

RESEARCH ARTICLE

Mechanical properties of sand tiger shark (*Carcharias taurus*) vertebrae in relation to spinal deformity

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SUMMARY

Approximately 35% of sand tiger sharks (*Carcharias taurus*) in public aquaria exhibit spinal deformities ranging from compressed vertebrae and loss of intervertebral space to dislocated spines with vertebral degeneration and massive spondylosis caused by excessive mineralization both within vertebrae and outside the notochordal sheath. To identify the mechanical basis of these deformities, vertebral centra from affected ($N=12$) and non-affected ($N=9$) *C. taurus* were subjected to axial compression tests on an MTS 858 Bionix material testing system, after which mineral content was determined. Vertebral centra from affected sharks had significantly lower mineral content and material behavior in nearly all variables characterizing elasticity, plasticity and failure. These mechanical deficiencies are correlated with size at capture, capture method, vitamin C and zinc deficiency, aquarium size and swimming behavior in public aquaria. Non-affected *C. taurus* had greater stiffness and toughness even though these properties are generally incompatible in mineralized structures, suggesting that the biphasic (mineralized, unmineralized phases) nature of chondrichthyan vertebrae yields material behavior not otherwise observed in vertebrate skeletons. However, vertebral centra from non-affected sharks had lower mineral content (33%), stiffness (167 MPa), yield strain (14%) and ultimate strength (16 MPa) than other species of sharks and bony vertebrates, indicating that biomechanical precautions must be taken in the husbandry of this species.

Key words: elasmobranch, spinal deformity, vertebrae, skeletal biomechanics, material properties.

Received 23 January 2013; Accepted 12 August 2013

INTRODUCTION

The sand tiger shark, *Carcharias taurus* Rafinesque 1810, is a large lamniform species found sporadically in temperate and tropical coastal waters throughout the world (Compagno, 2001). The conservation status of various populations ranges from ‘near threatened’ to ‘critically endangered’, with a global categorization of ‘vulnerable’ (Pollard and Smith, 2009). Dwindling populations are attributed to overfishing (directed and by-catch) and low reproductive output, with some stocks considered highly susceptible to extinction (Otway et al., 2004; Lucifora et al., 2009). *Carcharias taurus* attains sexual maturity relatively late in life (males: ~190 cm, 6–7 years; females: ~220 cm, 9–10 years), has a 12 month gestation period, reproduces every other year, and only produces two offspring per litter (Compagno, 2001; Lucifora et al., 2002; Gilmore et al., 2005; Goldman et al., 2006). Small litters in *C. taurus* are due, in part, to the unique phenomenon of intrauterine cannibalism in which the first embryo to attain feeding ability in each of the paired uteri consumes its siblings and additional ovulated eggs (Gilmore et al., 2005). Given these life history attributes, *C. taurus* is inherently incapable of quickly rebounding from population decline. Despite its troubled conservation status, *C. taurus* has become a commonly displayed exhibit species in public aquaria throughout the world [~202 individuals in 39 institutions (American Elasmobranch Society,

2006)] owing to its large size and menacing appearance, yet docile behavior.

Approximately 35% of *C. taurus* in public aquaria exhibit spinal deformities of varying severity. Many of these animals are eventually euthanized because of the gradual deterioration of their condition, thereby increasing pressure on wild stocks for exhibit specimens despite dwindling wild populations (Anderson et al., 2012). Hoenig and Walsh (Hoenig and Walsh, 1983), Berzins et al. (Berzins et al., 1998; Berzins et al., 2002) and Preziosi et al. (Preziosi et al., 2006) were the first to describe these spinal deformities, along with a variety of other skeletal tissue abnormalities including curled pectoral fins, gingival hyperplasia and permanently protruded upper jaws. A typical shark spine is composed of spool-shaped vertebral centra with concave ends, aligned end-to-end and separated by symmetrical intervertebral discs. Vertebral centra are comprised of areolar cartilage, a composite in which paired cones of concentrically deposited mineral are arranged tip-to-tip within a densely cellular matrix of unmineralized tissue, all of which are surrounded by a mineralized perimeter. In addition, centra are adorned dorsally by neural arches that protect the spinal cord, ventrally by hemal arches that protect the dorsal aorta, and laterally by rib cartilages (Kardong, 2006; Porter et al., 2007). Conversely, deformed spines in *C. taurus* were characterized by fractures, subluxations (partial spinal dislocations), compression and asymmetry of intervertebral discs,

and dramatic curvature in both the dorsal (scoliosis) and lateral (kyphosis) directions, generally between the pectoral girdle and first dorsal fin (Fig. 1). These conditions were often accompanied by distortion of neural and hemal arches, degeneration of vertebrae at the primary lesion site, excessive mineralization of the surrounding vertebrae resulting in spondylosis (fusion) of adjacent vertebrae and ribs into a permanently buckled conformation, and atrophy and fibrosis of the surrounding musculature. Microscopically, the injured tissues were characterized by the accumulation of granulocytes characteristic of an immune response and disorganized proliferation of hypertrophied chondrocytes indicative of ineffective healing; lack of remodeling may be a general characteristic of chondrichthyan cartilage, as is the case with mature articular cartilage in osteichthyan vertebrates (Ashhurst, 2004; Hall, 2005). Behaviorally, the affected sharks exhibited irregular swimming patterns and loss of forward thrust, and often sank to the bottom of their habitat (Hoenig and Walsh, 1983; Berzins et al., 1998; Berzins et al., 2002; Preziosi et al., 2006).

