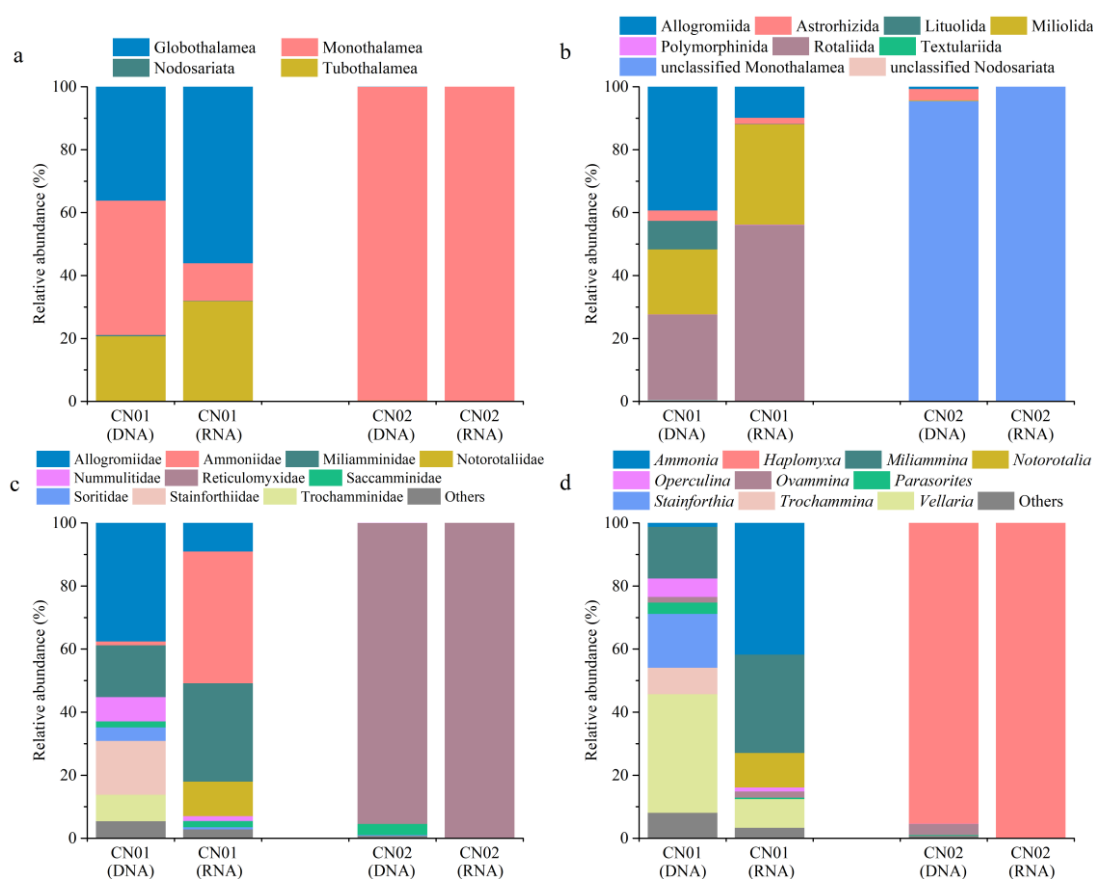


Figure 5. Total and active foraminiferal community composition in the sediment samples at the class (a), order (b), family (c) and genus (d) levels

In this study, a total of 33 and 25 foraminifera genera were identified using DNA-based and RNA-based HTS, respectively. At station CN01, the dominant genera included *Vellaria*, *Stainforthia* and *Miliammina* in total foraminiferal communities, and became *Ammonia*, *Miliammina* and *Notorotalia* in active foraminiferal communities (Fig. 5d). Discrepancies between the two assemblages might have resulted from many aspects. First, the total assemblage not only included dead individuals but was also the record of several generations, while the active assemblage represented only a single generation. On one hand, active assemblages based on a single generation might underestimate species richness compared to total assemblages based on several

