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The contribution of mineral to the material properties of vertebral cartilage from the smooth-hound shark *Mustelus californicus*

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Summary

Elasmobranch vertebral cartilage has a substantial mineral fraction (39–55%) and the arrangement of mineral varies among species. We examined vertebrae from one shark species, *Mustelus californicus*, to determine mineral content, the effect of mineral on material properties and the viscoelastic response of vertebral cartilage. We serially demineralized vertebrae and compressively tested them to failure at varying strain rates. Mineral in vertebral cartilage varies within individuals, intraspecifically and interspecifically; this is in contrast to bone, in which significant variation in mineral content is pathological or an interspecific effect. Within *Mustelus,* vertebrae with larger mineral fractions were significantly stiffer and stronger; however when variation is assessed across species, the structure has a larger effect. Shark vertebral cartilage did not show a substantial viscoelastic response at biologically relevant strain rates, validating the use of quasistatic testing for this material.

Key words: elasmobranch cartilage, mineral content, stiffness, strength, viscoelastic, elastic.

Introduction

Scale is critical when studying biological materials (Currey, 2005). For instance, we can study mechanics of the crystal structure in apatite, individual trabeculae in spongy bone, compact bone, or an entire long bone. At each of these levels we can test the mechanical properties of tissue and determine how these properties will influence the performance of the animal. This hierarchy presents opportunities to understand biological materials themselves as well as the arrangement of the materials within a structure.

The relationship between mineral content and properties of hard biological materials, particularly mammalian bone, has been explored in depth (Currey, 1999; Currey, 2002). Small changes in mineral content can have large effects on material properties in hard tissues, and those with larger mineral fractions are stiffer and stronger than those materials with less mineral. A biological example of this relationship is the rostrum of the Blaineville's beaked whale *Mesoplodon densirostris*, which is composed of 96% mineral, resulting in an incredibly stiff material (46 GPa) (Rogers and Zioupos, 1999; Zioupos et al., 1997). The fin whale *Balaenoptera physalus* tympanic bulla has 14% less mineral and is 35% less stiff (Currey, 1979). More dramatic still is the red deer antler *Cervus elaphus*, with 40% less mineral than *M. densirostris* and a 78% decrease in stiffness (Currey, 2002). However, the relationship between the amount of mineral and material properties is confounded in the aforementioned examples by testing different bones from different animals and thus different structures.

Structure (arrangement of mineral) is also a significant predictor of material properties. Lordosis of vertebrae in sea bass *Dicentrarchus labrax* L. resulted in structural changes in vertebral morphology; there was an increase in both bone volume and second moment of area (mm^4) in the lordotic compared to non-lordotic vertebrae (Kranenbarg et al., 2005a). Although there is a great deal of literature dedicated to understanding the influence of mineral amount and arrangement in bone, the nature of this relationship is not well known in other mineralized materials.

Elasmobranch vertebral cartilage is 'areolar'; it has a web-like infiltration of mineral in a hyaline cartilage matrix that varies in morphology by species (Moss, 1977; Ridewood, 1921). Portions of the mineral in elasmobranch vertebrae are arranged in elaborate patterns that vary by species, and these interspecific mineralization patterns are variable enough to be of systematic importance (Fig. 1) (Ridewood, 1921). For example, the mineral in the vertebrae of the shortfin mako *Isurus oxyrinchus* is arranged in plates around the centra, while vertebrae of the silky shark *Carcharhinus falciformis* are covered with a thick crust of mineral (Fig. 1) (Porter et al., 2006).

As in bone, mineral is a significant predictor of material properties in the cartilaginous skeletons of elasmobranchs (sharks, skates and rays), specifically in vertebral columns. Porter et al. examined six species of shark and one species of axially undulating (movement about the vertebral column) ray and found mineral contents ranging from 39–55% of dry mass