Previous studies have identified correlations between spinal deformities in *C. taurus* from public aquaria and capture methods, behavior and nutrition in aquaria, and aquarium design. Anderson et al. found that animals caught *via* net were more likely to develop spinal deformities than those caught by hook and line (Anderson et al., 2012). Affected sharks were caught at a smaller size than non-affected sharks (122 and 186 cm, respectively), had higher Fulton condition factors (body mass/length³), and had lower serum concentrations of vitamins C and E, potassium, and zinc as well (Anderson et al., 2012); deficiencies in vitamin C and zinc are known to affect collagen synthesis and crosslinking, as well as the skeletal mineralization process in other vertebrates (Starcher et al., 1980; Lall and Lewis-McCrea, 2007). Another study (Tate et al., 2013) found that affected sharks spend more time using powered swimming (greater swim-to-glide ratio) and have longer tail-beat durations than non-affected sharks, and that all *C. taurus* in public aquaria spend nearly 100% of their time swimming asymmetrically (turning as opposed to linear locomotion) regardless of spinal deformity. These behavioral correlates appear to be due to the spatial constraints imposed by aquaria, as aquarium length was negatively correlated with syndrome prevalence (Tate et al., 2013). Collectively, these results suggest that spinal deformity in *C. taurus* has a multifactorial etiology that often leads to euthanasia out of concern for animal welfare.

The purpose of the present study was to further explore the etiology of spinal deformity in *C. taurus* from public aquaria by examining the material properties and mineral content of vertebrae from affected and non-affected sharks. Specifically, we examined the hypotheses that (1) the stiffness, yield and failure properties of vertebrae from affected *C. taurus* are deficient, and (2) that this deficiency is associated with decreased mineralization of vertebrae.

MATERIALS AND METHODS

Specimen acquisition and imaging

Public aquaria holding *C. taurus* throughout the United States were requested to take part in a multi-stage survey to evaluate various hypotheses regarding the etiology of spinal deformity in this species; data were collected for both affected and non-affected sharks. Stage I of the survey collected historical, clinical and husbandry data, while Stage II of the survey collected spinal radiographs, blood samples for immunological and clinical chemistry analyses, and videography of swimming for behavioral analysis; these results are reported elsewhere (Anderson et al., 2012; Tate et al., 2013). Stage III of the survey entailed a necropsy of sharks that had been euthanized because of



Fig. 1. Sand tiger shark, *Carcharias taurus*, exhibiting significant spinal deformity.

severity of spinal deformity or those that expired from other causes (senescence, post-capture mortality). Upon death, spinal sections from 12 affected [217–269 cm total length (TL)] and nine non-affected (170–248 cm TL) sharks were shipped frozen to either The University of Tampa or The Florida Aquarium, where they were stored frozen. Clinical diagnoses of syndrome status were made by attending veterinarians and confirmed *via* necropsy and examination of spinal radiographs. The length of spinal sections varied from four vertebrae to complete spinal columns. To visualize the severity of deformity in an affected shark, several of these spines were imaged at the University Diagnostic Institute in Tampa, FL, on a Philips Mx8000 high-resolution X-ray computed tomography scanner (Philips, Amsterdam, The Netherlands) with a 1.3 mm slice thickness and 512×512 pixel field of view. Skeletal segmentation was then performed in Amira 5.4.2 (Visualization Sciences Group, Burlington, MA, USA). All stages of the survey were conducted in accordance with the guidelines of The Florida Aquarium's Animal Care and Use Committee.

Mechanical testing

Spinal sections were thawed to room temperature and the axial musculature was removed. All neural arches, hemal arches and ribs were removed from the spines, which were then separated into individual centra from which the intervertebral discs were removed. Length (0.01 mm), diameter (0.01 mm), mass (0.01 g) and volume (0.1 ml) were recorded and cross-sectional area was calculated for each centrum. For affected sharks, only centra at least four vertebrae away from an injury site, as verified by radiographs, were retained for mechanical testing. Injured vertebrae were not tested because of the *a priori* assumption that their irregular shapes and mineralization patterns would obfuscate the results of mechanical testing.

All centra were subjected to axial compression tests on an 858 Bionix material testing system with a 25 kN load cell (MTS Systems, Eden Prairie, MN, USA). Centra were compressed to 50% of their length at a rate of 7% centrum length s⁻¹ (Porter et al., 2007). Force and displacement were recorded and the data of only those centra that reached complete failure were retained for analysis ($N=4-52$ centra per individual). Force and displacement were converted into stress (force/cross-sectional area) and strain (displacement/centrum length) using Excel 2010 (Microsoft Corporation, Redmond, WA, USA). Stress–strain curves were then analyzed for stiffness (slope of linear elastic region of stress–strain curve), yield strength and yield strain (stress and strain at the point of transition from elastic to plastic material behavior where permanent deformation begins to occur), ultimate strength and ultimate strain (point of complete mechanical failure), plastic strength (ultimate strength minus yield strength);