generations (Kidwell, 2001). On the other hand, total assemblages can capture rare and patchily distributed species that have accumulated over several years (Murray, 2006). This might partly explain why the total assemblages exhibited higher species diversity and richness than active communities (Fig. 4), as well as in previous studies (Mendes et al., 2013; Li et al., 2021). Second, taxonomic composition biases could arise from the over-representation of metabolically active foraminifera relative to species responding more slowly to environmental changes (Lejzerowicz et al., 2013). Previous studies have shown that rotaliids tend to respond more rapidly and consume fresh incoming organic matter at a higher rate than monothalamous foraminifera (Gooday et al., 2008; Enge et al., 2011), which could have an impact on a higher proportion of Rotaliids because active cells produce more RNA molecules than resting cells and thus tend to saturate the resulting sequence data, especially after exponential PCR amplification (Lejzerowicz et al., 2013). This is consistent with our work, which revealed a higher relative abundance of Rotaliida in active assemblages compared with the that of the total communities (Fig. 5b). Third, the discrepancy between the total and active foraminiferal communities might result from taphonomic processes and postmortem transportation (Alves Martins et al., 2018, 2019). On one hand, dissolution of calcareous tests and degradation of agglutinated tests could cause differences in the percentages of calcareous and agglutinated foraminifera between total and active assemblages (Alve and Murray, 1997; Duros et al., 2012; Dessandier et al., 2018). Further studies are needed to determine the influencing conditions that cause variations in the relative abundance of individual species between total and living foraminiferal communities. On the other hand, the differences between the total and active assemblages might be influenced by hydrodynamic activity. Because active foraminifera are denser than detrital particles and empty tests of dead individuals, the latter are more easily suspended and transported by waves (Alves Martins et al., 2018, 2019). However, estimating the contribution of transported individuals to the total foraminiferal community was impossible because of the lack of sufficient evidence of the deposition of allochthonous individuals or the loss of autochthonous individuals. Lastly, changes in some of the main environmental factors, such as temperature, salinity, food supply, sediment type, and composition, might also make active communities different from total assemblages (Tarasova, 2006; Alves Martins et al., 2019). However, assessing the net effects of these factors when several environmental measurements are missing is difficult.

Heavy metals are one of the most widely distributed pollutants in coastal waters (Martinez-Colon et al., 2009), which have a deleterious effect on benthic organisms, especially foraminifera (Boehnert et al., 2020). In general, the decrease of species diversity and abundance of foraminifera can be regarded as an important indicator of ecological deterioration (Tarasova, 2006; Bouchet et al., 2020). In the present study, the contamination level of heavy metals at station CN02 was higher than that at station CN01 (Fig. 2), and the relatively lower species richness and diversity of foraminiferal assemblages were observed at station CN02 compared to those obtained at station CN01 (Fig. 4). The response of benthic foraminifera to environmental pollution was also expressed by morphological abnormalities and changes in chemical composition of the shell (Frontalini and Coccioni, 2008; Linshy et al., 2013; Ayadi et al., 2016; El-Kahawy et al., 2018; Boehnert et al., 2020; Cong et al., 2022). Unfortunately, they were not covered in this study. In addition, different species of benthic foraminifera have different tolerance to pollutants (Alve, 1991; Le Cadre and Debenay, 2006). Some with strong tolerance could rapidly reproduce to form dominant species, such as *Ammonia*

tepida, *A. parkinsoniana*, *Aubignyna perlucida*, *Bolivina striatula*, *Criboelphidium oceanensis*, *Eggerella scabra*, *Elphidium crispum*, *Hyanesina germanica*, *H. pacifica*, *Nonionella turgida*, *Protelphidium tuberculatum* (Frontalini and Coccioni, 2008; Coccioni et al., 2009); Some that are highly sensitive to environmental pollution declined in abundance or even extinct, such as *Adelosina* spp., *Buccella frigida*, *Cancris auriculus*, *Cribrostomoides subglobosus*, *Quinqueloculina* spp., *Uvigerina peregrina* (Valenti et al., 2008; Jorissen et al., 2009). This difference might cause changes in the abundance, community composition of benthic foraminifera (Tarasova, 2006; Klootwijk et al., 2021; Li et al., 2021). In this study, we found that Hg was at moderate potential ecological risks at station CN02 (Fig. 2), and the dominant genus was *Haplomyxa* at this station (Fig. 5). Therefore, we speculated *Haplomyxa* might have a tolerance for Hg. Future investigation needs to be conducted to decipher this speculative result. At station CN02, only one dominant genus was detected and contributed 95.44% and 100.00% of the total and active foraminifera abundance, respectively (Fig. 5). The following might also be related besides environmental factors. On the one hand, the taxonomic coverage in the foraminiferal gene database is incomplete (Lv, 2018; Qiao et al., 2022). The SSU rDNA sequences were lacking in the database for some foraminifera, which might be the reason of so many unclassified sequences in the HTS data of CN02 (RNA) (Table 4). Therefore, further efforts are required to develop the foraminiferal gene database to improve its taxonomic coverage. On the other hand, affected by environmental factors such as substrate type, food availability and predation, the benthic organisms inhabiting the intertidal zone are prone to aggregate on a small scale, which resulted in the patchy distribution of benthic organisms in the heterogeneous intertidal zone (Moodley et al., 1998; Braga et al., 2011; Zhang et al., 2019). As a result, coupled with the small size of benthic foraminifera, there might be a single species or a dataset with many “zero-counts” (Schlachter et al., 2008). Therefore, sampling should be taken seriously and based on theoretical principles or standard methods with a sufficient number of samples; otherwise, the collected samples will have a large deviation from the estimated results (Beukema and Dekker, 2012; Zhang et al., 2019).

