About the Crustiness of Poachers

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Abstract

Agonidae is a family containing 46 species of bottom dwelling fish. All of them possess heavy body armor, beneficial against predators and aggressive conspecifics. However, these types of useful traits usually come at a cost. We hypothesized that poachers pay for their armor in reduced maneuverability and increased body stiffness. In order to test our hypothesis, we investigated the natural flexibility of an *Agonidae* representative, *Agonopsis vulsa*, by analyzing videos of its flight response, and found that the fish are quite flexible. Additionally, we used a subset of sacrificed specimens in bending experiments to determine the body stiffness. The fish were bent in three different states (intact, with the plates removed, and with the muscle removed remaining only the vertebral column) to quantify the contributions of each layer to whole body stiffness. Finally, to quantify the protection gained from the armor, we looked at the material properties (stiffness) of the plates. We used material testing to determine the Young's modulus of the plates and compared it to the one of vertebral bone from the same specimens. We found no difference in Young's modulus between plates and bones. While the plates do contribute to stiffness in the fish, the extent to which they do was not as pronounced as expected. In addition, they contribute much more heavily to the stiffness of the body region than the tail region.

Introduction

People have been fascinated with armor for a long time and during history developed lots of different types for very different purposes. Looking at animal evolution, protection offered by body armor provide major advantages in many circumstances (from predation to fighting to abrasion) **(**Kruppert et al., 2020). Unlike humans, who have found it difficult to create something that provides protection without too much added bulk and weight, many animals have been very successful finding this balance. The fish family *Agonidae* (Poachers) are a good example in this regard. Poachers possess bony armor plates covering their entire body, with just a few exceptions having small gaps in this coverage (e.g. *Percis japonica*) and one species even having spine-bearing plates on their eyeballs (*Xenopyxis latifrons*). In the majority of species these plates each converge into a spine with exception of the ventral ones. Studying poacher anatomy and kinematics promises insight into general evolutionary patterns of animal body armor and may lead to advances in fields such as engineering. Poacher armor plates overlap and interlock with each other to provide more structural integrity. Their habitus vary slightly according to their position on the body. It has been observed that there are smaller, more flexible plates at the ventral side, as opposed to those found on the lateral and dorsal side of poachers (Bouilliart et al., 2014). Like other armored creatures, such as a seahorse, the poachers' protective armor contributes to the ability to withstand force (Porter et al., 2015). However, most beneficial traits come to a cost and in case of the poacher body armor we hypothesized a decreased body flexibility. Being bottom dwellers, the increase of weight that comes with their extensive armor most likely doesn't negatively influence their live style. Every creature has to be able to move for feeding, flight and mating purposes though. Poachers typically swim by moving their pectoral fins, and only use their caudal fins in flight responses (Nowroozi et al., 2009). When they are startled, though, they will demonstrate their escape response. This response usually is a sharp turn away from what scared them and retreat in the opposite direction.

Our study analyzes the relationship between body flexibility and the stiffness of the protective armor of poacher (in this experiment, *Agonopsis vulsa* was used). This was done in three different experiments. First, videos of the fish swimming were analyzed to find how much

A. vulsa will naturally and willingly bend in order to turn and avoid a threat. Second, the fish were bent using a material tester in order to see how much force was needed to bend the *A. vulsa* specimens to a certain angle, and how much stiffness was provided from the armor plates, muscle and bone. Third, material testing was used to determine the Young's Modulus of the individual plates and bone.

Methods

Specimen collection

All fish were collected in the Salish Sea close to San Juan Island. The majority of specimens were gathered on bottom trail surveys in the San Juan Channel on board the Kittiwake. The exceptions were two Rockhead Poachers (*Bathroganus swanii*) that were collected in a tide pool bail out at Deadman Bay, on the western shoreline of San Juan Island.

Kinematic analysis

The fish were videotaped table during a flight response to a sudden movement in close proximity. We used a GoPro Hero 4 mounted above a sea table that captured an area of 1.43 cm x 0.61 cm x 0.14cm. The sea table contained a plastic oval in the middle to provide a clear track for the fish to swim, and the camera was set with a linear frame of view at 1080 resolution and 30 frames per second. The area recorded had a white board laid on the bottom to increase contrast as well as a six-inch ruler for scale. A piece of particle board was leaned above the tank to block excess or irregular light, and an acrylic sheet was floated on top of the water to minimize ripples in the videos. Escape response trials were done for five *A. vulsa*, two alligator fish (*Anoplagonus inermis*), one sturgeon poacher (*Podothecus accipenserinus*), and two *Bathroganus swanii*. The fish were startled by quickly moving a net towards them, and the resulting turn was analyzed. Five videos were taken of each fish. The videos that were analyzed for this paper were of *A. vulsa*, all measuring about six inches in length. These videos were trimmed using MPEG studio and then processed in MATLAB to find the swimming speed (body length/second), tail beat frequency, tail beat period, stride length, tail beat amplitude, head amplitude, and amplitude of the body at three equally spaced points along the body. This data was used to find the maximum angle the fish voluntarily reached when startled.

