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Mechanical properties of nacre and highly mineralized bone

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We compared the mechanical properties of 'ordinary' bovine bone, the highly mineralized bone of the rostrum of the whale *Mesoplodon densirostris*, and mother of pearl (nacre) of the pearl oyster *Pinctada margaritifera*. The rostrum and the nacre are similar in having very little organic material. However, the rostral bone is much weaker and more brittle than nacre, which in these properties is close to ordinary bone. The ability of nacre to outperform rostral bone is the result of its extremely well-ordered microstructure, with organic material forming a nearly continuous jacket round all the tiny aragonite plates, a design well adapted to produce toughness. In contrast, in the rostrum the organic material, mainly collagen, is poorly organized and discontinuous, allowing the mineral to join up to form, in effect, a brittle stony material.

Keywords: bone; mineralization; nacre; mechanical properties; fatigue

1. INTRODUCTION

Many of the mechanical properties of bone are well known, and there is some information available concerning mother of pearl (nacre), as well as some other molluscan shell types. Ordinary bone, such as might be found in the long bones of bovines, contains much more organic material than does nacre, which only has about 1%. As a result it has not been possible to compare meaningfully the mechanical properties of these two skeletal types from different phyla, as one might wish to do in order to determine their advantages and disadvantages. Recently, however, work has been done on the very highly mineralized rostral bone of the ziphiid whale *Mesoplodon densirostris,* which has a mineral content similar to that of nacre, so it is possible to make comparisons. This paper reports on information already in the literature, adds new information on the fatigue behaviour of nacre from the pearl oyster *Pinctada margaritifera*, and discusses reasons for the differences and similarities seen.

2. MATERIAL AND METHODS

All three tissues we are considering here are biological examples of a composite mineralized matrix. They are combinations of an organic matrix, a mineral reinforcing phase and water. However, in the two bone examples (rostrum and *Bos*) the organic matrix is mainly (*ca.* 95%) collagen type I, whereas in nacre the organic phase is amorphous and consists mainly of proteins of very complex constitution. Figure 1 shows back-scattered scanning electron microscopy (SEM) images of transverse cross-sections of the bony tissues where the local mineralization levels can be appreciated, because more heavily mineralized areas appear lighter in this mode. Bovine bone has a structure called fibrolamellar, in which sheets of primary bone sandwich two-dimensional nets of blood vessels. There are usually some secondary osteons present. The rostrum is heavily remodelled and consists mainly of secondary osteons and remnants of the lamellar interstitial matrix. Nacre consists of layers of mineral plates of a very regular size (0.3–0.5 μm thick) stuck together by organic matrix.

Table 1 gives the sources of information for the various properties. We shall not repeat a description of the methods used if they have been given in those papers. A more detailed comparison of some aspects of mechanical behaviour and structure of *Mesoplodon* rostrum and bovine bone, based on the same specimens as here, is in Zioupos *et al.* (2000); relevant results are summarized from that work. There are differences in the tests on the bovine bone and rostrum on one hand, and nacre on the other. This is partly because the nacre specimens were necessarily smaller than the bone specimens. Also, most of the nacre tests were performed many years ago. However, all tests were standard mechanical tests on macroscopic specimens, and the differences are unlikely to have produced large spurious differences between the different specimen types. Furthermore the results are so clear that the differences introduced by the testing methods cannot have had an important effect on the conclusions. Similarly, because the results are so clear, we have not produced estimates of variability; these are mostly available in the source publications. To our knowledge, the fatigue tests have not been reported before.

(a) *Fatigue tests: rostrum and bovine bone*

It proved impossible to test *Mesoplodon* rostrum specimens in either tension or compression because they were so brittle. We were, however, able to conduct a series of three-point bending fatigue tests. Beam-shaped specimens, 50 mm long × 5 mm wide × 2 mm deep, were prepared from *Mesoplodon* rostrum and from the mid-diaphysis of a bovine femur, the animal being about 18 months old, with unfused epiphyses. The orientation of the specimens was in the longitudinal direction of the bone. The specimens were fatigued in a three-point bending rig (E-399-83 and D-790-86 American Society for Testing Materials standards) on an Instron 8511® servohydraulic machine (Instron, High Wycombe, UK), in Ringer's solution, at *ca.* 20 °C, under load control. A sinusoidal waveform and a frequency of 2 Hz were used. A compressive load range of ΔF (the minimum load being zero) produced an estimated maximum tensile stress at