It should be noted that the findings of the present study are based on only a single sampling survey conducted on sediment samples collected from intertidal zone near China Resources Wenzhou Power Plant, and consequently, we have not been able to assess the temporal changes in the benthic foraminiferal community structure. Accordingly, it is necessary to carry out continuous monitoring surveys to explore the relationship between the seasonal and long-term foraminiferal variations and environment changes in this region. Nevertheless, the findings of this study do provide a useful foundation for future studies on the benthic foraminifera community composition and diversity using high-throughput sequencing technology.

Conclusions

In this study, the concentrations and contamination level of heavy metals in intertidal sediments near China Resources Wenzhou Power Plant were investigated. The results showed low ecological risk level of heavy metals in survey stations and moderate contamination by Hg at the station close to the power plant. The diversity and community composition of total and active benthic foraminifera were revealed using HTS based on SSU rDNA and rRNA amplification. The results showed that the closer

to the power plant, the lower the diversity and richness of both total and active foraminifera. The total assemblages had higher values of species diversity and richness than those of active communities. a total of 33 and 25 foraminifera genera were identified using DNA-based and RNA-based HTS, respectively. The dominant genera included *Vellaria*, *Stainforthia* and *Miliammina* in total foraminiferal communities, and became *Ammonia*, *Miliammina* and *Notorotalia* in active foraminiferal communities at the station slightly away from the power plant; while only one dominant genus, *Haplomyxa*, was detected at the close to the power plant. We speculated *Haplomyxa* might have a tolerance for Hg, and future laboratory culture studies under controlled conditions needs to be conducted to decipher this speculative result.

Acknowledgements. This study was supported by the Science and Technology Planning Project of Zhejiang Marine Fisheries Research Institute (HYS-ZY-202101); Public Welfare Projects of Science and Technology Bureau of Zhoushan City (2022C31057).

