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THE MORPHOLOGY AND BIOMECHANICS OF JAW STRUCTURES IN
CHONDRICHTHYES

BY
JORDAN BALABAN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
BIOLOGICAL AND ENVIRONMENTAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2013

MASTER OF SCIENCE THESIS
OF
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2013

ABSTRACT

The skeletons of chondrichthyans (sharks, skates, rays, and chimeras) are composed entirely of cartilage, yet must still provide the skeletal support that bone does in other vertebrates. There is also an incredible range of diversity in the morphology of the cartilaginous skeleton of the feeding apparatus in Chondrichthyans. The goal of this research is to provide insight into the morphological evolution and biomechanical function of the cranial skeleton in chondrichthyans. Feeding style changes can occur with morphological changes in the skeletal elements of the shark feeding apparatus. In chapter one, to increase our understanding of how the feeding skeletal morphology has evolved with the of feeding style of sharks, the length, width, and angles of the elements of the feeding apparatus are measured in four species (white-spotted bamboo, *Chiloscyllium plagiosum*; spiny dogfish, *Squalus acanthias*; sandbar, *Carcharhinus plumbeus*; and dusky smoothhound, *Mustelus canis*). These species encompass a wide phylogenetic range, and include suction and bite feeders as well as two different orientations of the hyomandibula, the major jaw supporting element. A principle components analysis is used to identify relationships among the skeletal elements by species, and linear regressions are then used to test the effect of hyomandibula length on the other morphological variables. Strong relationships were discovered between the length of the hyomandibula and the lengths of all other skeletal elements and the angle of the hyomandibula. The bite feeders have longer elements and appear to maximize the size of the oral cavity, allowing larger prey to be swallowed. Suction feeders have shorter elements, which restrict the size of the oral cavity and mouth opening, but can concentrate suction forces. Based on the strong relationship between hyomandibula

length and angle on feeding morphology, the mechanical properties of the hyomandibular cartilages in the same four shark species is investigated in chapter two. Young's modulus, a measure of stiffness, and Poisson's ratio, a measure of three-dimensional shape change, of the hyomandibular cartilages are compared. While Poisson's ratio is similar among the species, Young's modulus increases with mineralization and is larger in the suction feeders. Though sharks have a cartilaginous skeleton, some species have higher mineralization of elements that are under higher stress.

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I thank my advisor, Dr. Cheryl Wilga for her generous guidance, support and patience throughout my time at URI. I would also like to thank my committee members Dr. Adam Summers, for his guidance on the mechanical properties chapter, and Dr. Holly Dunsworth for useful conversations in planning my morphology chapter. I thank Dr. Frederick Vetter, my committee chair, for help with the planning of the mechanical properties study. Thank you to Isabel Nowinowski and Mark Billard for help in collecting data. Thank you to my labmates Jason Ramsay, Rachel Nichols, Anabela Maia, and Stacey Sakai for their friendship and support. A special thanks goes to Jason Ramsay for generous conversations on the morphology chapter. To family and friends, thank you for support and encouragement.

PREFACE

This thesis is presented in manuscript format

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CHAPTER 1

“Morphology of the Feeding Apparatus in Four Shark Species”

by

Jordan P. Balaban^{1*}, Adam P. Summers², and Cheryl Wilga¹

prepared for submission to the Journal of Experimental Biology

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Abstract

Sharks have an incredible diversity of feeding mechanisms for a group with so few extant species. To understand the relationship between the morphology of the shark feeding apparatus and feeding style, length, width, and angle of the ten skeletal elements in the feeding apparatus are measured in four shark species (white spotted bamboo, *Chiloscyllium plagiosum*; spiny dogfish, *Squalus acanthias*; sandbar, *Carcharhinus plumbeus*; and dusky smoothhound, *Mustelus canis*). These species encompass a wide phylogenetic range, and include suction and bite feeders as well as two different orientations of the hyomandibula, the primary jaw support element. A principle components analysis is used to identify relationships among the skeletal elements by species, and linear regressions are then used to test the effect of hyomandibula length on the other morphological variables. Strong relationships were discovered between the length of the hyomandibula and the lengths of all other skeletal elements and the angle of the hyomandibula. Bite feeders have longer elements and appear to maximize the size of the oral cavity, allowing larger prey to be swallowed. Suction feeders have shorter elements, which restrict the size of the oral cavity and mouth opening, but can concentrate suction forces. Significant regressions of all skeletal elements may be of use for future paleontological studies.

Introduction

Sharks are known as effective predators, yet they have a relatively simple feeding apparatus compared to bony fishes (Motta & Huber 2012). The ten skeletal elements (Fig. 1) of the feeding apparatus are: paired palatoquadrate cartilages (upper jaw); paired Meckel's cartilages (lower jaw); the five elements of the hyoid arch (paired

Figure 1. Skeletal anatomy of the feeding apparatus of a lemon shark. BH-basihyal, CH-ceratohyal, CP-craniopalatine ligament, CR-chondrocranium, HY-hyomandibula, MA-Meckel's cartilage, PQ-palatoquadrate (after Motta and Wilga 1995).

hyomandibular cartilages dorsally, paired ceratohyal cartilages ventrally, and the single medial basihyal cartilage), and the chondrocranium (Gregory 1904, Wilga 2002, Wilga et al. 2011).

Despite this seeming morphological simplicity, sharks have an incredible diversity of feeding mechanisms, especially considering there are fewer than 500 extant species (Wilga 2008, Dean et al. 2004, 2005, Frazzetta 1988, Huber et al. 2005, Moss 1977, Motta & Wilga 1995, Motta & Wilga

2001).