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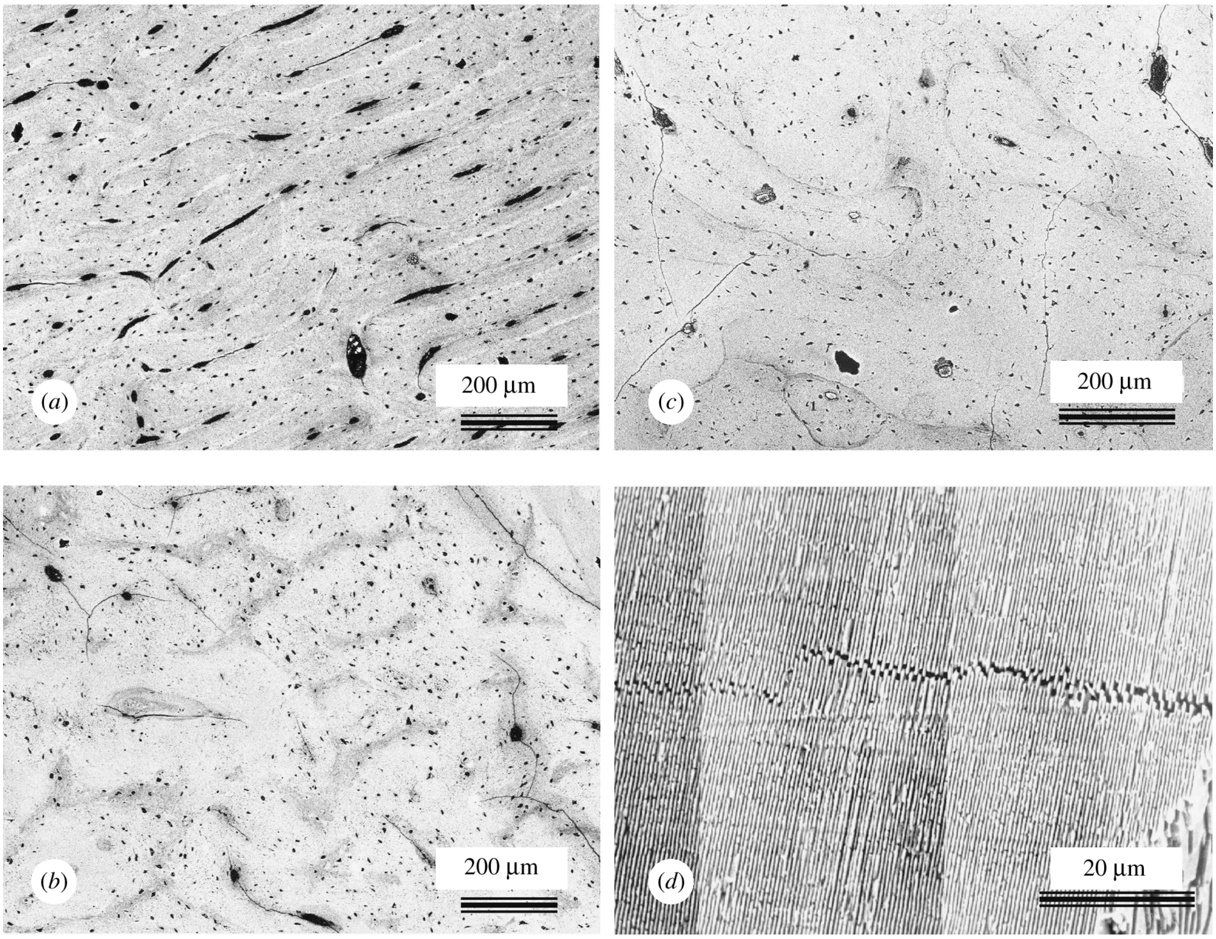