REFERENCES

- [1] Alve, E. (1991): Benthic foraminifera in sediment cores reflecting heavy metal pollution in Sorfjord, western Norway. – *The Journal of Foraminiferal Research* 21(1): 1-19.
- [2] Alve, E., Murray, J. W. (1997): High benthic fertility and taphonomy of foraminifera: a case study of the Skagerrak, North Sea. – *Marine Micropaleontology* 31: 157-175.
- [3] Alves Martins, M. V., Hohenegger, J., Frontalini, F., Laut, L., Miranda, P., Rodrigues, M. A., Duleba, W., Geraldès, M. C., Rocha, F. (2018): Heterogeneity of environments in a coastal lagoon mouth by the comparison between living and dead benthic foraminiferal assemblages (Ria de Aveiro Portugal). – *Estuarine, Coastal and Shelf Science* 213: 199-216.
- [4] Alves Martins, M. V., Hohenegger, J., Frontalini, F., Dias, J. M. A., Geraldès, M. C., Rocha, F. (2019): Dissimilarity between living and dead benthic foraminiferal assemblages in the Aveiro Continental Shelf (Portugal). – *Plos One* 14(1): e0209066.
- [5] Ayadi, N., Zghal, I., Aloulou, F., Bouzid, J. (2016): Impacts of several pollutants on the distribution of recent benthic foraminifera: the southern coast of Gulf of Gabes, Tunisia. – *Environmental Science and Pollution Research* 23(7): 6414-6429.
- [6] Bergamin, L., Di Bella, L., Ferraro, L., Frezza, V., Pierfranceschi, G., Romano, E. (2019): Benthic foraminifera in a coastal marine area of the eastern Ligurian Sea (Italy): Response to environmental stress. – *Ecological Indicators* 96: 16-31.
- [7] Beukema, J. J., Dekker, R. (2012): Estimating macrozoobenthic species richness along an environmental gradient: sample size matters. – *Estuarine, Coastal and Shelf Science* 111: 67-74.
- [8] Blasco, J., Drake, P. M. (2008): An integrative approach using benthos to evaluate environmental quality of the Sancti-Petri saltmarsh area. – *Marine Environmental Research* 66(1): 200-205.
- [9] Boehnert, S., Birkelund, A. R., Schmiedl, G., Kuhnert, H., Kuhn, G., Hass, H. C., Hebbeln, D. (2020): Test deformation and chemistry of foraminifera as response to anthropogenic heavy metal input. – *Marine Pollution Bulletin* 155: 111112.
- [10] Bouchet, V. M., Deldicq, N., Baux, N., Dauvin, J. C., Pezy, J. P., Seuront, L., Méar, Y. (2020): Benthic foraminifera to assess ecological quality statuses: the case of salmon fish farming. – *Ecological Indicators* 117: 106607.
- [11] Braga, C. F., Monteiro, V. F., Rosa-Filho, J. S., Beasley, C. R. (2011): Benthic macroinfaunal assemblages associated with Amazonian saltmarshes. – *Wetlands Ecology and Management* 19(3): 257-272.

- [12] Coccioni, R., Frontalini, F., Marsili, A., Mana, D. (2009): Benthic foraminifera and trace element distribution: a case-study from the heavily polluted lagoon of Venice (Italy). – *Marine Pollution Bulletin* 59(8-12): 257-267.
- [13] Cong, J. Y., Long, H. Y., Zhang, Y., Wang, N. (2022): Ecological environment response of benthic foraminifera to heavy metals and human engineering: a case study from Jiaozhou Bay, China. – *Chinese geology* 5(1): 12-25.
- [14] Dessandier, P. A., Bonnin, J., Kim, J. H., Racine, C. (2018): Comparison of living and dead benthic foraminifera on the Portuguese margin: understanding the taphonomical processes. – *Marine Micropaleontology* 140: 1-16.
- [15] Duros, P., Fontanier, C., De Stigter, H. C., Cesbron, F., Metzger, E., Jorissen, F. J. (2012): Live and dead benthic foraminiferal faunas from Whittard Canyon (NE Atlantic): Focus on taphonomic processes and paleo-environmental applications. – *Marine Micropaleontology* 94: 25-44.
- [16] El-Kahawy, R., El-Shafeiy, M., Helal, S. A., Aboul-Ela, N., El-Wahab, M. A. (2018): Morphological deformities of benthic foraminifera in response to nearshore pollution of the Red Sea, Egypt. – *Environmental Monitoring and Assessment* 190(5): 312.
- [17] Enge, A. J., Nomaki, H., Ogawa, N. O., Witte, U., Moeseneder, M. M., Lavik, G., Ohkouchi, N., Kitazato, H., Kučera, M., Heinz, P. (2011): Response of the benthic foraminiferal community to a simulated short-term phytodetritus pulse in the abyssal North Pacific. – *Marine Ecology Progress Series* 438: 129-142.
- [18] Frontalini, F., Coccioni, R. (2008): Benthic foraminifera for heavy metal pollution monitoring: a case study from the central Adriatic Sea coast of Italy. – *Estuarine, Coastal and Shelf Science* 76(2): 404-417.
- [19] Frontalini, F., Coccioni, R. (2011): Benthic foraminifera as bioindicators of pollution: a review of Italian research over the last three decades. – *Revue de Micropaléontologie* 54(2): 115-127.
- [20] Ge, L. (2006): Accelerate the construction of Wenzhou power grid. – *Zhejiang Economy* (13): 60-61.
- [21] Gomez-Leon, A., Rodriguez-Figueroa, G. M., Shumilin, E., Luisa Carreno, A., Sanchez, A. (2018): Abundance and distribution of benthic foraminifera as indicators of the quality of the sedimentary environment in a subtropical lagoon, Gulf of Check for California. – *Marine Pollution Bulletin* 130: 31-39.
- [22] Gooday, A. J., Nomaki, H., Kitazato, H. (2008): Modern deep-sea benthic foraminifera: a brief review of their morphology-based biodiversity and trophic diversity. – *Biogeochemical Controls on Palaeoceanographic Environmental Proxies* 303(1): 97-119.
- [23] Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., del Campo, J., Dolan, J., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W., Lara, E., Le Bescot, N., Logares, R., Christen, R. (2013): The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. – *Nucleic Acids Research* 41: D597-D604.
- [24] Hakanson, L. (1980): An ecological risk index for aquatic pollution control. A sedimentological approach. – *Water Research* 14(8): 975-1001.
- [25] Hu, Y. S. (2013): Research on power plant project financing risks evaluation and preventive measures. – Master's Thesis, North China Electric Power University.
- [26] Jorissen, F. J., Bicchi, E., Duchemin, G., Durrieu, J., Galgani, F., Cazes, L., Gaultier, M., Camps, R. (2009): Impact of oil-based drill mud disposal on benthic foraminiferal assemblages on the continental margin off Angola. – *Deep Sea Research Part II: Topical Studies in Oceanography* 56(23): 2270-2291.
- [27] Kidwell, S. M. (2001): Preservation of species abundance in marine death assemblages. – *Science* 294(5544): 1091-1094.
- [28] Klootwijk, A. T., Alve, E., Hess, S., Renaud, P. E., Sørli, C., Dolven, J. K. (2021): Monitoring environmental impacts of fish farms: comparing reference conditions of

