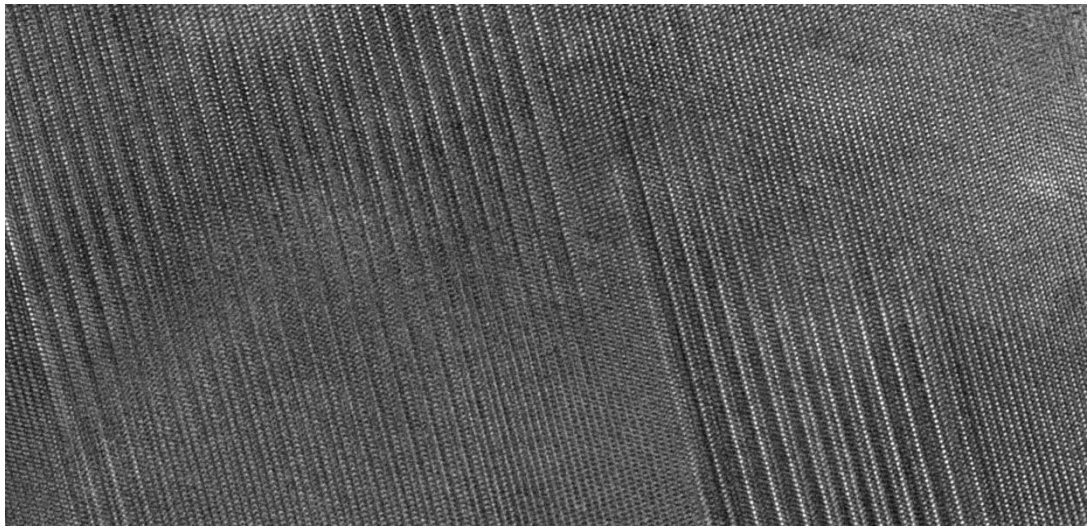
Nanostructure of biogenic aragonite: a study of otoliths and bivalve shells from a freshwater environment



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Introduction and aims of the study

Bivalve shells and otoliths grow during a lifetime of the individuals, they show small increments which are easily observable, hence otoliths and shells are excellent time-keepers.

Foreign elements (such as Sr, Ba, Hg, etc.) can be incorporated into the structure of aragonite, providing information about both the life history of the individuals and the geochemical evolution of their environments (Cerrato, 2000; Schulz-Mirbach et al., 2018).

Otoliths and bivalve shells have been examined by geochemical methods, but their nanostructures (especially those of otoliths) were poorly studied (Schulz-Mirbach et al., 2013).

In our study, we studied the samples on micro- and nanoscales. Here we present the preliminary results on the nanostructures of a *sagitta* otolith from a *pike perch* and of a *Dreissena* shell, focusing on the results of transmission electron microscopy.

Applied methods

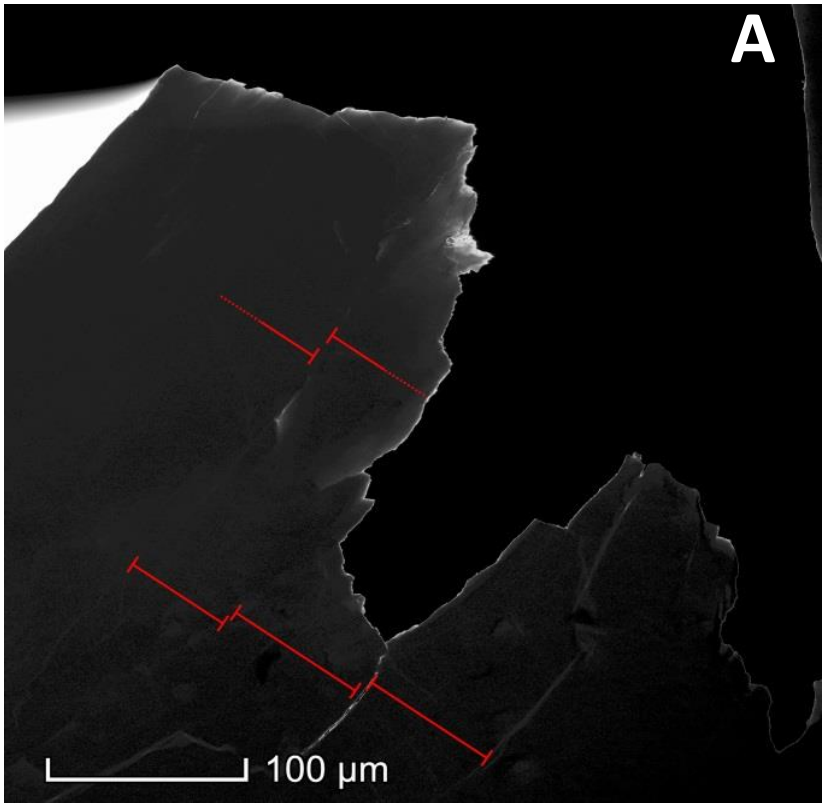
Otolith: an oriented section was cut perpendicular to the longest axis of the sagitta from a *pike perch*. The section was fixed onto a Cu grid and the central part ion-milled by an Ar-ion miller.
Bivalve shell: ground sample made from the shell of *Dreissena* and dropped gently onto a lacey Cu grid from ethanol suspension.