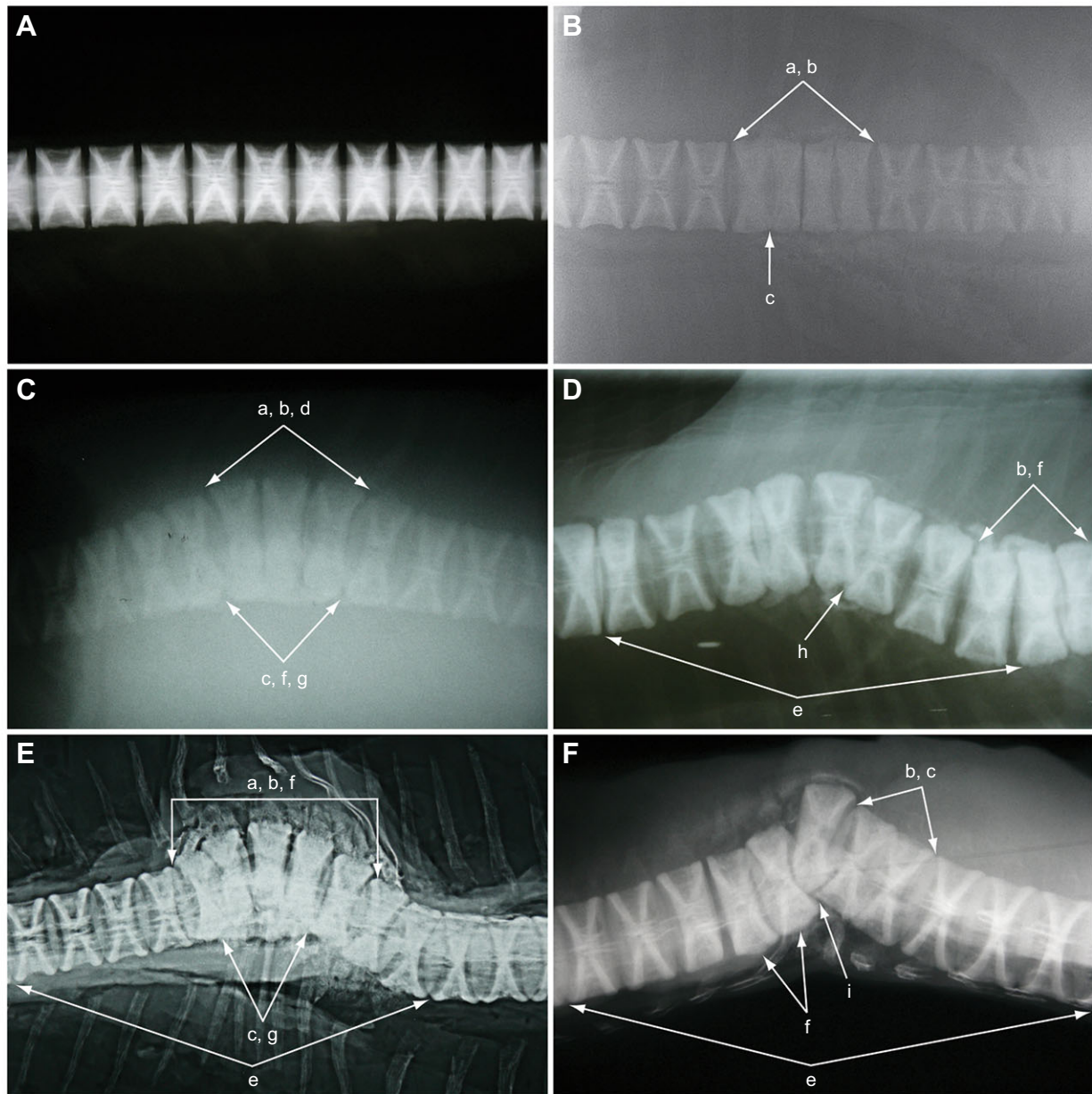


Fig. 2. Range of pathologies associated with spinal deformity in sand tiger sharks, *Carcharias taurus*, from public aquaria. (A) Non-affected spine exhibiting uniformity of vertebrae and intervertebral discs. (B) Less severe spinal deformity characterized by compressed vertebral centra and loss of intervertebral space. (C,D) Moderately severe spinal deformities characterized by compressed vertebral centra, loss of intervertebral space, minor or major spinal curvature, excessive mineralization within the vertebral matrix (endophytic idiopathic mineralization) and/or in the peripheral zone of the centrum outside of the notochordal sheath (exophytic idiopathic mineralization), spondylosis caused by excessive mineralization, and subluxation. (E,F) Very severe spinal deformities characterized by compressed vertebral centra, loss of intervertebral space, major spinal curvature, excessive mineralization within the vertebral matrix (endophytic idiopathic mineralization) and/or in the peripheral zone of the centrum outside of the notochordal sheath (exophytic idiopathic mineralization), spondylosis caused by excessive mineralization, and extreme subluxation of the spine. a, compressed vertebral centra; b, loss of intervertebral space; c, spondylosis; d, minor spinal curvature; e, major spinal curvature; f, endophytic idiopathic mineralization; g, exophytic idiopathic mineralization; h, subluxation; i, extreme subluxation.

representing the ability to resist ultimate failure following yield), and work of fracture (area under the stress–strain curve divided by cross-sectional area; representing the energy needed to cause complete mechanical failure) using LoggerPro 3.8.5 (Vernier Software and Technology, Beaverton, OR, USA).

Mineral content

Following mechanical testing, all vertebral centra were burned in a Thermolyne 1400 muffle furnace (Barnstead International, Dubuque, IA, USA) at 550°C for 8 h and then weighed (to the nearest

0.01 g). Mineral content was determined by comparing the pre- and post-burn mass of each vertebral centrum.

