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Material properties and biochemical composition of mineralized vertebral cartilage in seven elasmobranch species (Chondrichthyes)

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Summary

Elasmobranchs, particularly sharks, function at speed and size extremes, exerting large forces on their cartilaginous skeletons while swimming. This casts doubt on the generalization that cartilaginous skeletons are mechanically inferior to bony skeletons, a proposition that has never been experimentally verified. We tested mineralized vertebral centra from seven species of elasmobranch fishes: six sharks and one axially undulating electric ray. Species were chosen to represent a variety of morphologies, inferred swimming speeds and ecological niches. We found vertebral cartilage to be as stiff and strong as mammalian trabecular bone. Inferred swimming speed was a good, but not infallible, predictor of stiffness and strength. Collagen content was also a good predictor of material stiffness and strength, although proteoglycan was not. The mineral fraction in vertebral cartilage was similar to that in mammalian trabecular bone and was a significant predictor of material properties.

Key words: mineralized cartilage, stiffness, ultimate strength, collagen, proteoglycan.

Introduction

No less an authority than Steven Vogel asks us to imagine "*… how a shark manages with such a flimsy skeleton?*" (Vogel, 1988). This quote is emblematic of the perception that the skeleton of cartilaginous fishes is mechanically inferior to that of bony fishes. A strong argument against this common wisdom is that cartilaginous fishes perform at functional extremes. For example, whale sharks are the biggest fish in the sea (Gudger, 1941). They have skeletons many times larger than those of the largest bony fish. Also, shark skeletons endure tens to hundreds of millions of loading cycles in their transoceanic migrations (Bonfil et al., 2005; Boustany et al., 2002; Carey and Scharold, 1990). Furthermore, we suppose that in some sharks, the skeleton must resist the high loads that occur during burst swimming.

The vertebral column of a shark is subjected to a variety of loading regimes depending on swimming speed and mode. During anguilliform swimming, the left and right sides of individual vertebrae are alternately loaded in tension and compression and the rate and frequency of loading vary with swimming speed (Lindsey, 1978; Sfakiotakis et al., 1999). However, faster subcarangiform and thunniform swimmers exhibit less local lateral displacement (Gemballa et al., 2006). The rising intramuscular pressure imposes a different load by compressing the entire vertebral column in the cranial to caudal direction (Martinez et al., 2002; Vogel, 1988; Wainwright et al., 1978). Vertebral deformation under these loads must be very low to efficiently transfer these muscularly applied loads.

There is a well known relationship between loading regime and the properties of biological materials (Wainwright et al., 1976). For example, a weight bearing horse femur is more than three times as stiff as a mature red deer antler, though the latter is far tougher than the former (Currey, 1999). Although swimming is a 'low impact' mode of locomotion, it has been shown to increase bone density in humans (Falk et al., 2003). The loads imposed by long duration, fast swimming suggest that vertebrae of faster swimming sharks might be stiffer and stronger than slower swimmers.

The amount and arrangement of mineral are important determinants of material properties. Currey, examining a variety of bones, found that increasing bone mineral content by just 10% can nearly double the material stiffness (Currey, 1999). For example, bony fish vertebrae exposed to higher loads have more mineral and the mineral is arranged so that second moment of area is higher than in normal vertebrae (Kranenbarg et al., 2005a; Kranenbarg et al., 2005b). The mineral content and arrangement of elasmobranch vertebrae vary among species, and it has been suggested that additional calcification may develop in a response similar to bone modeling (Ridewood, 1921; Urist, 1962).

Differences in vertebral mineralization in elasmobranchs should have implications for the spine's ability to resist

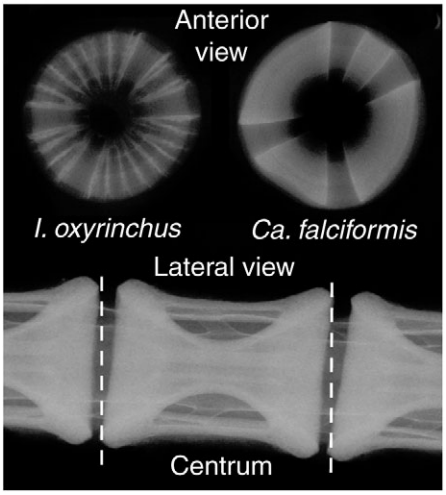

Fig. 1. Radiograph of an anterior view of a mako shark (*I. oxyrinchus*) and silky shark (*C. falciformis*) vertebral centra with excised neural and hemal arches. Shark vertebral cartilages vary in extent and pattern of mineralization (Ridewood, 1921). *I. oxyrinchus* has many plates of mineralization radiating from the centrum whereas *Ca. falciformis* has essentially solid mineral around the perimeter of the vertebra. The lateral view of gulper shark vertebrae (*Ce. granulosus*) illustrates the mineralized double cone configuration.

deformation from the loads imposed by swimming. Vertebral centra consist of a double cone of mineral with elaborations varying by taxa (Ridewood, 1921) (Fig. 1). For example, shortfin mako (*Isurus oxyrinchus*) vertebrae have flattened plates of mineral around the perimeter of the double cone, whereas the silky shark (*Carcharhinus falciformis*) has a continuous crust of thick mineral (Fig. 1). Cartilage mineralization in elasmobranch vertebral centra is 'areolar'; calcium phosphate hydroxyapatite is found in web-like patterns of varying density throughout the centra (Moss, 1977). By contrast, the rest of the skeleton is 'tesselated' consisting of tiny blocks of mineralized tesserae, sometimes occurring in multiple layers, on the surface of a hyaline cartilage skeletal element (Dean and Summers, 2006).

