

Functional Morphology of Elasmobranch Jaws: Testing the Strain Rate of the Hyomandibulae Cartilage

Final Draft

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Introduction:

This project explored and analyzed the strain and structure of elasmobranch jaws, particularly the hyoid arch. The cartilaginous element tested was the hyomandibula in four individuals each of the angel shark, *Squatina squatina*, spiny dogfish, *Squalus acanthias*, and sandbar shark, *Carcharhinus plumbeus*. We put them through trials that tested for strain and we performed geometric morphometric analyses on the results.

Chondrichthyans have a modified gill arch called the hyoid arch that they use during prey capture and mastication. In all our tested shark species, the cartilaginous hyoid arch expands the buccal cavity. This expansion functions to create negative pressure in the mouth to draw in prey for suction feeders, and allows a wide buccal area to take in as much prey as possible for bite feeders. The hyoid arch in elasmobranchs consists of the ceratohyal, basihyal, and hyomandibula cartilages. The hyomandibula is significant since it connects the ceratohyal to the chondrocranium, connecting functions of the upper and lower jaws.

Teleosts and elasmobranchs evolved different jaw structures, especially in the hyoid region: teleosts have craniums ossified with dermal bone, with the hyoid arch incorporated into the suspensorium and opercular series. The hyoid expands laterally in teleosts, as shown in previous research with *Amia calva* and *Micropterus salmoides* (Wilga 2008), while it compresses in elasmobranchs, except in mako and sandbar sharks (Wilga 2008). Lamniform and carcharhiniform sharks have long, posteriorly oriented hyomandibulae to make room for a wide buccal cavity. Previous research done on buccal pressure shows that lamniform and carcharhiniform sharks do not rely much on suction feeding, but rather on bite feeding (Wilga 2008). Suction feeders, in particular the white-spotted bamboo shark, laterally compress their buccal cavity to generate negative pressure to draw the prey in (Wilga & Sanford 2008). Even the very large megamouth shark uses negative pressure by pulling its basihyal cartilage posteriorly and ventrally rotating its hyoid arch to expand its buccal cavity (Tomita et al 2011).

All chondrichthyan skeletons are cartilaginous, yet many readily feed on bony teleosts. Some, like the Horned shark, *Heterodontus francisci*, are durophagous, even with cartilaginous jaws (Huber et al. 2005). Since cartilage is more malleable than bone, the question is brought up as to how chondrichthyans can feed on durable, hard prey with cartilaginous jaws. Hence, our project focuses on the strain levels of the hyoid arch

elements in sharks and rays. We tested the hyoid arch since it swings the lower jaw ventrally from the chondrocranium to expand the buccal cavity and experiences much of the pressure from mastication.

We also examined cartilage mineralization. One suggestion in literature was that the mineralization of cartilage makes it stiffer and therefore more resistant to strain (Porter et al. 2007). Mineralization occurs in chondrichthyan jaws as prismatic or globular calcification in the form of blocks called tesserae. They form around the perichondrium, the outer layer of the extracellular matrix (Dean and Summers 2006). Since the calcified tesserae add another layer to the cartilage, one of our hypotheses was that cartilage elements with more mineralization would be more resistant to strain than those that had a thinner layer of mineralization.

Our second hypothesis was that shorter, more square-like hyomandibulae would deform less than longer, thinner hyomandibulae. We obtained data on hyomandibulae shape by measuring their length and cross-sectional areas, and used length data to test this hypothesis. We predicted that long, thin specimens had more area to deform than short, squat specimens, leading to a higher Poisson's ratio of strain. Our third hypothesis was that mineralized area and hyomandibula cross-sectional area were correlated, since a thicker layer of mineralization would add to the extracellular matrix area of the cartilage. Significant correlations would suggest that larger hyomandibulae are more resistant to strain than smaller ones.