Figure 1. SEM micrographs of (*a*) bovine bone, (*b*) tympanic bulla of the fin whale *Balaenoptera physalus*, (*c*) rostrum. These images were taken in the back-scattered mode. The tympanic bulla is shown, for comparison with the rostrum, since it is a very highly mineralized bony tissue (Currey 1979). However, we do not consider it further in this paper. (*d*) Nacre. The nacre photograph was taken in standard mode (Currey 1977). It shows how a crack, running from right to left, pursues an extremely jagged course. Note the difference in scale. Bovine bone, tympanic bulla and rostrum have similar constituents, based on collagen fibrils impregnated with mineral. Nacre is made of aragonite plates of very regular 0.5 m thickness with organic matrix in between. On the other hand, rostrum and bulla and nacre have similar compositions.

Table 1. *Sources of information*

	bovine bone	rostral bone	nacre
composition	Zioupos *et al*. 2000	Zioupos *et al*. 2000	Harper 2000
Young's modulus	Zioupos *et al*. 1997	Zioupos *et al*. 1997	Currey 1976
bending strength	Zioupos *et al*. 1997	Zioupos *et al*. 1997	Currey 1976
work of fracture	this paper	this paper	Currey 1977
K_c	this paper	this paper	Jackson *et al*. 1988
hardness	Zioupos *et al*. 2000	Zioupos *et al*. 2000	this paper
fatigue	this paper	this paper	this paper

the outer layers of the beam of $\Delta s = 1.5 \times \Delta F \times L \times b^{-1} \times h^{-2}$, where L is the span between the two supports (40 mm), b is specimen breadth, h is specimen thickness and Δs is stress range.

First, the modulus of elasticity of each bone specimen was obtained by cycling a few times at a very low stress range. The modulus was used to determine the stress at which the specimens would be tested in fatigue. We arranged the tests so that there was an even distribution of stiffer and less stiff specimens at the various imposed fatigue stresses. Eighteen *Mesoplodon* and 11 bovine bone specimens were successfully tested in fatigue.

(b) *Fatigue tests: nacre*

There are two layers in the shell of the pearl oyster, of prisms and nacre. The prismatic layer was ground off before specimen shaping started. The specimens were necessarily smaller than the bone specimens, and were *ca*. 3 mm wide, 1 mm deep, had a gauge length of 35 mm, and had a high polish. They were loaded in an Instron 1122 table model machine under load control, with a triangular waveform, in three-point bending at 2 Hz. The specimens were immersed in water while being loaded.

Table 2. *The composition and mechanical properties of the three tissues*

('Along' in the case of the nacre specimens refers to cracks that travel between the layers of plates. This direction is termed 'between' in Currey (1977).)

	bovine bone	rostral bone	nacre
mineral (%)	65	96	98
organic (%)	25	1	1
water (%)	10	3	1
Young's modulus (GPa)	20	40	34
bending strength (MPa)	220	55	210
work of fracture (kJ m^{-2})			
normal	3.2	0.1	1.65
along	0.8–1.0	0.02–0.035[a]	0.15
K_c (MPa $m^{0.5}$)			
normal	6.0	1.5	4.5
along	2.2	0.7	—
hardness (kg m^{-2})	55–70	227	200
fatigue			
$\log_{10}$ stress			
unnormalized	$2.36-0.063 \log_{10} N$	$1.78-0.030 \log_{10} N$	$2.39-0.083 \log_{10} N$
normalized	$2.33-0.053 \log_{10} N$	$1.76-0.024 \log_{10} N$	—
unlogged stress			
unnormalized	$229 N^{-0.063}$	$60 N^{-0.030}$	$245 N^{-0.083}$
including non-failures	—	—	$251 N^{-0.090}$

[a] These values are estimates, calculated by use of the equation for critical strain energy density G (kJ m^{-2}) $= K_c^2$(MPa $m^{0.5}$) $\times E$(GPa), which is sufficiently satisfied for linearly elastic brittle materials. The critical strain energy density is derived from the maximum load that the work of fracture specimens reached.

It is unsatisfactory that the fatigue tests were not the same for all three tissues but this was necessitated by the necessarily smaller size of the nacre specimens, and the fact that the tests were carried out in different laboratories with different facilities.