- sediment geochemistry and benthic foraminifera with the present. – *Ecological Indicators* 120: 106818.
- [29] Kon, K., Shimanaga, M., Horinouchi, M. (2020): *Marine Ecology: Intertidal/Littoral Zone*. – In: Inaba, K., Hall-Spencer, J. (eds.) *Japanese Marine Life*. Springer, Singapore.
- [30] Le Cadre, V., Debenay, J. P. (2006): Morphological and cytological responses of *Ammonia* (foraminifera) to copper contamination: implication for the use of foraminifera as bioindicators of pollution. – *Environmental Pollution* 143(2): 304-317.
- [31] Lecroq, B. (2014): *Molecular Assessment of Benthic Foraminiferal Diversity*. – In: Kitazato, H., M. Bernhard, J. (eds.) *Approaches to Study Living Foraminifera*. Environmental Science and Engineering. Springer, Tokyo.
- [32] Lejzerowicz, F., Voltsky, I., Pawlowski, J. (2013): Identifying active foraminifera in the Sea of Japan using metatranscriptomic approach. – *Deep Sea Research Part II: Topical Studies in Oceanography* 86: 214-220.
- [33] Li, Q., Lei, Y., Morard, R., Li, T., Wang, B. (2020): Diversity hotspot and unique community structure of foraminifera in the world's deepest marine blue hole–Sansha Yongle Blue Hole. – *Scientific Reports* 10(1): 1-11.
- [34] Li, T., Cai, G., Zhang, M., Li, S., Nie, X. (2021): The response of benthic foraminifera to heavy metals and grain sizes: a case study from Hainan Island, China. – *Marine Pollution Bulletin* 167: 112328.
- [35] Linshy, V. N., Saraswat, R., Kurtarkar, S. R., Nigam, R. (2013): Experiment to decipher the effect of heavy metal cadmium on coastal benthic foraminifer *Pararotalia nipponica* (ASANO). – *Journal of the Palaeontological Society of India* 58(2): 205-211.
- [36] Lv, M. (2018): Preliminary study on molecular diversity of benthic foraminifera and its relationship to the environmental factors. – PhD dissertation, University of Chinese Academy of Sciences.
- [37] Martinez-Colon, M., Hallock, P., Green-Ruiz, C. (2009): Strategies for using shallow-water benthic foraminifera as bioindicators of potentially toxic elements: a review. – *The Journal of Foraminiferal Research* 39(4): 278-299.
- [38] Meadows, P. S., Campbell, J. I. (1988): *The Intertidal Zone*. – In: *An Introduction to Marine Science*. Springer, Boston, MA.
- [39] Medinger, R., Nolte, V., Pandey, R. V., Jost, S., Ottenwälder, B., Schlötterer, C., Boenigk, J. (2010): Diversity in a hidden world: potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. – *Molecular Ecology* 19(s1): 32-40.
- [40] Mendes, I., Dias, J. A., Schönfeld, J., Ferreira, Ó., Rosa, F., Lobo, F. J. (2013): Living, dead and fossil benthic foraminifera on a river dominated shelf (northern Gulf of Cadiz) and their use for paleoenvironmental reconstruction. – *Continental Shelf Research* 68: 91-111.
- [41] Moodley, L., Heip, C. H. R., Middelburg, J. J. (1998): Benthic activity in sediments of the northwestern Adriatic Sea: sediment oxygen consumption, macro- and meiofauna dynamics. – *Journal of Sea Research* 40(3-4): 263-280.
- [42] Moss, J. A., McCurry, C., Schwing, P., Jeffrey, W. H., Romero, I. C., Hollander, D. J., Snyder, R. A. (2016): Molecular characterization of benthic foraminifera communities from the Northeastern Gulf of Mexico shelf and slope following the Deepwater Horizon event. – *Deep Sea Research Part I: Oceanographic Research Papers* 115: 1-9.
- [43] Murray, J. W. (1991): *Ecology and Palaeoecology of Benthic Foraminifera*. – Longman Scientific & Technical, Harlo.
- [44] Murray, J. W. (2006): *Ecology and Applications of Benthic Foraminifera*. – Cambridge University Press, New York.
- [45] Murray, J. W., Bowser, S. S. (2000): Mortality, protoplasm decay rate, and reliability of staining techniques to recognize 'living' foraminifera: a review. – *The Journal of Foraminiferal Research* 30(1): 66-70.

