The Affect of Ontogeny on Structure and Function of Preopercular Spines in *Myoxocephalus polyacanthocephalus*

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Abstract

I investigated the affect of ontogeny on the structure and function of pre-opercular spines. By measuring how these spines matched up against the standard length of the animal. Spines were surgically removed from Myoxocephalus *polyacanthocephalus* and put through puncture tests to measure the required force to puncture a ratio of the spine length. We found that spine length grows isometrically to standard length and force to puncture showed a negative allometry. Meaning that larger individuals had the same size spine to body length ratio but the larger spines is more efficient at puncturing. To assess the structure Finite Elemental Analysis techniques were applied to three different size classes of spines. This analysis showed that smaller spines concentrate the Von Mises stress at the tip while the largest sine dissipates this stress down the dorsal medial ridge. The smallest class of spine also showed a much higher Von Mises stress than the larger spine.

Introduction

Understanding the affect of morphology on fitness is crucial in understanding the bigger picture of the organism's ecological role. Though little research has been done upon scorpaeniformes preopercular spines, there has been a substantial amount of functional morphology and biomechanics of canine tooth structure (Freeman and Weins, 1997; Freeman and Lemen, 2006). These structures are intended in similar uses, they are designed to puncture. The theory is that these structures are designed as defense mechanisms in predator-prey interactions (Cowan 1969). This theory leads to a general hypothesis that smaller individuals will require larger and stranger spines compared to the larger individuals. This study focuses on these ontogenetic affects upon the functionality and structure of the preopercular spines, specifically in the species *Myoxocephalus polyacanthocephalus* (Great Sculpin).

In *M. polyacnathocephalus* preopercular spines arrange themselves in a two-pronged spear pointing in the dorsal-posterior portion of the fish. Not much has been studied on the physical capabilities of these constitutive defenses. Though many spines are present upon the skeletal elements the preopercle spines are heavily accentuated in this particular species. The preopercular bone lies within the suspensorium and is closely with ligaments to both the quadrate as well as the hyomandibular (Datavo, 2013; Cowan, 1970). This set up of tightly connected tendons and ligaments gives the spine certain rigidity and limits the range of motion in respect to the rest of the skull elements.

Due to the enlarged spine and large range between juvenile and adult size, *M. polyacanthocephalus* is an ideal study species to look at scaling forces. I will use previous methods lain out by previous puncture measurement tests; some completed on teeth (Whitenack and Motta, 2009) and others on mammalian spines (Cho et. Al 2012). This will tell us how the puncture forces scale through ontogeny. However this tells us little on the composition or structure of the preopercles, using Finite Elemental Analysis we can observe how stress is dealt with across the different size classes of *M. polyacanthocephalus*. FEA along with scanning electron micrograph can give us a lot of information of how these spines are designed and how that design changes through ontogeny.

Materials and Methods

Puncture Forces

M. polyacanthocephalus for this study were collected in a beach seine on Friday Harbor, Washington (Jackson Beach 48°31'13.0"N 123°00'35.1"W). Fifteen specimens were chosen to encompass as large a size range as possible. Specimens were euthanized with MS-222 and then frozen for 24-96 hours. Each individual was thawed, measured and then had its pre-opercles surgically removed and measured both pre-opercular. One pre-opercle per individual (left or right) was chosen to complete puncture tests. The opercles were randomly decided using Research Randomizer v4.0 (randomizer.org). To determine a viable puncture depth the secondary spine of *M. polyacanthocephalus* was used as a biologically relevant stopping point. Measurements of the perpendicular intersection showed on average the secondary spine tapered off at 25% of the primary spine length, meaning the trial depth of the primary spine was set to 75% of the total length of that spine.

Samples of pre-opercular were epoxied into PLA plastic boxes designed using Autodesk 123d Design and printed using an ORION Delta Rapid Prototyper (SeeMeCNC inc., Goshen, India). These boxes were designed to be manipulated in a 500 N Synergie 100 Materials Testing System (MTS Systems Corporation, 14000 Technology Dr. Eden Prairie, Minnesota, USA) to both protect the spines from damage and allow for maneuverability of the spines to ensure a viable puncture angle. Mold Star 16 Fast Silicone Rubber (Smooth-On Inc., 2000 Saint John St. Easton, Pennsylvania) was used for puncture material to ensure consistency. Rubber was molded into 20x20 mm boxes with varying depths, also designed in 123d Design and printed on the ORION Rapid Prototyper.

