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Experimental assessment of benthic foraminifera as salinity bioindicators: Integrating morphological and eDNA approaches

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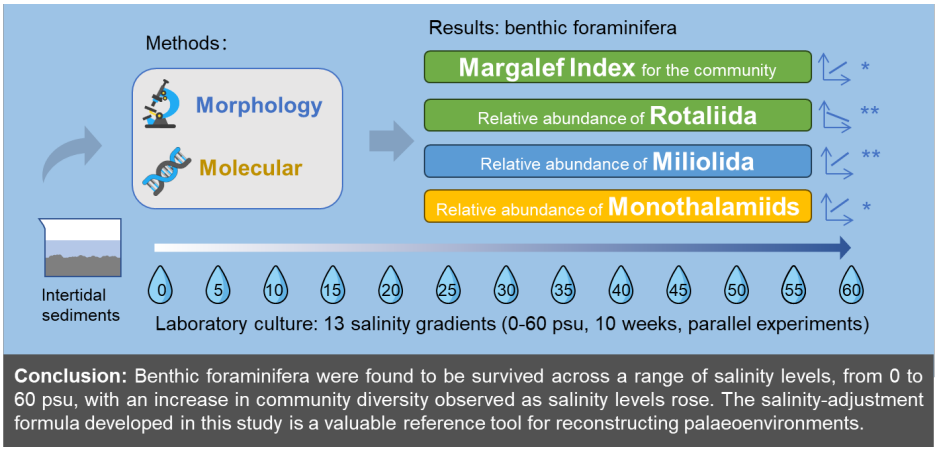
Abstract. Benthic foraminifera are extensively used as bioindicators for paleoenvironmental reconstruction, yet environmental DNA (eDNA) analysis provides a powerful lens to uncover their community diversity and environmental responses. Currently, quantitative experimental studies on their responses to salinity gradients are scarce. Here, sediments from the intertidal zone of Qingdao Bay were subjected to a 10-week controlled culture across 13 salinity levels (0–60 psu), and community dynamics were analysed using both morphological and eDNA approaches. Foraminifera exhibited high tolerance to extreme low (0 psu) and high (60 psu) salinities, and community diversity (Margalef index) increased significantly with salinity (p<0.05). The relative abundance of calcified Rotaliida declined with increasing salinity (p<0.01), allowing for the establishment of a robust linear regression model. The eDNA analysis revealed that soft-bodied Monothalamiids—often overlooked morphologically—reached up to 76.2% relative abundance (average 56.1%). In contrast, the salinity-driven increase in high-Mg calcite-shelled Milioliida was observed only through morphological analysis. These results demonstrate the distinct but complementary nature of morphological and molecular methods. This study addresses the scarcity of experimental constraints on salinity responses, offering a calibrated reference for applying foraminifera in both ecological assessment and paleo-reconstruction.



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1 Introduction

Foraminifera are cosmopolitan unicellular protists widely distributed across marine environments. Highly sensitive to environmental changes, they preserve extensive environmental information within their tests and community structures, making them valuable proxies for environmental monitoring and paleoclimatic reconstructions. (Bouchet et al., 2012; Ying et al., 2024). In coastal and shelf environments, seawater conditions are highly variable, and salinity is closely linked to local monsoons, precipitation, and continental runoff. The mixing of freshwater alters the physicochemical properties of seawater, thereby influencing the growth of foraminiferal populations and the preservation of their tests (Murray et al., 1999; Nigam et al., 1992; Sen Gupta, 1999). Field investigations into salinity have revealed the presence of living foraminifera in environments ranging from extremely low salinity levels (down to 0.1 psu in the Paraná River in South America) to hypersaline conditions (up to 92 psu in the saline-alkaline regions of southern Europe). However, both abnormally high and low salinities can induce morphological deformities, including changes in test size, increased proportions of abnormal individuals, loss of surface ornamentation, and delayed or even inhibited reproduction (Boltovskoy et al., 1991).

Laboratory culture experiments on salinity have traditionally focused on species-level responses, such as growth, reproduction, and test morphology, whereas the collective responses of entire benthic foraminiferal communities remain poorly understood. Bradshaw (1957) investigated the salinity tolerance of *Streblus beccarii* (Linné) var. *tepida* (Cushman) and found that the optimal range for reproduction and growth lies between 20 and 40 psu. When salinity dropped below 13 psu, the time required for reproduction doubled compared to normal conditions, and growth ceased at salinities above 67 psu. Similarly, Nigam et al. (2006) reported that both excessively high and low salinities adversely affect the growth rate of foraminifera; however, low salinity poses a greater threat than hypersaline conditions, as test dissolution tends to occur under reduced salinity.