Shark jaws are not fused to the cranium, and can protrude away from the cranium during a feeding event (Gregory 1904, Maisey 1980, Wilga 2001). The jaws are suspended posteriorly from the cranium by the hyomandibular cartilages, which articulate with the lower jaw, and by one to three anterior craniopalatine ligaments that connect the upper jaw with the cranium (Gregory 1904, Maisey 1980, Wilga 2008, Fig. 1). At rest, the hyomandibula projects laterally from the cranium, and may be oriented directly

Figure 2. Partial elasmobranch phylogeny including the orders of the species used in this study: bamboo, *C. plagiosum* (Orectolobiformes); sandbar, *C. plumbeus* and smoothhound, *M. canis* (Carcharhiniformes); and dogfish, *S. acanthias* (Squaliformes). L-lateral HY orientation, P-posterior HY orientation, A-anterior HY orientation. (Modified from Nelson 2006, Wilga 2008)

lateral, posterolateral, or anterolateral in different taxonomic groups (Moss 1977, Wilga

2008, Fig. 2). The distal tips of laterally oriented hyomandibulae are at a maximal

distance from the cranium at rest, and are moved medially as they swing ventrally during

jaw opening (Wilga 2008, 2010). Shark species in the orders Heterodontiformes,

Orectolobiformes and Squaliformes have short, laterally oriented hyomandibulae (Wilga

2008). Anteriorly oriented hyomandibulae are found only in batoids (skates and rays)

(Fig. 2, Wilga 2008). The ceratohyal and basihyal are associated with the branchial

arches or are absent in batoids (Miyake and McEachran, 1991), therefore are not included

in this study. Posteriorly oriented hyomandibulae are found in lamniform and

carcharhiniform sharks (Moss, 1977). Posteriorly oriented hyomandibulae are long and

swing ventrolaterally, which increases the width of the oral cavity, allowing large prey to

pass through with minimal obstruction (Moss 1977, Wilga 2008). Suction feeding sharks have short jaws with short, laterally oriented hyomandibulae, while bite feeding sharks have long jaws with long, posteriorly oriented hyomandibulae (Moss 1977, Wilga 2008).

The ceratohyal cartilages are medial to Meckel's cartilages and articulate with the hyomandibulae proximally and the basihyal distally. The coracohyoideus and coracoarcualis muscles depress the basihyal posteroventrally, which in turn depresses the ceratohyal posteroventrally (Marion 1905, Motta et al., 1997; Wilga and Motta 1998, 2000). In a study of 19 shark species, the ceratohyal of suction feeding sharks had a higher second moment of area (a measure of resistance to bending) than ram feeders (Tomita et al. 2011). The coracohyoideus and coracoarcualis muscles of suction feeders generate large forces to rapidly rotate the ceratohyal posteroventrally, expanding the oral cavity, which creates a large negative pressure to pull prey into the mouth. These forces place bending stress on the ceratohyal, and the large second moment of area helps to resist the forces. Aspect ratio (length/width) of an element may indicate the resistance to bending; ratios greater than 20:1 have greater bending and shear deformation (Spatz et al. 1996).

Basic morphological relationships among the elements of the shark feeding apparatus can provide a greater understanding into how a group of organisms with so few skeletal elements has evolved such functional diversity. To this end, the morphology of the skeletal elements involved in the feeding apparatus is quantified in four species of sharks to identify relationships in length, width, and hyomandibula angle among species with different feeding methods (bite, suction), hyomandibular cartilage orientation (lateral, posterior), and phylogenetic position. Several hypotheses will be addressed. 1)

The length of the hyomandibulae will be shorter (relative to cranial length) in suction feeding species with lateral hyomandibula orientation. 2) The length of the hyomandibula will vary in direct proportion with the length of the jaws, the length of the ceratohyal, and the angle of the hyomandibula. 3) The width of the basihyal and the intracranial distance between the hyomandibula articulations will vary in proportion with the other morphological variables and be larger in bite feeders (increasing mouth width for swallowing large prey items). 4) The aspect ratio (length/width) of the ceratohyal will be larger in suction feeders than bite feeders.

Methods

Species

Four species of sharks were used in this study: white-spotted bamboo, *Chiloscyllium plagiosum* (N=5); spiny dogfish, *Squalus acanthias* (N=6); sandbar, *Carcharhinus plumbeus* (N=7); and dusky smoothhound, *Mustelus canis* (N=7). These species encompass bite (sandbar and smoothhound) and suction (bamboo and dogfish) prey capture styles, posterior (sandbar and smoothhound) and lateral (bamboo and dogfish) hyomandibula orientations, and range throughout the elasmobranch phylogeny (Wilga 2008, Fig. 2). All measurements were taken from previously frozen animals.

Morphology

The following measurements were taken for each shark using either dial calipers or by taking digital images and taking measurements using the software program ImageJ (National Institutes of Health, Bethesda, MD, USA). The length of the hyomandibula

(HY), ceratohyal (CH), basihyal (BH), palatoquadrate (PQ), Meckel's cartilage (MC), and cranium (from the anterior nasal capsule to the posterior otic capsule) (CL), as well as the width of the HY CH, BH, and cranial distance between the left and right hyomandibular articulations (ICR) were measured. All measurements were taken from the left side of the shark. HY orientation was measured as the anterior angle between the midline of the longitudinal axis of the head and the midline of the longitudinal axis of the HY. HY angle was measured using a protractor or by taking digital pictures and using the angle tool in the software program ImageJ (National Institutes of Health, Bethesda, MD, USA). Aspect ratios of the HY, CH, and BH were calculated as the length divided by the width of each element.

Statistics

A principal components analysis was run to test for variation among the species. The following morphological measurements were included for analysis: length of the HY, CH, BH, MC, PQ, and ICR normalized to cranial length; aspect ratio of the HY, CH, and BH; and HY angle. Linear regressions were then run to test for the relationships between the morphological variables, using the normalized and non-normalized lengths of the HY, CH, BH, MC, PQ, and ICR, as well as the HY angle and aspect ratios of the HY, CH, and BH. To test hypotheses one and four ANOVAs were run to test for differences in CH aspect ratio and normalized HY length among species.

Results

Principal components one and two account for 86% of the variance among the

variables, while PC3 contributes 8% of total variance. All four species cluster in different areas of morphospace in a plot of PC1 by PC2 (Fig. 3). Since all length measurements were normalized to cranial length (CL), size was not a factor for any of the axes.

