Title: Stiffness without mineral: material properties and biochemical components of jaws and chondrocrania in the Elasmobranchii (sharks, skates, and rays)

Running Title: Cartilaginous jaw mechanics and biochemistry

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Abstract

Chondrichthyians (sharks, ratfish, and rays) can function at extremes (growing big, swimming fast, and eating hard-prey) suggesting their skeletons are experiencing loading regimes equal to or greater than those of other fishes. In most vertebrates, cartilage is a soft connective tissue serving two purposes; a low-friction bearing surface and contour filler; however, cartilaginous fishes maintain a skeleton made of cartilage throughout life. We examined material properties and biochemical components of cartilage from the jaws and/or chondrocranium of seven species of shark. For each species cylindrical plugs were drilled from the specimen, mineralized tesserae were removed, and plugs tested in compression to ten percent of initial thickness (ε=0.10) at 2mm/sec. Stiffness and strength varied significantly among species and in both cases the chondrocranial properties were greater than those of the jaws. After materials testing, cartilage plugs were lyophilized to obtain water content; then collagen and proteoglycan was measured with hydroxyproline and DMMB assays, respectively. Water content was greatest in the chondrocranial cartilage while collagen content was consistent between the jaws and chondrocrania. However, proteoglycan content was greater in the jaw cartilage. The average values for water and proteoglycan content were consistent with mammalian cartilage, while collagen content was much lower than mammalian cartilage. Material properties and biochemical components were also similar to the mineralized cartilage found in elasmobranch vertebral cartilage.

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Introduction

Skeletons are able to resist large stresses including those caused by growing big, swimming fast, and eating hard-prey. Embryonic vertebrates have cartilaginous skeletons; as they mature, most convert the skeleton into bone, an exception is the Chondrichthyian fishes (sharks, rays, and ratfish), which retain a cartilaginous skeletons through adulthood. Fossil evidence shows that Chondrichthyians abandoned a bony skeleton sometime after *Stethacanthus* 350 MYA (Coates *et al.*, 1998). For nearly 455 million years elasmobranchs (sharks, skates, and rays) have inhabited the oceans, sharing the environment and ecological niches with bony fish (Janvier 1996). Their survival alongside bony fish suggests there are benefits of a cartilaginous skeleton.

Links between between the material properties and function of skeletal elements are clear in bone. For example, a whale bulla is highly mineralized and therefore very stiff and brittle, which are essential characteristics for low-loss transmission of high frequency sound (Currey, 2002). Deer antlers grow astonishing fast, are poorly mineralized, but have a high collagen content and dense mesh of mineralized tissue. This makes antler strong and very tough, which is vital as male deer use the antlers for protracted and forceful discussions of dominance. A similar relation between material properties and skeletal function has been demonstrated in the mineralized vertebrae of cartilaginous skeletons. Porter *et al.* (2006) posited that vertebral centra would be stiffer and stronger, thereby contributing to whole body stiffness, in sharks with faster swimming speeds. More recently, flexural stiffness in propterygia of the pelvic girdle was greater in stingrays that were considered true punters (Macesic and Summers 2012).

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The cartilaginous skeleton of elasmobranchs is composed of two kinds of cartilage (Dean and Summers, 2006). Areolar cartilage, found in the vertebrae, is infiltrated with mineral. The remaining skeletal elements, including the jaws and chondrocranium, are called tessellated cartilage. Tiny hexagonal mineralized tiles (tesserae) cover the surface of the cartilage (Dean and Summers, 2006; Moss, 1977). Some elasmobranch species have multiple layers of tessarae, analogous to cortical thickening seen in bone, for added reinforcement under loading forces (Dingerkus et al. 1991; Summers et al. 1998). Areolar cartilage, a complex composite of mineralized and unmineralized tissue, is as stiff as trabecular bone though not as strong (Porter et al., 2006). The tessellated skeleton offers an opportunity to determine the material properties of unmineralized shark cartilage, as it is relatively easy to strip away the tesserae from any individual element.

The goals of the present study are four-fold 1) to determine material properties of unmineralized tessellated shark cartilage including stiffness, strength, yield strain and ultimate strain; 2) measure some basic compositional parameters of the cartilage including water, collagen, and proteoglycan content; 3) correlate the material properties with the composition of cartilage; and 4) quantify in these parameters variation among species and between two skeletal elements. We expect that this will give us some insight into the structure function relationship of shark skeletal cartilage.