Traditional studies have typically relied on morphological identification of foraminiferal taxa and statistical analyses of community composition to infer past or present environmental conditions. However, this approach is time-consuming and requires considerable taxonomic expertise. With the advancement of molecular biology, environmental DNA (eDNA) techniques have become increasingly popular. High-throughput sequencing (HTS) enables more efficient assessments of foraminiferal diversity, substantially reducing both time and labour costs (Pawlowski et al., 2020; Pawlowski et al., 2022). Recently, eDNA-based approaches for foraminiferal research have been successfully applied to monitor biodiversity and to further assess environmental conditions (Maeda et al., 2024; O'Brien et al., 2024). In addition, analyses of foraminiferal ancient DNA (aDNA) extracted from sediment cores provide valuable complementary information on species that fail to fossilize, thereby enabling the reconstruction of past biodiversity and offering a more comprehensive understanding of paleoenvironmental changes (Demianiuk et al., 2025; Pawłowska et al., 2014). However, applications of the eDNA approach to investigate the responses of foraminiferal communities to environmental changes under controlled laboratory conditions remain very limited, which constrains the potential of foraminifera as reliable indicators for tracking short-term and long-term environmental variability.

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In this study, sediment samples were used for culture experiments rather than single-species cultivation, allowing a more realistic simulation of the natural habitats of benthic foraminifera. Based on this setup, environmental salinity gradients were manipulated to assess the ecological effects of environmental variability under controlled conditions. Intertidal sediment samples were collected from Qingdao Bay and cultured for ten weeks under 13 salinity gradients ranging from 0 to 60 psu. Both morphological and eDNA approaches were employed to analyse community parameters and taxonomic composition, and their relationships with salinity were quantitatively examined. The objectives of this study are to evaluate the consistency between morphological and eDNA-based estimates of benthic foraminiferal diversity, to identify the differential responses of distinct taxonomic groups to salinity variation, and to establish a practical framework for linking community dynamics with environmental gradients, providing a valuable reference for modern environmental assessment and paleoenvironmental reconstruction.

2 Materials and methods

2.1 Sample collection and laboratory culture

Samples were collected from the intertidal zone of Qingdao Bay (36.03°N, 120.19°E, Fig 1), where the annual salinity typically ranges from 31 to 38 psu (Lei et al., 2017). At the time of sampling, the *in situ* water temperature was 17.8 °C, and seawater salinity, measured using a handheld refractometer with a precision of 1‰, was 33.1 psu. The surface seawater brought back to the laboratory showed a pH value of 8.21.

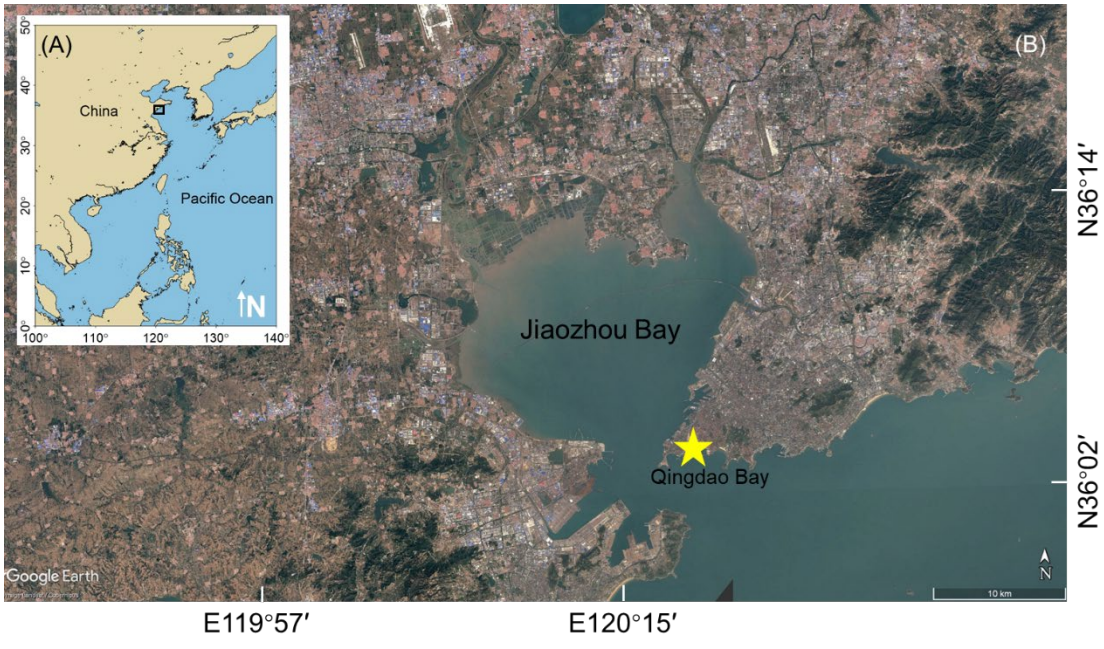

Figure 1. Map of the sampling area. (A) Regional map with a black rectangle indicating the location of the detailed view. (B) Detailed satellite view of the sampling site (yellow star) in Qingdao Bay. (Satellite imagery: © Google Earth. Map data: Google, Maxar Technologies).