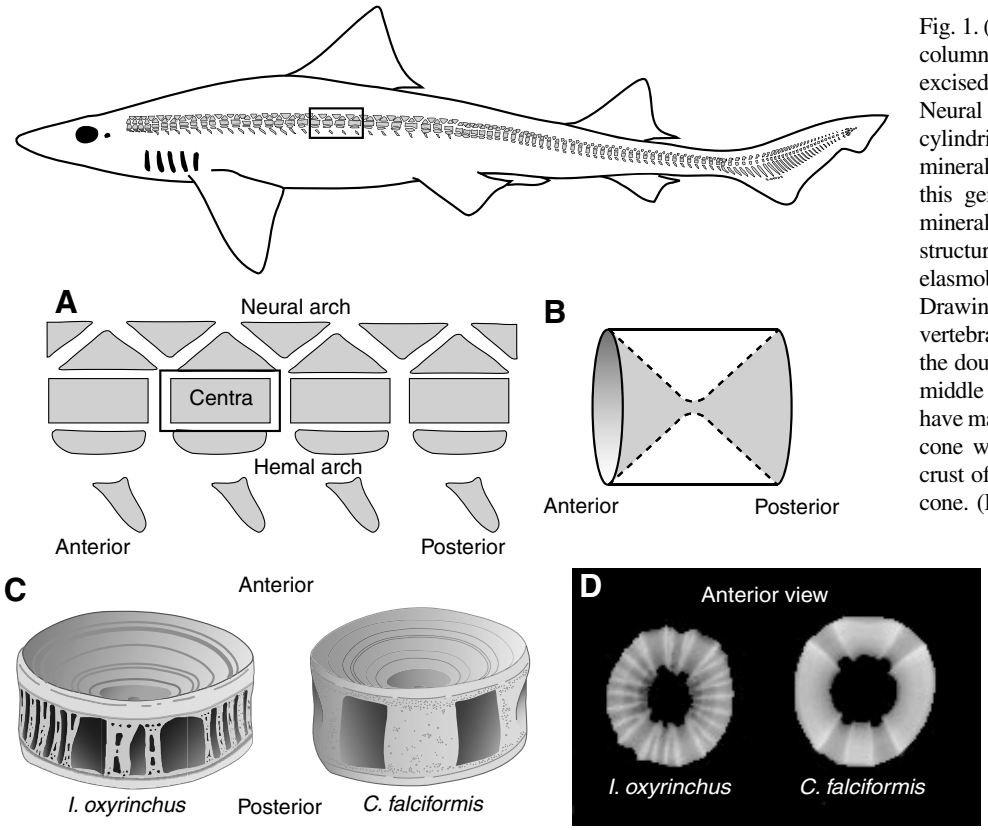

Fig. 1. (Top) Schematic of *M. californicus* vertebral column. Vertebrae used in these experiments were excised from under the first dorsal fin (boxed) (A). Neural and hemal arches were removed leaving cylindrical centra for materials testing. (B) The mineralized double cone structure is highlighted in this generalized elasmobranch vertebra. Intricate mineralization patterns branch off the double cone structure and the patterns vary extensively among elasmobranch species (Ridewood, 1921). (C) Drawings of 3/4 views of two species of shark vertebrae. The anterior surface is concave, part of the double cone structure, coming to a point in the middle of the centra. Mako (*I. oxyrinchus*) centra have many plates of mineral surrounding the double cone while the silky shark (*C. falciformis*) has a crust of mineral extending from the central double cone. (D) Anterior view radiographs of mako and silky vertebral centra with excised neural and hemal arches. The mako shark vertebra mineral is arranged in plates around the centra and relatively unmineralized cartilage fills the gaps between the plates. The silky shark vertebra has a highly mineralized sheath around the centra with less mineralized cartilages appearing where the neural and hemal arches are placed (Porter et al., 2006).

(Porter et al., 2006). In elasmobranch cartilage, a 16% increase in mineral content resulted in an 81% increase in ultimate strength and a 95% increase in stiffness. The seven species examined had a diversity of vertebral mineral patterns, such that the relationship between mineral structure and mineral amount was confounded.

Mineralized biological materials are described as anisotropic elastic solids up to their yield points and do not display substantial time-dependent behavior (Vogel, 1988; Vogel, 2003; Wainwright et al., 1976). This has been verified in bone tested using biologically relevant loading regimes (Currey, 1989). Cartilage, in contrast, is a viscoelastic material having both fluid and solid characteristics, and therefore displays strain-rate dependent mechanical and material properties. Unmineralized cartilage, such as bovine articular cartilage, becomes stiffer with increasing strain rate (Li et al., 2003; Park et al., 2004). Cartilaginous vertebrae of elasmobranchs also have a large mineral component and so may not exhibit the viscoelastic behavior seen in mammalian cartilage.

The goals of the present study were: (1) to determine the intraspecific variation in mineral content of vertebral cartilage in a single species; (2) to isolate the effect of mineral on the response to load of vertebral cartilage by serially removing mineral from vertebrae of the same morphology and comparing this to the effect of interspecific variation in mineral; (3) to compare the influence of mineral content on material properties in *Mustelus* to other elasmobranchs; and (4) to assess the viscoelastic behavior in elasmobranch cartilage by testing the strain rate dependence of vertebrae with and without mineral.

Materials and methods

Study animal

Mustelus californicus Gill 1864 (Carcharhiniformes: Triakidae), the gray smooth-hound shark, is commonly found along the California coast (Compagno, 2003). We used five adult animals (four males and one female who was not gravid) ranging from 76–81 cm in total length, caught off the coast of Southern California during the spring and summer of 2005 (Table 1).

Material properties

We removed at least 40 individual vertebrae from freshly frozen vertebral columns. We chose vertebrae from the region directly under the first dorsal fin to standardize for morphology and potential differences in material properties that may occur from the anterior to posterior end of the column (Fig. 1). We excised neural and hemal arches from the centra leaving an unadorned disk of mineralized cartilage. Vertebra mass, length (distance in mm from anterior surface to the posterior surface), and diameter of the anterior surface were measured.

A total of 204 vertebrae were separated into three groups; time zero (four vertebrae from each animal), control (16 vertebrae from each animal), and demineralized (at least 16 vertebrae from each animal). Time zero vertebrae were maintained in elasmobranch Ringers (Forster et al., 1972) at room temperature for no more than 2 h before being subjected to a uniaxial, unconfined compressive test to failure between two nonporous platens. Control centra were maintained in elasmobranch Ringers solution at 4°C with continual gentle stirring while suspended in tissue cassettes. These centra were

Table 1. *Summary of material properties and mineral content in M. californicus*

Individual	Total length (cm)	Sex	Mineral content (%DM)	(s.d./Range) ×100 (%)	Stiffness (MPa)	Strength (MPa)
1	79	Male	50.8±5.1	14.5	524.2±172.0	57.9±14.4
2	76	Male	47.5±1.6	4.6	566.3±271.0	50.8±13.7
3	81	Male	49.9±6.6	18.9	495.9±260.6	52.1±14.3
4	81	Male	50.9±5.5	15.7	424.1±119.1	44.8±13.4
5	79	Female	49.0±6.4	18.3	978.4±458.0	52.1±13.0