Statistical analysis

All variables failed the Shapiro–Wilk W test for normality and were therefore compared with a variety of statistical distributions to determine the appropriate statistical transformation to apply to each. Stiffness, plastic strength and work of fracture fit log-normal distributions, while yield strength, yield strain, ultimate strain and mineral content fit Poisson distributions. Log-normal-distributed

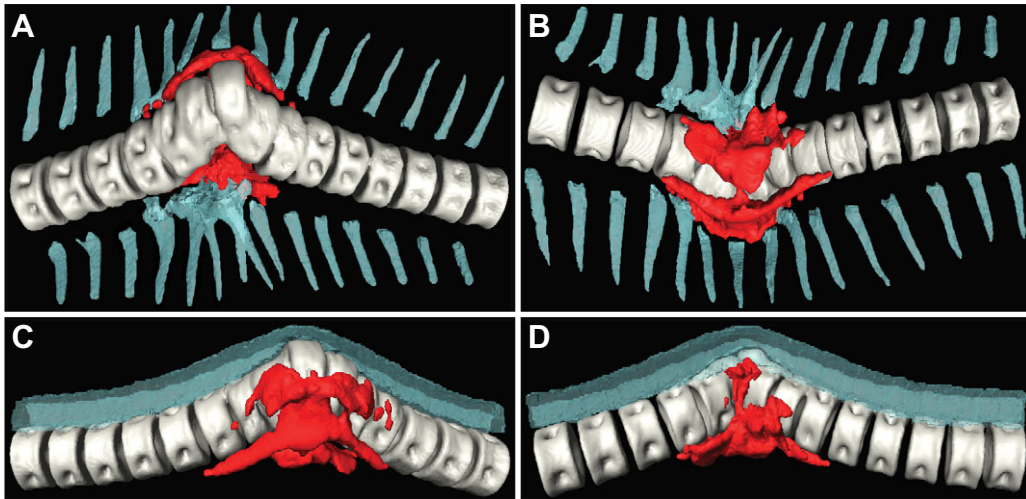


Fig. 3. Reconstructed CT scan illustrating the severity of spinal deformity in the sand tiger shark, *Carcharias taurus*, in (A) dorsal view, (B) ventral view, (C) right lateral view and (D) left lateral view. Vertebral centra are pictured in white, exophytic idiopathic mineralization is pictured in red, and weakly mineralized structures (ribs, neural arches) are pictured in translucent blue. For ease of viewing, A and B are pictured without neural arches, while C and D are pictured without ribs. Anterior is to the left in A, B and D, and to the right in C. Note overlapping, deformed ribs and vertebral centra fused together at the apex of the spinal lesion via idiopathic mineralization causing extensive spondylosis (illustrated by lack of intervertebral space between adjacent vertebral centra). Exophytic idiopathic mineralization distorted the hemal arches within the spinal lesion, causing partial occlusion of the dorsal aorta in this specimen.

variables were transformed using natural logarithms [$x'=\ln(x)$], Poisson distributed variables were square root transformed ($x'=\sqrt{x}$), and ultimate strength remained untransformed as it more closely approximated a normal distribution than any of the other distributions against which the data were tested (Zar, 1999). Following transformations, all variables other than yield strain passed Levene's test for homogeneity of variance and were further tested using parametric statistics; differences in yield strain between affected and non-affected sharks were assessed using the Wilcoxon rank scores test. A nested MANOVA was performed with health status as the fixed effect and individual nested within health status as the random effect, after which univariate nested ANOVAs were used to determine which variables differed with respect to health status. A principal components analysis based on a correlation matrix was then used to explore patterns of variation among variables. All principal components with eigenvalues ≥ 1.0 were retained for analysis, and variables with factor loadings ≥ 0.4 were considered to load heavily on a given axis. Previous studies have found considerable intra-individual variability in vertebral mineral content and identified mineral content as a predictor of material properties in shark vertebrae (Porter et al., 2006; Porter et al., 2007). Therefore, intra-individual variation in vertebral centra mineral content was determined for each spine [(standard deviation/range) $\times 100$] and linear regressions of all biomechanical variables with respect to mineral content were performed. Variation in mineral content between affected and non-affected sharks was compared using the Wilcoxon rank scores test. All statistical tests were performed in JMP 5.1.2 (SAS Institute, Cary, NC, USA) with a significance value of $P=0.05$.

RESULTS

Visual and radiographic assessment of affected and non-affected spines confirmed previous observations of spinal deformity in the collected specimens. Non-affected spines exhibited homogeneity in shape and alignment of vertebrae and intervertebral discs, and no signs of idiopathic mineralization (Fig. 2A). Affected spines exhibited a continuum of abnormalities with the least severe

consisting of vertebral bodies compressed and shifted from spinal alignment and partial loss of intervertebral space (Fig. 2B). Progression of the disorder resulted in severely compressed and fractured vertebral bodies, spondylosis and curvature (scoliosis and/or kyphosis) (Fig. 2C,D). The most severe cases involved extreme spondylosis of vertebrae, dramatic curvature (scoliosis and/or kyphosis), vertebral degeneration, and idiopathic mineralization both within vertebrae (endophytic mineralization) and in the peripheral zone of the centrum outside the notochordal sheath (exophytic mineralization) causing spondylosis of up to six adjacent vertebrae and their ribs (Fig. 2E,F, Fig. 3). In one of the most severe instances, fracture and idiopathic mineralization distorted a series of hemal arches to the extent that the dorsal aorta was partially occluded, possibly disrupting blood flow to the posterior half of the body (Fig. 3). The majority of spinal lesions occurred between the pectoral girdle and first dorsal fin, although injuries were observed anterior to the pectoral girdle and between the first and second dorsal fins in one and two individuals, respectively.