Elasmobranch vertebral cartilages have both an unmineralized and mineralized phase. The unmineralized phase is a gel consisting of water and proteoglycan in a matrix of collagen fibers; the second and third components are important contributors to material properties in mammals. Collagen is an important contributor to strength in cartilage (Currey, 2002). Elasmobranch cartilage contains one third type I collagen and the remaining portion is type II (Rama and Chandrakasan, 1984). Proteoglycans are the major noncollagenous organic component in elasmobranch cartilage (Michelacci and Horton, 1989). They add compressive strength to mammalian cartilage by increasing the swelling pressure through their hydrophilic interactions with water (Koob, 1989; Koob and Vogel, 1987). Furthermore, the presence of proteoglycans has been shown to inhibit calcification in elasmobranch cartilage (Gelsleichter et al., 1995; Takagi et al., 1984).

The present study provides the first description of the material properties and biochemical components of mineralized elasmobranch cartilage from a diversity of species that exhibit a wide variety of swimming speeds and lifestyles. The goals of this study were: (1) Measure the material stiffness (Young's modulus), ultimate strength and yield strain of vertebral centra in compression; (2) quantify the water, collagen, proteoglycan (PG) and mineral content of the mineralized cartilage; and (3) determine if the biochemical composition of the cartilage significantly contributes to it's material properties. We hypothesized, firstly, that the fastest swimming elasmobranchs will have the greatest compressive strength and material stiffness. Second, elasmobranch cartilage will have similar water, collagen and PG contents to mammalian cartilage. Finally, the biochemical components of elasmobranch cartilage will have significant positive relationships with material properties.

Materials and methods

Experimental animals

Species were sampled from the two major lineages of sharks, Galea and Squalea, as well as the Batoidea, the dorsoventrally flattened elasmobranchs (Fig. 2). Species were sampled from four orders and five families: Carcharhiniformes (Carcharhinidae and Sphyrnidae), Lamniformes (Lamnidae), Squaliformes (Centrophoridae) and Torpediniformes (Torpedinidae). We collected data from seven species of cartilaginous fishes: shortfin mako (*Isurus oxyrinchus* Rafinesque 1810), smooth hammerhead [*Sphyrna zygaena* (Linnaeus 1758)], silky shark [*Carcharhinus falciformis* (Müller and Henle 1839)], sandbar shark

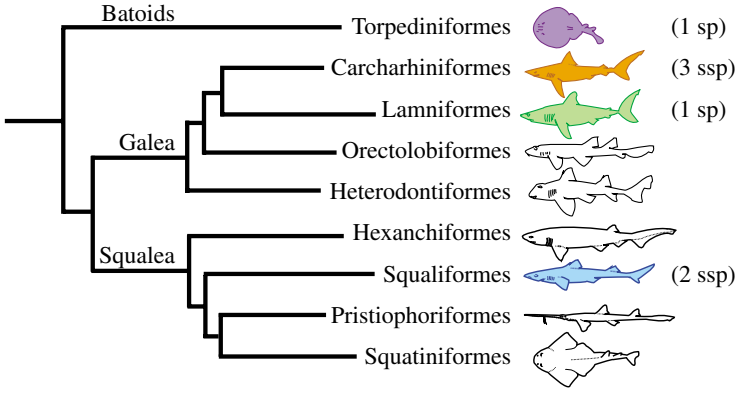

Fig. 2. Phylogeny of a diverse group of seven species varying in their ecology and inferred swimming speeds [phylogeny is adapted from Maisey et al. (Maisey et al., 2004)]. The ordinal color scheme is maintained in this paper. We sampled from the batoids and both lineages of sharks. The number to the right of the icon is the number of species we used from each order.

Table 1. *Summary of material properties and biochemical constituents of the mineralized vertebral centra of seven elasmobranch species*

		Water		Mineral		Proteoglycan		Collagen		Ultimate strength		Stiffness		Yield strain	
Species	Individuals	*N*	% WM	*N*	% DM	*N*	% DM	*N*	% DM	*N*	MPa	*N*	MPa	*N*	ϵ
I. oxyrinchus	4	33	43.7	10	39.2	27	28.4	29	20.9	32	11.9	32	329.4	28	0.12
S. zygaena	1	20	42.5	10	48.8	16	25.9	14	26.8	20	23.8	20	523.4	20	0.13
Ca. falciformis	3	20	52.9	10	49.3	14	22.7	15	25.8	19	24.3	19	563.9	19	0.10
Ca. plumbeus	1	19	45.8	10	46.9	5	24.2	5	24.5	19	23.7	19	396.9	19	0.15
Ce. granulosus	1	19	47.2	10	55.1	9	12.0	9	22.8	19	20.8	19	425.8	19	0.10
Ce. sp. A	1	20	43.0	10	50.9	10	19.2	10	20.7	20	14.7	20	323.0	20	0.06
T. californica	2	29	26.4	10	39.0	8	19.6	10	17.4	29	4.6	29	25.5	29	0.22

Values shown here are the mean for each species and the sample size for each assay.
DM, dry mass; WM, wet mass.

