Exploring Exoskeletons of the Cold Using Micro CT

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Aims

A considerable body of work has considered the biological processes and adaptations of Antarctic invertebrate species but there has been little investigation of exoskeleton structure or material characteristics. This work aimed to study the exoskeletons of a variety of species based in Anatarctica. In the case of the Antarctic Clam (*Laternula elliptica*) the aim was to look at damaged shells and identify any features indicating signs of self repair. In the case of the Antarctic sea urchin (*Sterechinus neumayeri*), the aim of the work was to study the shells and spines and compare them to a temperate species (*Psammechinus miliaris*) and identify any differences.

Method

S. neumayeri and *L. elliptica* were collected by SCUBA divers from 3-10m depth at South Cove, Rothera Point, Adelaide Island, Antarctic Peninsula (67°34’ S, 68°08’ W). They were maintained in the aquarium at the British Antarctic Survey-run UK Rothera Research station before being transported to the UK (British Antarctic Survey, Cambridge) and held in a recirculating aquarium, prior to sampling. The temperature of the water in all aquarium systems was maintained at 0°C. Animals were fed white fish twice weekly.

P. miliaris were collected by SCUBA divers from a site 3-10m deep at Rubha Garbh, Loch Creran, Scotland (56°30’ N 5°22’ W). They were transferred to flow through aquaria at the Scottish Association for Marine Sciences, Oban and subsequently to a recirculating aquarium system at the School of Ocean Sciences, University of Bangor, Wales. Water in the recirculating system was fed from the Menai Straits via a header tank and kept at ambient temperatures, which varied seasonally from 10°C-16°C. Animals were fed an artificial diet comprising mussels, seaweed and alginic acid.

In all cases the animals were sacrificed according to National Laws and local rules and regulations. The samples were then cleaned and stored for later use or immediately sectioned so that spines and shells could be separated for characterization.

In the case of Micro CT, samples were secured to the sample holder using dental wax and scanned using either a Skyscan 1172 (Bruker Micro CT) or Skyscan 1272 (Bruker Micro CT). In all cases samples were scanned with a voltage of 50kV, stepsize of 0.2° with frame averaging of 2 and various pixel sizes. The resulting projections were processed into 3D data sets using a full cone beam Feldkamp reconstruction algorithm with NRecon software (Bruker CT). Images were subseuquently viewed in 3D using CT Vox (Bruker CT) where in the case of the sea urchins a transfer function was applied to see the difference in mineral content.

In the case of the sea urchin further comparisons were made using TGA (Thermo Gravimetric Analysis) to allow the amount of organic and inorganic content to be determined.

Results

Figure 1 comprise of images of an Antarctic shell from the bivalve mollusk *Laternula elliptica*. Figure *1a* is the complete shell demonstrating the gross shape and surface structure. The cross-section presented in *Figure 1b* demonstrates a relatively homogeneous through-thickness structure.

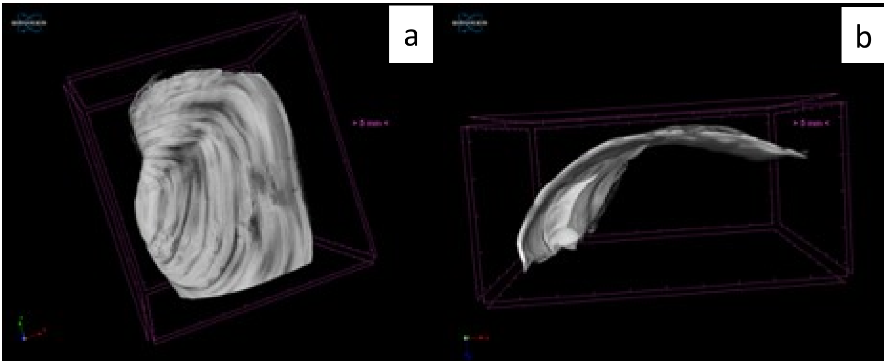

Figure 21: Images of an Antarctic clam shell. The whole section (a) and a cut through section (b)

In contrast *Figure 2* is an image of a damaged shell. The damaged part is clearly identifiable in *Figure 2a* towards the centre of the shell. The surface structure was highly disrupted but the damage was not enough to kill the clam, it survived and was brought back to the UK before being sacrificed. When we look at the cross section of the shell, in *Figure 2b*, we can see the broken fragments but also some thinner sections of organic matter that appear across the top of the shell. This is likely to be a new layer of shell that has formed, like a callous that forms over bone. This is even clearer in *Figure 3*. It is known from other experiments and observations [1]that *L. elliptica* can survive iceberg impacts and repair their shells and this is the first detailed description and materials analysis of such repairs.