MANOVA identified significant differences among biomechanical variables (Pillai's trace, $F_{180,3672}=13.6667$, $P<0.0001$). Subsequent ANOVAs identified significant differences between affected and non-affected sharks such that the vertebral centra of affected sharks had lower stiffness ($F_{1,19}=9.7042$, $P=0.0020$, 143.5 versus 168.6 MPa), yield strength ($F_{1,19}=32.0628$, $P<0.0001$, 9.6 versus 10.5 MPa), ultimate strength ($F_{1,19}=53.3267$, $P<0.0001$, 14.7 versus 16.3 MPa), ultimate strain ($F_{1,19}=53.7959$, $P<0.0001$, 19.8% versus 25.0%), work of fracture ($F_{1,19}=26.8872$, $P<0.0001$, 22.2 versus 29.6 Jm⁻²) and mineral content ($F_{1,19}=32.6601$, $P<0.0001$, 30.9% versus 33.4%) than those of non-affected sharks (Table 1). Wilcoxon rank scores test indicated that vertebral centra of affected sharks had significantly lower yield strain than those of non-affected sharks as well ($\chi^2_1=4.8535$, $P=0.0267$, 11.4% versus 13.8%; Table 1).

Principal components analysis generated three principal components with eigenvalues greater than 1.0 (77.14% of variance explained): ultimate strain, plastic strength and work of fracture loaded heavily on PC1; stiffness, yield strength and ultimate

Table 1. Summary of material properties and results of statistical comparisons of vertebral centra of sand tiger sharks (*Carcharias taurus*) from public aquaria with respect to health status

	Affected	Non-affected	$F_{1,19}$	χ^2_1	P
Stiffness (MPa)	143.5±5.5	168.6±7.6	9.7042		0.0020
Yield strength (MPa)	9.6±0.2	10.5±0.3	32.0628		<0.0001
Yield strain (%)	11.4±0.0	13.8±0.0		4.8535	0.0276
Ultimate strength (MPa)	14.7±0.3	16.3±0.2	35.3276		<0.0001
Ultimate strain (%)	19.8±0.0	25.0±0.0	53.7959		<0.0001
Plastic strength (MPa)	5.1±0.2	5.8±0.3	0.0524		0.8190
Work of fracture (J m ⁻²)	22.2±1.0	29.6±1.3	26.8872		<0.0001
Mineral content (%)	30.9±0.4	33.4±0.4	32.6601		<0.0001

Affected data represent 228 vertebral centra from 12 individuals. Non-affected data represent 227 vertebral centra from nine individuals. Bold P -values indicate statistically significant differences between affected and non-affected individuals.

strength loaded heavily on PC2; and yield strain loaded heavily on PC3 (Table 2). Mineral content had significant, albeit very weak, linear relationships with stiffness ($P=0.0010$, $r^2=0.0237$), ultimate strength ($P<0.0001$, $r^2=0.1116$), ultimate strain ($P<0.0001$, $r^2=0.1029$), plastic strength ($P<0.0001$, $r^2=0.1155$) and work of fracture ($P<0.0001$, $r^2=0.2045$). Average variability in mineral content was 30% in affected sharks (24–36% range), which was significantly greater than the 25% mineral content variability found in non-affected sharks (17–28% range; $\chi^2_1=4.8641$, $P=0.0274$).

DISCUSSION

Sand tiger shark spinal deformity

Vertebral centra from affected *C. taurus* in public aquaria had significantly lower mineral content and material properties in nearly all variables characterizing elasticity, plasticity and failure. Therefore, the vertebral centra of non-affected sharks are stronger, better able to resist deformation, and tougher. Stiffness was correlated with yield strength and ultimate strength, whereas toughness (i.e. work of fracture) was correlated with ultimate strain and plastic strength, and is often associated with the ability to resist crack propagation during post-yield behavior (Currey, 2008). Affected and non-affected sharks exhibited comparable plastic strength, suggesting some degree of similarity in post-yield behavior perhaps because of the ability of the unmineralized phase of the vertebrae to dampen fracture energy from the mineralized phase, as has been observed in viscoelastic composites in which the constituent materials differ greatly in stiffness and damping capacity (Chen and Lakes, 1993; Brodt and Lakes, 1995). Tessellated elasmobranch cartilage (mineralized cartilage surrounding unmineralized matrix) is a viscoelastic composite bearing such properties and likely behaves in this manner. However, the material properties of the individual mineralized and unmineralized phases of areolar cartilage in elasmobranch vertebral centra are unknown and the extent to which areolar cartilage behaves as other viscoelastic composites is speculative (Dean and Summers, 2006; Summers and Long, 2006; Dean et al., 2009b). Regardless, these data suggest that there is a continuum of material properties in the vertebrae of *C. taurus* in public aquaria and that individuals with lesser material properties are at greater risk for developing spinal deformity. Furthermore, the stiffness, strength and work of fracture of all *C. taurus* vertebrae from individuals in public aquaria are lower than those of most bony vertebrate skeletal elements, indicating that special care must be taken in the husbandry of this and other cartilaginous fishes (Currey, 2002).