Materials and Methods

Study Organisms

Species were sampled from two shark lineages (Galea and Squalea) and the Batoidea, the dorsoventrally flattened elasmobranchs, from four orders and five families: Carcharhiniformes (Carcharhinidae and Sphyrnidae), Lamniformes (Lamnidae), Squaliformes (Dalatiidae), and

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Rajiiformes (Myliobatidae) (Fig. 1). We collected data on material properties and biochemistry of cartilage from nine species of cartilaginous fishes (Table 1): shortfin mako (*Isurus oxyrinchus* Rafinesque 1810), smooth hammerhead (*Sphyrna zygaena* (Linnaeus 1758)), silky shark (*Carcharhinus falciformis* (Müller and Henle 1839)), sandbar shark (*Carcharhinus plumbeus* (Nardo 1827)), oceanic whitetip (*Carcharhinus longimanus (Poey 1861),* bull shark (*Carcharhinus leucas* (Müller and Henle 1839)), Greenland shark (*Somniosus micocephalus* (Block and Schneider 1801)), Pacific sleeper shark (*Somniosus pacificus* Bigelow and Schroeder 1944), and smooth-tailed mobula (*Mobula thurstoni* (Lloyd 1908)).

The requiem sharks (Carcharhiniformes) are galeomorph sharks with broad diets found both in and off shore (Compagno, 2003). In this study, the four members of the Carcharhinidae include near shore (*C. plumbeus*), pelagic species (*C. falciformis*), reef-associated oceanodromous species (*C. longimanus*), and a reef-associated amphidromous species (*C. leucas*). These sharks are all feeding generalists, and their diets all bony fishes. Hammerheads (*S. Zygaena* : Sphyrnidae) are found near shore and well off shore to depths of 200m (Kajiura et al., 2003). Another galeomorph shark, the shortfin mako (*I. oxyrinchus:* Lamnidae: Lamniformes) is regionally endothermic, and a high speed predator of marlin, tuna, and other pelagic bony fishes (Block and Carey, 1985; Wolf et al., 1988). Makos are believed to be the fastest swimming shark and range to depths of 150m in the pelagic zones of the oceans (Carey and Teal, 1969). The squalimorph sharks in this study were both sleeper sharks (*Somniosus*: Dalatiidae: Squaliformes), relatively sluggish, bottom dwelling animals (Compagno, 1984). They are found in benthopelagic marine habitats and are feeding generalists. The species we examined here are *So. microcephalus* (Greenland shark) and *So. pacificus* (Pacific sleeper shark).

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Finally, the smooth-tailed mobula (Mylibatidae: Rajiformes: Batoidea) is a pelagic, oscillatory swimmer found to depths of 100m and is a planktivorous filter feeder (misty 2013).

Material Properties

Jaw material testing

Silky (*C. falciformis*), sandbar (*C. plumbeus*), shortfin mako (*I. oxyrinchus*), and smooth hammerhead (*Sp. zygaena*) shark heads were collected at a Mexican fishery and stored on ice, then frozen at -30°C. Heads were shipped on ice and stored at -30°C. Each jaw was removed from the animal and cleaned of excess tissue. While the tissue was frozen at least ten plugs (and as many as fifteen) of cartilage were removed from the jaw using either an eight or ten mm diameter trephine drill head barrel (Salvin Dental Specialties, Inc.) in a drill press by MicroLUX Power Tools. Diameter of the trephine drill head depended on the size of the specimen. We obtained a piece of plexiglass with a 10mm hole drilled in the middle. Each plug was placed in the center of this hole and the sides of the plug were leveled with a single edge razor blade to remove the mineralized layer or tesserae found around jaw cartilage (Dean and Summers 2006). Plugs of cartilage were placed in a sealed tube with elasmobranch Ringers and were stored at -32ºC until material testing (Forster et al., 1972).

Tubes with cartilage plugs were placed in a cold-water bath to thaw. Once thawed, plugs were individually measured for thickness and placed in a small rectangular bag with 4-6ml of elasmobranch Ringers to maintain moisture during material testing. Each small plastic bag containing a cartilage plug was taped to a vertical platen on EnduraTEC LM2 TestBench (EnduraTEC). Plugs were tested in unconfined uniaxial compression to failure between two non-porous platens and each tests consisted of compressing the plug at 2 mm/sec (e = 10%).

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Data were captured using Wintest (EnduraTEC 2002) and transferred into Notepad (Microsoft). Notepad data files were loaded into Matlab (Student Release, 2002) and stress strain curves were generated using a custom script. We determined material properties from the stress strain curves and determined the ultimate strength (MPa), stiffness (MPa), yield strength (MPa), and yield strain (e) for each sample. We also calculated the strength:stiffness ratio (Currey, 2002; Porter and Long, 2010).