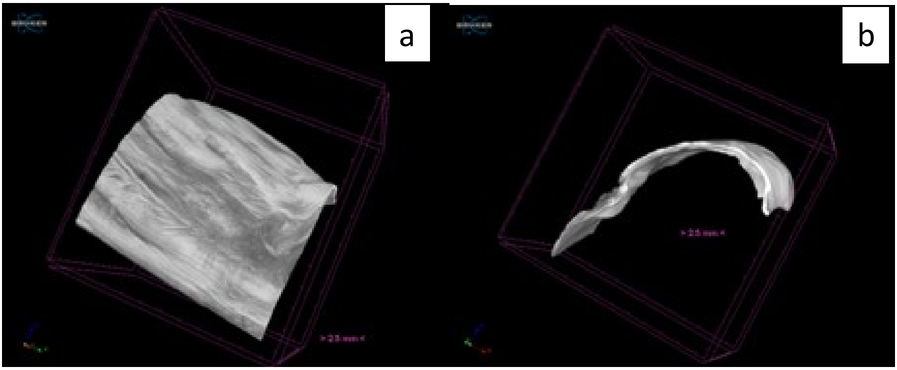

Figure 22: Images of a damaged Antarctic clam shell. A large section of shell (a) and a cut through of the damaged shell (b).

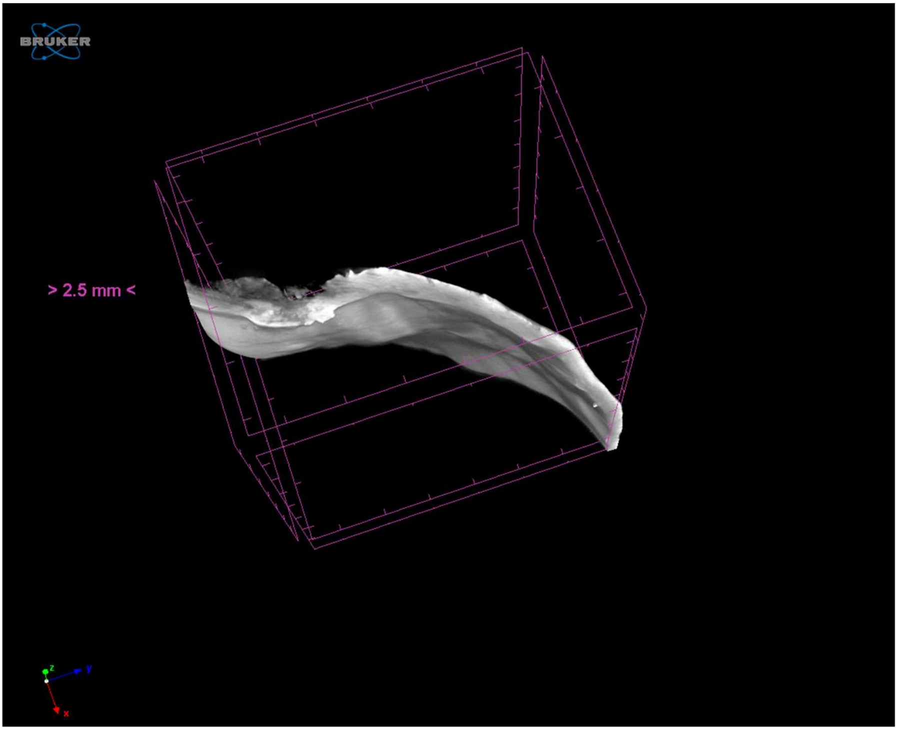

Figure 23: A close up image of the cut through of the damaged Antarctic clam shell

The comparison between the sea urchin *S. neumayeri* from Antarctica and the temperate *P. miliaris* identified large differences in the structure of the Spines. *Figure 4* is a spine from the Antarctic sea urchin. This spine is composed of struts of mineral ($CaCO_3$) with clear spaces between the struts, and these spaces are filled with organic material. When this was compared to a spine from the temperate sea urchin very clear differences were immediately apparent (*Figure 5*). There is much less mineral present in the whole structure in the Antarctic spine compared to the temperate one and the organics content of the spine from the Antarctic species is much higher than that of the temperate species.