DM, dry mass. Values are means ± s.d.

treated similarly to the time zero centra, but control centra were tested during the time course of the experiment (at the same times as the demineralized treatments). Concurrently, we demineralized the remaining vertebral centra from the five vertebral columns with a chelating agent, ethylenediaminetetraacetic acid (EDTA). Centra were immersed in 4 l elasmobranch Ringers with 83 mmol l^{-1} EDTA, minimum ratio of one vertebra to 100 ml of EDTA. Vertebrae in solution were incubated in a cold room at 4°C under the same conditions described above for the control vertebrae.

Demineralized samples were x-rayed daily to qualitatively determine mineral loss. We determined mineral loss by comparing the radio-opaque, highly mineralized portions, of the vertebrae to x-ray films from previous days. We subjected a subset of four demineralized vertebrae and four control vertebrae from each animal to materials testing at intervals of 39, 87, 135 and 279 h. The diameter and length of each vertebra were measured with a dial caliper to the nearest 0.01 mm. Control and demineralized vertebrae pairs were randomly assigned to a strain rate group (1, 5, 10 or 20% of their length s^{-1}) and were tested in a compressive test to failure using a MTS Mini Bionix 858 (Eden Prairie, MN, USA) with a 5 kg load cell.

Using published values for tail beat frequencies and silhouettes of fast starts in sharks to determine curvature (Domenici et al., 2004; Graham et al., 1990), and assuming the vertebral column acts as a uniform beam, we calculated the strain rate that we expected vertebrae to experience. This upper bound on strain rate is approximately 7% s^{-1}, so we chose experimental strain rates above and below this value. The strain rate we calculated is in agreement with literature values for human spinal column connective tissue (Stokes, 1987).

Compression testing resulted in load–displacement (N, mm) curves, which were analyzed using a custom script written in Matlab version 7.0 R.12 (The Mathworks Inc., Natick, MA, USA). We generated stress–strain curves and measured stiffness, ultimate strength, yield strength and yield strain for each vertebra. The above variables provide information regarding the response of mineralized cartilage to compressive loads. Stress was calculated using the cross-sectional area of the anterior surface of the vertebrae. Stiffness is the material's ability to resist compression and was measured as the linear region of the stress–strain curve before the material yielded. Ultimate strength is the maximum stress that can be applied to the material before it fails or breaks (Currey, 2002; Vogel, 2003; Wainwright et al., 1976). Yield strength and strain are measured at the clear inflection point seen in stress–strain curves of mineralized tissues, where the material transitions from elastic to plastic behavior and begins to permanently deform. Stress–strain curves from each vertebra were analyzed three times using the Matlab script to ensure accurate estimation of the above properties.

We determined mineral content after material testing by ashing vertebrae at 400°C for at least 8 h to obtain the mineral mass. Preliminary experiments established that 8 h was sufficient time to completely ash the vertebral sample from this species of shark. We calculated percent mineral content by dividing the mineral mass by the dry mass of each vertebra.

Statistical analyses

Stiffness and strength were analyzed using a two-way ANOVA in JMPIN (SAS Institute, Cary, NC, USA) with mineral content and strain rate as effects (Zar, 1999). We examined the overall effect of mineral content on stiffness and strength using linear regression models. We examined the viscoelastic properties by binning our data in two groups; vertebrae with greater than 45% mineral content (the vertebral mineral content (%) found in *M. californicus*) and vertebrae with less than 15% mineral. We are testing the unmineralized cartilaginous component of the tissue when examining vertebrae with less than 15% mineral content. We compared strain rate dependence in fully mineralized and demineralized vertebrae with an analysis of variance (ANOVA).

Results

Mineral content

Mean mineral content among individual *M. californicus* used in this experiment was not significantly different (P=0.24) and vertebrae had 49.5% mineral by dry mass in their cartilage (Fig. 2 and Table 1). These data were collected from vertebrae in the time zero and control groups, which were never exposed to EDTA. The range of mineral found in the vertebrae of one animal varies widely or very little, depending on the individual. We examined the variation among individuals by calculating the relationship between the standard deviation and the whole range of mineral found in these vertebrae [(s.d./mineral range)×100] (Table 1). The smallest variation was 4.6% while the largest was 18.9%.

Vertebral mineral content decreased significantly with time in EDTA ($F_{4,118}$=108.964; P<0.001) (Fig. 3B). The greatest loss of mineral at the above-mentioned EDTA concentration occurs between 87 and 135 h after initial exposure.