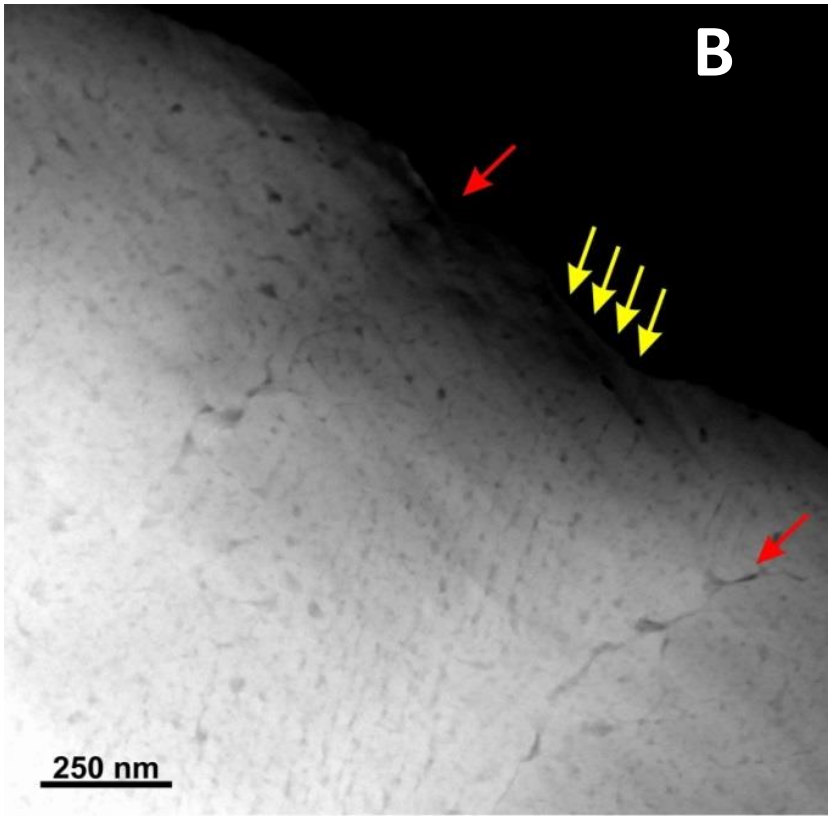
For a mineralogical characterization we examined the polished surface of the otolith sample by Raman spectroscopy (using a Renishaw spectrometer), before embedding the sample.

The microstructure and the growth increments of the otolith sample were observed using scanning electron microscopy (SEM), using an Apreo LoVac SEM.

The structural and compositional properties of the otolith and bivalve shell samples were studied using a Talos F200X G2 scanning transmission electron microscope (STEM).