Chondrocranium material testing

We tested the material properties of cartilage from fresh frozen chondrocrania of the oceanic whitetip (*C. longimanus*), bull shark (*C. leucas)*, Greenland shark (*So. micocephalus*), pacific sleeper shark (*So. pacificus)*, and smooth-tailed mobula (*M. thurstoni*). At least ten cartilage plugs were removed from each chondrocrania using a the 8 or 10mm diameter trephine drill head barrel and drill press (as described above) and placed in a beaker with elasmobranch Ringers. Cartilage plugs were then tested in a compressive test to failure between two nonporous platens at a 2mm/sec strain rate using a MTS Mini Bionix 858 with a 5kg load cell. Stress strain curves were analyzed in Excel and the material properties described above were determined.

Water Content

After testing material properties each specimen was maintained in elasmobranch ringers for compositional testing. We measured the wet weight of each plug, then minced the tissue to ensure the cartilage was completely desiccated. The plugs were lyophilized for more than 24 hours then we measured the dry mass. We calculated water content by subtracting the dry mass from the wet mass of each plug, and dividing by the wet mass. The dried cartilage was divided

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into two samples, which were placed in separate 2 ml centrifugation tubes for collagen and proteoglycan assays.

Collagen Content

We assumed the collagen is similar to other vertebrate collagens and used a hydroxyproline assay to determine content (Bergman and Loxley 1963; Porter et al., 2006). A 50 mg sub-sample was hydrolyzed in 1.5 ml 6 M HCl in a 100°C heat block overnight, then speed-vac'ed and re-suspended in 1.5 ml ddH_2O. The oxidant solution with 1 unit 7% Chloramine-T and 4 units acetate/citrate buffer (57 g sodium acetate (3 H_2O), 37.5 g trisodium citrate (2 H_2O), 5.5 g citric acid (H_2O), 385 ml isopropanol, made up to 1 L, pH 6.0) and Ehrlich's reagent (2 units *p*-dimethylamino-benzaldehyde (2 g aldehyde, 3 ml 60% perchloric acid) and 13 units isopropanol) and the hydroxyproline standard (400 ppm 1-hydroxyproline) were premixed and stored at 4°C. We added 5 µl of diluted sample, 45 µl of ddH_2O, 100 µl of isopropanol, and 50 µl of oxidant solution to a 2ml tube and incubated for 4 minutes at room temperature. Then 625 µl of Ehrlich's reagent solution was added and capped tubes were incubated for 60°C for 25 minutes. Immediately after incubation, 300 µl of each sample was pipetted into a 96 well microplate. A *µQuant*™ spectrophotometer was used to assay the samples at A_{558} (KC*junior*™ software). We calculated the collagen content by assuming that 10% of the dry mass was hydroxyproline (Bergman and Loxley, 1963).

Proteoglycan Content

A second 50 mg aliquot of jaw cartilage samples were lyophilized and then incubated at 60°C for 30 minutes along with papain (10 µl papain: 10 ml papain buffer, 0.2 M Na acetate, 4 mM

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EDTA, 20 mM cysteine, 6.0 pH) following Porter *et al.* (2006) and Summers *et al.* (2003) for use in elasmobranchs. After incubation, 1 ml of papain was added to each tube for digestion overnight in a 60°C heat block. Before use, the papain was inactivated in a 100°C heat block for approximately one hour. Samples were then diluted as necessary and assayed using a standard 1, 9-dimethyl-methylene blue (DMMB) assay as described by Templeton (1988).

Statistical Analyses

Statistical comparisons of material properties and biochemical componets were analyzed using Anova ($P<0.05$) and post hoc comparisons between species were made using a student's t test in JMP software version 5.0.1.a (SAS Institute Inc., Cary, NC, USA). Data sets were tested for normality using a Shapiro-Wilk W test, and those, which were not normal, were log transformed so they were normally distributed (Zar, 1999; Sokal and Rolf, 1995). Data in the figures and table are the untransformed values for ease in interpretation. Regressions were analyzed using simple linear models and fit with a power curve.