Methods:

All tests and measurements were run in Laboratory 8 of Friday Harbor Laboratories. Cheryl Wilga provided chondrichthyan jaws in vials of elasmobranch Ringer's Solution, separated into the left and right sides. The tested species were four individuals each of the angel shark, spiny dogfish, and sandbar shark. The dogfish and angel sharks were caught in Narragansett Bay and the sandbar sharks were caught in Delaware Bay. We cleaned a fourth species, the white-spotted bamboo shark, but could not test them due to mechanical difficulties. We took out the key cartilaginous elements of the hyoid arch: the ceratohyal, basihyal, and hyomandibula. We did this by cleaning away the attached muscles, tendons, ligaments, and skin. We put them in elasmobranch Ringer's solution so that the cartilage would not dry out. We used the Materials Testing System Synergie 100 to test strain. It put a load on the cartilage and we put sonometric crystals on the cartilage to record the change in width and depth. The crystals transmitted signals to each other and these signals appeared on the computer software SonoLab (Wilga and Sanford 2008). For the cartilage to stay upright during strain testing, we secured it to two washers using fast-setting putty. After the putty set, we glued the crystals on the cartilage, making sure they did not touch each other or the putty. We glued two crystals on the convex side of the cartilage and one crystal on the concave side, on the thinner end of the cartilage. The latter crystal was glued approximately across one of the crystals on the convex side. This way, the crystals across from each other would send signals recording width change, while the two crystals on the same side would transmit signals recording length change.

We placed the cartilage and crystals into a container of elasmobranch Ringer's solution so the cartilage did not dry out. We connected the wires to the cables that transmitted to the software. We used another software called TestWorks 4 to start the MTS and recorded graphs of measurement changes. We started the MTS, triggering the head to go down and press the cartilage from the top. It pressed up to 50 Newtons and automatically stopped when it hit 50N. We saved the transmissions on SonoLab to make a copy on SonoSoft, a software program we used to get information for our Excel data sheet.

We encountered some obstacles, mainly with sonometric crystal transmission and fusing the putty with the cartilage. Often when we put the crystals in the Ringer's solution to acclimate them to the environment, the crystals transmitted too much noise to make readable data. Usually we reduced noise by increasing and decreasing the Inhibit Delay option on SonoLab to reduce or increase the number of transmissions between crystals. Otherwise, the problem may have been that one sonometric crystal wire was damaged and needed to be replaced. Another way to reduce noise was to manipulate the data in SonoSoft. The other main problem was attaching the putty to the cartilage and keeping the washers parallel to each other. We solved this by drying the cartilage as much as possible and used small forceps to attach the putty to the cartilage.

To obtain cross-sectional area and mineralization data, we cut the cartilage in half with a scalpel. Then we cut 5mm away from the middle cut to get a slice of cartilage, 5mm in width. To measure the area and mineralization, we placed the slice on a light table. A microscope looking down on the light table connected to a computer showed the live viewing on the computer monitor. The mineralization was at the outer rim of the extracellular matrix and appeared darker than the ECM. We then used the outline option in the software to trace the inner edge of mineralization, then we outlined the outer edge to obtain mineralized area. The outer edge outline also calculated the cross-sectional area, which includes the mineralized area. We calculated mineralized area by subtracting the area between the inner edge outline and the outer edge outline from the cross-sectional area. We ran statistical analysis with the software JMP and tested our hypotheses with the Analysis of Variance (ANOVA) test.

Results:

Species	indiv	HY length mm	XS area m2	% area mineralized	XS area mm2	YM MPa	HYM poisson's ratio at -0.4% strain	HYM poisson's ratio at peak strain	HYM strain at peak	Stress at crystals MPa (N/m2)
angel	1	41.28	0.00006637	25.3	66.37	92.04	0.2160	0.3694	-0.0077	0.7533
angel	2	35.02	0.00006836	31.4	68.36	68.84	0.0218	0.0376	-0.0406	0.7314
angel	3	40.88	0.00004565	22.4	45.65	59.36	0.03386	0.09312	-0.12433	1.0954
angel	4	45.64	0.00009454	27.9	94.54	70.23	0.07944	0.23252	-0.03509	0.5289
dogfish	1	19.94	0.00002914	14.4	29.142	97.68	0.04505	0.60097	-0.09515	1.7157
dogfish	2	20.51	0.00003859	12.1	38.586	66.33	0.00616	0.17004	-0.14341	1.2958
dogfish	3	20.07	0.00003156	10.5	31.559	70.25	0.0625	0.33255	-0.12277	1.5843
dogfish	4	20.56	0.00002492	15.7	24.921	145.90			0.00959	2.0063
sandbar	1	27.03	0.00003260	14.8	32.6	55.36	0.00483	0.1739	-0.3086	1.5338
sandbar	2	34.32	0.00005111	9.2	51.106	46.18	0.34722	0.61889	0.01031	0.9784
sandbar	3	31.85	0.00003818	8.4	38.177	58.83			-0.00107	1.3097
sandbar	4	31.76	0.00003933	10.8	39.331	56.62	0.26667	0.32381	-0.00536	1.2713

Data table showing the results of strain and mineralization of angel sharks, dogfish, and sandbar sharks (4 individuals each). The hyomandibulae Poisson's ratio at -0.4% strain and poisson's ratio at peak strain for dogfish 4 and sandbar shark 3 had no value since they were both 0. Due to time constraints, we only tested the hyomandibulae of 3 elasmobranch species.

Effect of Cartilage Mineralization on Cross Sectional Area of Hyomandibula

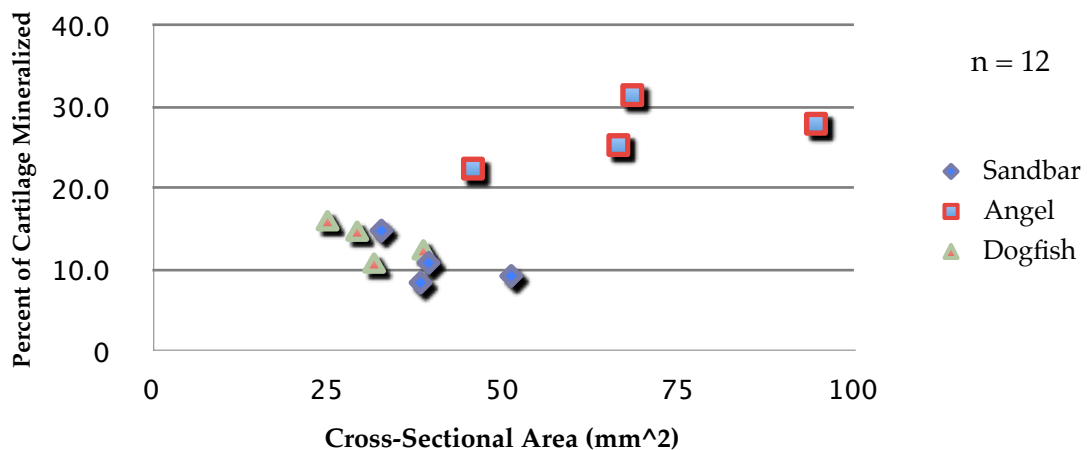


Fig. 2. The effect of mineralization on cross-sectional hyomandibula area. Since mineralization added another layer to cross-sectional area, we predicted that more mineralized area will result in a larger cross-sectional area. We rejected the null hypothesis since the ANOVA test showed that cross-sectional area and percent of cartilage mineralization were correlated: when the area increased, the mineralization increased.

