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Mechanical properties of deep-sea molluscan shell

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1. Introduction
The Patellogastropoda have been considered to represent a sister group of all other living gastropods, and thus are also considered the oldest gastropod group. All living patellogastropods are limpets with cap-shaped shells, typically occurring on intertidal rock substrates, except for a few living in the deep sea. The crucial question is whether the cap-shaped shells originated as a result of their adaptation to intertidal environments, or whether this shape represents a primitive feature. The patellogastropods also have the most complex shell microstructures of all gastropods (Fuchigami and Sasaki 2005). This shell complexity has often been used as evidence for their shallow water origin. Comparisons of the mechanical properties of homologous shell layers (i.e. layers having an identical microstructure, crystallographic texture and mineralogy) in patellogastropods living in shallow and deep water environments can shed light not only on the influence of water depth on the mechanical properties of biogenic carbonates, but also on patellogastropod phylogeny.

Here, we use a combination of nanoindentation and electron backscatter diffraction (EBSD) to test whether there is a difference in the mechanical properties of biogenic carbonates growing both in deep sea and in shallow water environments. The aims of the study are (1) to determinate the hardness \( H \) and reduced elastic modulus \( E_r \) in homologous layers of patellogastropod shells and (2) to analyse the influence of water depth on the values of \( H \) and \( E_r \).

2. Methods
Three different patellogastropod species from Japan were selected for this study: (1) the deep-sea limpet Pectinodonta orientalis from the Hyuga Basin, captured from 1092–1094 m, (2) Nipponacmea concinna from the coast near Kinkazan, Miyagi Prefecture, and (3) Limalepeta lima from the continental shelf near Kushiro, Hokkaido, captured from 50–100 m. The aragonitic concentric crossed lamellar (cCL) structure, forming the M + 1 layer in all of the above-mentioned species, and the calcitic semi-foliated (SF) structure, forming the M + 2 layer in P. orientalis and L. lima, were studied (see data in Fuchigami and Sasaki 2005).

Patellogastropod shells were separately embedded in epoxy resin. Subsequently, the samples were cut along the sagittal plane across the shell apex, and then polished using progressively finer polishing media (SiC, diamond and 0.05 μm colloidal silica).

The EBSD analysis was carried out using a Nordlys detector connected to a CamScan scanning electron microscope. Measurements of each of the shell layers were carried out in a regular grid with 15 μm spacing. The crystallographic orientations were evaluated by Channel 5 software (HKL Technology) and were plotted on a stereographic projection (Figure 1). For measurements of the mechanical properties in homologous layers only, areas having identical crystallographic textures were selected.

A Hysitron TI 950 TribolIndenter® nanomechanical test instrument was used to carry out quasi-static indents with a Berkovich probe on selected shell layers. The displacement-controlled indents at maximum indentation depth of 100 nm followed a trapezoidal loading function of \( 5 \times 2 \times 5 \) s. Reduced elastic modulus \( E_r = S/2(\pi A)^{1/2} \), where \( S \) is the contact stiffness and \( A \) is the contact area, was determined from indentation curves according to Oliver and Pharr method using the TriboScan software.

3. Results and discussion
The EBSD analysis revealed identical textural patterns and a similar orientation of the crystallographic axes in...
the aragonitic cCL structure of the M + 1 layer in all three patellogastropods. In contrast, the M + 2 layer with calcitic SF structure is rather variable. This layer is developed only in the deep-sea P. orientalis and shallow water L. lima. In both species, the M + 2 layer has the same textural pattern, but the [0001] axes are oriented differently in different parts of their shells, varying from a direction perpendicular to the sagittal plane (Figure 1(A), (B)) to a direction parallel with it (Figure 1(C), (D)).

The hardness of the calcitic SF structure only measured in areas with [0001] axes of calcite parallel to the sagittal plane is about 4.4(2) GPa in L. lima, and about 4.1(3) GPa in the deep-sea P. orientalis. The reduced elastic modulus is identical in both species, at about 76(4) GPa (Figure 2). The hardness of the aragonitic cCL structures is similar (4.3(3) GPa in N. concinna, 4.2(3) GPa in L. lima and 4.4(4) GPa in P. orientalis). Values of the reduced elastic modulus are slightly more variable (85(3) GPa in N. concinna, 83(3) GPa in L. lima and 81(3) GPa in P. orientalis).

The calcitic and aragonitic shell layers have a very similar hardness, but their reduced elastic modulus differs significantly (Figure 2). It is noteworthy that both the measured $H$ and $E_r$ values differ significantly from the mechanical properties of inorganic aragonite and calcite. The aragonitic cCL structure has significantly lower hardness values, as well as a reduced elastic modulus, than inorganic aragonite (Figure 2). On the other hand, the calcitic SF structure has slightly, but significantly, higher values of its reduced elastic modulus. However, values of its hardness are almost double those in inorganic calcite (Figure 2). Similar observations on brachiopod biocalcite were published by Merkel et al. (2009).

The analysis of the effect of water depth on $H$ and $E_r$ values in biocarbonates revealed the lack of a close relationship. A statistically significant difference was found only between the reduced elastic modulus in the aragonitic cCL layer of N. concinna and deep-sea P. orientalis, and between the hardness of the calcitic SF layer of L. lima and deep-sea P. orientalis. On the other hand, there was no difference between the reduced elastic modulus in the aragonitic cCL layer of L. lima and deep-sea P. orientalis.

4. Conclusions

1. Our analyses revealed that bioaragonitic layers have significantly lower $H$ and $E_r$ values than inorganic aragonite; however, compared with inorganic calcite, the biocalcitic layers have distinctly higher $H$ values, and similar $E_r$ values.

2. The water depth has no or only a weak influence on the mechanical properties of the biogenic carbonates of the deep-sea limpet P. orientalis. This finding may support the hypothesis of an early settlement in the deep-sea environment by the patellogastropods (Nakano and Ozawa 2004).

![Figure 1](image1.png) Results of the EBSD analysis of the M + 2 layer in the deep-sea P. orientalis.

![Figure 2](image2.png) Results of the nanoindentation analysis, showing a relationship between the mechanical properties of biogenic aragonite and calcite (i.e. hardness and reduced elastic modulus; average of 15 measurements for each sample); as well as the relationship between the mechanical properties of those carbonates and water depth of their formation.
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