(c) *Toughness*

Bone fracture toughness (FrT) specimens had dimensions (width and length) conforming to ASTM E-399-83 standards (width twice the thickness, length between supports eight times the thickness). All faces of the samples were polished by progressively finer grades of carborundum paper and brought to a mirror finish by use of Buehler micropolish powder® (Buehler, Coventry, UK). The final dimensions were prepared to a 0.01 mm accuracy. The whole preparation was under water at room temperature.

FrT was assessed using the critical stress intensity factor (K_c) at the initiation of a macrocrack. A notch was cut in the K_c specimens by the use of the finest Exakt band-saw blade (Mederex, Frome, UK), which had a width of just under 100 μm. The notch cut across the full width of the specimens and its depth was approximately half the thickness of the specimens, providing a notch length/width ratio (a/W) of *ca.* 0.5 according to standards. The length of the notch was measured by both a travelling microscope and vernier callipers to an accuracy of 0.01 mm. The width of the notch was *ca.* 100 μm and its tip was semi-circular with a minimum radius just under 50 μm. Stereomicroscopic observation showed that, in all bone samples, the load at which the notch started growing was the maximum load, after which the rapid decrease in load was caused by the growth of the crack.

The K_c-values we report here come from the formulae

$$K_c = 0.031623 \times f(a/W)\, P_C S\, B^{-1}\, W^{-3/2}\,, \qquad (1)$$

$$f(a/W) = 1.5(a/W)^{0.5}[1.99-(a/W)\,(1-a/W) \times \{2.15-3.93\,(a/W+2.7(a/W)^2\}] \times (1+2a/W)^{-1}(1-a/W)^{-3/2}\,, \qquad (2)$$

where B is the specimen thickness, S the span between the supports, W the specimen width and a the notch length. P_c was the critical load for crack growth. The displacement rate in our tests was 2 mm min^{-1} and was chosen so as to give a rate of increase of stress intensity in the range 0.55–2.75 MPa $m^{0.5}$ s^{-1}. We obtained estimates of K_c for bovine and rostrum specimens in two directions, the crack travelling either normal to the grain, or parallel to it. Values for K_c for nacre were taken from the careful experiments of Jackson *et al.* (1988).

Work of fracture (W_f) tests of bone specimens were carried out on square cross-section specimens with a chevron notch (a triangular ligament area remaining intact) (Tattersall & Tappin 1966) prepared by use of the diamond band-saw. The ligament tip was orientated so that the crack travelled transversely across the structural elements. Bending was applied at a cross-head speed of 0.1 mm min^{-1}. The span of supports was 31.6 mm. In these tests fracture starts at the tip of the ligament, the ever-increasing width of the fracture front allowing a stable fracture progression. The work done to complete the fracture was divided by twice the ligament area to yield the work needed to create a unit of new fracture surface. The nacre specimens had a deep slot cut in them, rather than a chevron slot. All bone and nacre specimens broke in a controlled fashion, which was the purpose of the triangular ligament and the slot.

3. RESULTS

The principal results are shown in table 2. The mineral, organic and water percentages are weight per

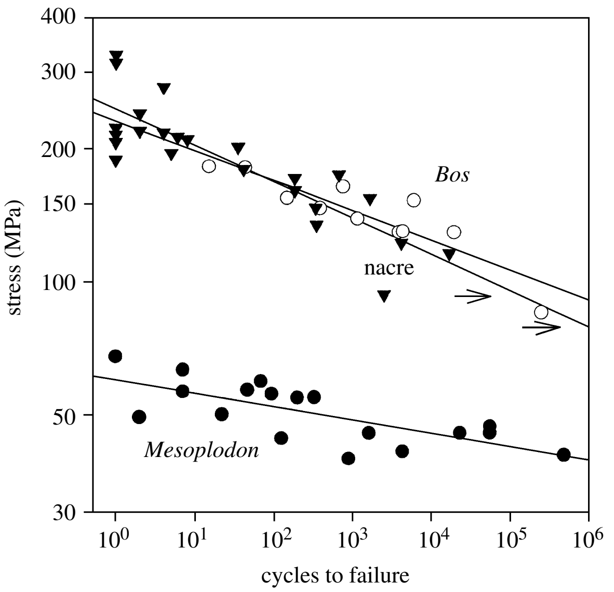

Figure 2. *S–N* diagrams of the three tissues. Open circles, *Bos*; solid triangles, nacre; solid circles, *Mesoplodon* rostrum. The two arrows indicate the nacre specimens that had not failed when the experiment had to be stopped. The arrow tips show the maximum stress and the cycles completed at termination.