Figure 3. Plot of the first and second principle components

Ceratohyal (CH) and hyomandibula (HY) aspect ratios are the only variables that load negatively on PC1 (Table 1), while all other variables load positively and are close together. *Chiloscyllium plagiosum* and *S. acanthias* load highly negative on PC1. *M. canis* loads closer to zero on the positive side of PC1 while *C. plumbeus* loads highly positive. Basihyal (BH) length loads highly negative on PC2, while BH and HY aspect ratios load highly positive, and all other variables load close to zero (Table 1). *C. plagiosum* and *M. canis* overlap on PC2, and they range from loading close to zero

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Variable	PC1	PC2	PC3
BH width	0.193	-0.647	0.466
BH aspect ratio	0.318	0.400	-0.116
CH length	0.366	-0.018	-0.032
CH aspect ratio	-0.306	0.007	0.401
HY length	0.342	-0.078	0.265
HY aspect ratio	-0.159	0.609	0.605
PQ length	0.357	0.127	0.111
MC length	0.351	0.103	0.176
ICR	0.349	0.006	0.230
HY angle	0.346	0.133	-0.274
Eigenvalues	7.335	1.238	0.756
% variance explained	73	12	8

Correlation matrix

	HY angle	ICR	MC length	PQ length	HY AR	HY length	CH AR	CH length	BH AR
BH width	0.304	0.551	0.457	0.439	-0.447	0.602	-0.373	0.534	0.076
BH AR	0.870	0.790	0.845	0.905	-0.163	0.760	-0.687	0.851	
CH length	0.939	0.934	0.913	0.949	-0.441	0.894	0.838		
CH AR	-0.880	-0.691	-0.727	-0.726	0.428	0.616			
HY length	0.791	0.894	0.905	0.918	-0.382				
HY AR	-0.400	-0.314	-0.269	-0.290					
PQ length	0.905	0.913	0.936						
MC length	0.853	0.940							
ICR	0.831								

Table 1. Component loadings and correlation coefficients generated by PCA on morphological variables. AR- aspect ratio, Inter CR- distance on cranium between HY articulations.

up to highly positive. *S. acanthias* and *C. plumbeus* also overlap on PC2, though *S. acanthias* ranges from slightly to highly negative, while *M. canis* ranges from around zero to highly negative.

Figure 4.

Linear regressions of the HY length normalized to cranial length to:
a) other normalized lengths, and b) HY angle.

Most of the variation in the lengths of all elements and in the angle of the HY (HYA) is explained by the length of the hyomandibula (HY). The linear regressions of the length normalized HY to all other lengths are as follows: $[HY = 0.0628 + (0.603 * ICR), (R^2 = 0.82, p < 0.001)]$, $[HY = 0.0117 + (0.436 * MC), (R^2 = 0.85, p < 0.001)]$, $[HY = -0.00362 + (0.429 * PQ), (R^2 = 0.84, p < 0.001)]$, $[HY = -0.0830 + (1.171 * BH), (R^2 = 0.38, p = 0.001)]$, $[HY = -0.00452 + (0.632 * CH), (R^2 = 0.78, p < 0.001)]$, and $[HY = 0.0869 + (0.00212 * HYA), (R^2 = 0.67, p < 0.001)]$ (Fig. 4).

The width of the basihyal (BH) explains some of the variation in the lengths of the other elements, but not in the angle of the hyomandibula (Linear regressions: HY $[HY = -0.0830 + (1.171 * BH), (R^2 = 0.38, p = 0.001)]$, $[BH = 0.259 + (0.180 * ICR), (R^2 = 0.27, p = 0.010)]$, $[BH = 0.254 + (0.116 * MC), (R^2 = 0.220, p = 0.02)]$, $[BH = 0.253 + (0.110 * PQ), (R^2 = 0.20, p = 0.028)]$, $[BH = 0.239 + (0.189 * CH), (R^2 = 0.25, p = 0.012)]$. However, much of the variation in the lengths of all elements, and the angle of the hyomandibula, can be explained by the intracranial distance between hyomandibula articulations (ICR) (Linear regressions: $[ICR = -0.0523 + (0.674 * MC), (R^2 = 0.9, p < 0.001)]$, $[ICR = -0.0690 + (0.654 * PQ), (R^2 = 0.87, p < 0.001)]$, $[ICR = 0.0382 + (0.00342 * HY \text{ angle}), (R^2 = 0.72, p < 0.001)]$, $[CH = 0.132 + (0.888 * ICR), (R^2 = 0.88, p < 0.001)]$, and $[HY = 0.0628 + (0.603 * ICR), (R^2 = 0.82, p < 0.001)]$).

Normalized HY lengths were different among species (ANOVA, $F=49.77$, $p < 0.001$). *C. plumbeus* was the largest (0.392 ± 0.001) followed by *M. Canis* ($0.288 \pm < 0.001$), *C. plagiosum* ($0.265 \pm < 0.001$), and *S. acanthias* ($0.252 \pm < 0.001$) (the latter three are not different from one another). The aspect ratio of the CH (CHAR) is different among the species (ANOVA, $F=22.59$, $p < 0.001$). *C. plagiosum* has the lowest CHAR

(4.38 ± 0.24). The next lowest CHAR is in *S. acanthias* (5.33 ± 0.24). The CHAR of *M. canis* and *C. plumbeus* were not different from one another but were lower than the other two species at 5.94 ± 0.16 and 6.11 ± 0.01 , respectively.

Discussion

The length of the hyomandibula accounts for most of the variation in the skeletal morphology of the shark feeding apparatus. Bite capturers (*C. plumbeus* and *M. canis*) have longer elements relative to head length, than the suction capturing *C. plagiosum* or the intermediate capturing *S. acanthias*. These longer elements have been noted previously and likely allow for larger bites (Moss 1977, Wilga 2008). Bite feeders also have posteriorly oriented (larger angle) hyomandibulae, which are longer because of the orientation (Fig. 2). The ceratohyal cartilage, which is medial to and parallels the lower jaw, necessarily lengthens with the jaws.