Results

Young's modulus (MPa) varied significantly among species ($F_{6,150}=81.73$; $P< 0.0001$; Fig 2A; Table 1). The stiffness of chondrocrania was greater than that of the jaws ($P<0.05$). In particular, the chondrocrania cartilages from *C. leucas* were over an order of magnitude stiffer than the jaw cartilage. Ultimate strength (MPa) also varied significantly among species ($F_{6,150}=29.01$; $P< 0.0001$; Fig 2B; Table 1). *C. leucas* strength was also significantly larger than the other species chondrocrania and jaw cartilages ($P<0.05$). The strength:stiffness ratio for jaw cartilage varied significantly among species ($F_{3,114}=5.7$; $P=0.0011$; Table 1). The *I. oxyrhinchus*

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cartilage had the largest ratio and was similar to *C. plumbeus* (Table 1). *C. falciformis* and *S. zygeana* were significantly lower than *I oxyrhinchus*. The strength:stiffness ratio for chondrocrania also varied significantly among species ($F_{2,16}$=8.02; *P*=0.0004; Table 1). *C. longimanus* was significantly greater than *S. pacificus* (Table 1).

Water content (%WM) varied significantly among species ($F_{4,143}$=5.185; *P*= 0.0006; Fig 3A, Table 1). *So. microcephalus* samples from the chondrocranium were composed of 90% water, significantly greater than the jaw specimens (*P*<0.05). Collagen content (%DM) also varied significantly among species ($F_{6,150}$=65.28; *P*< 0.0001; Fig 3B). *M. thurstoni* chondrocrania had more than twice the collagen content of the other species chondrocrania and jaws (P<0.05). Proteoglycan content (%DM) varied significantly among species ($F_{6,150}$=87.25; *P*< 0.0001; Fig 3C). *S. zygaena* had the greatest proteoglycan content while *So. microcephalus* and *M. thurstoni* had the least (*P*<0.05).

In chondrocrania there is a significant relation between stiffness and strength (R^2=0.97; *P*< 0.0001; Fig. 4A). Stiffness also increases with strength in mineralized elasmobranch vertebral cartilage (R^2=0.59; *P*< 0.0001; Fig. 4A) and mammalian bone (R^2=0.99; *P*< 0.0001; Fig. 4A). Both chondrocrania and vertebrae have greater strength-to- stiffness ratio than bone. However, there is no significant relation among stiffness and strength of elasmobranch jaw cartilage (Fig. 4B).

We pooled data from all species to examine the relationships between material properties and biochemical components. We found that as collagen content increases both stiffness (*P*<0.0001, R^2=0.149) and strength (*P*=0.0065, R^2= 0.627) decreases. The strength-to-stiffness ratio increases significantly as collagen content increases (*P*=0.04, R^2=0.035) as well as water content (*P*=0.009, R^2=0.057). As proteoglycan content increases strength in jaw cartilage will

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also increase significantly (P=0.0021). Proteoglycan content decreases significantly as collagen content increases for both jaws and chondrocrania (P<0.0001, R^2=0.079).

Discussion

Sharks are often referred to as 'living fossils' because they have a body plan that is readily recognized from fossils hundreds of millions of years old (Janvier, 1996; Coates *et al.*, 1998). There is a tendency to consider these types of lineages as 'primitive' or unchanged, and hence of low variability. Of course this teleological thinking is flawed, but it persists and so it is particularly gratifying to see that the unmineralized cartilaginous skeleton, the most basic building block of the shark, is comparable to bone in it variability in material properties and composition (Table 1, Fig. 2 and 3). Furthermore, the amount of mineral varies greatly and is a principal determinant of material properties in bone (Currey, 2002). In cartilage, we are seeing variation in compositional characteristics that are normally minor players in bony skeletons. Collagen in bone might vary between 85-90%, whereas in this study it ranges from 9-45% DW. Proteoglycan content has long been associated with the material properties of articular cartilage (15-25% DW; Koob and Vogel, 1987) and here we show variation (12-61% DW; Table 1; Fig 3) on the order seen across all types of mammalian cartilage, though our sampling has hardly been more than synoptic.

While the properties and content of unmineralized cartilage vary widely, the driving forces for the variation are not clear. For example, the jaws of sharks are subject to relatively large, dynamic, and cyclic loading, and the chondrocranium is likely far less stressed (Wroe *et al.*, 2008). This leads naturally to the hypothesis that jaw cartilage would be stiffer and stronger than chondrocranial cartilage, but this is emphatically not the case. Across all the species we