- [46] Parsaian, M., Shokri, M. R., Pazooki, J. (2018): The response of benthic foraminifera to aquaculture and industrial pollution: a case study from the Northern Persian Gulf. – *Marine Pollution Bulletin* 135: 682-693.
- [47] Pawlowski, J. (2000): Introduction to the molecular systematics of foraminifera. – *Micropaleontology* 46(S1): 1-12.
- [48] Pawlowski, J., Holzmann, M. (2002): Molecular phylogeny of foraminifera - a review. – *European Journal of Protistology* 38(1): 1-10.
- [49] Pawlowski, J., Holzmann, M. (2008): Diversity and geographic distribution of benthic foraminifera: a molecular perspective. – *Biodiversity and Conservation* 17: 317-328.
- [50] Pawlowski, J., Fontaine, D., Silva, A. A. D., Guiard, J. (2011): Novel lineages of Southern Ocean deep-sea foraminifera revealed by environmental DNA sequencing. – *Deep Sea Research Part II Topical Studies in Oceanography* 58(19-20): 1996-2003.
- [51] Pawlowski, J., Lejzerowicz, F., Esling, P. (2014): Next-generation environmental diversity surveys of foraminifera: preparing the future. – *Biological Bulletin* 227(2): 93-106.
- [52] Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apothéloz-Perret-Gentil, L., Beja, P., Boggero, A., Borja, A., Bouchez, A., Cordier, T., Domaizon, I., Feio, M. J., Filipe, A. F., Fornaroli, R., Graf, W., Herder, J., Hoorn, B., Jones, J. I., Sagova-Mareckova, M., Moritz, C., Barquín, J., Piggott, J. J., Pinna, M., Rimet, F., Rinkevich, B., Sousa-Santos, C., Specchia, V., Trobajo, R., Vasselon, V., Vitecek, S., Zimmerman, J., Weigand, A., Leese, F., Kahlert, M. (2018): The future of biotic indices in the ecogenomic era: integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems. – *Science of the Total Environment* 637-638: 1295-1310.
- [53] Pochon, X., Wood, S. A., Keeley, N. B., Lejzerowicz, F., Esling, P., Drew, J. Pawlowski, J. (2015): Accurate assessment of the impact of salmon farming on benthic sediment enrichment using foraminiferal metabarcoding. – *Marine Pollution Bulletin* 100(1): 370-382.
- [54] Qiao, L., Fan, S., Ren, C., Gui, F., Li, T., Zhao, A., Yan, Z. (2022): Total and active benthic foraminiferal community and their response to heavy metals revealed by high throughput DNA and RNA sequencing in the Zhejiang coastal waters, East China Sea. – *Marine Pollution Bulletin* 184: 114225.
- [55] Saraswati, P. K. (2021): Foraminifera—witness of the evolving Earth. – *Foraminiferal Micropaleontology for Understanding Earth's History*.
- [56] Schlacher, T. A., Schoeman, D. S., Dugan, J., Lastra, M., Mclachlan, A. (2008): Sandy beach ecosystems: key features, sampling issues, management challenges and climate change impacts. – *Marine Ecology* 29(S1): 70-90.
- [57] Schönfeld, J., Alve, E., Geslin, E., Jorissen, F., Korsun, S., Spezzaferri, S. (2012): The FOBIMO (FORaminiferal BIO-MONitoring) initiative—towards a standardised protocol for soft-bottom benthic foraminiferal monitoring studies. – *Marine Micropaleontology* 94: 1-13.
- [58] Segev, E., Smith, Y., Ben-Yehuda, S. (2012): RNA dynamics in aging bacterial spores. – *Cell* 148(1-2): 139-149.
- [59] Shen, L., Xia, Y. L., Chen, L. J. (2022): Medium-and long-term power supply and demand forecast and countermeasure in Zhejiang Province. – *Energy of China* 44(7): 66-73.
- [60] Suokhrie, T., Sarawat, R., Nigam, R. (2017): Foraminifera as Bioindicators of Pollution: A Review of Research over the Last Decade. – In: Kathal, P. K., Nigam, R., Talib, A. (eds.) *Micropaleontology and Its Applications*. Scientific Publishers, Jodhpur.
- [61] Tappan, H., Loeblich, A. R. (1988): Foraminiferal evolution, diversification, and extinction. – *Journal of Paleontology* 695-714.
- [62] Tarasova, T. S. (2006): Environmental impacts on the benthic foraminiferal fauna in nearshore ecosystems. – *Russian Journal of Marine Biology* 32(1): S11-S20.