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Sediment samples were cultured in clean, sterilized glass crystallizing dishes (90 mm in diameter) under 13 salinity gradients of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 psu. The dishes were incubated in a temperature-controlled chamber maintained at 18 °C, with a light: dark cycle of 12 h:12 h and an illumination intensity of approximately 6000 lx. Seawater with different salinities was prepared using in situ seawater as the base. Prior to salinity adjustment, the seawater was filtered through a 0.45 μm membrane to remove impurities. High-salinity water was obtained by evaporative concentration in an oven, whereas low-salinity water was prepared by dilution with distilled water (Bradshaw, 1955; Dissard et al., 2010). During the culture experiment, an overlying water volume approximately equal to that of the sediment was maintained in each crystallization dish, which was covered with a gas-permeable membrane to prevent salinity fluctuations caused by evaporation. Throughout the 10-week culture, half of the overlying water was replaced every three days, and 20 μL of concentrated algal suspension was added as food at the same interval. The algal suspension of *Phaeodactylum tricornutum* Bohlin used for feeding was pre-concentrated by centrifugation, homogenized, aliquoted into sterile 1.5 mL centrifuge tubes, and stored at −20 °C until use. During the culture period, the pH of the overlying water was regularly monitored and maintained at 7.7 ± 0.16. After 10 weeks of culture, half of each sediment sample was used for morphological analysis, while the other half was reserved for eDNA extraction.

2.2 Sample processing by morphology

For morphological analysis, the samples were fixed for 48 hours in a solution of 95% ethanol mixed with 1 g/L Rose Bengal to distinguish living from dead foraminiferal individuals (Lei et al., 2017; Schönfeld et al., 2012). Subsequent morphological processing and analysis were conducted following ISO 23040:2021. Species identification was conducted based on existing literature plates (Lei et al., 2016), with pre-treatment viability determined by observing staining patterns. The identification of all living foraminiferal individuals in the selected samples was conducted to the level of species, and these were then grouped for quantitative analysis. In addition, community parameters were calculated, including total abundance, Margalef diversity index, Shannon–Wiener index, and Simpson index.

2.3 Sample processing by eDNA metabarcoding

For sediment samples used in DNA extraction, 0.25 g replicates were collected from three distinct locations within each sample. This was done in order to minimize errors arising from sample heterogeneity. The sample was then processed using the QIAGEN (Germany) DNeasy PowerSoil Kit (12888-100), yielding 100 mL of environmental DNA solution. The process of polymerase chain reaction (PCR) was conducted utilizing the foraminifera-specific primers s14F3 and s17, which were designed to target the 37f hypervariable region of the foraminifera SSU ribosomal DNA. Each PCR reaction volume of 25 μL contains 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 thermal cycling profile implemented a two-stage amplification strategy: an initial denaturation at 94°C for 90 s, followed by 35 cycles of denaturation (94°C, 60 s), annealing (55°C, 60 s), and extension (72°C, 45 s). This was immediately

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followed by an additional 10 cycles with modified timing (30 s denaturation, 30 s annealing, and 120 s extension) to ensure complete amplification.

Amplicons were purified using a DNA Gel Extraction Kit and verified via 1% agarose gel electrophoresis. Library construction was carried out using the Illumina TruSeq DNA PCR-Free Library Preparation Kit, followed by quantification with Qubit. Sequencing was conducted on the Illumina NovaSeq 6000 platform to generate paired-end reads.

Raw paired-end reads were demultiplexed based on unique barcodes and merged using FLASH (v1.2.7) (Magoc and Salzberg, 2011). Data quality was inspected using FastQC (Brown et al., 2017). To ensure high-quality datasets, low-quality sequences (quality score < 30) were filtered out using QIIME (v1.9.1) (Bokulich et al., 2013). Finally, the denoising pipeline Unoise3 (Edgar, 2016) was employed to generate Operational Taxonomic Units (OTUs) from the clean data.

Perform a correlation analysis on the statistical results of the species populations using SPSS. Conduct linear fitting with a 95% confidence interval using Origin.