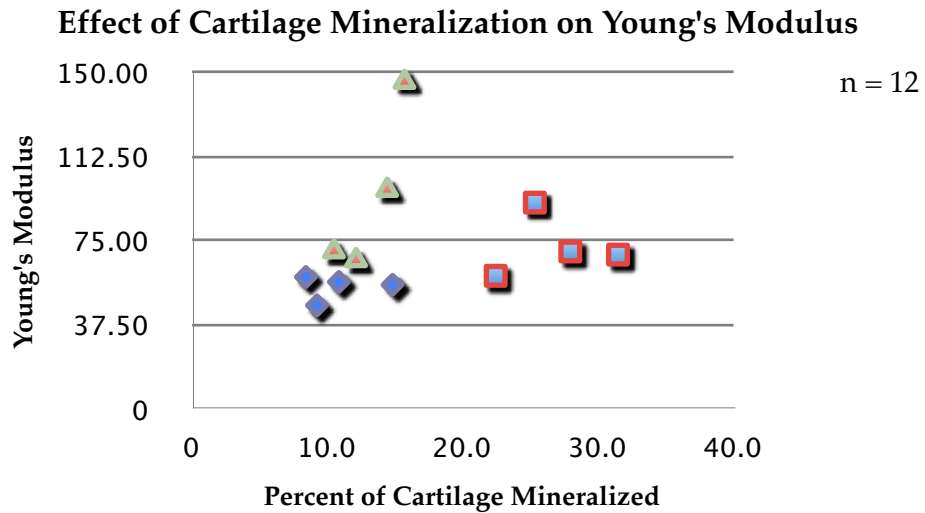


Fig. 1. The effect of mineralization on the Young's Modulus values. We predicted that a larger mineralized area would result in a high Young's Modulus, signifying that it would be more resistant to strain. We accepted the null hypothesis since the ANOVA test showed no correlation between cartilage mineralization percentage and Young's Modulus value.

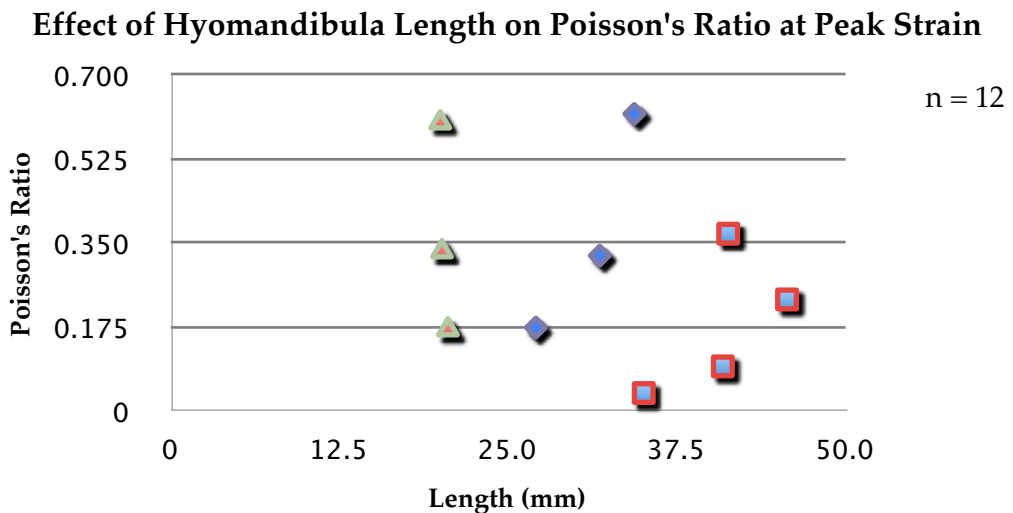


Fig. 3. The effect of hyomandibula length at peak strain. One of our hypotheses was that shorter hyomandibula would deform less than longer hyomandibula. There were 3 individuals each instead of 4 for dogfish and sandbar sharks because their data cells did not have values. We accepted the null hypothesis since the ANOVA test showed no correlation between hyomandibula length and Poisson's Ratio at peak strain.