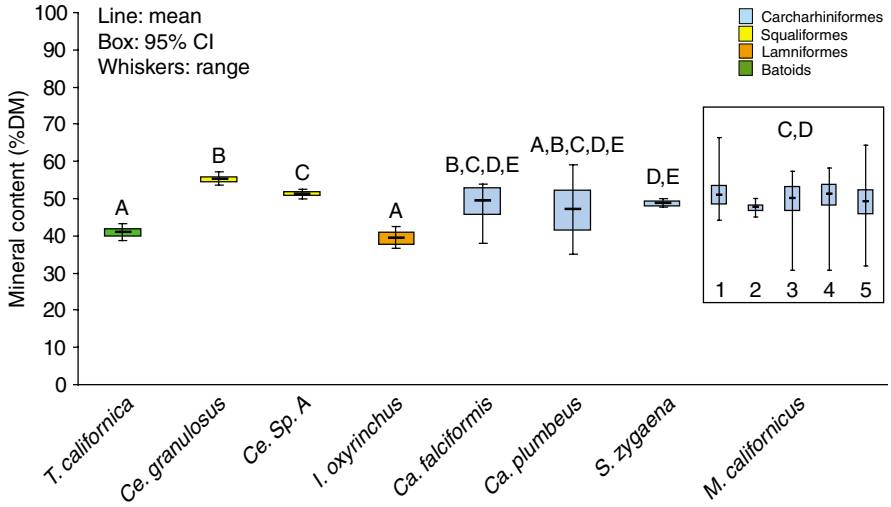

Fig. 2. Mineral content as % dry mass (DM) in eight species of elasmobranch, including one axially undulating batoid, the electric ray. There are significant differences among species ($F_{7,159}$=15.061; P<0.001). We tested material properties of 20 vertebrae each from five gray smooth-hounds *M. californicus* (boxed). Letters above the box and whisker plot denote significant differences and species are color-coded by order. N=10, except for *M. californicus* (N=100).

Material properties

Strength increased significantly with mineral content in vertebrae that had never been treated with EDTA, whether compared across several species (R^2=0.58; P<0.001) or looking exclusively at *M. californicus* (R^2=0.112; P<0.001) (Fig. 4A). Stiffness increased significantly with mineral content only across species where both mineral and morphology were varying (R^2=0.604; P<0.001) (Fig. 4B) (Porter et al., 2006).

Our two-way ANOVA model, using mineral content and strain rate from vertebrae demineralized with EDTA as effects, was significant for the material properties of stiffness ($F_{4,197}$=25.8546; P<0.001) and strength ($F_{4,197}$=105.2814; P<0.001). Mineral content has a significant effect on both stiffness and strength (P<0.001) while strain rate was only a significant effect in the strength model (P<0.001).

Strength and stiffness increase significantly as mineral content in the vertebral cartilage increases (R^2=0.64; P<0.001 and R^2=0.36; P<0.001, respectively) (Fig. 5A,B). At biologically relevant mineral contents (approximately 50% mineral), stiffness values vary greatly.

Viscoelastic effect

The effects of strain rate were variable: strain rate does not affect the failure strain of mineralized shark vertebrae (P=0.20) (Fig. 6A). However, yield strength significantly increases with increasing strain rate ($F_{3,94}$=4.729; P<0.01) (Fig. 6B), and vertebrae tested at 1% s^{-1} yielded at lower stresses than those vertebrae tested at 10% s^{-1} and 20% s^{-1}.

Strength is a strain rate dependent material property in mineralized vertebral cartilage but not in demineralized cartilage (Table 2). Fully mineralized vertebrae are stronger at every strain rate than vertebrae with less than 15% mineral content (Fig. 7). Strength of fully mineralized vertebrae increases significantly as strain rate increases ($F_{3,96}$=4.978; P<0.001). Mineralized vertebrae (greater than 45% mineral content) tested at 10% and 20% of their length s^{-1} were stronger than those tested at 1% s^{-1}. Vertebrae with less than 15% mineral in their structure were not strain rate dependent in compression (P=0.142).

Stiffness is a strain rate dependent material property in demineralized cartilage but not in mineralized cartilage (Table 2). Fully mineralized vertebrae are also significantly stiffer than demineralized vertebrae (Fig. 8). Increasing strain rate during compressive testing did not influence stiffness of mineralized *Mustelus* vertebrae (P=0.818). Stiffness in vertebrae with less than 15% mineral increased as the strain rate increased ($F_{3,30}$=10.693; P<0.001).

Discussion

Mineral content in elasmobranch vertebral cartilage varies within individuals, intraspecifically and interspecifically; while in bone, variation is largely interspecific (Currey, 1999; Currey, 2002). Material properties of vertebrae from the gray smooth-hound *M. californicus* are strongly influenced by the mineral content. We can now compare the effect of mineral content to that of structure and determine how it influences material properties. We tested the viscoelastic response of mineralized elasmobranch cartilage and found that material properties were not influenced by the biologically relevant strain rates tested in this study. Our results suggest that, at these strain rates, elasmobranch vertebral cartilage is acting as an elastic solid, validating interpretations from quasistatic testing.

Mineral variation

The amount of mineralization in elasmobranch vertebral cartilage shows variation at three levels of organization: within individual, intraspecific and interspecific (Fig. 2; Table 1). At the low end, within individual variation is just 5% (Individual 2, 50–45%), while at the high end, the 20 vertebrae from individual 5 varied over 32% (64–32%), nearly the entire range for the species (35%) (Fig. 2). The gray smooth-hound is exceptionally variable in its mineralization; not only did the range of mineralization exceed that of the other seven elasmobranch species previously studied, but it also exceeded the range of all seven combined (24%) (Porter et al., 2006). This is in contrast to the pattern in mammals where variation is nearly exclusively at the interspecific level (Currey, 2002). For example, mean mineral content from 25 bovine femurs was 66.7% and the standard error was only 0.17 (Currey, 1979). Interspecific variation in mineral content is similar in mammals