[*Carcharhinus plumbeus* (Nardo 1827)], gulper shark [*Centrophorus granulosus* (Bloch and Schneider 1801)], and an axially undulating torpedo ray (*Torpedo californica* Ayres 1855). A second gulper shark (*Centrophorus* sp.) from an undescribed species was also used and is referred to as *Ce. sp. A* (Table 1).

The requiem sharks (Carcharhiniformes) are fast swimming, maneuverable sharks found both in and off shore (Compagno, 2003). The two members of the Carcharhinidae we sampled are a near shore (*Ca. plumbeus*) and a pelagic species (*Ca. falciformis*). Hammerheads (Sphyrnidae) are very flexible, fast swimming sharks, and are found near shore and well off shore to depths of 200 m (Kajiura et al., 2003).

The shortfin mako (*I. oxyrinchus* Lamniformes: Lamnidae) is a regionally endothermic, high-speed predator of marlin, tuna and other pelagic bony fishes (Block and Carey, 1985; Wolf et al., 1988). It is believed to be the fastest swimming shark (Carey and Teal, 1969). Makos inhabit the pelagic zone of the world's oceans to 150 m.

Gulper sharks (Squaliformes: Centrophoridae: *Centrophorus* sp.) are relatively sluggish, bottom dwelling sharks capable of short bursts of high-speed swimming (Compagno, 1984). They are found in bathydemersal marine habitats below 200 m in depth.

Finally, the Pacific electric ray (Torpediniformes: Torpedinidae) is a slower swimming, demersal, marine species off the Californian coast. It is capable of delivering a dangerous electric shock, and it is remarkable among rays and skates for using its caudal fin for axial undulation.

Material properties

We removed vertebrae from the region of the first dorsal fin from fresh and freshly frozen vertebral columns. There is evidence that freezing tissues before testing has no effect on the material properties of the specimen (Panjabi et al., 1985). We excised neural and hemal arches from the centra leaving an unadorned disk of mineralized cartilage (Fig. 1). Centra volume was obtained by water displacement to 0.1 ml. Centra were massed to 0.01 mg and each centrum was digitally photographed with a scale bar and the cross-sectional area (the anterior/caudal surface) was calculated using ImageJ software (NIH).

Centra were kept in elasmobranch Ringers solution (Forster et al., 1972) for no more than 2 h before being subjected to material testing. Using an MTS Mini Bionix with a 50 kg load cell we performed a quasi-static compressive test to failure at a strain rate of 2 mm s^{-1} on at least nineteen (and as many as 33) vertebrae from each species (Table 1). Cartilage stiffness is greater at higher strain rates and we were interested in testing the limits of elasmobranch mineralized cartilage (Li et al., 2003). The resulting load-displacement curves were analyzed with Matlab v 12 (Mathworks Inc.). Stress–strain curves were generated and ultimate strength, material stiffness (Young's modulus) and yield strain were obtained for each vertebra. Ultimate strength is the maximum stress that can be applied to a material before it fails, and material stiffness is the ratio of stress to strain in the elastic region of the stress–strain curve. Yield strain (ϵ) is the percentage length change of the material at which irrevocable shape change occurs (Vogel, 2003). We calculated stress using the cross-sectional area of the vertebra, this intentionally ignores the structural heterogeneity of the vertebrae. We do not have sufficient information on the properties of the mineralized and unmineralized phases nor on their distribution to avoid this simplification. Data from each vertebra were analyzed three times to ensure accurate estimation of material properties.

An important *caveat* regarding our measure of stress is that we assumed the vertebrae were a homogeneous material; the entire cross section of the vertebrae bore the compressive force. This is plainly not the case as there are distinct inhomogeneities in mineral distribution among species (Fig. 1). However, the interaction between the mineralized and unmineralized portions of the vertebrae is probably quite complex and not amenable to simple modeling as a layered composite. In our results it is very probable that the stress in the mineralized parts of the vertebrae is higher than our reported values whereas in the unmineralized regions we have overestimated this parameter. This work is intended to set the stage for further examination of the role of the mineralization of the vertebrae in responding to load.

Compositional analysis

After material testing, centra were lyophilized for 24 h and massed again to obtain dry mass (organic material + mineral content). Water content was calculated by subtracting dry mass from wet mass and dividing by wet mass. A sub-sample of 10 lyophilized vertebrae from each species was placed in a 450°C furnace for 24 h to remove the organic portion of the dry mass. After 24 h the vertebrae were removed from the furnace and the ash-free dry mass was recorded. Mineral content was calculated by dividing the ash-free dry mass by the dry mass.

Collagen content was determined using a hydroxyproline assay (Bergman and Loxley, 1963). Dried vertebrae were homogenized in a Thomas Scientific tissue mill with 0.20 mm mesh size. Once homogenized, approximately 50 mg (to the nearest mg) of each sample was acid hydrolyzed in 1500 μl of 6 mol l^{-1} hydrochloric acid at 100°C for 18 h. Samples were dried under vacuum to remove the HCl and were resuspended in 1500 μl distilled water. Samples (10 μl) were diluted 1:100 with distilled water and 200 μl of isopropanol were added to each sample. Then 100 μl of 7% choloramine-T were added before incubation for 4 min at room temperature. After adding 1.25 ml of Ehrlich's reagent and incubating at 60°C for 25 min, 300 μl were plated and the absorbance at 558 nm was measured with a Bio-Tek μQuant™ spectrophotometer. Samples were measured against a standard 400 p.p.m. hydroxyproline solution of (trans-4-hydroxy-L-proline; Arcos Organics, NJ, USA) which was diluted to generate a standard curve. Collagen concentration was calculated assuming 10% hydroxyproline (Miller, 1971). Each centra had three to five replicate samples for the hydroxyproline assay which were averaged for analysis.