3 Result

3.1 Morphological analysis of benthic foraminifera

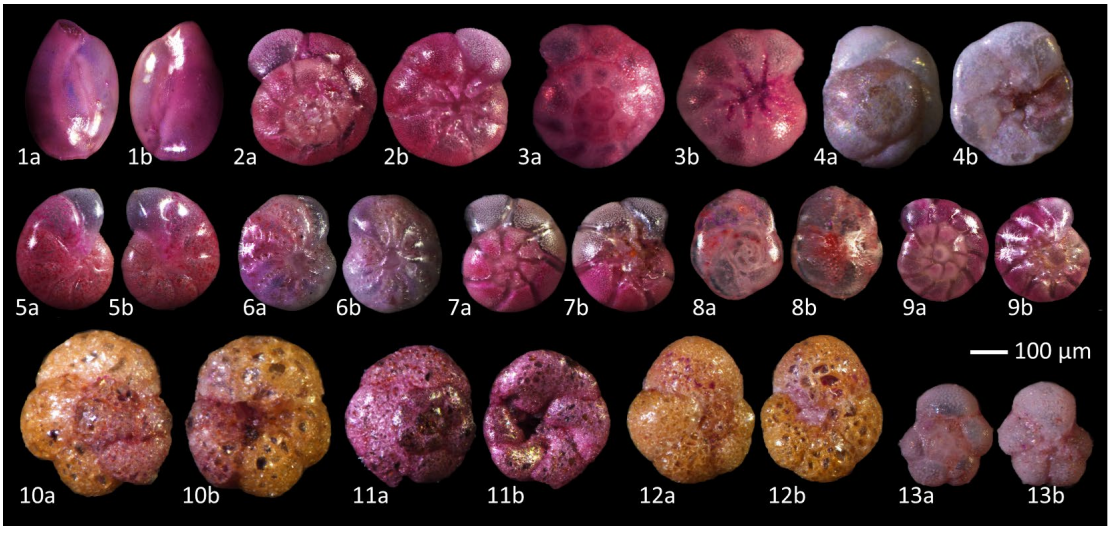

Figure 2. Micrographs of benthic foraminifera. Note: 1. *Quinqueloculina seminula*, 2. *Ammonia tepida*, 3. *Ammonia sobrina*, 4. *Rosalina vilardeboana*, 5. *Cribrononion gnythosuturatum*, 6. *Elphidium macellum*, 7. *Ammonia aomoriensis*, 8. *Buccella frigida*, 9. *Ammonia beccarii*, 10. *Ammoglobigerina globigeriniformis*, 11. *Trochammina squamata*, 12. *Trochammina inflata*, 13. *Murrayinella globosa*.

In this study, traditional morphological methods identified a total of 9,077 foraminiferal specimens belonging to 13 species, of which 7,217 were stained live specimens (see Fig. 2). Foraminifera species numbers exhibited slight fluctuations across salinity gradients, with the highest species count of 13 recorded at the 55 psu salinity gradient and the lowest count of 10 species documented at 10 psu. The relative abundance and species number of different taxonomic composition across

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salinity levels is demonstrated in Fig. 3 (A/B). The Rotaliida group is comprised of nine species, which account for 66.52% of the total. The relative abundance of these species peaked at 15 psu, where they constituted 84.70% of the total. The Miliolida group comprised one species, accounting for 30.59% of the total. At its most prevalent relative concentration, it attained 45.20% at a salinity gradient of 55 psu. The Textulariida group was comprised of three species, accounting for 2.89% of the total. Their maximum relative abundance was recorded as 5.59% at a salinity gradient of 30 psu.

The dominant species in the community were *Quinqueloculina seminula*, *Cribronion gnythosuturatum*, *Ammonia aomoriensis*, *Ammonia beccarii*, and *Ammonia tepida*. The combined abundance of these five dominant species accounted for 89.18% of the total foraminifera count. A Spearman correlation analysis was conducted between the relative abundances of the three taxonomic groups and salinity (Table 1). The results demonstrated a statistically significant negative correlation between the relative abundance of Rotaliida and salinity ($p < 0.01$). A regression model (Fig. 4A), $Y = -0.5x + 81.44$, was developed to describe the relationship between the variables ($p < 0.01$, $R^2 = 0.69$). The relative abundance of the Miliolida group showed a significant positive correlation with salinity ($p < 0.01$).