Results
Structure of otoliths



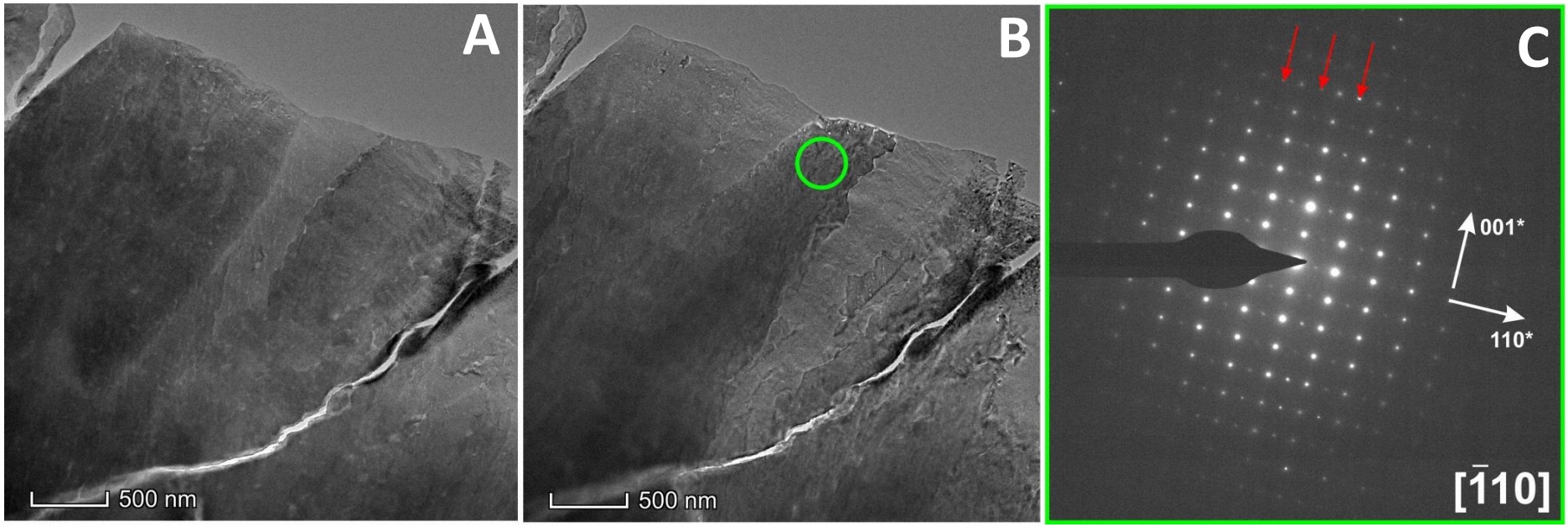


- We observed zones (increments) on different scales, the largest zones had 40-50 µm width (Fig. A).

- Holes with a few tens of nm in diameter were identified within the increments; they were elongated in the same direction (Fig. B, yellow arrows). At the borders of the increments we observed longer and wider holes (Fig. B, red arrows).