weight of the fully hydrated tissue. The value of 'organic' for nacre is taken as the mean of the values for *Unio pictorum* and *Atrina pectinata* determined by Harper (2000) and the values for 'water' and 'organic' are assumed to be equal.

The Young's modulus and bending strength were determined in three-point bending. 'Normal' and 'along' refer to the direction of crack travel relative to the predominant grain of the specimen. Hardness is Vicker's microhardness. The diagonals of the indentations were of the order of 10–50 μm and we were able to target indentations at the solid mineralized matrix, avoiding areas of increased microporosity. Hardness was measured on dry specimens. The fatigue equations are of the form: $\log_{10}$ stress (MPa) as a function of $\log_{10}$ cycles to failure (N). This is an illogical way of modelling the data, because clearly the cycles to failure are a function of the applied stress, rather than vice versa. However, this way of doing things is unfortunately deeply embedded in the fatigue literature. Analysis of the bones included a normalization by adjusting the stress values Δs by use of the particular modulus of elasticity of each specimen: $\Delta s(E) = \Delta s E_a / E_s$ where E_s is the modulus of elasticity of each specimen and E_a is the mean elastic modulus value of all *Mesoplodon* or bovine specimens. This adjustment performs a normalization to equivalent strain values. The onset of microfracture in bone depends on the macroscopic strain (Zioupos *et al*. 1994). This normalization was not performed on the nacre specimens. The equations are also given unlogged. Two of the nacre specimens did not fail before the experiment had to be stopped. The table shows the small difference between the regression equations which do, and which do not include these two specimens treated as if they had failed at the cycle at which the experiment was stopped.

Rostral bone and nacre are very similar in their composition (except, of course that the mineral in bone approximates to carbonate apatite, dahllite, while the mineral in nacre is calcium carbonate in the form of aragonite). Bovine bone has much more water and organic material, mainly collagen, than the other two materials. The Young's modulus of nacre is intermediate between the values for the bones. Nacre has a bending strength about equal to that of bovine bone. The work of fracture of the rostral bone is extremely low normal to the grain, nacre is much higher, although when the crack travels between the layers of mineral the value falls considerably. The K_c-values for the rostrum are about one-quarter that of the bovine bone, but the anisotropy is similar for the two bony tissues. We have no information of the value for K_c for nacre when the crack travels between the layers, so we can say nothing about the amount of anisotropy in nacre.

The fatigue behaviour of rostral bone is markedly different from the other two tissues (figure 2). The monotonic bending strength of this bone is much lower than that of the other tissues. Therefore, of course, the whole set of *S–N* points for rostrum is lower than those of the other two. Nevertheless, rostral bone showed standard fatigue behaviour, with an increase in the number of cycles to failure as the stress was reduced. Statistical comparison of the slopes showed no significant difference between those for *Bos* and nacre, but both were significantly steeper than the slope for *Mesoplodon*. It is apparent from table 2 that normalizing the data changed the equations describing the results rather little. Therefore, the fact that the nacre results were not normalized is not, we think, a concern.

4. DISCUSSION

Compared with bovine bone, the bony material of the rostrum of *Mesoplodon densirostris* has very little organic material or water, a much higher Young's modulus and hardness, but a much lower bending strength, toughness and fatigue strength. Compared with bovine bone, nacre, like the rostrum, has very little organic material or water, and has a markedly higher Young's modulus and hardness. However, its bending strength is about the same, its work of fracture is about half in the normal direction, and much less in the 'easy' direction, and is far higher than that of the rostrum; K_c is similar, as is the fatigue behaviour. In short, the high mineral content of the rostrum bone is associated with a great loss of strength and toughness compared with bovine bone but this is not the case for nacre. Why is this?