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examined there was not a single example of a jaw that was stiffer than any chondrocranium and there is only one example of a jaw that is stronger than a chondrocranium (*C. plumbeus* is stronger than *So. pacificus*; Table 1, Fig. 2). Along these same lines, collagen content increases as stiffness and strength decrease but there no clear relationships between proteoglycan or water content and either stiffness or strength. This is very much at odds with the literature on bone and mineralized areolar shark cartilage (Currey, 2002; Porter *et al.*, 2007; Porter *et al.*, 2006; Macesic and Summers, 2012). We view these unexpected relationships as evidence that the story of mineralized cartilage is very complex. It is likely that the resolution lies in assessing the entire composite skeletal element in the context of its actual functional milieu. In the case of the unexpectedly weak and flaccid jaws, it seems likely that we are looking at the wrong properties to understand the function of the unmineralized tissue. Surely we would get different results if we did materials testingat different orientations. Consider that in the propterygia of skates - the stiffness is due primarily to the mineralized rind of around the element (Macesic and Summers, 2012). If the same is true in the jaws, then the unmineralized core is free to assume some other role. Since damping (ability to absorb strain energy) is usually inversely related to stiffness, it is possible that the core of the jaws is serving to damp out the high frequency and amplitude strains associated with feeding (Vogel, 2003). The chondrocranium does not bear these loads and so the core is under no selective pressure to increase damping ability. We believe that material and structural tests that reflect the *in vivo* stress and strain patterns and loading regimes will be more informative than simple quasi-static measures of load and displacement.

On stiffness and strength - 'the two properties together describe a solid about as well as you can reasonably expect two figures to do' (Gordon 1968). For all the shortcomings and difficulties with estimating Young's modulus and ultimate strength, they are very useful qualities

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for qualitative discussion of a biomaterial. There is often a clear relationship between these two parameters, and that relationship is dictated by microstructure, crack stopping adaptations and the number and distribution of flaws in the material (Vogel, 2003; Curry 2002; Wainwright *et al.*, 1978). In bony tissue there is a strong correlation between stiffness and strength, across two orders of magnitude of stiffness, which strength is about 1% of the stiffness (Currey, 2002). In the vertebrae of sharks a more complex relationship emerges, in which a least squares fit suggests they are 5 times stronger for a given stiffness, but the data are far more scattered (Porter and Long, 2010). In this case the data dispersion is likely due to the microscale architecture of the complex mineral phase of the tissue (Porter *et al.*, 2006; Porter *et al.*, 2007). The data we have described here for chondrocrania show a similar dispersion to the bone data (fig 4) but with a 30 fold higher strength for a particular stiffness compared to bone. The slope of the relationship is the same as for bone and both are steeper than the relationship for vertebrae. We expect that this tight correlation between the two properties is driven by the homogeneity of the unmineralized composite cartilage. Potential fracture flaws include the lacunae for chondrocytes and cartilage canals, both of which are evenly distributed through the tissue (Dean et al., 2009, Dean *et al.*, 2010). The jaw tissue presents an altogether different picture that suggests further investigation may be fruitful. There is no clear relationship between stiffness and strength for the pooled data, and when broken down by species it appears there might be some species which have an inverse trend (fig. 4). These data suggests there may be some architectural factors at the microscale that dictate the response of jaw tissue to loads.

Perhaps the most troubling aspect of the data we have presented here is the lack of a clear relationship between any biochemical parameter and our two materials properties. Of course, without a mineral phase we have lost the principal determinant of stiffness and strength in other

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skeletal tissues, but there are certainly good correlations between biochemistry and material properties of unmineralized articular cartilage and also mineralized elasmobranch cartilage (Koob 1989; Koob and Vogel, 1987; Porter et al., 2006; Macesic and Summers, 2012). We propose several factors that might explain this unexpected finding. First, we may simply be looking at the wrong properties, that is, stiffness and strength are affected by so many factors that can act counter to each other that perhaps a good relationship is obscured. Some weak evidence for this is seen in the significant relationship between both water and collagen content and the ratio of strength-to-stiffness. The poor explanatory power of the relationship, seen by low R^2 values, makes it clear that there are other important, and thus far unmeasured, aspects of composition that are dictating response to load. Second, our testing regime is standardized to laboratory temperatures and some viscoelastic solids are notoriously temperature sensitive. Though cartilaginous fishes are generally ectotherms, we did test a regional endotherm in this study. Also of the species tested here, the range of temperatures of the habitat varies by more than 20ºC. Perhaps the properties at the biologically appropriate temperatures are more closely related to the composition of the tissue. Either of these possibilities points the way to further investigation of this complex composite material.