Results

Nanostructure of otoliths

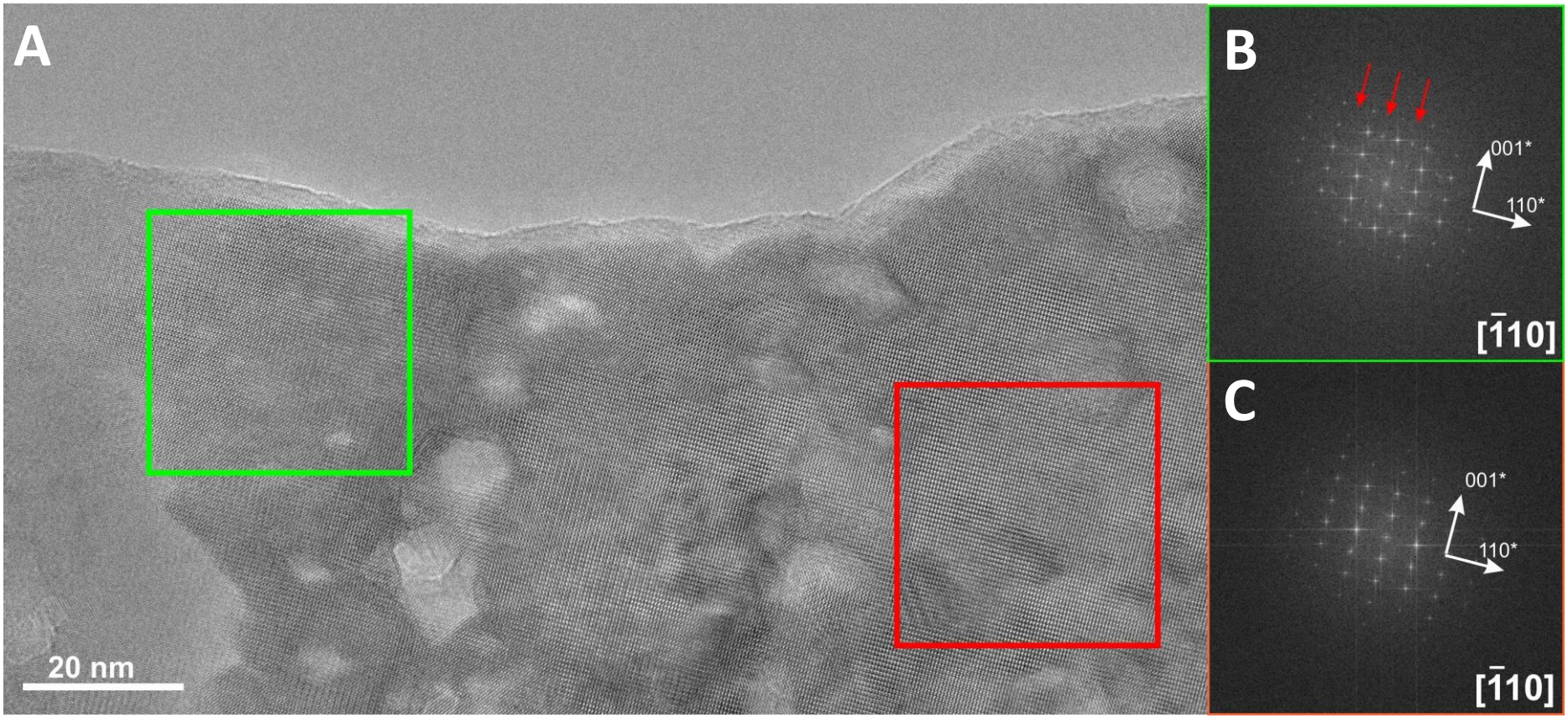


- The ‘daily increments’ contained smaller, a few hundred-nm large units (sections) that seemed to be single crystals (Fig. A).

- The sections showed large orientational differences (Figs. A, B) between their same zone axes (around 10°), and multiple periodicity with respect to aragonite (Fig. C, extra reflections marked by red arrows).

- As the result of the multiple periodicity, we observed similar diffraction patterns reported by Gavryushkin et al. (2019), and interpreted as resulting from twinning of aragonite.