Physical testing was carried out using the Materials Testing System. Each spine was run through five trials, each trial used untested rubber boxes. The MTS was arranged to drive a pre-opercular spine in the negative z-axis into an unconstrained box filled with silicone rubber. Using Test Works (MTS Systems Corporation, 14000 Technology Dr. Eden Prairie, Minnesota, USA) the MTS was programmed to record the force (N) to press the spine in to the rubber up to 75% of the spine length and then recede back to its original position. In the smallest individual (3.014 cm Total Length) the spine was unable to puncture the rubber and was therefore not included in the puncturing data set. Data was extracted and plotted using log-log plots to assess the allometry and scaling and linear regressions in R.

Spine Morphology and FEA

Spines not used for puncturing data were used for structural assessment. Two spines were prepared for SEM. One spine was cut into cross sections using a Hurricane Laser (Full Spectrum Laser LLC, 6216 S Sandhill Rd. Las Vegas, Nevada, USA) to assess the spine and pre-opercular core; the remaining pre-opercle was left uncut to assess the surface of the bone and spine. Samples were fixed in formalin for two hours, and then placed in a series of ethanol baths starting with 50% and progressing through 70%, 95% and then 100% for at least one hour for each bath. Sample was then prepared for and placed in a JEOL JCM-500 Benchtop SEM (JEOL Inc., 11 Dearborn Road, Peabody, MA, USA).

Of the remaining pre-opercular samples three were μCT scanned using a Skyscan 1170 Micro-CT (Micro Photonics, 1550 Pond Road Allentown, PA USA). Spines were chosen to cover the widest available size differentiation. The μCT scans were converted into .jpg files and then imported into Amira (FEI Visualizations Sciences Group, Burlington, MA, USA). Once in Amira spines were sectioned and isolated using the *LabelField* function, the largest of the three pre-opercle was cropped due to an inability for the computer program to import the full scan. The lower half of the pre-opercle was removed because of a priority of leaving the primary spine intact. The surfaces generated in Amira contain an unnecessary number of polygons, and a reduction in the number of polygons usually has no or only little effect upon the end surface model (Kleinteich et al. 2011). Therefore I reduced the amount of polygons to 102,000 polygons in order to simplify the surface. Using Amira functions the surfaces were edited to remove remaining intersections and a mesh file (IDEAS.unv) was created for analysis in the FEA program.

Finite element analysis was completed on FEBio(Musculoskeletal Research Laboratories, Salt Lake City, Utah, USA). The three separate meshes contained 200,000 to 1.2 million tetrahedral elements depending on size of mesh. Each mesh was given Isotropic-Elastic material with a Young's modulus of 8.4 GPa's and a Poisson's ratio of 0.3. The Young's modulus was a value determined by Horton and Summers (2009) on acellular bon in *M. polyacanthocephalus*. The remaining values have been used in previous FEA studies of non-mammalian skulls (Kleinteich et al. 2011, Whitenack et al. 2011).

Within the Preview application of FEBio the pre-opercle was arranged to put the direction of the primary spine in the positive-z direction. A *Fixed Displacement* in the x,y, and z direction was applied to the nodes that connected the pre-opercle to the hyomandibular as well as the nodes that connect the pre-opercle to the quadrate. A *Nodal Force* of 10 N was applied in negative-z direction on the tip of each of the primary spines. The amount of nodes was scaled according to the surface area of each mesh recorded in Amira. The file was exported and run through FEBio and analyzed in the Postview application. The Von Mises stress was recorded for each pre-opercle and images were taken of the visual representations to assess the dissipation of the effective stress.