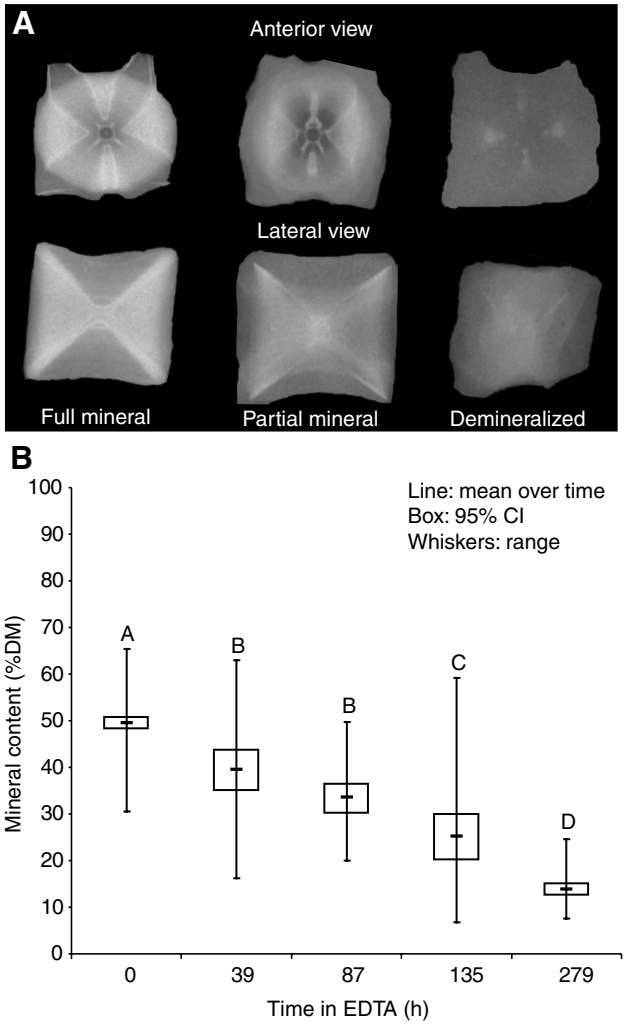

Fig. 3. Vertebral mineral content during serial demineralization. (A) Vertebrae from anterior and lateral views. Fully mineralized vertebra show the morphology described in Fig. 2. A partially demineralized vertebra contains approximately 25% mineral content by dry mass (DM). A demineralized vertebra has mineral arranged in disjointed fragments and it consists of approximately 10% mineral by dry mass in the cartilage. (B) Mineral content (%) decreases significantly with prolonged immersion in EDTA ($F_{4,118}$=108.94; P<0.001). After 279 h in EDTA, vertebrae had approximately 72% of their original mineral content removed. Data is shown in box and whisker plots and letters above the boxes denote significant differences. N=20, except for 0 h (N=100).

(37%) and elasmobranch vertebrae (35%). An exemplar low mineral content value from mammalian tissues is that of reindeer antler (59%), and bone can be as mineralized as Blaineville's beaked whale rostrum (96%) (Currey, 1979; Rogers and Zioupos, 1999).

The high intraspecific variation we see in *Mustelus* may be caused by one or more than one characteristic of elasmobranch vertebral cartilage. Smooth-hounds are relatively short lived sharks (~10 years) compared to the other species we have studied (~20–60 years) (Compagno, 1984). As sharks age, their mineral content may asymptotically increase towards a maximum dictated by the biochemistry of the cartilaginous matrix (Dingerkus et al., 1991). As an intriguing aside, the single female we tested had stiffer vertebrae than the male sharks, even though all five animals were approximately the same size (Table 1). This could be related to hormonal changes that may be contributing to sexual dimorphisms noted in elasmobranchs, or physiological differences associated with reproductive cycle (Kajiura et al., 2005). Furthermore, *M. californicus* is a rapidly growing shark; females have been found to reach maturity after 2–3 years while males are mature after 1–2 years. Rapid and differential growth rates between sexes may potentially influence mineral content in the cartilaginous axial skeleton (Yudin and Cailliet, 1990).

The physiological and mechanical factors mediating mineralization in elasmobranch cartilages are largely unexplored, though we do know that it is a 'deposition only' system (Dean and Summers, 2006). Hypermineralization in the form of trabeculae does not appear to develop as a direct response to stresses imposed on the skeleton, though trabeculae appear in regions where they will experience high stresses (Summers, 2000). Within a species the mineralization patterns are incredibly conserved, but they do vary ontogenetically (Ridewood, 1921). We controlled for this uncertainty by testing mature animals of similar total lengths (Table 1).

Structure, the microscopically visualized mineral distribution in a cartilaginous matrix within shark vertebrae is consistent within a species, so the variation we found in the material properties of the fully mineralized smooth-hound vertebrae is largely due to the amount of material (Fig. 2) (Ridewood, 1921). In previous work on seven shark species, vertebrae varied in both qualities – structure and amount of material. By integrating these data sets we could begin to tease apart the effect of shape independently of the effect of material amount. We compared the effect of natural mineral content variation in a single species to the confounded influence of mineral arrangement and amount across the seven species tested earlier (Porter et al., 2006) in addition to *M. californicus* tested here. For example, in *M. californicus*, varying mineral content by 10% does not increase the stiffness as we might predict based on bone models (Currey, 2002). However, when we tested multiple structures from eight species, increasing mineral content by 10% increased stiffness by 110 MPa (Fig. 4) (Porter et al., 2006). Likewise, increasing mineral content by 10%, also increases strength by 44% when varying structure and mineral content (multiple species) but only 32% for just mineral content (within smooth-hound). Mineral arrangement has a greater ability to influence material properties than the amount. Our interpretation, that structure matters more than mineral content, is consistent with the data from mammalian bone, where a 7.4% difference in mineral content between antler and bovine femur (small change of mineral amount for wildly different mineral arrangement) yields a 27% increase in bending strength and a 45% increase in stiffness (Currey, 1979). In other words, structure (arrangement of mineral) trumps material (amount of mineral) in determining response to load.