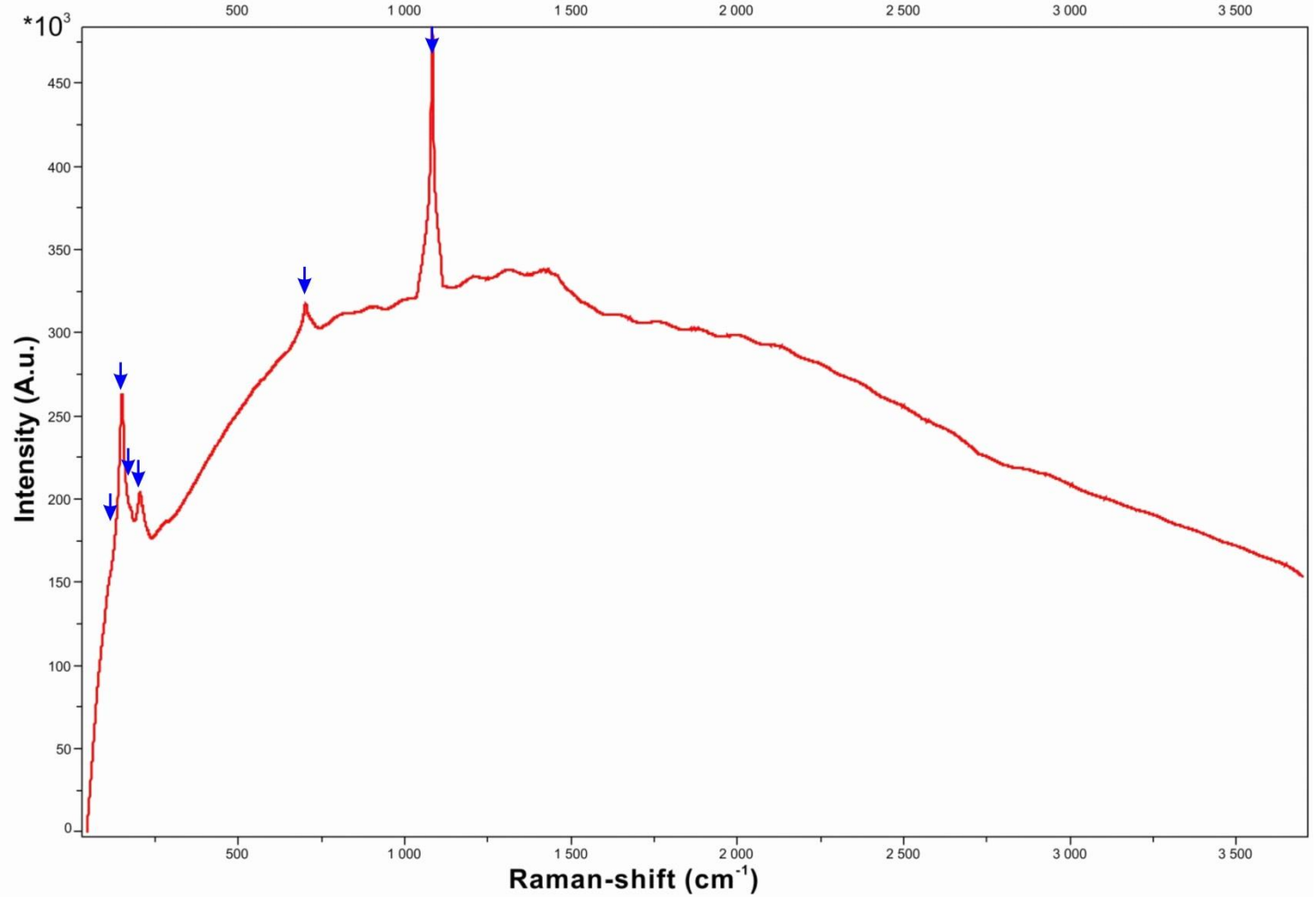
Results
Nanostructure of otoliths



- Fast Fourier transforms (FFTs) of the boxed areas in Fig. A revealed that the periodicity changed even within small (50 nm) distances (reflections marked by red arrows are present in Fig. B but absent in Fig. C).