The reason lies in differences in the microstructure of the two tissues. The mineral of the rostrum is similar to 'ordinary' bone mineral in its elemental constitution. Zylberberg *et al*. (1998) and Rogers & Zioupos (1999) have shown that the crystallites of the rostrum are much larger than normal in bone and are very well aligned. They have very regular plate-like shapes and the long mineral columnar formations seem to displace the sparse collagen fibres into a quasi-hexagonal pattern around them. Compared with the situation in 'ordinary' bones this reversal of roles between collagen and mineral probably explains why the rostrum is so brittle—in effect it comprises a nearly homogeneous ceramic mass with no interconnecting, and separating, matrix.

On the other hand nacre, although also having a very low organic content, has this organic material arranged in a highly precise and adaptive manner, allowing it to be quite surprisingly tough. Jackson *et al.* (1988, 1990) have shown that nacre is superior to most other artificial composite ceramics in stiffness, strength and toughness. Pearl oyster nacre consists of flat sheets of aragonite, *ca.* 0.5 μm thick. Between each layer of mineral is a very thin layer of organic material, mainly protein. The precise structure of the proteins seems to determine such things as which crystallographic species is laid down, and indeed to determine the shapes of the crystals (Addadi & Weiner 1997; Walters *et al.* 1997).

Apart from its role in determining the structure of the mineral, the organic layer acts as a powerful toughening device. As a crack travels down through and round the layers of crystals the plates spring apart, and in doing so extend the organic sheets, so that the plates have chewing-gum-like connections. Work must be done in extending knowledge concerning this organic material. Smith *et al.* (1999) have cloned and expressed one of the principal proteins, lustrin A, from the shell of the abalone (*Haliotis* sp.) and, using atomic force microscopy, have shown it has a very characteristic load-deformation curve. After the yield point it has a saw-toothed shape, with the load increasing and then periodically dropping sharply as the strain increases. This periodicity is apparently caused by the sequential unwrapping of periodic regions in the protein. The result is that a large strain can be accommodated without very high loads being required, even though the original load-deformation curve is quite steep. This is an ideal property for such an energy-absorbing filler between the mineral sheets. Furthermore there is evidence that this unwrapping can self-heal if the strain is reduced.

The maximum toughening effect brought about by the organic matrix is achieved when the matrix operates in a shear mode. When loaded so that the crack travels across the sheets (figure 1*d*) the nacre shows crack deflection, fibre- (aragonite crystal-) pull-out and matrix crack bridging (Jackson *et al.* 1988; Wang *et al.* 1995). However, when loaded so that the crack passes between the sheets the toughening produced by the crack having to break through, or travel round the ends of the sheets is absent, and the work of fracture in this direction is, indeed, very low (table 2). The works of fracture in the two directions have a ratio of about 15:1. This is a much higher ratio of the toughness in the two directions than is seen in the bony specimens (table 2).

The mixture of mineral, organic material and water found in bone results in the ability of bone to have a variety of mechanical properties that fit it for the particular mechanical environment in which it finds itself. Increasing mineral makes bone stiffer and stronger in bending, but reduces its toughness. Different bone tissues seem to be set at a particular point to optimize their mechanical properties (Currey 1999). If the bone becomes very highly mineralized, however, there is a large increase in the Young's modulus, but a catastrophic decline in bending strength and toughness. This is found in the highly mineralized tympanic bulla of the whale, as well as in the rostrum of *Mesoplodon*. The remaining organic material is not well situated to have any effect on the toughness of the bone, whose properties resemble that of a naturally occurring mineral—stiff but brittle. The mystery of the rostrum is why it is so highly mineralized (Zioupos *et al.* 1997). We have no idea of the adaptive reason, if any, for the production of such a brittle material.

Nacre, in contrast, has organic material beautifully arranged to produce a material of quite respectable toughness (though it is highly anisotropic in this property). The mystery of nacre is why it seems never to be modified to have a rather larger amount of organic material, in which case it would be very tough and strong, while still remaining quite stiff.

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