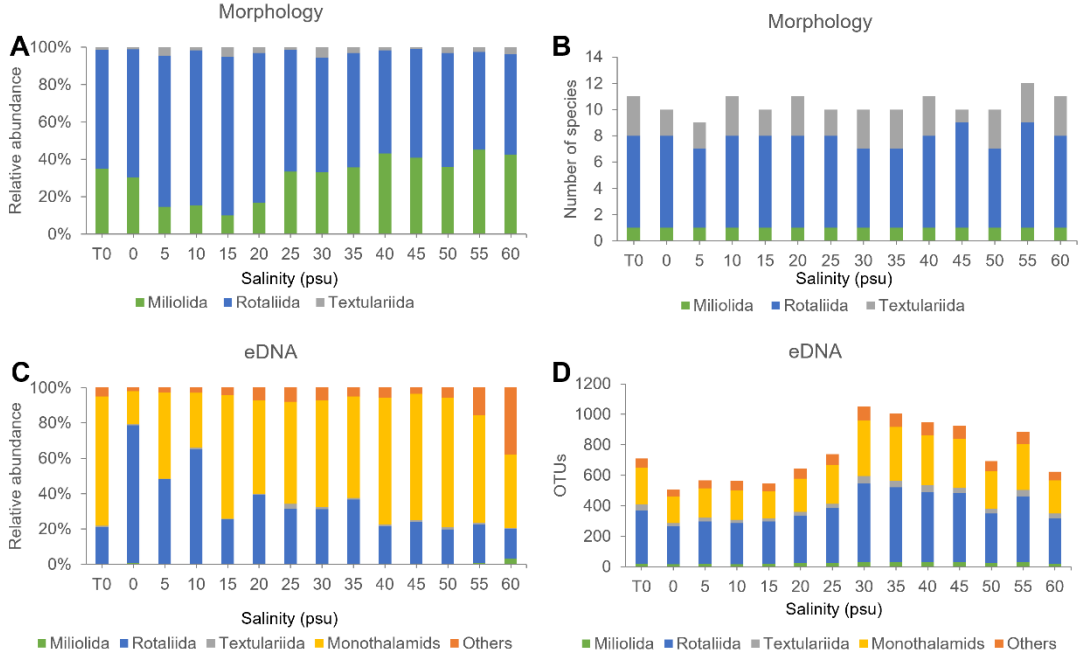

Figure 3. Culture experiment results: relative abundance of taxa (A/C) at various salinities using morphological and eDNA methods, the number of species identified by morphological methods (B) and alongside the number of OTUs per taxon in the eDNA method (D).

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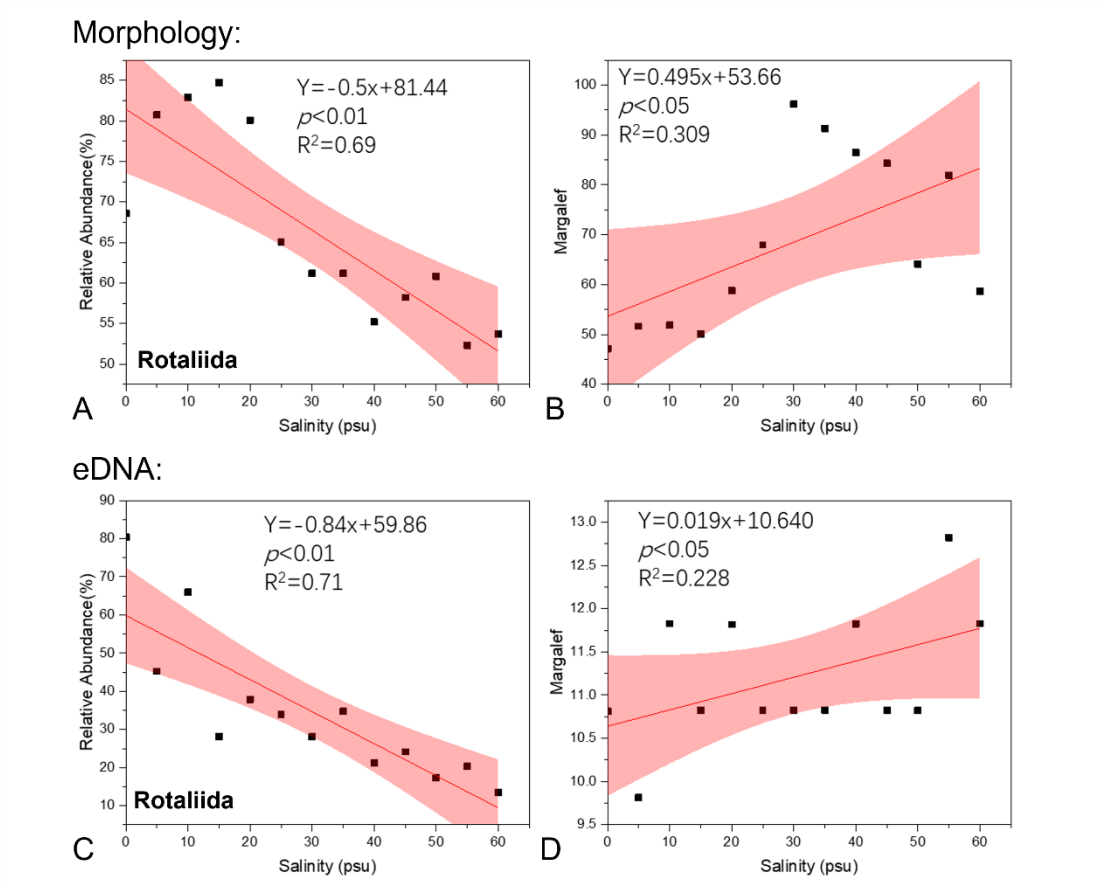

Figure 4. Regression function fitting plots for the relative abundance of Rotaliida (A, C) and the Margalef diversity index of the foraminifera community (B, D) against salinity gradients in this study's morphological and eDNA methods, with the pink area indicating the 95% confidence interval.

Based on morphological statistical results, the Spearman correlation analysis between benthic foraminiferal community diversity indices and salinity is presented in Table 2. The results indicate that the Margalef index exhibits a significant positive correlation with salinity, while the Simpson index of the community shows a significant negative correlation with salinity. Consequently, a linear regression function was established between the Margalef index and salinity gradient (Fig. 4B): $Y = 0.495x + 53.66$ ($p < 0.05$, $R^2 = 0.309$).

Table 1 Spearman correlation coefficients (R values) between foraminiferal taxa detected by morphological and eDNA methods and salinity gradients. Bold text indicates statistically significant correlations (*p < 0.05, ** p < 0.01).