In Fig. 1, the dogfish and sandbar sharks had similar mineralized areas, while the angel sharks had larger mineralized areas. Nevertheless, the ANOVA tested negative for correlation between the two variables ($F = 0.0239$, $p = 0.6369$). Hence, we accepted the null hypothesis. These statistical results were surprising since previous research showed that a highly mineralized hyomandibula would be more resistant to strain, thus resulting in a high Young's modulus (Balaban et al. 2013). Perhaps the analysis showed no correlation since the data point for the fourth dogfish specimen had a particularly high Young's modulus of 145.90 MPa, while the Young's modulus of the other three dogfish specimens were significantly smaller in value. Figure 4 however showed a positive correlation between mineralized area and cross-sectional area, in that as the percentage of mineralization increased, the cross-sectional area increased. These results communicated that a large hyomandibula with a large cross-sectional area will most likely have a highly mineralized area. Lastly, our final hypothesis was that as hyomandibula length increased, the Poisson's ratio at peak strain would also increase. The findings however showed that there was no significant correlation between the two variables. We found these results surprising since we predicted that short, squat cartilage would deform less than those that are long and thin. We expected long hyomandibulae to buckle under a load because there was more area for it to deform. Perhaps the lack of one dogfish data point and one sandbar data point interfered in the analysis.

Discussion:

One study tested mineralization in the smooth-hound shark's vertebral cartilage (Porter et al. 2007). However, this study concluded that the actual structure of the minerals had more effect on strain strength rather than their mass and area alone. One structure in mineralized cartilage is the tesserae of calcified cartilage (Dean & Summers 2006). Several layers of tesserae are present in the corners of jaws in large sharks and in the jaws of durophagous sharks; hence, they appear to help stiffen the cartilage for feeding (Dean & Summers 2006). More research needs to be done in this field since it is still unknown as to how and when this character evolved.

A similar study tested how mineral structure affected cartilage strain in relation to mineral content (Porter et al. 2007). Mineral content signified the amount of mineral present in the cartilage, while mineral structure referred to the actual arrangement of the minerals. The writers increased mineral content by 10% and cartilage stiffness did not increase; however, when they increased mineral content by 10% across eight species with multiple mineralization structures, cartilage stiffness increased by 44%. Hence, future research could focus on how certain mineral structures could increase cartilage strain rate in elasmobranch jaws.

Cartilage strain testing is not exclusive to elasmobranchs: one study used live miniature pigs to test the deformation of the nasal septal cartilage during feeding (Al Dayeh et al. 2009). The experiments tested whether the septal cartilage functioned as a support structure or as a shock absorber during mastication. In animals with a bony skeleton, cartilage is thought to absorb and distribute stress to protect fragile or less

flexible bones (Al Dayeh et al. 2009). The results showed that the strain was compressive overall. The strain was higher in the septum than in nearby bones; hence, the cartilage was most likely taking mastication pressure off of the nearby bones. However, strain distribution was not uniform: the anterior septal cartilage experienced significantly more strain than the posterior, septo-ethmoid region of the cartilage. The anterior region was thicker than the posterior region, implying that the anterior was able to take on more load (Al Dayeh et al. 2009). In our project, we assumed that most strain would be contained in the region with the thinnest area of the cartilage since we applied pressure on one end of the cartilage.

Another aspect about the septum cartilage strain study was how they measured strain rate. The writers used differential variable reluctance transducers (DVRTs) attached to the cartilage with barbed broaches to measure strain. They drilled a hole in the ethmoid bone and placed a DVRT in it, with half its length on the bone and half on the cartilage. They used electromyography with fine wire electrodes to measure mastication patterns over time, since they worked with live specimens. The writers state that the instruments functioned well for strain testing during mastication; hence, along with sonomicrometry, the use of DVRTs and electromyography would be a reliable method of measuring strain rate in cartilage and could perhaps be used in live specimen testing for elasmobranchs.

Related studies have been conducted on elasmobranch bite force, particularly in white sharks and horn sharks (Wroe et al. 2008; Huber et al. 2005). White sharks are known to feed on bony prey while horn sharks are primarily durophagous; hence, a big question is how cartilaginous jaws are able to withstand eating bony, hard prey. Since the upper and lower jaws experience the most occlusion to the prey, testing the strain of both jaws would help scientists understand elasmobranch jaw mechanics.