While affected *C. taurus* vertebral centra had lower mineral content than those of non-affected sharks, mineral content was barely

correlated with material properties. Significant, though sometimes weak, relationships have been identified between mineral content and stiffness in shark vertebrae and numerous other vertebrate endoskeletal materials (Currey, 2002; Currey, 2008; Porter et al., 2006; Porter et al., 2007). The lack of predictability between mineral content and material properties in this study may be associated with vitamin C and zinc deficiencies observed in *C. taurus* from public aquaria, as these metabolites affect both the quantity and quality of collagen and mineralization in skeletal elements (Anderson et al., 2012). Vitamin C is an enzymatic cofactor involved in the cross-linking of polypeptide subunits into tropocollagen triple helices, which are subsequently bundled into collagen fibrils and fibers that serve as the scaffold for deposition and orientation of mineral in connective tissues (Hulmes, 2002; Holmes and Kadler, 2006; Lall and Lewis-McCrea, 2007); the orientation of mineral can affect stiffness, as seen in the anisotropic behavior of bone (Currey, 2002; Currey, 2008). Zinc is an enzymatic cofactor that supports the activity of collagenase and numerous metalloenzymes (e.g. alkaline phosphatase, tyrosine phosphatase), thereby affecting the maintenance of the collagen scaffold of connective tissues and the mineralization process (Starcher et al., 1980; Yamaguchi and Fukagawa, 2005). Consequently, the quantity and orientation of collagen and mineral, and degree of collagen cross-linking, can affect the strength and stiffness of skeletal elements (Boskey et al., 1999; Rath et al., 2000; Porter et al., 2006; Currey, 2008); greater work of fracture in non-affected vertebral centra may be related to the biochemical integrity of the organic phase for these reasons. As a result, the typical relationship between mineral content and material

Table 2. Results of principal components analysis on material properties of vertebral centra of sand tiger sharks (*Carcharias taurus*) from public aquaria

	Principal component		
	1	2	3
Eigenvalue	2.8550	2.1676	1.1487
Percent variation	35.6878	27.0956	14.3582
Stiffness	-0.22621	0.51343	0.30038
Yield strength	-0.05932	0.65580	-0.03165
Yield strain	0.18330	0.25221	-0.74022
Ultimate strength	0.34130	0.40608	0.33733
Ultimate strain	0.50670	0.00458	-0.27925
Plastic strength	0.40786	-0.24189	0.34462
Work of fracture	0.51147	0.13823	0.02643
Mineral %	0.33245	-0.01246	0.22273

Bold values indicate those that loaded heavily on a given principal component.

Table 3. Material properties and mineral content of elasmobranch vertebrae

Species		Mineral content (%)	<i>E</i> (MPa)	ϵ_{yield}	σ_{ult} (MPa)
<i>Carcharias taurus</i> (affected)	Sand tiger shark	31	144	0.11	15
<i>Carcharias taurus</i> (non-affected)	Sand tiger shark	33	167	0.14	16
<i>Carcharhinus falciformis</i>	Silky shark	49	564	0.10	24
<i>Carcharhinus plumbeus</i>	Sandbar shark	57	370	0.15	24
<i>Centrophorus granulosus</i>	Gulper shark	55	426	0.10	21
<i>Isurus oxyrinchus</i>	Shortfin mako shark	39	330	0.12	12
<i>Mustelus californicus</i>	Smooth-hound shark	50	598		52
<i>Sphyrna zygaena</i>	Smooth hammerhead shark	49	523	0.13	24
<i>Torpedo californica</i>	Pacific electric ray	39	26	0.22	5

E, stiffness; ϵ_{yield} , yield strain; σ_{ult} , ultimate strength. Data from species other than the sand tiger shark *Carcharias taurus* are from Porter et al. (Porter et al., 2006; Porter et al., 2007).

properties may be compromised in organisms suffering from nutritional deficiencies that affect skeletal homeostasis. Perhaps further research into the biochemistry and mechanics of chondrichthyan cartilage will determine whether the lack of correspondence between mineral content and material properties is ubiquitous or unique to the current investigation (Dean, 2011).

Deficiencies in vitamin C and zinc cause a variety of connective tissue disorders including spinal curvature, vertebral deformities and reduced vertebral mineral content in scorbutic teleosts (Lim and Lovell, 1978; Silverstone and Hammell, 2002; Lall and Lewis-McCrea, 2007) and delayed maturation, defective mineralization and malformed bones in zinc-deficient rhesus monkeys, cattle and chickens (Hidiroglou, 1980; Starcher et al., 1980; Leek et al., 1984). These pathologies correspond with the gross anatomy of spinal deformity in *C. taurus*, as well as the quantity and variation in mineral content of their vertebrae. *Carcharias taurus* in public aquaria have the lowest vertebral mineral content of any species of cartilaginous fish that has been investigated (Table 3) and intra-individual variability in mineral content was considerable in both affected (30%) and non-affected (25%) sharks. Considerable intra-individual variability in vertebral mineral content has been identified in other species of sharks, although average intra-specific variability is generally lower. The greatest intra-specific variability found in one comparative study was 14% for the smooth-hound shark *Mustelus californicus* (Porter et al., 2007).

Additional risk factors associated with spinal deformity among *C. taurus* in public aquaria include size at capture, capture method and aquarium size. The average size at capture of affected *C. taurus* is 122 cm TL, which corresponds to approximately 1 year of age (Goldman et al., 2006), and those sharks captured *via* net instead of hook-and-line are five times more likely to develop spinal deformity in aquaria. Fortunately, only 8% of *C. taurus* caught *via* hook-and-line develop these deformities (Anderson et al., 2012). It is likely that the spines of young sharks are less able to withstand the mechanical stress of capture, perhaps indicating ontogenetic variation in the mineralization of vertebrae, and that this fragility is exacerbated by the stress of being hauled out of the water by net. The capture process may induce subclinical trauma that is subsequently compounded by nutritional deficiencies (described above) and altered locomotive behavior in aquaria. *Carcharias taurus* in public aquaria spend nearly 100% of their time engaged in powered, asymmetrical swimming (turning as opposed to linear locomotion) because of the spatial constraints of aquaria, whereas wild sharks alternate between nearly equal periods of powered swimming and gliding and have no constraints on linear locomotion (Klay, 1977; Powell et al., 2004; Tate et al., 2013). Incidence of spinal deformity is higher