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Figures and Legends

Table 1. Summary of material properties and biochemical constituents of elasmobranch tesselated cartilage

			Water		Collagen		Proteoglycan		Stiffness		Strength		Strength / Stiffness	
Species	**Individuals**	**Specimen**	**N**	**% WM**	**N**	**% DM**	**N**	**% DM**	**N**	**MPa**	**N**	**Mpa**	**N**	**Ratio**
Carcharhinus falciformis	3	J	54	86.07	54	15.07	54	32.07	48	62.55	48	8.91	48	0.2622
Carcharhinus plumbeus	2	J	18	83.05	18	11.81	18	43.84	15	78.79	15	41.96	15	0.5406
Sphyrna zygaena	3	J	13	83.7	13	8.97	13	61.35	15	48.22	15	9.14	14	0.1953
Isurus oxyrinchus	4	J	42	86.51	42	17.79	42	34.67	50	20.05	50	7.59	41	0.6766
Carcharhinus leucas	1	C							6	775.97	6	171.89	6	0.2257
Carcharhinus longimanus	1	C							4	172.06	4	44.86	4	0.2622
Somniosus microcephalus	1	C	20	90.35	10	13.44	10	12.6						
Somniosus pacificus	1	C			10	13.76	20	38.46	9	116.34	9	22.04	9	0.1965
Mobula thurstoni	1	C			10	44.75	9	14.8						

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

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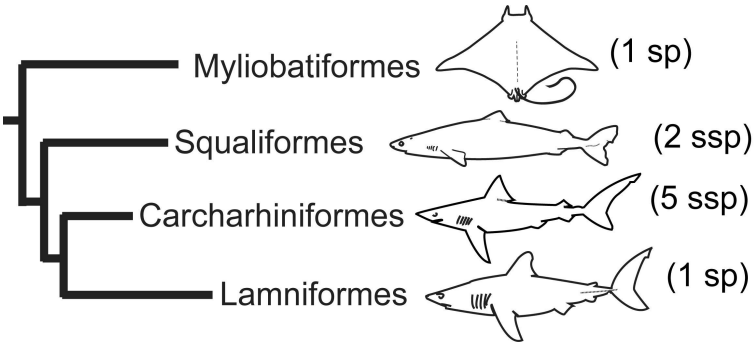

Figure 1: A phylogeny showing the major groups sampled. We have representative species from both major lineages of sharks (Galeomorph and Squalimorph) and batoids. The numbers indicated to the right of the icon represent the number of species sampled from each order. This phylogeny is adapted from Aschilman *et al.*, 2012.

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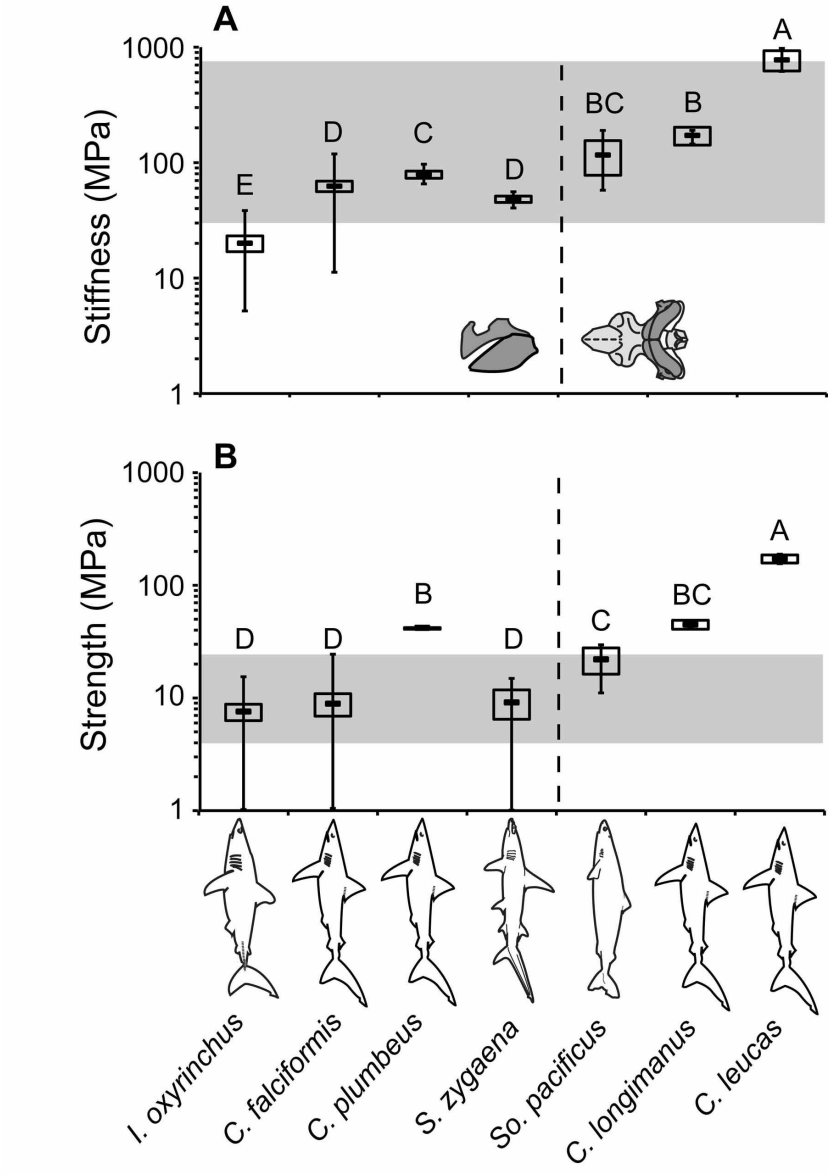