This is a *caveat* to the interpretation of the EDTA results. As EDTA chelates the mineral there is the possibility that differences in diffusion distance will lead to changes in the hard tissue morphology. Though our radiographs do not appear to

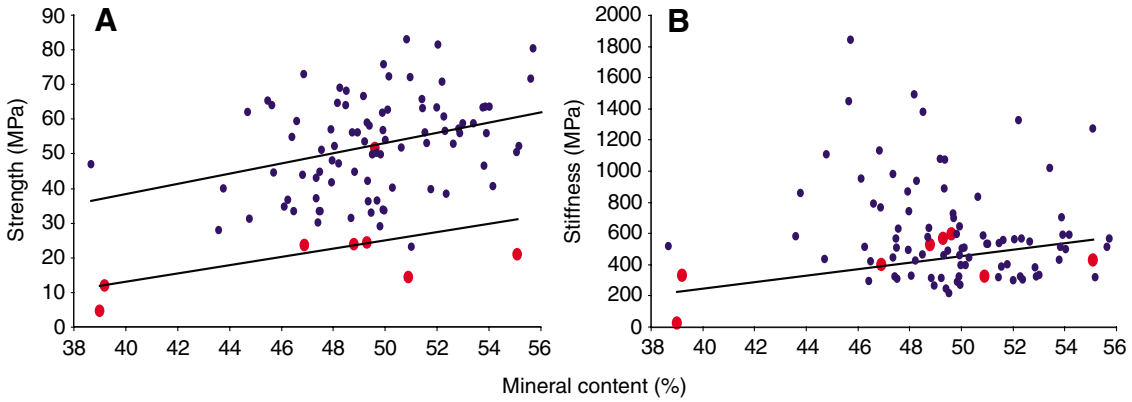

Fig. 4. Arrangement of mineral (structure) in a cartilaginous matrix contributes more to the material properties of elasmobranch vertebral cartilage than the amount of mineral. (A) Strength increases with both mineral amount (blue; R^2=0.112; P<0.001) in *M. californicus* vertebrae and mineral arrangement (red; R^2=0.580; P<0.001) in eight elasmobranch species. Increasing mineral from 40% to 50% will increase strength over a range of morphologies 44% (red) but only 32% over the range of mineral amount (blue). (B) Stiffness only increases with respect to mineral arrangement (red) within the vertebral cartilage (R^2=0.604; P<0.001). The natural variation (blue) of mineral contents found in *M. californicus* vertebrae are presented this regression. Mineral morphology (red) is shown as mean mineral content and strength or stiffness for *M. californicus* and for each of seven species previously examined (Porter et al., 2006). Regression statistics were calculated using all data points from each species rather than the mean value shown in the figure.

show substantial changes in mineral arrangement until the content drops below 20%, even small changes could have an effect on properties. This is made clear in observations of changes in material properties with mineralization pattern in bony fish vertebrae (Kranenbarg et al., 2005a; Kranenbarg et al., 2005b). An effective way to rule out this possible confounding effect would be to make micro computed toniography (micro-CT) scans of each vertebra before testing to memorialize the exact hard tissue arrangement (Kranenbarg et al., 2005b; Summers et al., 2004). We remain confident of the EDTA results in light of the similar relationship between material properties and mineral content seen when fully mineralized vertebrae of *M. californicus* are compared with each other.

The relationships we propose here are in accordance with expectations from other mineralized hard tissues (Currey, 1999). Stiffness and strength increase with mineral content and they will show a nearly linear relationship to each other. There are of course exceptions, suggesting that there may be a premium mineral content for some skeletal tissues. High stiffness does not always mean high strength, especially in tissues with extremely high mineral contents, because they become brittle. A fin whale tympanic bulla with 86% mineral has an extremely high stiffness (31.3 GPa) and low strength (33 MPa) compared to a bovine femur having 67% mineral with lower stiffness (13.5 GPa) but higher strength (247 MPa) (Currey, 1999).

Table 2. *Strain rate dependence of mineralized and demineralized elasmobranch vertebral cartilage*

	Mineralized vertebrae	Demineralized vertebrae
Mineral content (%DM)	>45%	<15%
Stiffness	–	SRD
Ultimate strength	SRD	–

SRD, strain rate dependent.

Viscoelastic response

Determining the response of viscoelastic materials to load is complicated because they show a time dependent response absent in elastic materials. When a quasistatic test, appropriate for elastic materials, is performed on a viscoelastic material it reveals information valid only for the selected strain rate. This is a drawback, but there is a real advantage to quasistatic testing: the results are easily interpreted and the testing equipment and analysis are relatively simple. Though virtually every biological material is viscoelastic to some extent, many of them, including bone, function as nearly purely elastic materials at biologically relevant strain rates. Typically, unmineralized mammalian cartilage does not act as an elastic solid and there is an extensive literature on dynamic testing of cartilage. However, we did not find substantial strain rate dependency in the material properties of mineralized shark vertebral cartilage at biologically relevant strain rates, validating the interpretations from quasistatic testing for this material.