Results

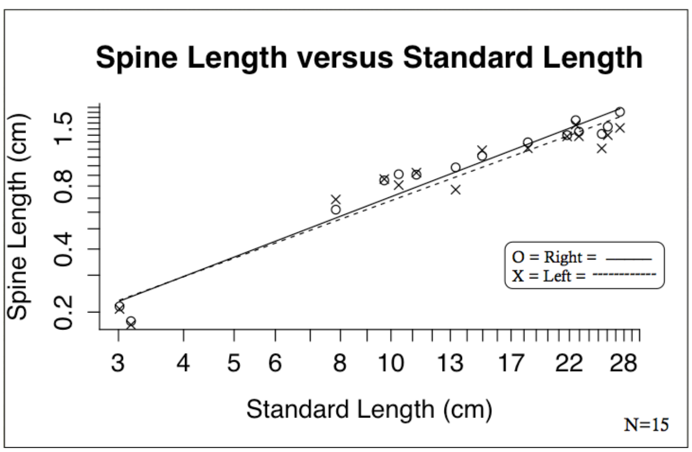


Figure 1. Results of morphometric data on preopercular spines in *M. polyacanthocephalus.* Spine Length (cm) plotted against Standard Length (cm) on log axis. Right and Left preopercle spine length plotted as O and X respectively. Dotted line is the linear regression of left spines and has an equation of y= 0.912x-2.48, R^2=0.925. Solid line is linear regression with equation of y=0.957-2.54, R^2= 0.959.

<u>Puncture Forces</u>

Measurements of the pre-opercular spines show a nearly isometric growth as seen in figure 1. Both right and left linear regressions have slopes of 0.957 and 0.912 respectively (linear regression, R^2=0.96 and 0.93, P-value= $2.06e^{-10}$ and $1.1e^{-8}$ respectively). The data shows a slight negative allometry due to the slopes being less than one. This concludes that larger individuals tend to have slightly smaller spine length to total length ratio. In puncture tests, the force required to reach 75% of the spine length is not isometric, as seen in figure 2. The slope of the linear regression is 1.658, which when taking into account the scaling forces of force and the displacement of a volume it results in being negatively allometric (linear regression, R^2=0.974, P-value=$2.2e^{-16}$). The smallest individual acquired for the study (Total Length=3.014) was unable to puncture the silicon rubber and shows a size limit to the method of testing.

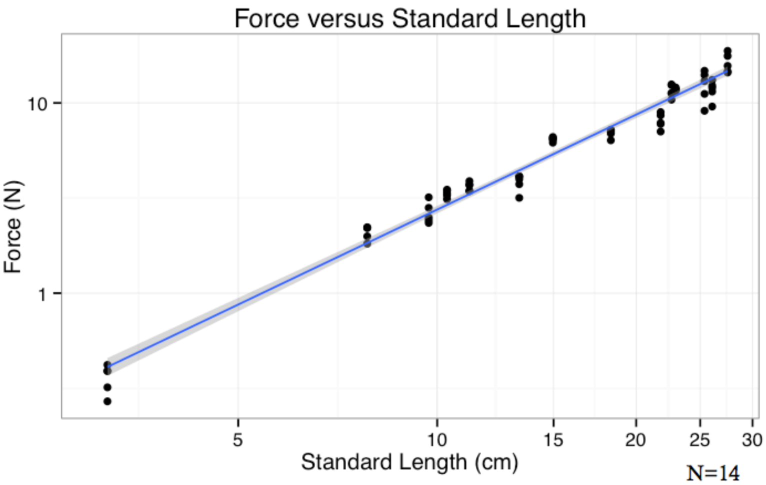


Figure 2. Results of MTS testing. Force (N) required to puncture 75% of spine length plotted against Standard Length (cm), both in log axis. Five trials were completed per spine, shading of line accounts of standard error. Linear regression equation is y= 1.66x-2.806, R^2=0.974. Larger spines are able to puncture more efficiently than smaller spines.

Spine Morphology and FEA

In SEM analysis, the pre-opercular spine showed highly regularized trabeculations in the main areas of the bone (figure 3a). These canals are seen throughout the pre-opercle but are quickly lost when reaching the base of the spines. The individual pre-opercle shown in figure 3b was a sample taken from the smallest individual of our study. Analysis of sections of larger spines also in SEM showed similar trabeculated bones in the pre-opercle core and a lack of trabeculae in the spines. These were supported by µCT scans of the three different size class of spines. Each spine showed hollow canals running along the center of the pre-opercle but solid bone within the pre-opercular spines (figure 3c).