	Rotaliida	Milioliida	Monothalamiids	Textulariida
Morphology	**- 0.896****	**0.874****	\	0.060
eDNA	**- 0.912****	- 0.033	0.544	-0.016

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Table 2 Spearman correlation coefficients between alpha diversity of foraminiferal communities detected by morphological and eDNA methods and salinity gradients. Bold text indicates statistically significant correlations (*p < 0.05, ** p < 0.01).

	Margalef	Shannon-Wiener	Simpson	Pielou
Morphology	**0.619***	0.538	**-0.874****	0.407
eDNA	**0.517***	0.319	0.269	0.044

3.2 Environmental DNA results of benthic foraminifera

In this eDNA experiment, the amplified fragments utilized for sequencing exhibited an approximate length of 380 base pairs. Subsequent to the implementation of quality control measures, the results yielded a total of 773,819 DNA sequences, which were annotated into 1,977 OTUs and matched to 103 species. Rarefaction analysis was performed on the eDNA data (Fig. 5A). The curves for all treatment groups leveled off as the number of reads increased, suggesting that the sampling effort was adequate to represent the benthic foraminiferal diversity. The number of OTUs in samples at different salinities is shown in Fig. 3D. The results obtained from the study indicate that the total number of foraminiferal OTUs increased initially and then decreased under experimental conditions ranging from 0 to 60 psu, with a peak observed near 30 psu. Moreover, the trend was more pronounced in the low-salinity range (0–30 psu) compared to the high-salinity range (35–60 psu).

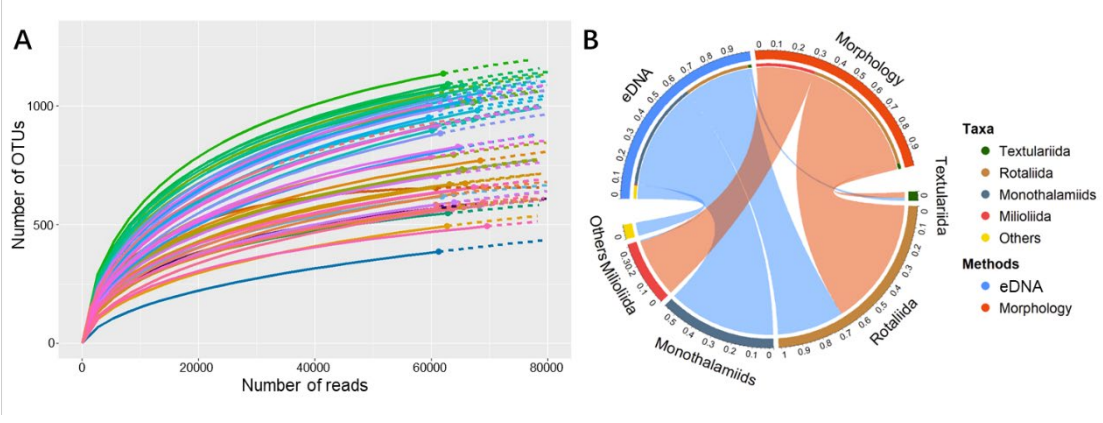

Figure 5. (A) Rarefaction curves for eDNA samples, plotting the number of OTUs against sequencing reads. (B) Chord diagram comparing the community composition of benthic foraminifera obtained by molecular (eDNA) and morphological approaches.

In terms of taxonomic composition (Fig. 5B), the eDNA data detected Monothalamiids, a group typically overlooked by morphological analysis. Additionally, a portion of taxonomically unresolved taxa were grouped as 'Others'. Monothalamiids exhibited the highest read count, accounting for 56.06% of the total. The maximum relative abundance recorded for this group was 76.19%, which was achieved under the 50 psu salinity gradient (Fig. 3C). The Rotaliida constituted 34.65% of the total, achieving a maximum relative abundance of 80.34% under the 0 psu salinity gradient. The Textulariida class accounted for 1.67% of the total, with a maximum relative abundance of approximately 3.43% at the 30 psu salinity gradient. The Miliolida class constituted 0.66% of the total, exhibiting a maximum relative abundance of 4.70% at the 10 psu salinity gradient.

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As the salinity gradient increases, there is a concomitant decrease in the proportion of Rotaliida, accompanied by a slight increase in the proportion of Monothalamiids. A Spearman correlation analysis was conducted between each group and salinity, revealing a highly significant negative correlation ($p < 0.01$) between Rotaliida relative abundance and salinity (Table 1). As shown in Fig. 4C, the following linear regression equation was established: the regression equation can be expressed as $Y = -0.84x + 59.86$ ($p < 0.01$, $R^2 = 0.71$).

Spearman's correlation analysis based on eDNA data revealed a significant positive correlation between the Margalef diversity index and salinity (Table 2). Consequently, a regression model was established to quantify this relationship (Fig. 4D), yielding the following equation: $Y = 0.019x + 10.640$ ($p < 0.05$, $R^2 = 0.228$).