The length of the hyomandibula explained a significant amount of variation in the width of the basihyal and the intracranial width between the hyomandibula articulations (ICR). Although the regression of the hyomandibula to the basihyal was significant, the R^2 values were low (0.38), indicating that much of the variance in the width of the basihyal is due to some factor other than the length of the hyomandibula. However, much of the variance in the intracranial width between hyomandibula articulations was due to the length of the hyomandibula ($R^2 = 0.82$). These are likely functionally coupled and relate to feeding style. Bite feeding sharks have long, posteriorly oriented hyomandibulae and long jaws to help bite and swallow large prey items (Moss 1977, Wilga 2008, this study). A relatively wider cranium and basihyal would create a wider gape to allow larger

prey to be consumed. Conversely, suction feeders have short, laterally oriented hyomandibulae with short jaws that are occluded laterally during suction, and that create a small mouth opening that can concentrate suction pressure (Moss 1977, Wilga 2008).

The aspect ratio of the ceratohyal cartilages is lower in the two species that can use suction to capture (*C. plagiosum* and *S. acanthias*) than those that always bite capture (*C. plumbeus* and *M. canis*) ($F=22.59$, $p<0.001$). In a previous study of 19 shark species, suction feeders were found to have ceratohyal cartilages that are more resistant to bending than sharks with other feeding styles (Tomita et al. 2011). Although this study uses a different metric (aspect ratio rather than second moment of area), the ceratohyal cartilages with lower aspect ratios (shorter and thicker) were found in the suction feeding species. Bending forces on short, squat elements also shear the elements (Spatz et al. 1996), and low aspect ratio ceratohyals resist bending forces better than the relatively gracile higher aspect ratio ceratohyals of bite feeders.

Most of the elements in the species studied here have significant linear relationships to one another. This indicates that variation in any of the morphological variables measured here will be reflected in most of the other variables. This is potentially significant for paleontological research because other than teeth, fossil data from sharks is limited because cartilage does not fossilize well (Shimada 2005). It is difficult to formulate hypotheses about the feeding behaviors of extinct species without complete specimens, which are rare. However, with fossilized cranial, hyomandibular, ceratohyal, palatoquadrate, or Meckel's cartilages, the lengths of the other cranial elements may be predicted using the regression equations. A complete list of regressions on the morphological data can be found in appendix A.

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CHAPTER 2

“Mechanical Properties of the Hyomandibula Varies by Feeding Style in Four Shark Species”

by

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Abstract

The skeletons of chondrichthyans (sharks, skates, rays, and chimeras) are composed entirely of cartilage, yet must still provide the skeletal support that bone does in other aquatic vertebrates. Understanding the mechanical properties of shark cartilage will provide insight on how sharks have survived for hundreds of millions of years with a skeleton that cannot heal, and that is composed of considerably less stiff material than bone. Mechanical properties were measured in the hyomandibular cartilage, the primary jaw supporting element, of four species of sharks (white-spotted bamboo, *Chiloscyllium plagiosum*; spiny dogfish, *Squalus acanthias*; sandbar, *Carcharhinus plumbeus*; and dusky smoothhound, *Mustelus canis*). These species encompass a wide phylogenetic range, and include suction and bite feeders as well as two different orientations of the hyomandibula. Stiffness and three-dimensional shape change are compared using Young's modulus and Poisson's ratio. Although Poisson's ratio is similar among the species, Young's modulus increases with mineralization and is higher in the specialized suction feeder. Though sharks have a cartilaginous skeleton, some species have higher mineralization of elements that are under higher stress.

Introduction

The skeletons of chondrichthyans (sharks, skates, rays, and chimeras) are composed entirely of cartilage and must provide the stiff skeletal support that bone does in other vertebrates. Bone has two main advantages over cartilage: it can remodel and repair. In remodeling, the cortical layer thickens in areas of high stress or trabecular rods are created in the planes where force is applied to reinforce bone strength (Thompson, 1917; Goldstein, 1987). Though cartilage cannot remodel or repair (Kemp & Westrin, 1979), shark jaws must resist substantial forces generated during feeding. The chondrichthyan jaw is strengthened by the uniquely layered composition of the cartilage. In the non-vertebral skeleton of chondrichthyans, an inner core of unmineralized hyaline cartilage is surrounded externally by a mosaic of mineralized hexagonal tiles called tesserae (Kemp & Westrin, 1979; Dean and Summers, 2006). In tessellated cartilage, the thickness of the tesseral layer can lead to differences in the functional properties of different skeletal elements (Summers 2000).

The vertebral centrum of sharks has areolar calcification, which forms in concentric rings and permeates the entire cartilaginous structure (Moss 1977, Dean & Summers, 2006, Porter et al. 2006). Minerals are distributed throughout areolar cartilage, and thus may have different properties than tessellated cartilage, which is only mineralized in the outer layer. The vertebral centra of several shark species have a Young's moduli between 323 and 563 MPa (Porter et al. (2006). Young's modulus is a measure of stiffness, which is calculated by testing a structure in compression or tension. The slope of the linear portion of a plot of force per unit area (N/m^2 (Pa)), or stress, placed on the structure by the resultant strain (change in length/original length) is a

measure of Young's modulus, whereby the larger the modulus, the stiffer the structure, and the greater resistance to shape change. Young's modulus for bony tissue is between 10-20 GPa, while values for non-shark cartilage range from 0.21 to 2.6 MPa (Currey 2010, Korhonen et al. 2002, Jin & Lewis 2004, Stolz et al. 2004).

The propterygia, a skeletal element associated with the pelvic girdle in batoids, has tessellated cartilage (Dean & Summers 2006, Macesic & Summers 2012). Batoids that punt (use the propterygia to push off of the sea floor) have stiffer propterygia than those of non-punters (Macesic & Summers 2012). Young's modulus was not measured in the propterygia because they were tested in bending, rather than compression or tension (Macesic & Summers 2012), however flexural stiffness ranged from 140-2533 MPa. Higher mineralization leads to stiffer areolar and tessellated cartilage (Porter et al. 2006, Macesic & Summers 2012).

When a cube is compressed in one dimension, it will expand in the other two dimensions. The ratio of compression along one axis and expansion in the other two is called Poisson's ratio. There is a wide range of values for Poisson's ratio in biological tissues, with cartilage ranging from 0.15 to 0.503 (Korhonen et al. 2002, Jin & Lewis 2004), and bone ranging from 0.09 to 0.5 (Shahar et al. 2007, Wirtz et al. 2000). There are no measured values for Poisson's ratio in shark cartilage.