In Finite Elemental Analysis of three different size classes of pre-opercular spines we see a high amount of Von Mises stress where the load was applied; however, larger pre-opercles showed a better dissipation of these effective stresses by dissipating the stress down the dorsal medial ridge of the primary spine all the way to the connection of the spine at its base to the rest of the pre-opercal (Figure 4c). Small and Medium size class showed a moderate amount of dissipation of the Von Mises stress down the medial side (figure 4a and b); however, the two smaller size classes also concentrated the stress upon the tip at much higher numbers than the largest spine. The smallest spine showed a maximum Von Mises stress of 3.297 MPa, the medium size spine had a maximum Von Mises stress at 2.51 MPa and largest size class spine had a maximum Von Mises stress of 1.644 MPa.

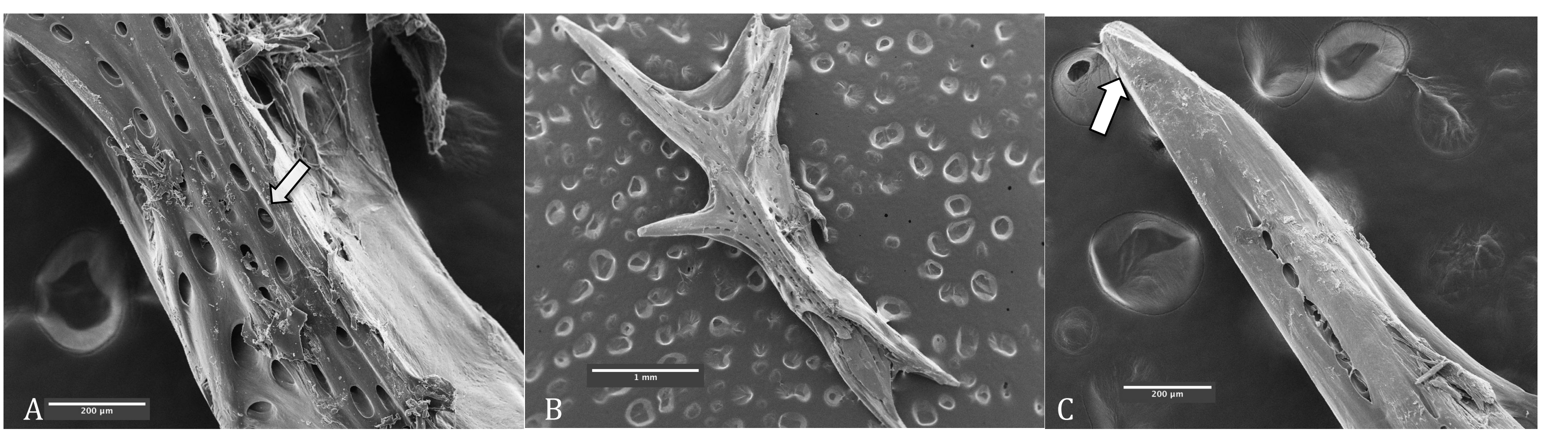
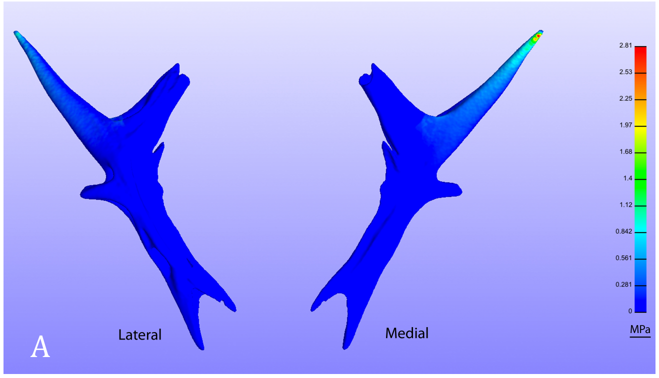
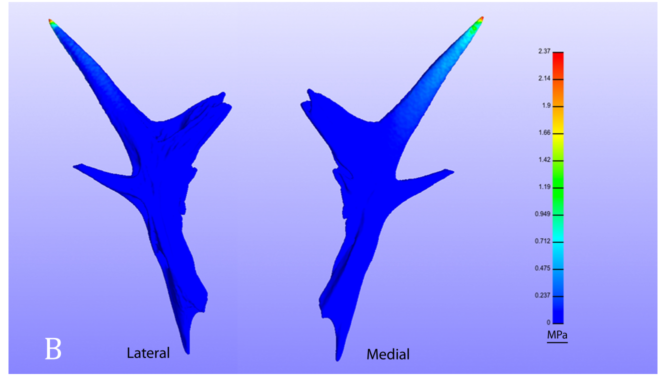


Figure 3. A) SEM of Trabeculations in the Pre-opercal. Arrow is outlining canal system and its progression through the lower bone. B) Overview of entire preopercal. C) Close-up of primary spine. Arrow is pointing out solid tip lacking trabeculae.