- [63] Valenti, D., Tranchina, L., Brai, M., Caruso, A., Cosentino, C., Spagnolo, B. (2008): Environmental metal pollution considered as noise: effects on the spatial distribution of benthic foraminifera in two coastal marine areas of Sicily (Southern Italy). – *Ecological Modelling* 213(3-4): 449-462.
- [64] Vilela, C. G., Batista, D. S., Neto, J. A. B., Ghiselli Jr, R. O. (2011): Benthic foraminifera distribution in a tourist lagoon in Rio de Janeiro, Brazil: a response to anthropogenic impacts. – *Marine Pollution Bulletin* 62(10): 2055-2074.
- [65] Xu, Z. Q., Ni, S. J., Tuo, X. G., Zhang, C. J. (2008): Calculation of heavy metals' toxicity coefficient in the evaluation of potential ecological risk index. – *Environmental Science and Technology* 31(2): 112-115.
- [66] Yang, S. M., Wu, J. L. (2000): Study on network structures of Wenzhou power system. – *Zhejiang Electric Power* (1): 5-7.
- [67] Youssef, M., El-Sorogy, A., Al-Kahtany, K., Mohsen, S. (2021): Benthic foraminifera as bio-indicators of coastal marine environmental contamination in the Red Sea - Gulf of Aqaba, Saudi Arabia. – *Bulletin of Environmental Contamination and Toxicology* 106(6): 1033-1043.
- [68] Zhang, T., Zhao, F., Wang, S., Zhang, T., Liu, J., Gao, Y., Zhuang, P. (2019): Estimating the macrobenthic species richness with an optimized sampling design in the intertidal zone of Changjiang estuary. – *Acta Oceanologica Sinica* 38(2): 114-124.
- [69] Zhang, Y. M. (2012): Current situation and countermeasure analysis of electricity demand in Zhejiang province. – *China Electric Power* 8: 72-74.
- [70] Zhang, Z. Z., Li, S. L., Dong, Y. X., Wang, Q. H., Xiao, F., Lu, J. (2005): Deposition rate and geochemical characters of sediments in Zhejiang offshore, – *Marine Geology and Quaternary Geology* 25(3): 15-24.
- [71] Zhuang, W., Zhou, F. (2021): Distribution, source and pollution assessment of heavy metals in the surface sediments of the Yangtze River Estuary and its adjacent East China Sea. – *Marine Pollution Bulletin* 164: 112002.