Shark jaws are not fused to the cranium, and can protrude away from the cranium during a feeding event (Gregory 1904, Maisey 1980, Wilga 2001). The jaws are suspended posteriorly from the cranium by hyomandibular cartilages that articulate with Meckel's knob of the lower jaw and anteriorly by one to three craniopalatine ligaments

that connect the upper jaw with the cranium (Gregory 1904, Maisey 1980, Wilga 2008, Fig. 1).

The orientation of the hyomandibula may affect the orientation of the forces on the cartilage when the hyomandibula swings ventrally during feeding (Huber 2006, Wilga 2008, 2010). At rest, the hyomandibular cartilages can be directed laterally,

Figure 1. Skeletal anatomy of the feeding apparatus of a lemon shark. BH-basihyal, CH-ceratohyal, CP-craniopalatine ligament, CR-cranium, HY-hyomandibula, MC-Meckle's cartilage, PQ-palatoquadrate. (after Motta and Wilga 1995).

anterolaterally, or posteolaterally with respect to the midline (Moss 1977, Wilga 2008, Fig. 2). The distal tips of laterally and anterolaterally oriented hyomandibulae move medially as they swing ventrally during jaw opening, whereas posterolaterally oriented hyomandibulae move anterolaterally as they swing ventrally (Wilga 2008). Horn sharks, *Heterodontus francisci*, which have laterally oriented hyomandibulae, capture

prey using suction and is durophagous (eats hard bodied prey) (Moss 1977, Wilga 2008).

The hyomandibulae of horn sharks are thought to be loaded in tension during biting, opposing the forces generated in the lower jaw (Huber 2006). In lemon sharks, *Negaprion brevirostris*, which have posterolaterally oriented hyomandibulae, the hyomandibulae are thought to be loaded weakly in compression when the jaws are not protruded, but loaded with stronger compression when the upper jaws are protruded when bite feeding (Wilga 2008, Huber 2006).

The mechanical properties of the hyomandibular cartilages are measured in compression by calculating Young's modulus and Poisson's ratio. This will provide an understanding of how tessellated shark cartilage responds to compressive forces, and to give insight into the material properties of shark cartilage. The mechanical properties of

Figure 2. Partial elasmobranch phylogeny including the orders of the species used in this study: bamboo, *C. plagiosum* (Orectolobiformes); sandbar, *C. plumbeus* and smoothhound, *M. canis* (Carcharhiniformes); and dogfish, *S. acanthias* (Squaliformes). L-lateral HY orientation, P-posterior HY orientation, A-anterior HY orientation. (Modified from Shirai 1996, Wilga 2008)

the hyomandibular cartilage are compared among four shark species with two different hyomandibula orientations (lateral vs posterior) and two different feeding styles (bite vs suction). The percent of cross-sectional area that is mineralized is also compared to test whether mineralization influences the mechanical properties. Three hypotheses will be tested. 1) Young's modulus of the hyomandibular cartilage will increase as mineralization increases. 2) Bite feeders will have a higher modulus than suction feeders.

3) Laterally oriented hyomandibulae will have a higher modulus than posteriorly oriented hyomandibulae. 4) Poisson's ratio will be similar among the species.

Methods

Animals

Four shark species were used in this study (Fig. 2) that vary by feeding mode, hyomandibula orientation, and phylogenetic position: white-spotted bamboo sharks are suction feeders with laterally oriented hyomandibulae [*Chiloscyllium plagiosum*; Hemiscylliidae, Orectolobiformes; $N=3$]; spiny dogfish are generalist feeders with laterally oriented hyomandibulae [*Squalus acanthias*; Squalidae, Squaliformes; $N=4$] and dusky smoothhounds [*Mustelus canis*; Triakidae, Carcharhiniformes; $N=5$], and sandbar

Figure 3. Setup of materials testing machine. The HY is secured between two circular metal plates with Loctite repair putty. Sonometric crystals are secured to the dorsal and ventral sides of the HY for Poisson's ratio analysis. The entire HY is submerged in elasmobranch rinsers solution for testing.

sharks [*Carcharhinus plumbeus*; Carcharhinidae, Carcharhiniformes; $N=3$] are bite feeders, both with posteriorly oriented hyomandibulae (Fig. 2). Right and left hyomandibular cartilages were used for the analysis and useable data was collected for six bamboo,

six spiny dogfish, nine smoothhound, and five sandbar hyomandibulae. All

hyomandibulae were dissected from previously frozen whole animal specimens. Freezing does not alter the mechanical properties of vertebrate skeletal tissue (Macesic and Summers, 2012; Porter et al., 2006).

Young's modulus

The mechanical properties of each hyomandibular cartilage was measured using a materials testing machine (MTS Synergie 100, software TestWorks 4, version 4.08 B) to measure compressive strength (Fig. 3). The hyomandibula was placed upright on a metal disc and glued in place with hardening putty (Loctite repair putty, multi-purpose). Another disc was placed on top of the hyomandibula and glued in place with putty. A large flat plate was placed on the top disc to ensure that the putty hardened with the discs parallel to one another. The apparatus was placed into elasmobranch ringer's solution (Forster et al., 1972) for at least 30 minutes to allow the putty to set. Once set, the putty had an elastic modulus of 3.19 GPa, which is three orders of magnitude higher than the modulus of articular cartilage and about one order of magnitude higher than that of previously tested shark cartilage (Korhonen et al. 2002, Jin & Lewis 2004, Stolz et al. 2004, Porter et al. 2006, Macesic & Summers 2012). The hyomandibulae were then placed in the materials testing machine so the discs were parallel with two metal plates and the hyomandibulae could be loaded on the longitudinal axis. The top plate was connected to a force transducer. The hyomandibulae were compressed at a rate of 0.5 mm/s until the slope of the linear portion of the ascending force curve was apparent (50 to 100 N of force). Data was collected at a frequency of 120 Hz. Compression tests were conducted five times (2 pre-conditioning trials, and three test trials), with three minutes between trials. The hyomandibulae were kept in ringer's solution at all times after

Figure 4. Dorsal and cross-sectional view of the hyomandibular cartilages of each of the four species in this study. Black lines represent where cuts were made to measure cross sectional area. The images on the right show the reflected cross-sections of each element.

excision except for a short period (no more than five minutes) used to attach the sonometric crystals (see Poisson's Ratio section below).