The results of this study bring up provoking ecological and morphological implications as well: previous studies showed that the white-spotted bamboo shark, a suction feeder, had a significantly higher mineralization percentage when tested against two bite feeding species and one generalist species (Balaban et al. 2013). The writers suggest that the bamboo shark had more mineralization than the other species because of their suction-feeding style, where they would need very stiff hyomandibulae to withstand the forces and negative pressure coming into the buccal cavity during feeding. This finding brought up certain questions, such as when this morphological change occurred phylogenetically. This research suggests that at a point in evolution, the orientation of hyomandibulae branched out into various orientations among different groups of elasmobranchs; for instance, squaliforms and batoids are typically generalist feeders and have laterally directed hyomandibulae (Wilga 2008).

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References:

- 1) Al Dayeh, A. A., Rafferty, K. L., Egbert, M., Herring, S. W. "Deformation of Nasal Septal Cartilage During Mastication." *Journal of Morphology* 270.10 (2009): 1209-18. Print.
- 2) Dean, M. N., and A. P. Summers. "Mineralized Cartilage in the Skeleton of Chondrichthyan Fishes." *Zoology* 109.2 (2006): 164-68. Print.
- 3) Huber, D. R., Eason, T. G., Hueter, R. E., Motta, P. J. "Analysis of the Bite Force and Mechanical Design of the Feeding Mechanism of the Durophagous Horn Shark *Heterodontus Francisci*." *Journal of Experimental Biology* 208.18 (2005): 3553-71. Print.
- 4) Lauder, G. V., and L. E. Lanyon. "Functional-Anatomy of Feeding in the Bluegill Sunfish, *Lepomis-Macrochirus* - *In vivo* Measurement of Bone Strain." *Journal of Experimental Biology* 84.FEB (1980): 33-&. Print.
- 5) Porter, M. E., T. J. Koob, and A. P. Summers. "The Contribution of Mineral to the Material Properties of Vertebral Cartilage from the Smooth-Hound Shark *Mustelus Californicus*." *Journal of Experimental Biology* 210.19 (2007): 3319-27. Print.
- 6) Swartz, S. M. "Strain Analysis as a Tool for Functional-Morphology." *American Zoologist* 31.4 (1991): 655-69. Print.
- 7) Tomita, T., Sato, K., Suda, K., Kawauchi, J., Nakaya, K. "Feeding of the Megamouth Shark (Pisces: Lamniformes: Megachasmidae) Predicted by Its Hyoid Arch: A Biomechanical Approach." *Journal of Morphology* 272.5 (2011): 513-24. Print.
- 8) Wilga, C. A. D. "Evolutionary Divergence in the Feeding Mechanism of Fishes." *Acta Geologica Polonica* 58.2 (2008): 113-20. Print.
- 9) Wilga, C. D., and C. P. Sanford. "Suction Generation in White-Spotted Bamboo Sharks *Chiloscyllium Plagiosum*." *Journal of Experimental Biology* 211.19 (2008): 3128-38. Print.
- 10) Wroe, S., Huber, D. R., Lowry, M., McHenry, C., Moreno, K., Clausen, P., Ferrara, T. L., Cunningham, E., Dean, M. N., Summers, A. P. "Three-Dimensional Computer Analysis of White Shark Jaw Mechanics: How Hard Can a Great White Bite?" *Journal of Zoology* 276.4 (2008): 336-42. Print.

- 11) Rafferty KL, Herring SW, Artese F. "Three-dimensional loading and growth of the zygomatic arch". *J Exp Biol.* 2000;203:2093–2104.
- 12) Balaban, Jordan, "The Morphology and Biomechanics of Jaw Structures in Chondrichthyes" (2013). Open Access Master's Theses. Paper 130.
<http://digitalcommons.uri.edu/theses/130>