A second 50 mg sub-sample of each vertebra was used to determine the PG content (Farndale et al., 1986). Samples were digested with papain extract and buffer at 60°C for 12 h, then heated to 100°C to denature the enzyme. Samples were centrifuged at approximately 13 800 ***g*** for 10 min and the resulting pellet was washed three times with 99% ethanol. The pellet was then resuspended in 400 μl 0.05·mol l^{-1} sodium acetate, pH 7.4 and 5 μl of the resuspended sample was diluted 1:25 with distilled water. 1000 μl of dimethylmethylene blue (DMMB) indicator solution was added to the sample and the absorbance was measured immediately with a Bio-Tek μQuant™ spectrophotometer at 525 nm. Samples were measured against a standard of 0.2 mg ml^{-1} chondroitin sulfate-6 (Seikagaku Coroporation, Tokyo, Japan), which was diluted to generate a standard curve.

Statistical analysis

The scarcity of fresh material of some species (i.e. *Centrophorus* ssp.) made a two-stage analysis of variance necessary. Our first model assessed variation between species with each vertebra taken as an independent sample. A second analysis performed on the subset of species with multiple individuals (*Torpedo*, *Isurus* and *Ca. falciformis*) tested whether there was significant variation among individuals of a species. This second analysis was a nested ANOVA with [vertebral centra] nested within [individual animal] to show that variation in material stiffness and ultimate strength were due to variation between vertebral centra rather than different animals (Sokal and Rohlf, 2001; Zar, 1999). There remains the possibility that we were not able to measure a systematic difference in material properties along the vertebral column. We have insufficient material for such a comparison and doubt it would show significance given the small number of vertebrae sampled relative to the number of precaudal vertebrae in these species.

Data were analyzed in SPSS v12.0 (SPSS Inc. 2003. SPSS 12.0 FPR Windows Student Version; SPSS Inc., Chicago, IL, USA) using ANOVA (P<0.05). Comparisons among species were made using a Games Howell *post hoc* test in the SPSS 12.0 data pack, which tests comparisons and does not assume equal variances. The relationship between material properties and biochemical constituents was tested using linear regression analyses in SPSSv.12.0 (SPSS Inc. 2003). In the box and whisker plots of material and compositional data where the box represents the ±95% confidence intervals, the mean for each species is represented by the bold horizontal line, and the whiskers are the ranges obtained for each species.

Results

Material properties

There were significant interspecific differences in ultimate strength (the stress at which the vertebral centra failed) among species ($F_{6,151}$=182.79; P<0.001) (Fig. 3A and Table 1). *T. californica* was less strong than all the shark species (P<0.001), and of the shark species the mako was the least strong. All the charcharhinid species (*S. zygaena*, *Ca. falciformis* and *Ca. plumbeus*) had similar strengths. There were differences between the squalid species: *Ce. granulosus* is stronger than *Ce. sp. A* (P<0.001).

We found significant differences in material stiffness among the seven elasmobranch species ($F_{6,151}$=54.43, P<0.001) (Fig. 3B and Table 1). *T. californica* was more than an order of magnitude less stiff than all the shark species (P<0.001). *Ce. sp. A* and *I. oxyrinchus* were the sharks with the lowest stiffness, and they were approximately 17 times stiffer than *T. californica*.

Yield strains were significantly different among groups ($F_{6,147}$=27.576; P<0.001) (Fig. 3C and Table 1). Yield strain in *T. californica* vertebral centra was significantly greater than the shark species examined here (P<0.007). *Ce. sp. A* had the lowest yield strain of all the sharks and was nearly four times lower than *T. californica* (P<0.044). Strain was the only material property that showed a significant [individual animal] effect.

Regardless of species the vertebrae failed in a similar manner: the anterior and posterior mineralized cones developed two types of cracks: circumferential cracks that divided the centrum into annuli, and radial cracks running from the center of the cone to the outer edge. The cones that form the central 'spindle' collapsed outwards, flattening and

becoming more oblique. The *I. oxyrinchus* vertebrae showed an additional fracture modality in the plates of calcification between the cones (Fig. 1). Each plate failed in Euler buckling, generally at the midpoint of the plate when viewed laterally. These multiple failures led to a distinctive chevron pattern in the formerly parallel plates.

Compositional analysis

There were significant differences in water content between species ($F_{6,153}$=70.485; P<0.001) (Fig. 4A and Table 1). *T. californica* had less water in the vertebral cartilage than all shark species (P<0.001). *Ce. granulosus* had greater water content than *Ce. sp. A*.

Mineral content varied significantly among species ($F_{6,63}$=27.831; P<0.001) (Fig. 4B and Table 1). All the carcharhinid (*S. zygaena*, *Ca. falciformis* and *Ca. plumbeus*) vertebral cartilage had similar mineral content, as did *T. californica* and *I. oxyrinchus*. *Ce. granulosus* had more mineral than *Ce. sp. A* (P<0.001).