The hyomandibulae were then bisected at the narrowest point to measure the cross sectional area at the region of maximal stress (Fig 4). To ensure that all hyomandibulae within a species was cut along the same plane, the cut was made at a fixed percentage of hyomandibular cartilage length from the proximal end. One half of the bisected hyomandibula was placed in modeling clay and arranged so the cut surface was parallel to the table. The cross-section was then digitally photographed using a Zeiss dissecting

scope (Stemi 2000-C, Jena, Germany) with a top mounted Spot Insight color camera (IN-320, Sterling Heights, MI, USA). A reference ruler was placed in each image at the same height as the specimen for scaling purposes. The total cross sectional area was calculated using the software program ImageJ (National Institutes of Health, Bethesda, MD, USA), and the cross sectional area of the mineralized layer alone was measured by subtracting the cross-sectional area of the unmineralized core cartilage from the total cross-sectional

Figure 5. Representative stress/strain curve of a hyomandibula. Trials one and 2 are preloading trials, while trials 3, 4, and 5 are experimental.

area. A stress/strain curve was plotted using the force data, the surface area, and the strains calculated from the material tests. The elastic modulus was calculated as the slope of the linear portion of the curve. The modulus of each element was measured as the average of the last three compression trials (Fig. 5). Cross-sectional aspect ratio was then measured using ImageJ (National Institutes of Health, Bethesda, MD, USA) as the anteroposterior length of each cross-section divided by the dorsoventral width.

Poisson's ratio

Figure 6. Dorsal and cross-sectional view of a smoothhound, *Mustelus canis*, HY with sonometric crystals attached.

Sonomicrometry crystals were attached to the hyomandibular cartilages for the compression tests (Fig. 6). Sonomicrometry (Sonometrics corp.) uses ultrasound signals to determine the distance between pairs of piezoelectric

crystals. A sonometric crystal was glued on the dorsal and the ventral surfaces of the hyomandibula, the same distance from the proximal end of the hyomandibula, using cyanoacrylate glue (E-Z Bond Instant glue, thin (5 CPS viscosity)). Since the speed of sound through cartilage is approximately the same as that of seawater (Del Grosso & Mader 1972, Toyras et al. 2003), sonometric crystals were used to measure expansion of the cartilage in the horizontal direction while being compressed longitudinally.

Statistics

T-tests, one-way ANOVAs, and linear regressions were used to compare Young's Modulus, mineralization levels, aspect ratios, and hyomandibula length within and among species. Linear regressions were run to determine the effects of size and mineralization on Young's modulus and the effect of size on mineralization.

Data were analyzed using SigmaPlot (v. 11.0, Systat Software, San Jose, CA, USA). The mean values for the three compression tests for each left and right hyomandibulae were used in the statistical analyses for Young's modulus and Poisson's

ratio. Paired t-tests were used to test for differences between species in both of these measures.

Results

Young's modulus

Mean Young's Modulus of the hyomandibular cartilages ranged from 56.42 to

Figure 7. Plot of Young's modulus to hyomandibula (HY) length. Regression line is drawn for the smoothhound, *Mustelus canis*

140.13 MPa and differed among species (ANOVA: $H=14.064$, $P<0.01$; Fig. 7). The modulus of the hyomandibulae of *C. plagiosum* (140.135 ± 13.272 MPa), the suction feeder, was larger than the other species. However, the bite (*M. canis* 65.62 ± 5.499 MPa, *C. plumbeus* 70.013 ± 6.114 MPa) and generalist (*S. acanthias* 56.418 ± 6.813 MPa)

feeders were similar. Hyomandibula length had an effect on Young's Modulus in *M.*

canis (Young's modulus = $-41.156 + (131.660 * \text{HY length})$, ($R^2=0.802$, $P=0.001$); Fig. 7).

Mineralization

Figure 8. Plot of Young's modulus to percent of cross-sectional area that is mineralized. B=Bamboo shark, *C. plagiosum*, D=dogfish, *S. acanthias*, M=smoothhound, *M. canis*, S=sandbar, *C. plumbeus*. Regression is for all data combined.

Percent cross-sectional mineralization differed among the species (ANOVA, $F=74.812$, $P<0.001$; Fig. 8). The suction feeder, *C. plagiosum*, had a higher percentage of cross-sectional mineralization than all other species ($32.89 \pm 1.334\%$). The generalist *S. acanthias* (21.536 ± 0.944) had greater mineralization than the other two species. The percentage of cross-sectional mineralization of the bite feeding species, *M. canis* (16.469 ± 0.666) and *C. plumbeus* (15.612 ± 0.417), were similar. Percent cross-sectional

area mineralized has an effect on the Young's Modulus among the species (Young's modulus= $-5.807+(4.108 \times \% \text{ Cross-section mineralized})$, ($R^2=0.59$, $p < 0.001$); Fig. 8).

Cross-sectional aspect ratio

The aspect ratio of the cross sectional area differed among the species (ANOVA, $F=313.88$, $p < 0.001$). The cross-sectional aspect ratio is higher in the generalist species, *S.*

Figure 9. Poisson's ratio by species. Black lines are the median and the boxes range from the 25th to the 75th percentile. Values for cartilage and bone are ranges from (Korhonen et al. 2002, Jin & Lewis 2004, Shahar et al. 2007, Wirtz et al. 2000)

Poisson's ratio

Data for Poisson's ratio in *C. plagiosum* and *C. plumbeus* was not used for analysis because the noise level was too high to detect the signal. Poisson's ratio is similar between the generalist, *S. acanthias* (0.168 ± 0.026), and the bite feeder, *M. canis* (0.099 ± 0.014) (Fig. 9).