Figure 2: Material properties of cartilage found in shark jaws and chondrocrania. (A) Material stiffness, as measured by Young's modulus of elasticity was significantly different among the species ($F_{6,130}$=81.73; P<0.001). The chondrocrania of *C. leucas* and *C. longimanus* were significantly stiffer than chondrocrania from other species (P<0.001), and those chondrocrania were also stiffer than chondrocrania and jaw cartilage sampled here (P<0.001). (B) Ultimate strength (MPa) of cartilages from seven elasmobranch species showing significant differences ($F_{6,130}$=29.01; P<0.001). The gray box represents the range of data from the mineralized vertebral centra from elasmobranchs (Porter *et al.*, 2006). The lines represent the means for each species, the boxes are the 95 % CI, and the whiskers are the maximum and minimum from each species. Letters above the box and whisker plot denote significant differences between species.

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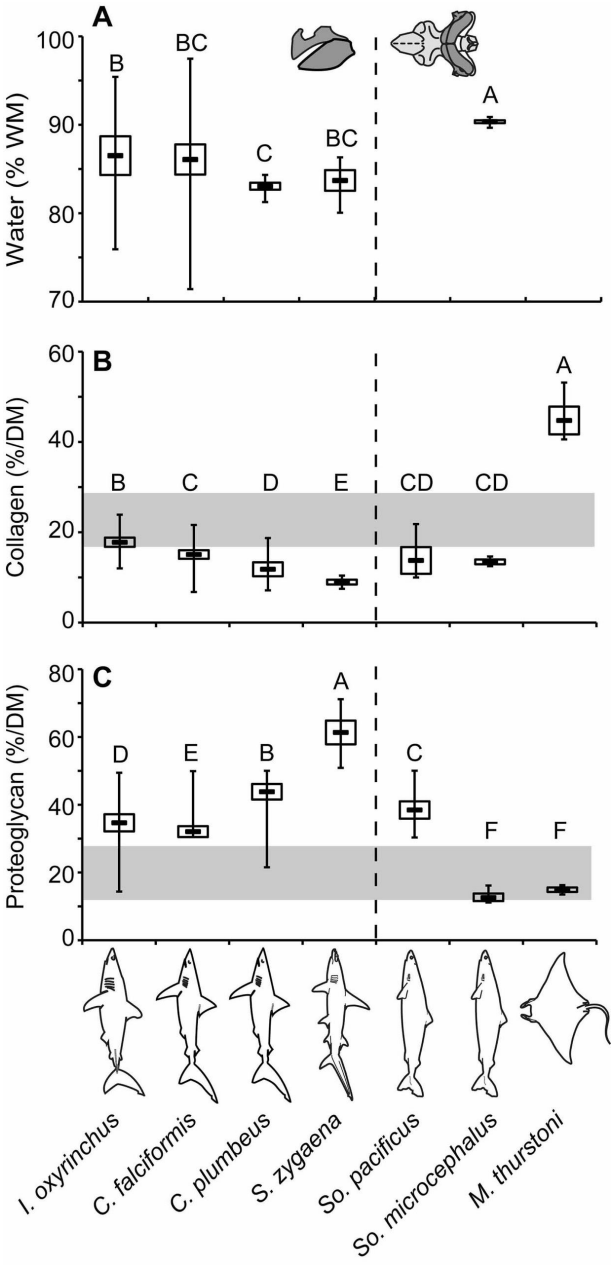