We found significant differences in PG content between species ($F_{6,82}$=10.5310; P<0.001) (Fig. 4C and Table 1). *Ce. granulosus* vertebrae are lower in PG content than the other species (P=0.019). The *I. oxyrinchus* and carcharhinids (except *Ca. falciformis*) had more PG than the squalids (*Ce. granulosus* and *Ce. sp. A*) and *T. californica* (P=0.027). *I. oxyrinchus* had more than twice the PG content of the *Ce. granulosus*.

Collagen content in the vertebral centra varied between species by nearly 10% ($F_{6,85}$=4.054; P=0.001) (Fig. 4D and Table 1). The carcharhinid sharks (*S. zygaena*, *Ca. falciformis* and *Ca. plumbeus*) had significantly greater collagen concentrations (24.5–26.8%) than *T. californica* (17.4%) (P=0.009).

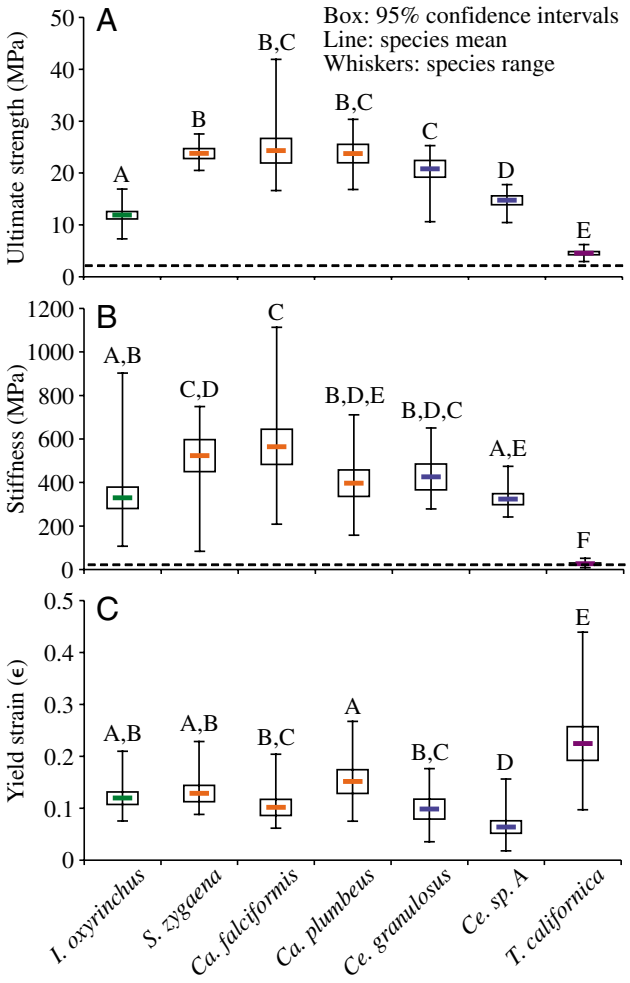

Fig. 3. Material properties of mineralized cartilage in shark vertebral centra. (A) Ultimate strength (MPa) of vertebral cartilages from seven elasmobranch species showing significant differences ($F_{6,151}$=182.8; P<0.001). The broken horizontal line represents the lower limits of trabecular bone (Rho et al., 1994). Letters above the box and whisker plot denote significant differences between species. (B) Material stiffness was significantly different among the species ($F_{6,151}$=54.4; P<0.001). *T. californica* was less stiff than all shark species (P<0.001). The horizontal line shows the lower limits of stiffness for trabecular bone (Hodgskinson and Currey, 1992). (C) Yield strain was significantly different among the species ($F_{6,147}$=27.6; P<0.001). *T. californica* had the greatest yield strain of all species (P<0.007).

Correlating biochemical components and material properties

Biochemical constituents were significant predictors of material properties of elasmobranch vertebrae. Water, mineral and collagen content all significantly increased the ultimate strength of this cartilage (adjusted R^2=0.451, 0.342 and 0.175 respectively; P<0.001) (Fig. 5). PG content, although differing among species had little predictive power. Water, mineral and collagen content were all significant predictors of increasing material stiffness (adjusted R^2=0.446, 0.243 and 0.218, respectively; P<0.001), however, PG content was not (Fig. 6).

Discussion

Material properties of vertebrae from these seven species of cartilaginous fishes are similar to the lower ranges of mammalian trabecular bone, most notably in material stiffness and ultimate strength. The cartilage from these vertebrae differs drastically from mammalian cartilage in their large mineral fraction. Our results suggest that areolar mineralized cartilage is a comparable skeletal building material to mammalian trabecular bone. These results offer a plausible, if incomplete, explanation for the ability of chondrichthyan fishes to perform well at functional extremes with a skeleton made of cartilage.

Material properties

Stiff skeletons will transfer energy more efficiently at all swimming speeds, so high speed fish should have the stiffest skeletons (McHenry et al., 1995). Animals with stiff bodies are best able to resist skeletal deformation from the forces of tendon pulling on them during swimming. Our data supported this hypothesis: the carcharhinids (396–563 MPa) were stiffer than the squalids (322–425 MPa), which were in turn stiffer than *T. californica* (25.5 MPa) (Fig. 3B and Table 1).

However, contrary to our hypothesis, *I. oxyrinchus* vertebral centra fall in the middle range of material stiffness values.