Discussion

Mineralization plays the greatest role in increasing the stiffness of the hyomandibular cartilages in the four species studied here. *C. plagiosum* has a higher

acanthias (4.85 ± 0.14), than all other species. The bite feeding species, *C. plumbeus* (3.24 ± 0.02), has the next highest cross-sectional aspect ratio, which is larger than the other two species. The aspect ratio of the bite feeder, *M. canis* (1.77 ± 0.02), and the suction feeder, *C. plagiosum* (1.65 ± 0.01), are similar.

Young's modulus (140 MPa) than the other three species (56-70 MPa) (Fig. 7). This stiffness appears to be due to a higher degree of mineralization and a rounder cross-section, which may prevent buckling (Fig. 8). Though the modulus of the hyomandibular cartilage in *S. acanthias* was similar to that of *M. canis* and *C. plumbeus*, *S. acanthias* hyomandibula have a higher mineralization level. *S. acanthias* were expected to have a higher Young's modulus than either of the bite feeders because of this mineralization; however, the relatively high cross-sectional aspect ratio likely decreases compressive strength in *S. acanthias* and increases the potential for buckling.

Mineralization and compressive strength are directly related in shark vertebrae (Porter et al., 2006), as are mineralization and bending strength in the pelvic fin propterygia of batoids (Macesic and Summers, 2012). The values of Young's modulus for shark vertebrae range from 323 to 563.9 MPa (Porter et al., 2006), which is higher than that for the hyomandibulae in bamboo sharks. However, vertebrae likely experience higher levels of cyclical compressive stress from anguilliform swimming. Torpedo rays, which swim using axial undulation like sharks, are benthic (live on the sea floor), and swim more slowly than the shark species (Porter et al. 2006). The mean Young's modulus for torpedo ray vertebrae is 25.5 MPa, which is lower than the modulus for hyomandibular cartilage (56-140 MPa). However, the mean Young's modulus of the vertebrae in sandbar sharks, *Carcharhinus plumbeus*, is 396.9 MPa (Porter et al. 2006), which is larger than that of the hyomandibulae (70.013 MPa). Stiff vertebrae help transfer energy from muscles to swimming motions (Porter et al. 2006), and may contribute to selection for higher mineralization in vertebrae. However, the mineralization pattern of vertebrae differs from that of the non-vertebral endoskeleton, such as the hyomandibula.

Vertebral cartilage may be stiffer because areolar cartilage is not mineralized only around the outer edge like non-vertebral cartilage, but rather minerals are distributed throughout the structure (Dean & Summers 2006).

Bony tissues have a Young's modulus between 10-20 GPa, (Currey 2010), which is two orders of magnitude greater than the average maximum value (140 MPa) for shark hyomandibulae, and over an order of magnitude greater than shark vertebrae (0.5 GPa). Young's moduli for bovine articular cartilage ranges from 0.29 to 1.79 MPa (Korhonen et al. 2002; Jin & Lewis 2004), and from 0.21 to 2.6 MPa for porcine articular cartilage (Stolz et al. 2004). Unmineralized shark cartilage is expected to have a similar modulus as articular cartilage. The mineralized outer layer of tessellated cartilage likely has a higher modulus than the unmineralized core, yet the relatively harder tesserae probably interacts with the relatively softer unmineralized core to give the whole element a value between articular cartilage and bone.

Within the tesseral layer are mineralized polygonal tesserae connected by collagenous fibers (Kemp & Westrin 1979, Dean & Summers 2006). Under compression, the tesserae abut one another and stress is distributed throughout the tesseral layer, while the fibers allow the tesserae to separate when in tension (Liu et al. 2010). The interactions within the tesseral layer as well as between the tesseral layer and the unmineralized core may provide tessellated shark cartilage with a pliable supportive element that is unlikely to crack yet is strong enough to resist forces incurred during feeding. This is a particularly important property for cartilage because of the limited ability to repair. In homogenous materials, once a crack forms the sharp end of the crack acts as a force multiplier so minimal energy is required to continue the crack until the structure fails

(Vogel 2003). However, shark cartilage is not homogenous. Brittle, crack sensitive tesserae are surrounded by more pliable fibers, all of which surround a soft, pliable core (Kemp & Westrin, 1979).

Poisson's ratio of *S. acanthias* (0.168) and *M. canis* (0.099) are similar. Poisson's ratio for bovine articular cartilage, which is not mineralized, ranges from 0.15 to 0.503 (Korhonen et al. 2002; Jin & Lewis 2004). Poisson's ratio for equine cortical bone ranges from 0.09 to 0.19 (Shahar et al. 2007), while human femoral cortical bone ranges from 0.2 to 0.5, and cancellous bone ranges from .01 to 0.35 (Wirtz et al. 2000). The wide overlap in the range of values for non-mineralized cartilage and bone indicates that the hyomandibulae of *S. acanthias* and *M. canis* have a similar Poisson's ratio to other biological tissues.

Conclusion

Young's modulus is greater in hyomandibular cartilages with a relatively higher percentage of cross-sectional mineralization than those with less mineralization. There appears to be a relationship to feeding style in the four species studied here, where the suction feeding species has a stiffer hyomandibula than the bite feeders or the generalist. One possible reason is that stiff elements can resist larger forces and transmit energy from muscle to movement effectively (Porter et al. 2006). Suction feeders rapidly expand the buccal and pharyngeal cavities to generate negative pressure that draws prey into the mouth (Lauder 1983, Wilga & Sanford 2008). Though the hyomandibulae of bite feeders were hypothesized to have a higher Young's modulus, the large forces generated during suction may necessitate stiffer hyomandibular cartilages than those of bite feeders.

Analyses of additional shark species with different feeding styles will shed more light on the relationships between feeding style, mineralization, and stiffness of the hyomandibular cartilages.