Figure 3: Biochemical composition of jaw and chondrocrania cartilage from seven species of elasmobranch. (A) Water content (% WM) is significantly different among species ($F_{4, 143}$=5.185; P=0.006). (B) There were significant differences in collagen content, expressed as percentage of dry mass, among species ($F_{6,150}$=65.28; P<0.001). Overall, the collagen content of the species examined ranged from nearly 45 % (*M. thurstoni*) to only 9% (*S. zygaena*). (C) Proteoglycan (PG) content, expressed as percentage of dry mass (DM), varied among species ($F_{6,159}$=87.25; P<0.001). The highest PG content was 61% found in *S. zygaena* and the lowest was only 12% found in *So. microcephalus*. The gray box represents the range of data from the mineralized vertebral centra from elasmobranchs (Porter *et al.*, 2006). The lines represent the means for each species, the boxes are the 95 % CI, and the whiskers are the maximum and minimum from each species. Letters above the box and whisker plot denote significant differences between species.

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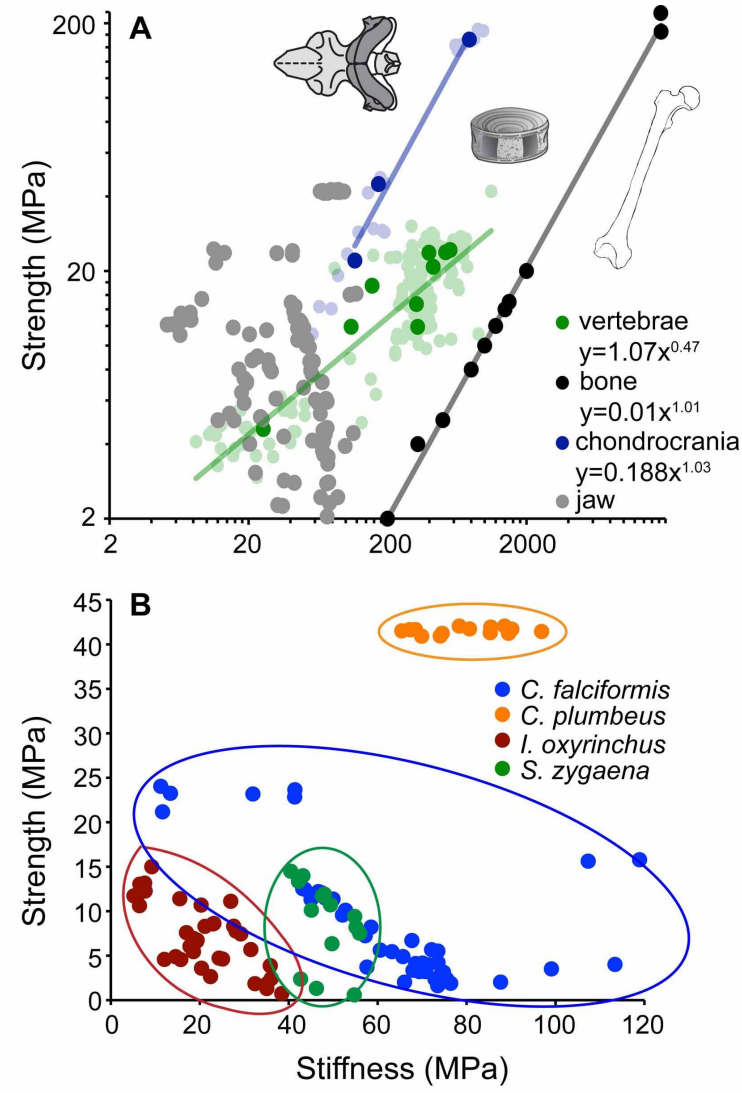

Figure 4: Comparative mechanical properties of skeletons. (A) The tessellated chondrocranium cartilage of elasmobranchs is stronger in compression, at a given Young's modulus, than the both elasmobranch mineralized vertebral cartilage and mammalian bone. Elasmobranch data points include chondrocranium data from the present study (blue) and also previously published values on mineralized cartilaginous vertebrae from *Isurus oxyrinchus, Sphyrna zygaena, Carcharhinus falciformis, Carcharhinus plumbeus, Centrophorus granulosus, Centrophorus sp*., *Mustelus californicus,* and *Torpedo californica* (Porter et al., 2006, 2007; and Porter and Long, 2010). We plotted a power fit for chrondrocrania and vertebrae are generated from raw data points while the means for each species are shown in dark blue and green, respectively. The power fit for mammalian bone was generated using values in the literature (Currey, 2002; Currey, 1999; Wainwright et al., 1976). (B) A clear stiffness and strength relation is not found in the elasmobranch jaw cartilage.

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