The material stiffness of elasmobranch cartilage falls into the range of mammalian bone which varies from 4 MPa in trabecular bone to 34 100 MPa found in a fin whale ear bone (Currey, 1999) (Fig. 3B). The stiffness of trabecular bone, the primary component of mammalian vertebrae, ranges from 4 to 350 MPa in humans and 35 to 7000 MPa in non-human models (horse and bovine) (Hodgskinson and Currey, 1992). More specifically, trabecular bone from human and ewe lumbar vertebrae range from approximately 600 to 3000 MPa (Mitton et al., 1998; Rho et al., 1994). The variation in elasmobranch vertebral material stiffness is less than a quarter of that found in trabecular bone of mammalian vertebrae.

We further supposed that the vertebrae of swiftly swimming sharks must be strong to withstand the forces exerted on them. Two factors contribute to this increase in force on the vertebral column. First, as an animal swims faster, the forces exerted directly by the muscles increase in amplitude and frequency (Coughlin and Rome, 1996). Second, the internal pressure of a shark increases with swimming speed, compressing the axial skeleton (Martinez et al., 2002; Wainwright et al., 1978). Indeed, we found that ultimate strength of the vertebrae did follow a pattern similar to our findings for stiffness: the carcharhinids (23.7–24.3 MPa) had the strongest centra followed by the squalids (14.7–20.8 MPa), and *T. californica* (4.5 MPa) (Fig. 3 and Table 1). The makos (*I. oxyrinchus*), the fastest swimming sharks, again did not support our hypothesis. It is important to note that vertebral cartilage does appear to fail in nature, so compressive strength is a biologically relevant property (Fig. 7).

Elasmobranch vertebral cartilage is comparable to mammalian bone in ultimate strength, which can range from 25 MPa to 340 MPa, with weight-bearing limbs approaching the high end of this range (Currey, 1999) (Fig. 3A). Cezayirlioglu et al. (Cezayirlioglu et al., 1985) reported ultimate compressive strength values of the human tibia and femur (206 and 213 MPa, respectively) that were ten times higher than average elasmobranch vertebral cartilage (17 MPa). A better comparison is with trabecular bone in human lumbar vertebrae (3.2 MPa) and sheep vertebrae (23.4 MPa) (Mitton et al., 1998; Rho et al., 1994) – and our data for shark vertebrae overlap this range.

Data from the fastest shark, the shortfin mako (*I. oxyrinchus*)

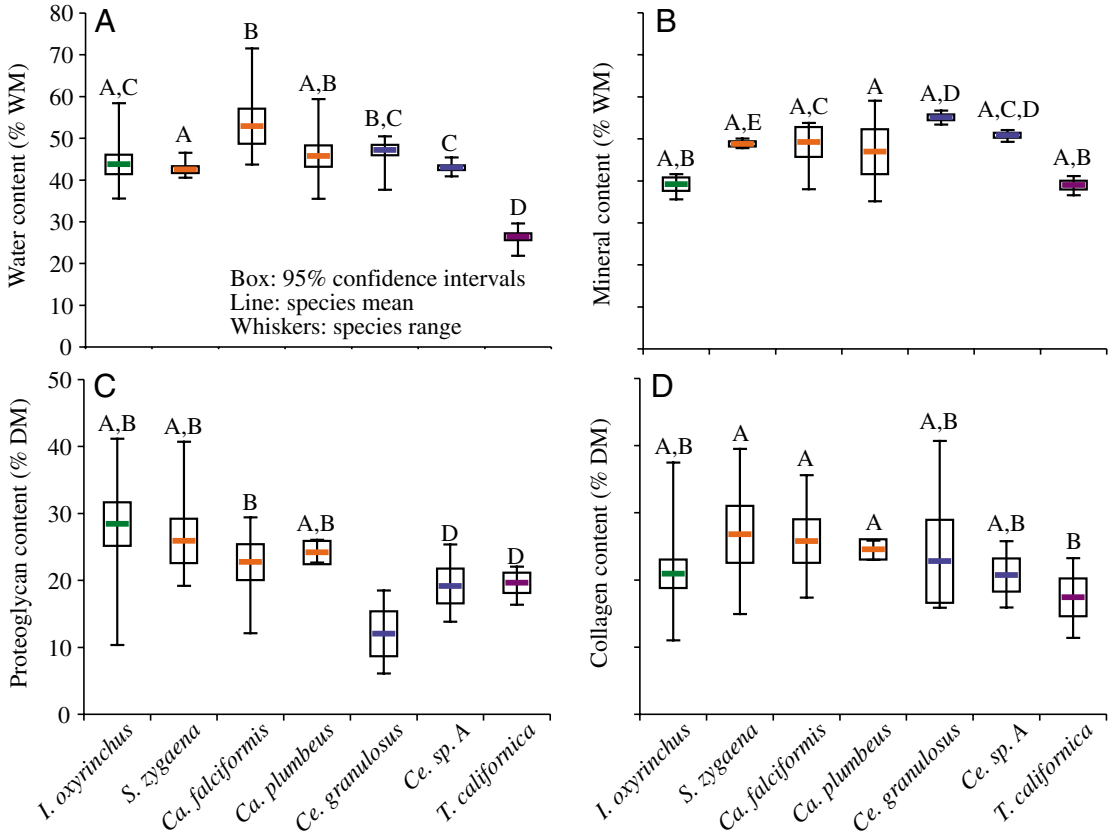

Fig. 4. Biochemical composition of vertebrae from seven species of elasmobranch. Letters above the box and whisker plot denote significant differences between species. (A) Water content (% WM) is significantly different among species ($F_{6,153}$=70.483; P<0.001). (B) Mineral content (% DM) varied significantly among species ($F_{6,63}$=27.836; P<0.001). (C) Proteoglycan (PG) content, expressed as percentage of dry mass (DM), varied among species ($F_{6,82}$=10.531; P<0.001). The highest PG content was 28% found in *I. oxyrinchus* and the lowest was only 12% found in *Ce. granulosus*. (D) There were significant differences in collagen content, expressed as percentage of dry mass, among species ($F_{6,85}$=4.054; P=0.001). Overall, the collagen content of the species examined ranged from 17% (*T. californica*) to 27% (*S. zygaena*).