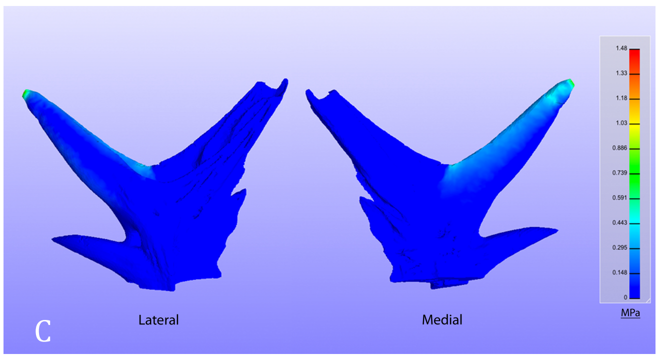


Figure 4. A) Smallest Pre-opercal spine, Maximum Von MIses Stress= 3.297 MPa , Stress is concentrated at tip with some dissipation of stress down the medial length of the spine. B) Medium Pre-opercal spine, Maximum Von Mises Stress = 2.51 MPa, Stress is concentrated at tip with some dissipation of stress down the medial length of the spine. C) Largest Pre-opercal spine, Maximum Von Mises Stress= 1.644 MPa, Stress is dissipated much more readily. Higher concentration down dorsal-medial ridge than smaller spines.

Discussion

Most of the stabbing data collected is non-intuitive - the larger the spine, the more efficient it becomes at penetration relative to its body size. The slope of the regression line shown in figure 2 shows the penetration force is scaling to the power of 1.7. However, if one was talking about the scaling factors of a volume compared to a length you should find that it has a scaling at a power of 3 (Gould, 1965). Due to the method in which the spine is displacing its volume within the rubber medium you would expect a scaling power to be to the third, but that's not what we see. The possible mechanical reasoning for this could be a shift in the aspect ratio or a change in apical sharpness. A way to measure this is explained in paper by Freeman and Weins (1997) where they measured sharpness of bat canine teeth. Preliminary data does not seem to support this but more investigation must occur before it can be disregarded as a possibility. Another possible explanation is lateral compression of these spines to change shape into more of a blade shape than a cone, this could give an advantage in puncturing through a material by limiting the surface area relative to body size and reducing its friction.

The isometric growth of the pre-opercular spines also is contrary to the original hypothesis, which indicates that selective pressures are selecting for effective spines as a larger individual. *M. polyacanthocephalus* is primarily a benthic fish, but there is little literature on its natural predators or habitat of choice. Therefore not much can be inferred as to why these fish keep large spines as they age.

Some interesting SEM photographs show highly regularized trabeculations within the bone. These trabeculae form a similar structure to cranial lateral line systems shown in a paper written by Bird and Webb (2014). These trabeculae from a series of canals that run the entire length of the pre-opercle but cease as they begin to move up the spine. More histological work must be carried out however to confirm the hypothesis that these are cranial lateral line systems.

The Finite Element Analysis however does support the original hypothesis. When the spines are small you concentrate all the force on the tip which, if in the instance of predation occurring, if any part of the pre-opercle is damaged it will only be the tip of the spine. In the larger spine however it dissipates the stress much more evenly, which is not great because if and when the spine meets too great of a force the breaking point will end up being at the base of the spine. Therefore, the smaller spines can actually handle large forces relative to their body size in a much more efficient way where they minimize the amount of stress and concentrate it at one point.

Our understanding of how preopercular spines in *M. polyacnathocephalus* works has been reopened. The length of the spines grows isometrically in respects to body size, and larger individuals have more efficient spines compared to body length than smaller individuals. Much more research must be accomplished in this regard; however, in FEA we determined that smaller individuals concentrate Von Mises stress at the apical point of the spine. Larger spines dissipate stress more readily and overall have much less Von Mises stress than smaller preopercles.

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