4 Discussion

4.1 Investigation into the salinity adaptability of benthic foraminifera communities

The formation of foraminiferal community structures is driven by multiple environmental factors, including food supply, temperature, salinity, pH, and dissolved oxygen. These factors influence survival, reproduction, and shell preservation, thereby shaping sedimentary records (Gooday, 2003). For planktonic foraminifera, community dynamics are more strongly governed by hydrological conditions and variations in primary productivity. In contrast, for benthic foraminifera, particularly within marginal sea environments, abiotic factors such as temperature and salinity frequently exert a dominant influence (Chaabane et al., 2024; Groeneveld et al., 2018). This study, which was conducted through multi-gradient salinity culture experiments on intertidal sediments, found that both molecular and morphological methods could detect viable foraminifera even under extreme low-salinity (0 psu) and high-salinity (60 psu) conditions. Furthermore, the Margalef index demonstrated a significant increase in community diversity with rising salinity, indicating that salinity is a key driver influencing community structure, with high salinity promoting greater complexity in community composition.

Foraminifera exhibit a remarkably broad tolerance of salinity levels. In the Burullus Lagoon, large populations of *Ammonia tepida* flourish across a salinity range of 1.5 to 14.6 psu, accounting for over 97 per cent of the total foraminiferal community (Orabi et al., 2017). *Ammonia beccarii* has been found in lagoons in southern California with a salinity range of 15–68 psu and in salt marshes along the southern European coast with a salinity range of 7–92 psu. It exhibits no discernible morphological variation in response to environmental conditions (Boltovskoy et al., 1991). However, changes in salinity levels often result in the deformation or dissolution of foraminifera shells, as well as alterations to the structure of the community (Amao et al., 2018; Saraswat et al., 2015). In environments with low salt concentrations, there is a demonstrable decline in the feeding and metabolic efficiency of foraminifera. Conversely, the collective of foraminifera exhibits a higher degree of tolerance to physiological stress induced by elevated levels of salinity. The present study also revealed a significant positive correlation between the Margalef index of foraminiferal communities and salinity, with the diversity of benthic foraminifera increasing as salinity rises. In order to apply salinity parameters to paleoenvironmental reconstructions, multiple experimental

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runs are required. Increases in both density and breadth of experimental setups, in conjunction with extended cultivation periods, would yield more precise results.

4.2 The Relationship Between the Biomineralizations of Two Calcareous Foraminiferal Groups and Salinity

The biomineralization mechanisms of different foraminiferal taxa are closely linked to their ecological environments (Weinkauf et al., 2013). In conditions of reduced seawater salinity, there is an increase in the solubility of carbon dioxide, resulting in calcium carbonate being placed in a low-concentration, unsaturated state. Porcelaneous foraminifera (Miliolida), being evolutionarily primitive, utilize supersaturated seawater as a mother liquor (Bentov et al., 2006). The formation of acicular calcite crystals within cellular vesicles is a direct process, and these crystals are assembled at the site responsible for shell formation through a process of disordered precipitation (Nooijer et al., 2009). At higher salinities, the solubility of carbon dioxide is known to decrease, leading to calcium carbonate supersaturation. This environment is conducive to the formation of Miliolida, characterized by compact structures and disordered calcium carbonate crystal arrangements. Given the relatively elevated magnesium-to-calcium ratio characteristic of seawater, calcite formed under the shell-building mechanism of Miliolida exhibits elevated magnesium content (Nooijer et al., 2009). The magnesium content of different species of hyaline foraminifera (Rotaliida) varies considerably. In seawater of moderate salinity, where calcium carbonate approaches saturation or is slightly supersaturated, Rotaliida can gradually transform seawater into vesicles (Bentov et al., 2009), chemically modifying the seawater within the cell. This process involves the pumping out of hydrogen ions and magnesium ions, thereby raising the solution's pH. This process has been shown to facilitate the formation of more robust, less soluble, and orderly arranged low-magnesium calcite. This process has its origins in calcification genes that are unique to Rotaliida (Toyofuku et al., 2017; Ujiié et al., 2023).

It can be deduced from the findings of this experiment that, in conditions of low salinity, there was a decline in the abundance of foraminifera. Among these, the high-magnesium calcite shells of porcelaneous Miliolida were prone to dissolution, whereas the low-magnesium calcite shells of hyaline Rotaliida resisted dissolution more readily, leading to an increase in the relative abundance of Rotaliida. At higher salinities, the formation of shells was found to be more favorable for Miliolida, thereby explaining the higher relative abundance observed at elevated salinities in classical morphological analyses. Research undertaken hitherto has indicated that hyaline Rotaliida, for example *Elphidium* and *Ammonia*, exhibit a high level of tolerance to low-salinity environments, whereas porcelaneous Miliolida become more prevalent in highly saline conditions (Amao et al., 2018; Charrieau et al., 2018; Orabi et al., 2017).