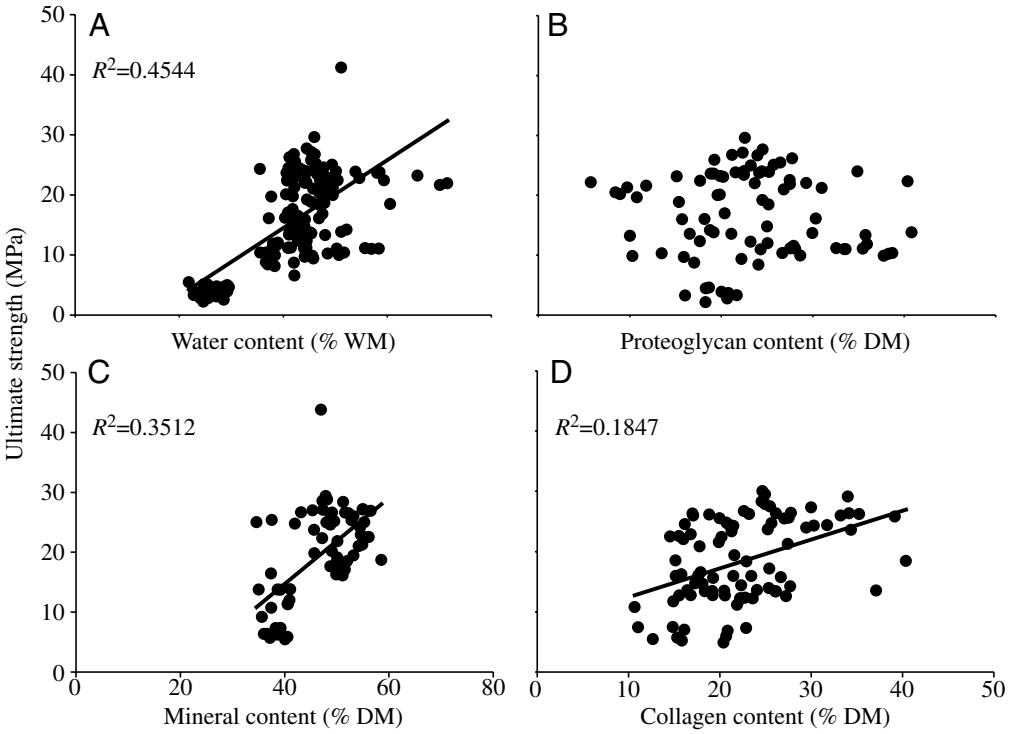

Fig. 5. Linear regressions of biochemical content on ultimate strength from seven species of elasmobranch. Ultimate strength of the shark vertebral cartilage was significantly correlated to water, mineral and collagen content (P<0.001) (A,C,D), but not proteoglycan content (B). Each point represents the material test and subsequent biochemical assay on a single vertebra.

a regionally endothermic, piscivorous fish, did not support our hypotheses. Perhaps, the root of this incongruity is in the recently described novel force transmission mechanism makos use to propel themselves through the water (Donely et al., 2004; Donely and Shadwick, 2003). Like tunas, and in contrast to other sharks, makos have extremely long myoseptal tendons that carry much of the muscular load directly to the tail (Gemballa et al., 2006; Shadwick and Gemballa, 2006). This arrangement allows the body to remain rigid during swimming with only the tail oscillating.