Yield strain, which is strain rate dependent in bone (human and bovine models), is not strain rate dependent in fully mineralized vertebrae (Fig. 6A) (Carter and Caler, 1985; Currey, 1988). Increased mineral adds complexity to the mineralized structure in vertebrae; the presence and subsequent failure of a mineralized structure within a cartilaginous matrix could account for the presence of a yield point, and also why ultimate strength is not strain rate dependent in the absence of mineral.

The relatively small strain rate dependence of ultimate strength agrees with findings in human and bovine bone (Fig. 7; Table 2) (Carter and Caler, 1985; Carter and Hayes, 1976). We can best describe this using the ‘Cumulative Damage’ model, which describes the time dependent characteristics of human

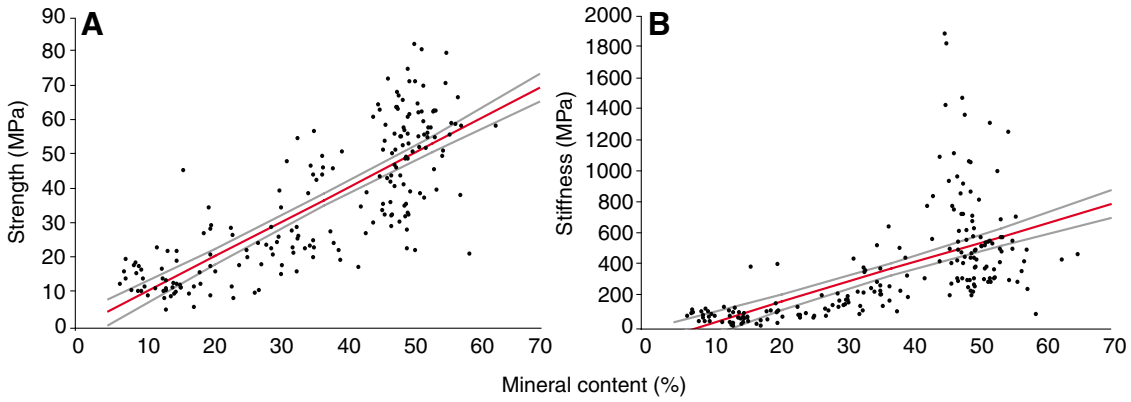

Fig. 5. Linear regressions of mineral content on material properties in vertebral cartilage from *M. californicus*. (A) Strength (MPa) increases significantly as mineral content increases (R^2=0.64; P<0.001). (B) Stiffness (MPa) increases significantly with increased mineral content (R^2=0.36; P<0.001). The red line is the regression line and the gray lines bounding it are the 95% CI. These regressions include data from control vertebrae and those that were demineralized in EDTA.

bone (Carter and Caler, 1985). This model suggests that when bone is loaded to a stress that might not normally break it, and is then held at this stress, damage is accumulated in the form of cracks and will eventually fracture the bone. When vertebrae are tested at a low strain rate the mineral has time to accumulate damage, explaining the strength differences we see between faster and slower strain rates. We point out that ultimate strength differences in mineralized vertebrae are likely not biologically relevant in light of the extensive overlap of values obtained at each strain rate.

The stiffness of mineralized and demineralized vertebrae have strain rate dependencies that are similar to bone and cartilage, respectively (Fig. 8; Table 2). Stiffness in demineralized vertebrae was rate dependent but did not vary in mineralized vertebrae (Fig. 8). Stiffness in reindeer antler and bovine bone is also not strain rate dependent (Currey, 1988; Currey, 1989), but mammalian cartilage, empirically and theoretically is highly strain rate dependent (Li et al., 2003; Li and Herzog, 2004).

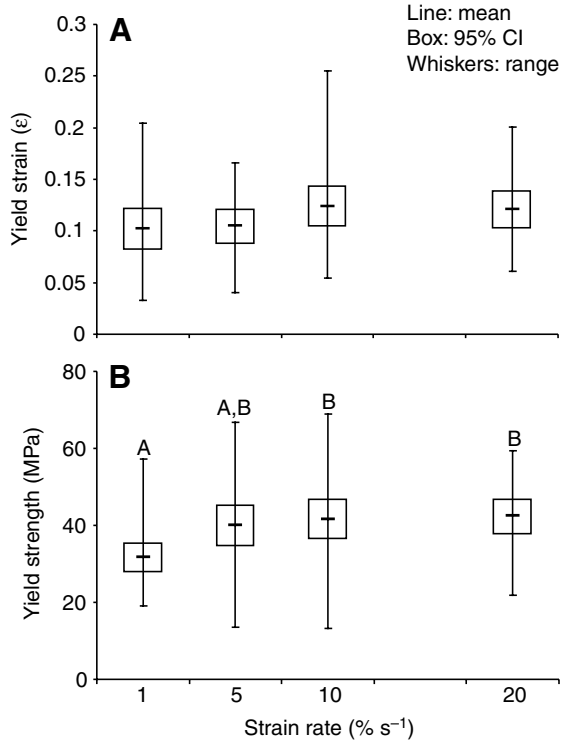

Fig. 6. Failure strain and yield strength of mineralized *M. californicus* vertebrae. (A) Failure strain (%) did not vary with strain rate (P=0.20). (B) Yield strength (MPa) in mineralized vertebrae varied significantly among the strain rates tested here ($F_{3,94}$=4.729; P<0.01). Yield strength of vertebrae tested at strain rates of 1% s^{-1} was significantly lower than vertebrae tested at higher strain rates (10% s^{-1} and 20% s^{-1}).

