## Synchrotron stories: Illuminating the past through foraminiferal imaging

In the past two centuries, human activities have significantly impacted coastal regions, affecting vulnerable areas subjected to natural hydrographic changes and increasing anthropogenic pressures [1]. Rising atmospheric carbon dioxide levels (pCO<sub>2</sub>) lead to ocean acidification (OA), as well as contribute to higher temperatures, increased water stratification, and the expansion of oxygen-depleted zones (hypoxia or deoxygenation), and results in the degrading of coastal benthic ecosystems.

To understand these changes' severity and potential outcomes, we turned to paleoenvironmental records, specifically marine sediment archives and their calcite microfossils. In our study, we used 3D reconstructions of foraminiferal shells, focusing on the species Elphidium clavatum in the Öresund region, a transition zone between the North Sea, Skagerrak, and the Baltic Sea, highly vulnerable to hydrographic changes and human impacts [1]. Foraminifera are single-celled microorganisms, often with a shell of calcite making them highly useful in environmental and climate reconstructions. Employing a synchrotron lightbased µCT approach, we provide a historical context from the 1800s to the present day, offering insights into environmental changes through morphological patterns [2].

Detailing morphological changes based on synchrotron light-based  $\mu$ CT has only been used to a limited extent in micropaleontology; we are providing one of the very first  $\mu$ CT-based time series. We used foraminiferal samples from the study by Charrieau *et al.* [1], and we particularly focused on *Elphidium clavatum*, to unravel environmental changes in the Baltic Sea entrance over the past 200 years. This species is especially interesting since it dramatically changes its abundance over the investigated period. We carefully selected 16 sediment layers, each representing a snapshot in time, and chose a total of 124 foraminiferal specimens for detailed tomographic analysis.

To capture the intricate details of these microscopic specimens, we employed advanced imaging techniques at SPring-8 **BL47XU**. Synchrotron light-based  $\mu$ CT, with a voxel size of 0.5  $\mu$ m and 1800 projections, provided unprecedented clarity in our 3D reconstructions. These images were then processed using open-source software Fiji and MeshLab (Fig. 1).

The challenging task of measuring the thickness of foraminiferal shells was tackled using a novel approach. Instead of traditional 2D image analyses which only use parts of the shell, we automatically computed the thickness of the entire shell from the image stack, using the BoneJ plugin in Fiji. This is a novel approach, not used before in micropaleontology. Moving beyond traditional analyses, we explored the relationships between the surface/volume ratio, pore density, and thickness from 3D reconstructions using MeshLab (Fig. 2). Pore patterns, a relatively unexplored aspect, were examined using a topological tool to estimate the number of pores throughout the entire shell.

Our analysis of 124 foraminiferal specimens revealed profound changes in foraminiferal abundance, reflecting the impact of natural shifts and anthropogenic pressures over the investigated time period. Long-term morphological trends in *Elphidium clavatum*, such as a ~28% decrease in shell average thickness and a ~91% increase in the number of pores, illustrate the pronounced change in coastal environments due to multiple stressors such as ocean acidification, deoxygenation, and warming (Fig. 3). Large variability in morphological patterns at the same age level indicates contrasting environmental conditions, while lower variability suggests stability.

Examining variations over the centuries, we noted



Fig. 1. Illustration of the stepwise image processing of a shell. (A) Visualization of raw images. (B) Segmentation. (C) Binary images resulting from the segmentation. (D) Thickness map generated by the BoneJ plugin. (E) 3D reconstruction of a shell.



Fig. 2. Relationships between the shell surface/volume ratio and the pore density. Values adjusted between 0 and 1.

asynchronous changes between foraminiferal abundance and morphological patterns. In the early industrial period, despite stable foraminiferal abundances, pore density fluctuated broadly, indicating significant natural variations. From the 1940s, synchronous increases in both abundance and morphological variability suggest more pronounced seasonal environmental shifts and increased anthropogenic impact. In the early 21st century, a stable abundance alongside significant morphological variations signifies a persistent multi-stressor scenario. The large degree of morphological variability might be linked to maximizing the chances of species survival during more adverse conditions.

Our project not only underscores the significance of using a 3D time series of calcifying microfossils from geological archives to quantify the impacts of anthropogenic climate change and natural variability but also introduces an enhanced efficiency in 3D postdata analysis. The method is a valuable complement to traditional foraminiferal assemblage studies, providing a more detailed and nuanced understanding of the evolving dynamics in coastal environments. By decoding the intricate language of foraminiferal morphological changes, our study contributes critical insights into the effects of both human influence and natural forces on these delicate ecosystems, furthering our knowledge of the complex interactions shaping our coastal regions.



Fig. 3. 3D time series of the (A) Average thickness and (B) Number of pores. Boxplots are shown with colored individual data points per estimated year, the red diamond indicates the mean. The dashed line is the shells' abundance [2]. The morphological values (y-scale) are adjusted (0-1). The bold letters (a, b) indicate significant differences. A regression line (black line) with a 95% confidence interval (gray area) represents a significant long-term trend.

Helena L. Filipsson\* and Constance Choquel

Department of Geology, Lund University, Sweden

\*Email: Helena.Filipsson@geol.lu.se

## References

[1] L. M. Charrieau et al.: Biogeosciences 16 (2019) 3835.
[2] C. Choquel, D. Müter, S. Ni, B. Pirzamanbein, L. M. Charrieau, K. Hirose, Y. Seto, G. Schmiedl, H. L. Filipsson: Front. Earth Sci. 11 (2023) https://doi.org/10.3389/feart.2023.1120170

(41