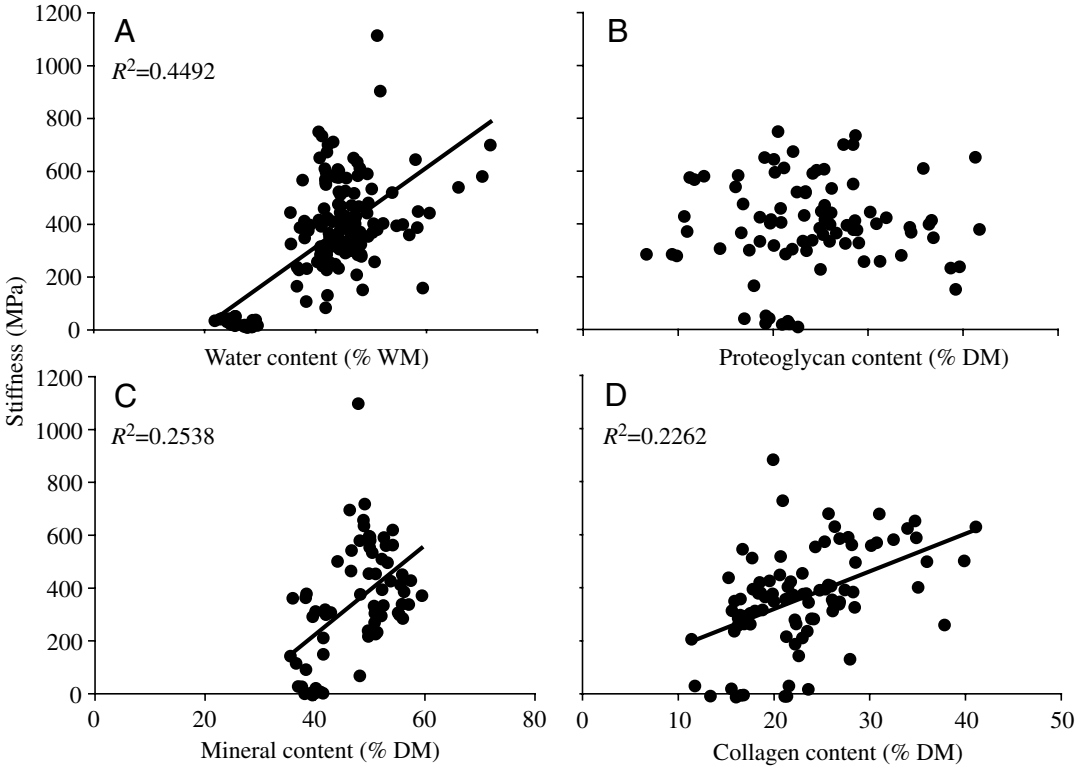

Fig. 6. Linear regressions of biochemical content on material stiffness from seven species of elasmobranch. Proteoglycan content (B) is the only biochemical component that appears to not influence material stiffness. Each point represents a single material test and subsequent biochemical assays on a single vertebra.

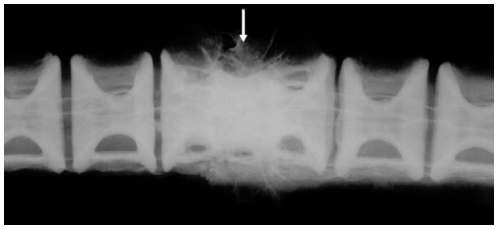

Fig. 7. Radiograph of a portion of a vertebral column from *Ce. granulosus* (Gulper). Two vertebrae appear to be fused together (white arrow) as a possible result of material failure. This may also be the result of some pathology, but we suspect not based on the overall health of the rest of this vertebral column. Spinal deformities in elasmobranchs generally affect much larger portions of the column (Heupel et al., 1999). This may be an example of healing in a cartilaginous skeleton, which is contrary to information in the literature (Ashhurst, 2004).

Biochemical composition

Collagen volume fraction is a good predictor of stiffness in cartilage tissue culture from rabbits, and as collagen content increases so does the tensile stiffness of condylar cartilage from human femurs (Kempson et al., 1973; Simha et al., 1999). We also found collagen to be a predictor of both material stiffness and ultimate strength – a 25% increase in collagen content corresponds to a 53.8% increase in material stiffness and a 38.7% increase in strength (Figs 5 and 6).

The surprisingly large PG component in mineralized elasmobranch vertebrae (Fig. 4C) can be accounted for if it is restricted to the non-mineralized portion of the vertebrae. This is likely because PGs are partially or completely removed during the cartilage calcification process and the presence of PGs seems to inhibit calcification (Gelsleichter et al., 1995; Takagi et al., 1984). In contrast to mammalian cartilage and tendon, which has a similar PG component (15–25% dry mass), we found the material properties of elasmobranch vertebrae are not predicted by PG content (Koob, 1989; Koob and Vogel, 1987; Venn and Maroudas, 1977) (Figs 7 and 8). This suggests that the mineralized portion of the vertebrae dominates the material properties of the vertebrae in compression. Although the mineral is probably important under most testing conditions, we propose that dynamic testing rather than the quasi-static results presented here would reveal a link between PG content and material properties.

The presence of a large mineral fraction is the most striking difference between elasmobranch and mammalian cartilages. Shark vertebral cartilage is more like mammalian bone, the mineral content of which can range from 54% in trabecular bone to 94% in compact bone; whereas mammalian cartilage is essentially void of mineral (Currey, 2002) (Fig. 4B). Mineral content is a great predictor of stiffness and strength in mammalian bone (Currey, 1999; McCalden et al., 1997). For instance, a 20% loss in mineral content corresponded to a 35% decrease in strength, and a 60% mineral loss reduced strength by 75% (Shah et al., 1995). Similarly, in elasmobranch vertebrae, a 20% decrease in mineral content corresponded to a decrease of 55.6% in strength and decreased material stiffness by 59.0%. Again, these results suggest that the mineralized structures dominate the compressive properties of elasmobranch vertebrae.

Summary

We have examined the material properties and biochemistry of the mineralized cartilage found in the vertebrae of seven elasmobranch species. This tissue behaves similarly to mammalian trabecular bone in material stiffness and ultimate strength. Collagen contents are more similar to mammalian bone than to mammalian cartilage, and these vertebrae have mineral fractions equaling that of mammalian bone. That vertebral cartilage has bone-like stiffness and strength makes it unlikely that decreased functional demands were a selective force in the abandonment of a bony skeleton by cartilaginous fishes (Coates et al., 1998). The material properties of the tessellated elasmobranch skeleton may hold similar performance surprises and should be a focus of future experimental study.

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