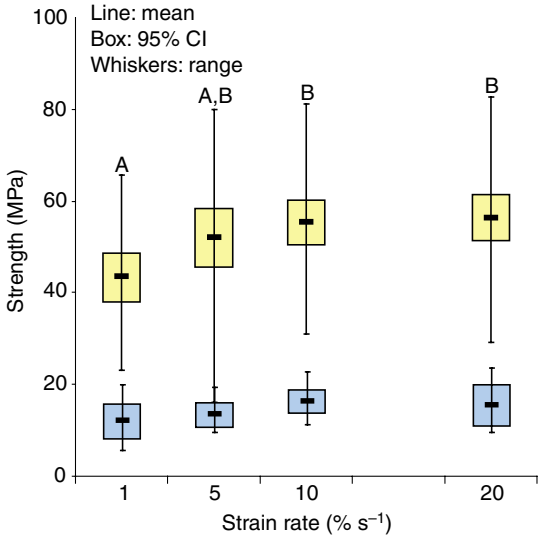

Fig. 7. Strength (MPa) of mineralized vertebrae (yellow) (approximately 50% mineral by dry mass) and demineralized vertebrae (blue) (<15% mineral by dry mass) at various strain rates (% s^{-1}). We found mineralized vertebrae were significantly stronger than demineralized vertebrae at all strain rates (P<0.001). Strength of mineralized vertebrae increases significantly with increasing strain rate ($F_{3,96}$=4.978; P<0.01). Strength does not differ with strain rate in the demineralized vertebrae (P=0.142). Letters above the box and whisker plot denote significant differences.

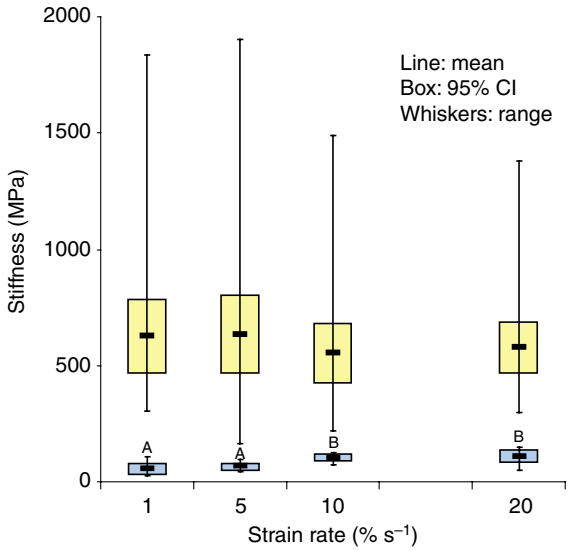

Fig. 8. Stiffness (MPa) of mineralized (yellow) (approximately 50% mineral by dry mass) and demineralized vertebrae (blue) (<15% mineral by dry mass) at various strain rates (% s^{-1}). Mineralized vertebrae are stiffer than demineralized vertebrae (P<0.001). Stiffness does not vary significantly with strain rate in mineralized vertebrae (P=0.818). However, demineralized vertebrae had significantly lower stiffness values at 1% and 5% strain than they did at 10% and 20% strain ($F_{3,30}$=10.693; P<0.001). Letters above the box and whisker plot denote significant differences.

Understanding vertebral response to load is important for understanding vertebral column function and the effect of structure on swimming mechanics. As a shark undulates through the water, one side of the vertebral column will be loaded in compression and the other will be loaded in tension. Cyclical loading occurs during swimming and will vary between animals employing different swimming styles. Vertebral column loading in anguilliform swimmers will be very different than in thunniform swimmers, suggesting that swimming speed can also influence vertebral column loading. Additionally, thunniform swimming sharks have musculotendinous systems that transmit forces farther along the body, placing the vertebral column in compression, while in slower swimming sharks the vertebral column will be loaded in tension and compression on opposite sides of the animal simultaneously (Donely et al., 2004; Gemballa et al., 2006; Shadwick and Gemballa, 2006). As in bone, we have shown the mineral found in shark vertebral cartilage is an important predictor of material properties (Figs 7 and 8) (Currey, 2002).

Conclusions

We examined mineral variation, the effect of mineral content on material properties, and viscoelastic responses of cartilaginous vertebrae from one shark species, *M. californicus*. We found mineralization varies within individuals, within this species and among species. The amount of mineral has large effects on the material properties, but this effect is overshadowed by the even larger influence of structure, or organization of the mineral, on the material properties of elasmobranch vertebrae. Many of the material properties examined here were not strain rate dependent at biologically relevant strain rates; validating the interpretations from quasistatic tests on this tissue. The importance of mineral in bony skeletons has long been discussed in the literature and the effects of varying mineral on material properties are well known, especially in mammalian skeletons. Only recently have we begun to understand the mechanics of cartilaginous skeletons, presenting many opportunities to examine the effects of sex, age, structure and ecological niche.

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