in smaller aquaria, in which turning radii are reduced (Tate et al., 2013). We therefore hypothesize that spinal fractures originate on the side of the vertebral column to which *C. taurus* constantly turns (compressive side), causing the fractured vertebrae to slip towards and possibly rupture outward from the opposite (tensile) side of the spinal column. Behavioral data were provided for only two of the affected sharks in this study, both of which corroborate this idea (see Materials and methods, Specimen acquisition, Survey Stage II). Injury sustained *via* repetitive asymmetrical behavior has also been observed in thoroughbred racehorses, which run in the counter-clockwise direction and are more likely to develop dorsal metacarpal disease in their left forelimbs (Palmer, 2002). Unfortunately, without data from wild-caught *C. taurus* it is impossible to determine whether deficiencies in material properties predispose certain individuals to spinal deformity or whether these mechanical deficiencies are a product of any of the above-mentioned factors in the aquarium environment.

Elasmobranch skeletal materials

Vertebral centra of *C. taurus* from public aquaria have comparable yield strain but lower stiffness and ultimate strength than nearly all other elasmobranch species that have been investigated (Porter et al., 2006; Porter et al., 2007) (Table 3). It must be noted that these other elasmobranch species were wild-caught and that inclusion of data from wild-caught *C. taurus* may paint a wholly different picture of this species' mechanical properties. A reasonably strong relationship between mineral content and stiffness was found when including multiple species (Fig. 4), consistent with interspecific studies of bone (Currey, 2002), despite the lack of a relationship among *C. taurus* from public aquaria. This hints at differences in intra-individual, intra-specific and inter-specific variability in mineral content alluded to in the previous section. In effect, elasmobranch mineralized cartilage has greater variability in mineralization, and greater variability in stiffness at any level of mineralization, than bone (Porter et al., 2007). Vertebral centra of *C. taurus* from public aquaria were also unique in that they exhibited a positive, albeit non-significant, relationship between mineral content and ultimate strength (Fig. 4), their ultimate strength was considerably greater than their yield stress (i.e. the vertebrae exhibited 'plastic strength'), and greater stiffness, toughness and mineral content all co-occurred in vertebral centra from non-affected *C. taurus*. Conversely, bony structures tend to have higher mineral content (~59–96%), exhibit negative relationships between mineral content and strength, mineral content and toughness, and stiffness and toughness, and have near equivalence of yield stress and ultimate stress (Currey, 1999; Currey, 2002; Wegst and Ashby,

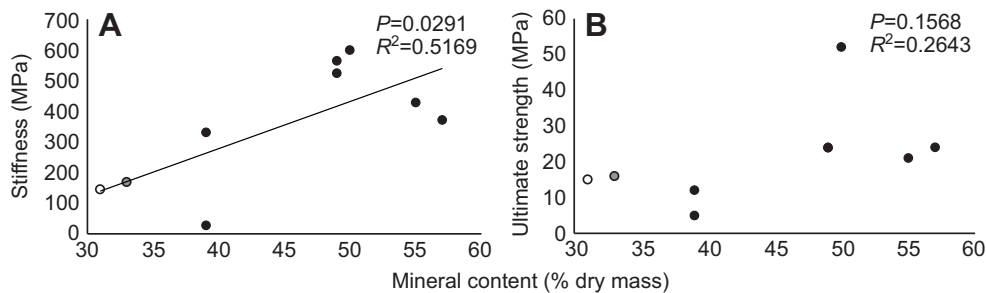


Fig. 4. (A) Vertebral stiffness (MPa) versus mineral content (% dry mass) and (B) ultimate strength (MPa) versus mineral content (% dry mass) for eight species of chondrichthyan fishes (circles), including affected (open circles) and non-affected (shaded circles) *Carcharias taurus*. Data for species other than the sand tiger shark are from Porter et al. (Porter et al., 2006; Porter et al., 2007). See Table 3 for mineral content, stiffness and ultimate strength data.

2004; Meyers et al., 2008). These findings corroborate the assertion that the composite nature (paired mineralized and unmineralized phases) and comparatively lower mineral content of some elasmobranch skeletal elements yield emergent mechanical properties that would not otherwise be possible in more densely mineralized structures such as bone, where the inorganic phase dominates (Dean et al., 2009a; Dean et al., 2009b; Liu et al., 2010; Macesic and Summers, 2012). Perhaps this unique pairing of properties is because the unmineralized phase dampens fracture energy from the mineralized phase, possibly preventing microfractures from coalescing (Chen and Lakes, 1993; Brodt and Lakes, 1995; Hansma et al., 2005; Dunlop et al., 2011).

Elasmobranch vertebrae (stiffness=26–598 MPa) are at the low end of the continuum of skeletal material properties in the cartilaginous fishes (Porter et al., 2006; Porter et al., 2007). Unmineralized jaw cartilage has a stiffness of 29–56 MPa, whereas its mineralized counterpart has a stiffness of 4050 MPa (Summers and Long, 2006; Wroe et al., 2008; Jagnandan and Huber, 2010). The dentine and enameloid of shark teeth have stiffnesses ranging from 23,000 to 28,000 MPa and from 69,000 to 76,000 MPa, respectively (Whitenack et al., 2010). Shark vertebrae are outperformed by cortical bone in stiffness (3200–46,000 MPa), yield strength (131–272 MPa) and ultimate strength (27–271 MPa), but are capable of tolerating much greater strain (ultimate strain=0.2–11.4% for cortical bone). Lastly, shark vertebrae overlap the range of stiffness for calcified cartilage from the mammalian epiphyseal plate (0.1–5700 MPa) and cancellous bone (4–7000 MPa) (Hodgkinson and Currey, 1990; Mente and Lewis, 1994; Currey, 2002; Laasanen et al., 2003; Kemp et al., 2005). Although shark vertebrae behave similarly to these latter tissues, it must be noted that tests of shark vertebrae involve whole structures with multiple materials, whereas the aforementioned data are based on individual skeletal materials.