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Appendix A

The following are the significant regression equations for:

Data of all species combined normalized to cranial length:

$$[ICR = -0.0523 + (0.674 * MC), (R^2 = 0.90, p < 0.001)],$$

$$[ICR = -0.0690 + (0.654 * PQ), (R^2 = 0.87, p < 0.001)],$$

$$[PQ = 0.188 + (1.323 * ICR), (R^2 = 0.87, p < 0.001)],$$

$$[HY = 0.0869 + (0.00212 * HY \text{ angle}), (R^2 = 0.67, p < 0.001)],$$

$$[ICR = 0.0382 + (0.00342 * HY \text{ angle}), (R^2 = 0.72, p < 0.001)],$$

$$[MC = 0.166 + (0.00485 * HY \text{ angle}), (R^2 = 0.76, p < 0.001)],$$

$$[MC = 0.138 + (1.332 * ICR), (R^2 = 0.90, p < 0.001)],$$

$$[PQ = 0.147 + (0.00537 * HY \text{ angle}), (R^2 = 0.84, p < 0.001)] ,$$

$$[PQ = 0.188 + (1.323 * ICR), (R^2 = 0.87, p < 0.001)],$$

$$[PQ = 0.0781 + (0.952 * MC), (R^2 = 0.89, p < 0.001)],$$

$$[CH = 0.0965 + (0.00364 * HY \text{ angle}), (R^2 = 0.89, p < 0.001)],$$

$$[CH = 0.132 + (0.888 * ICR), (R^2 = 0.88, p < 0.001)],$$

$$[CH = 0.0765 + (0.611 * MC), (R^2 = 0.83, p < 0.001)],$$

$$[CH = 0.0326 + (0.633 * PQ), (R^2 = 0.91, p < 0.001)],$$

$$[BH = 0.259 + (0.180 * ICR), (R^2 = 0.27, p = 0.010)],$$

$$[BH = 0.254 + (0.116 * MC), (R^2 = 0.22, p = 0.021)],$$

$$[BH = 0.253 + (0.110 * PQ), (R^2 = 0.20, p = 0.028)],$$

$$[BH = 0.239 + (0.189 * CH), (R^2 = 0.25, p = 0.012)],$$

$$[ICR = 0.0382 + (0.00342 * HY \text{ angle}), (R^2 = 0.72, p < 0.001)],$$

$$[MC = 0.166 + (0.00485 * HY \text{ angle}), (R^2 = 0.76, p < 0.001)],$$

$[PQ = 0.147 + (0.00537 * HY \text{ angle}), (R^2 = 0.84, p < 0.001)],$
 $[HY = 0.0628 + (0.603 * ICR), (R^2 = 0.82, p < 0.001)],$
 $[HY = 0.0117 + (0.436 * MC), (R^2 = 0.85, p < 0.001)],$
 $[HY = -0.00362 + (0.429 * PQ), (R^2 = 0.84, p < 0.001)],$
 $[HY = -0.0830 + (1.171 * BH), (R^2 = 0.38, p = 0.001)],$
 $[HY = -0.00452 + (0.632 * CH), (R^2 = 0.78, p < 0.001)],$
 $[HY = 0.0869 + (0.00212 * HY \text{ angle}), (R^2 = 0.67, p < 0.001)],$
 $[CH \text{ AR} = 0.302 - (0.00106 * HY \text{ angle}), (R^2 = 0.77, p < 0.001)],$
 $[BH \text{ AR} = -0.115 + (0.00649 * HY \text{ angle}), (R^2 = 0.78, p < 0.001)],$
 $[BH \text{ AR} = -0.283 + (1.768 * CH), (R^2 = 0.60, p < 0.001)],$
 $[BH \text{ AR} = -0.0891 + (2.207 * HY), (R^2 = 0.48, p < 0.001)],$
 $[BH \text{ AR} = -0.297 + (1.223 * PQ), (R^2 = 0.67, p < 0.001)],$
 $[BH \text{ AR} = -0.223 + (1.198 * MC), (R^2 = 0.64, p < 0.001)],$
 $[BH \text{ AR} = -0.0663 + (1.617 * ICR), (R^2 = 0.58, p < 0.001)],$
 $[HY \text{ AR} = 0.456 - (0.834 * BH), (R^2 = 0.21, p = 0.027)].$

Raw data of all species combined:

$[HY = -0.0135 + (0.623 * CH), (R^2 = 0.89, p < 0.001)],$
 $[HY = -0.147 + (0.978 * BH), (R^2 = 0.67, p < 0.001)],$
 $[HY = -0.0305 + (0.429 * PQ) R^2 = 0.91, p < 0.001)],$
 $[HY = -0.0454 + (0.462 * MC), (R^2 = 0.91, p < 0.001)],$
 $[HY = 0.272 + (0.670 * ICR), (R^2 = 0.89, p < 0.001)],$
 $[BH = 0.799 + (0.462 * CH), (R^2 = 0.70, p < 0.001)],$

$[BH = 0.917 + (0.294 * PQ), (R^2 = 0.62, p < 0.001)],$
 $[BH = 0.965 + (0.306 * MC), (R^2 = 0.57, p < 0.001)],$
 $[BH = 1.159 + (0.449 * ICR), (R^2 = 0.58, p < 0.001)],$
 $[CH = 0.0516 + (0.672 * PQ), (R^2 = 0.96, p < 0.001)],$
 $[CH = 0.111 + (0.708 * MC), (R^2 = 0.91, p < 0.001)],$
 $[CH = 0.552 + (1.041 * ICR), (R^2 = 0.92, p < 0.001)],$
 $[PQ = 0.139 + (1.044 * MC), (R^2 = 0.94, p < 0.001)],$
 $[PQ = 0.863 + (1.510 * ICR), (R^2 = 0.91, p < 0.001)],$
 $[MC = 0.775 + (1.420 * ICR), (R^2 = 0.94, p < 0.001)],$
 $[HY \text{ angle} = 48.668 + (19.322 * ICR), (R^2 = 0.67, p < 0.001)],$
 $[HY \text{ angle} = 37.493 + (13.616 * MC), (R^2 = 0.71, p < 0.001)],$
 $[HY \text{ angle} = 39.155 + (12.592 * PQ), (R^2 = 0.71, p < 0.001)],$
 $[HY \text{ angle} = 46.368 + (26.007 * HY), (R^2 = 0.60, p < 0.001)],$
 $[HY \text{ angle} = 39.679 + (18.476 * CH), (R^2 = 0.69, p < 0.001)].$