Foraminifera, indicator organisms of great utility in the field of geology, are frequently employed to deduce paleo-oceanic temperatures by means of their shell magnesium-calcium ratios. Significant variations in the responses of different foraminiferal taxa are observed across salinity gradients. In circumstances where species assemblages are divergent, the resultant community changes may manifest distinct patterns. Therefore, after controlling for temperature effects, foraminifera also serve as amplifiers of salinity variations, enabling salinity determination based on specific species assemblages.

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Furthermore, when employing foraminifera as paleo-oceanic thermometers in geological studies, we recommend conducting faunal composition analyses beforehand to enhance the accuracy of results.

4.3 Traditional morphology and molecular biology methods

This study employed a combined approach of traditional morphology and molecular biology methods (eDNA) to examine the relationship between benthic foraminiferal communities and salinity response. The findings from both methodologies concurred on several conclusions: the diversity of foraminifera exhibited a positive correlation with salinity, while the relative abundance of Rotaliida taxa demonstrated a significant negative correlation with salinity.

It has been demonstrated that molecular biological methods are a powerful tool for describing species diversity. Firstly, while morphological analysis in this experiment identified only 13 species, the eDNA methods possesses considerable sensitivity, detecting 103 species and thereby better reflecting the diversity within the foraminiferan group. Secondly, under normal circumstances, the organic shells of Monothalamiids are relatively soft and prone to damage during morphological preparation. Moreover, the absence of distinct morphological characteristics and the low probability of fossilization result in their current exclusion from traditional morphological studies, due to the absence of relevant knowledge for morphological identification (Schönfeld et al., 2012). Molecular methods can detect foraminiferal groups with organic shells that are missed by traditional morphological methods. Furthermore, these groups constitute dominant taxa in molecular biology analyses, accounting for a significant proportion of species across all salinity gradients. Therefore, compared to morphological identification, it has the advantages of requiring fewer samples and being more sensitive. Furthermore, the automatic assignment of sequences to OTUs can reduce the human error inherent in traditional morphological methods, such as species identification based on shell morphology, where abnormalities or damage to the shell can make species identification difficult.

On the other hand, morphological methods can provide valuable complementary insights to eDNA approaches. For this experiment, to avoid controversies arising from primer bias and sampling volume (Pawlowski et al., 2014), statistical analyses were conducted based on the number and proportion of OTUs across different taxa. The bar chart of OTU proportions shows that foraminifera within the porcelaneous Miliolida group make up a very small proportion, and changes induced by salinity gradients in this group are not reflected. A statistical analysis of classical morphology reveals that, although the foraminiferal assemblages within porcelaneous Milioliida are relatively monotypic, their numbers are substantial and they constitute a high proportion of the total biological count. Traditional morphological methods can overcome the limitations of molecular biology when studying of Milioliida. Moreover, while traditional morphology enables species identification, it also allows damage and deformities in foraminiferal shells to be observed, providing insight into the specific environmental impacts on foraminifera. This facilitates the analysis of the causes behind alterations in foraminiferal community composition, which is a capability absent in molecular biology.

This study integrates morphological and eDNA methods to comprehensively elucidate foraminiferal community responses to salinity gradients. However, both approaches are subject to inherent uncertainties when determining viability. Morphological identification is susceptible to factors such as staining efficiency and subjective judgement (Bernhard et al.,

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2006; Fontanier et al., 2002), while eDNA is unable to distinguish genetic signals originating from living, dead, or environmental residues (Pawlowski et al., 2014). This common limitation hinders the precise characterization of active community structures. To address the challenge of distinguishing living foraminifera, environmental RNA (eRNA) has emerged as a more promising tool for indicating active communities in ecological surveys, owing to its rapid extracellular degradation which enables the accurate representation of living individuals (Chen et al., 2025; Qiao et al., 2025). Although the application of eRNA in foraminiferal research remains in its infancy and methods lack standardization, the future development and optimization of this technology—particularly when integrated with laboratory culture systems—will directly elucidate the impacts of salinity variations on active foraminiferal communities. Consequently, this will build upon the present study to provide mechanistic insights of greater ecophysiological significance.

5 Conclusions

This study involved collecting intertidal sediments for salinity gradient culture experiments. A combination of traditional morphological and molecular biological methods revealed that benthic foraminifera can survive under salinity conditions ranging from 0 to 60 psu. The Margalef diversity index for benthic foraminifera increased markedly with increasing salinity, while the relative abundance of Rotaliida taxa decreased significantly. A series of functional relationships were identified. Moreover, the relative abundance of the Milioliida group, which dominates in morphological analyses, exhibits a positive correlation with salinity. Molecular methods employing high-throughput sequencing have detected a diverse array of Monothalamiids groups, whose relative abundance exceeds 50%. The salinity-related linear functions established by this research institute have the potential to provide effective reference and data support for the reconstruction of ancient marine environments.

Author contributions

YC and YL conceptualized the study and curated the data, and YC carried out the investigation and methodology. YC and FW developed the software and created the visualizations, with FW performing the formal analysis. YC prepared the original draft, and YL reviewed and edited the manuscript. YL also acquired funding, provided resources, and supervised the project.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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