A significant issue regarding the material properties of and damage to cartilaginous tissues is the extent to which these tissues are capable of healing. Mature articular cartilage in bony vertebrates does not heal, although epiphyseal plate cartilage does exhibit remodeling if tissue trauma occurs early in development (Hall, 2005). Ashhurst (Ashhurst, 2004) found that experimental lesions of pectoral fin rays in dogfish (*Scyliorhinus* spp.) produced a fibrous matrix but that no integration of new and old tissues occurred. Porter et al. (Porter et al., 2006) anecdotally reported spondylosis as evidence of healing, although the tissue structure was not investigated to determine whether newly deposited mineral was integrated into the existing tissue. Preziosi et al. (Preziosi et al., 2006) found that deformed *C. taurus* vertebrae exhibited disorganized proliferation of hypertrophied chondrocytes and extensive mineralization both within and peripheral to the matrix of the original vertebrae and concluded, like Ashhurst (Ashhurst, 2004), that the tissue did not heal. The extensive idiopathic

mineralization found in the vertebrae of *C. taurus* with spinal deformity suggests that these sharks are capable of callus formation, similar to the bones of osteichthyan vertebrates, but lack the subsequent remodeling phase of the healing process (Ashhurst, 2004; Hall, 2005). Given that the areolar cartilage of elasmobranch vertebrae has lower performance limits (i.e. yield strength, ultimate strength) than most other vertebrate skeletal materials and that this cartilage apparently has limited healing ability, great care must be taken in the husbandry of cartilaginous fishes, and further research into the healing of chondrichthyan cartilage is warranted.

Conclusions

The emergent picture of spinal deformity in *C. taurus* from public aquaria is one of multifactorial syndrome origin. Subclinical trauma induced during capture, irregular swimming behavior and nutritional deficiencies, and decreased material stiffness, yield and failure properties are all associated with spinal deformity. Without material properties data from wild-caught *C. taurus* it is impossible to know whether pre-existing low material performance contributes to capture-induced trauma or is the result of the aforementioned nutritional deficiencies. Given the considerable intra-individual variability in the current data set, it is likely that spinal deformity in *C. taurus* from public aquaria originates *via* a weakest link scenario: a single mechanically deficient vertebrae is damaged early in life and unique aspects of aquarium husbandry may then promote the development of pathologies associated with the disorder. Thus, it is possible that non-affected sharks are candidates for spinal deformity given the right set of stressors. Owing to the prevalence of this disorder and imperiled conservation status of *C. taurus*, we encourage public aquaria housing this species to consider the mechanical properties of the spine in their husbandry procedures, as well as other recently published husbandry recommendations (Anderson et al., 2012; Tate et al., 2013). Collectively, these precautions may ensure the health of *C. taurus* in public aquaria and reduce pressure on wild stocks for exhibit specimens.

ACKNOWLEDGEMENTS

This work is dedicated to the memory of Geri Rau for inspiring a spirit of discovery and nurturing all fascinations, no matter how bizarre. We would like to thank the many people and institutions that made this study possible. Aquaria that contributed spinal sections for biomechanical analysis include the Adventure Aquarium (Camden, NJ, USA), Downtown Aquarium (Denver, Colorado, USA), Florida Aquarium (Tampa, FL, USA), Henry Doorly Zoo and Aquarium (Omaha, NE, USA), Moody Gardens Aquarium Pyramid (Galveston, TX, USA), Mystic Aquarium (Mystic, CT, USA), New York Aquarium (New York, NY, USA), Ripley's Aquarium (Myrtle Beach, SC, USA), SeaWorld Orlando Theme Park (Orlando, FL, USA), South Carolina Aquarium (Charleston, SC, USA), Tennessee Aquarium (Chattanooga, TN, USA) and Underwater Adventures Aquarium (Bloomington, MN, USA). N. Crane, T. Gage, W. Frusher, S. Bhansali and P. Motta of the University of South Florida, and P. Gignac and R. Walsh of Florida State University were instrumental in the collection of material properties data. S. Coy, K. Heym and P. Holmes of The Florida Aquarium (Tampa, FL, USA) performed and developed radiographs. M. Melendez of University Diagnostic Institute (Tampa, FL, USA) facilitated CT scans used in the generation of Fig. 3.

AUTHOR CONTRIBUTIONS

D.R.H., P.A.A. and I.K.B. are responsible for the conception and design of this study. D.R.H., D.E.N., C.M.S., P.A.A. and I.K.B. are responsible for its execution, interpretation, drafting and revision.

COMPETING INTERESTS

No competing interests declared.

FUNDING

Funding for this study was provided by a grant awarded to I.K.B., P.A.A. and D.R.H. from the Association of Zoos and Aquariums Conservation Endowment Fund and Disney Worldwide Conservation Fund (grant no. 08-856), a grant awarded to I.K.B. from the Jacarlene Foundation, a grant awarded to P.A.A. from The Bernice Barbour Foundation, and a research fellowship awarded to D.E.N. from The University of Tampa College of Natural and Health Sciences. P.A.A. was supported by charitable contributions from The Spurlino Foundation and an anonymous donor.

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