Results

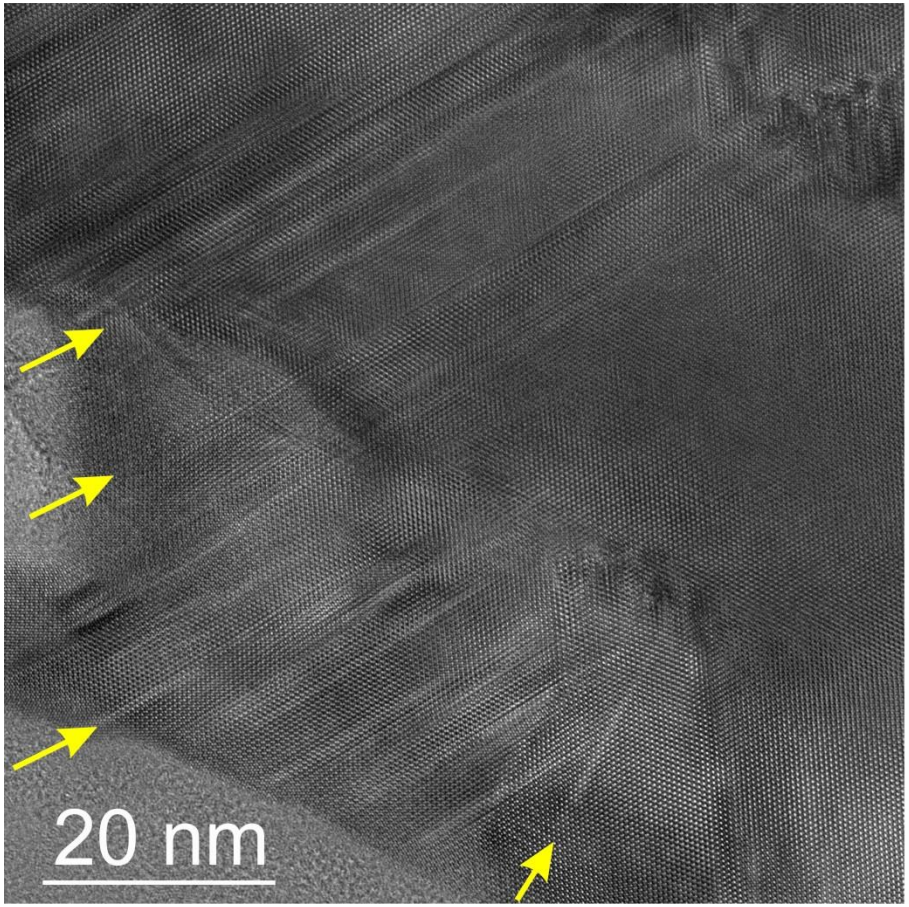
Characterization by Raman spectroscopy

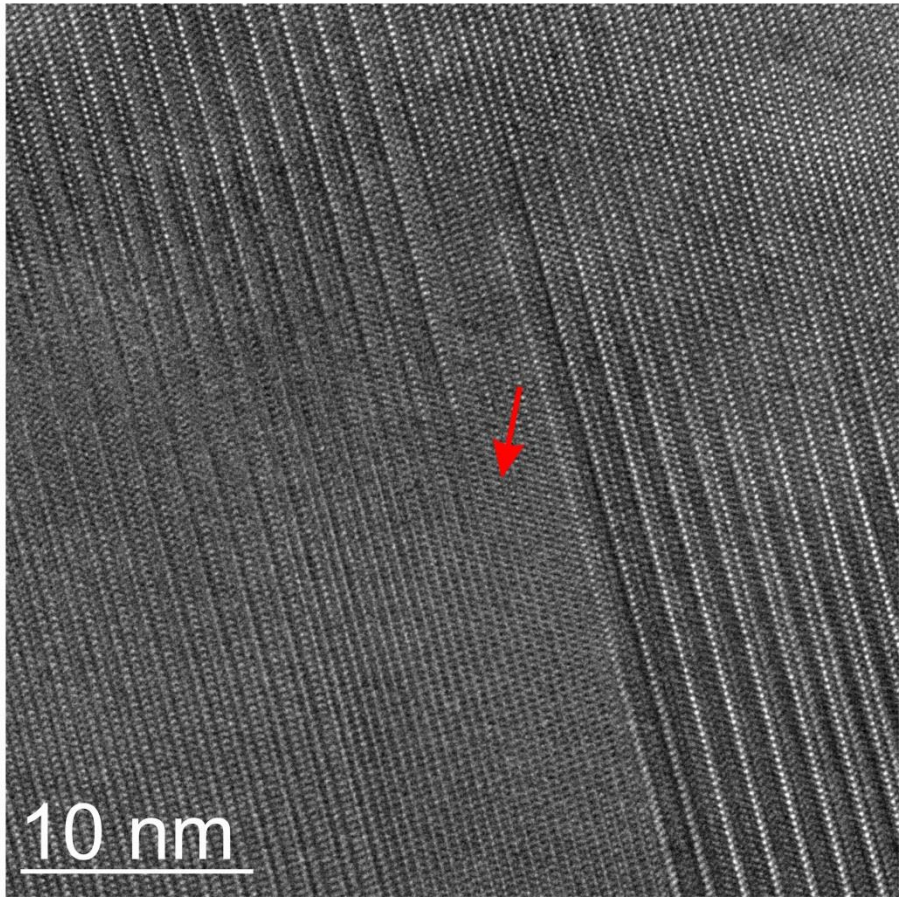


- We detected the Raman-active bands of aragonite (blue arrows) with a notably high background.

Results

Nanostructure of bivalve shells





The bivalve shells contained much larger single crystalline parts than the otolith sample.
The shells also show single and multiple aragonite periodicities, and HRTEM images (A, B) reveal that the structural units with multiple periodicity occur in zones (yellow arrows). Interestingly, these zones can “dissipate”, gradually terminate within a few tens of nanometers (red arrows), without causing any apparent strucural deformation.

Discussion

Both samples contained aragonite crystals and despite their different origins, both samples had similar properties.

The central region of the *sagitta* showed increments on different scales – from a few tens of µm wide increments to a few hundred nm large sections. The increments had asymmetrical shapes, and elongated holes separated them from one another. We observed large (around 10°) deviations between the same zone axes of the increments and even between the sections. In contrast, the bivalve shell contained much larger parts with uniform crystallographic orientations.

We found nm-large holes within the sections of the otolith and measured a high luminescent background with spectroscopy methods. We suggest that we detected the remnants of organic materials within the holes.

The observed twinning in otolith was similar to that reported by Gavryushkin et al. (2019), and could be the result of stacking faults derived from the rotation of carbonate groups. In the Dreissena shell we also observed twinned zones, plus multiple periodicities that ‘dissipated’ within a few tens of nm.

Thank you for Your interest!

References:

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Acknowledgements:
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