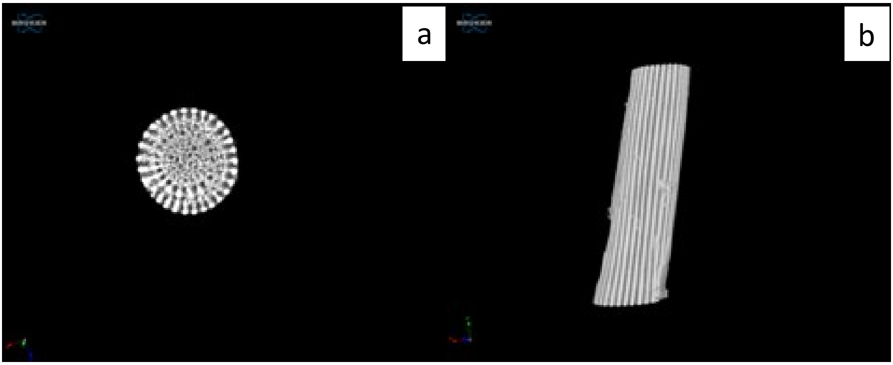

Figure 24: Images of Antarctic sea urchin spines with the cross-section (a) and the full length (b)

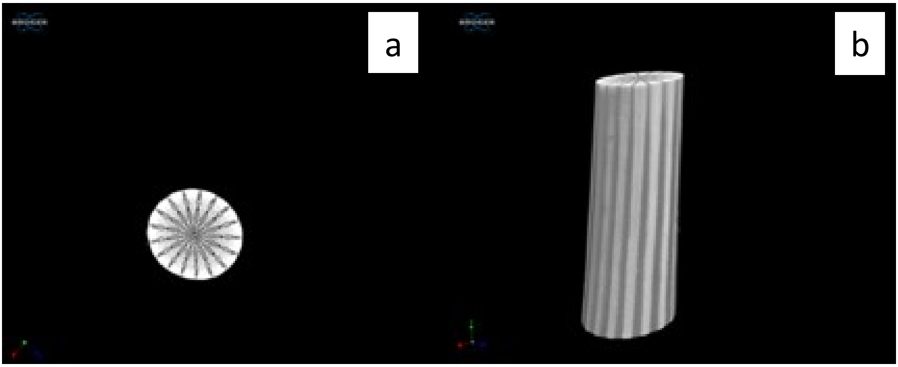

Figure 25: Images of UK sea urchin spines with the cross-section (a) and the full length (b)

When TGA was carried out on the spines from the temperate and Antarctic sea urchins the difference in their inorganic content was very clear, *Figure 5*. There was a lot more inorganic matter in the temperate species of sea urchin than that observed in the Antarctic one. This may well be due to the fact that producing a skeleton costs a much larger proportion of the animal's energy budget in Antarctica than at lower latitudes[1] and they have adapted by producing spines with less mineral.

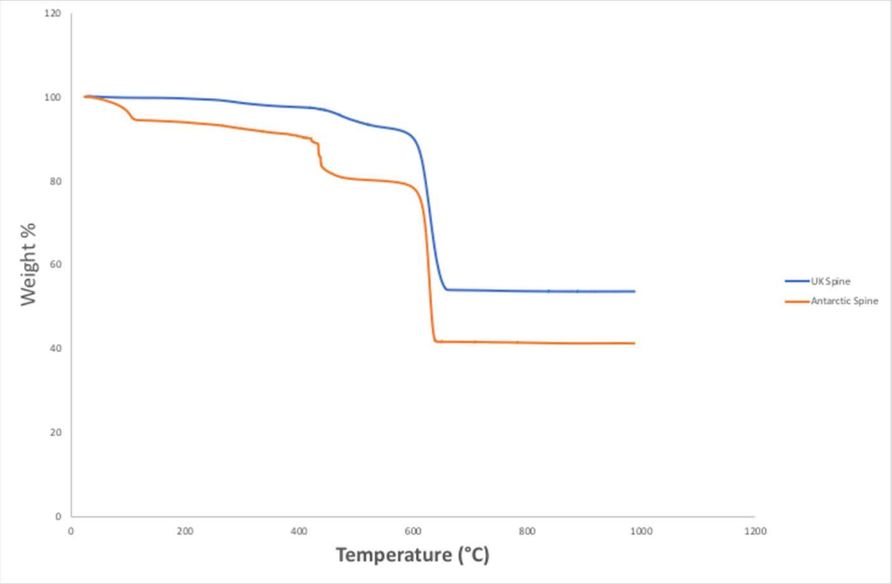

Figure 26: TGA data for Antarctic and UK sea urchin spines

Conclusion

It has been shown that Micro CT is a tool that allows the materials aspects of skeletons of Antarctic species to be studied in great detail. It can also be used to identify differences in the skeletons produced between species from polar-regions and those from warmer climates. This might lead to the discovery of new structures to be exploited in structural engineering. By combining this knowledge with biological techniques it might be also possible to identify proteins that cause these differences in structure and that have adapted to work in colder climates.

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References

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