Bending trials

In order to prepare the fish for bending, three *A. vulsa* were sacrificed following IACUC protocol: They were placed in a tank that contained four liters of seawater and one gram of MS-222 (equals 250mg/L) for one hour. They were frozen immediately afterwards.

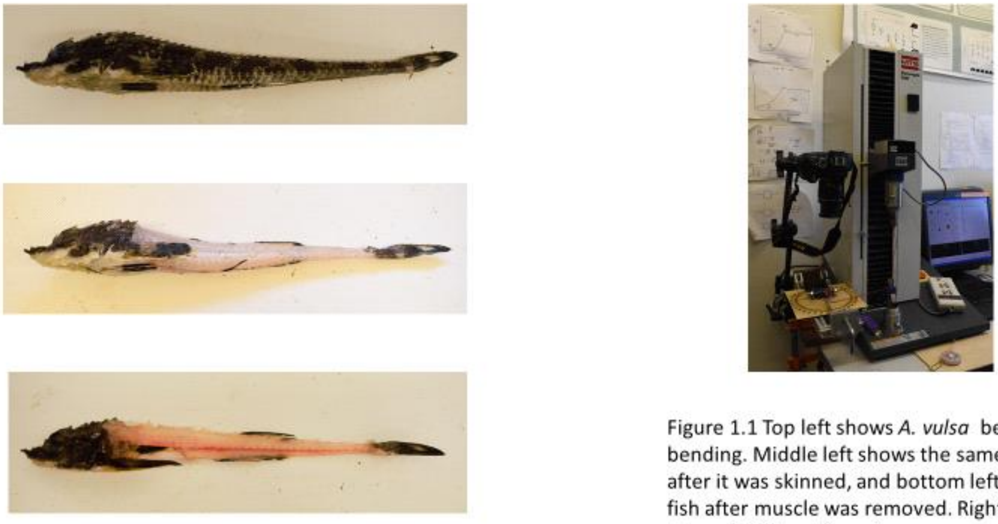

Figure 1.1 Top left shows *A. vulsa* before bending. Middle left shows the same fish after it was skinned, and bottom left shows fish after muscle was removed. Right side shows MTS bending setup.

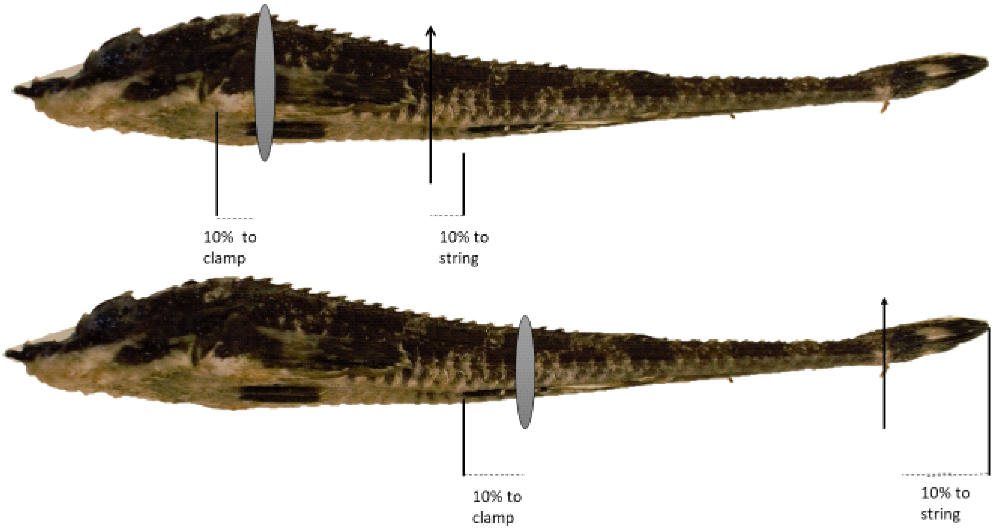

Figure 1.2 Shows attachment to MTS for bending. Both clamps and string were attached at 10% of the region's length.

On the day testing was to take place, the fish were completely thawed and then bent using an MTS Synergie 100 material tester (Fig. 1.1). In the first run, the head end of the fish was clamped, and a string was tied to the posterior end of the mid-body region (placement determined by the fins). This string went through pulleys to the load cell of the material tester. The placement of the clamp and the string were determined by measuring the body region and placing both the clamp and string 10% of the total body length in from the measured area (Fig.1.2). The fish was bent until a max force of 8N was reached, or the fish was about to be damaged from the force. The same method was used for a second run that measured the bending force of the tail. For this run, the posterior end of the mid-body region was clamped and the tail end of the fish was attached to the string. The force necessary to bend the fish was measured (five times for each of the two regions of each fish). Bending trials were performed on the whole fish, and then the armor plates were removed and measurements were taken again. Finally, the muscle was removed, and measurements were taken a third time. Each trial was videotaped using a Nikon D5300 set at 1920x1080, 60p and photos of each fish were taken before trials began, after skin removal, and after muscle removal using the same camera. The data gathered was statistically analyzed via an ANOVA using Rstudio (R core team 2017).

Plate collection and SEM

Plates were taken from previously frozen fish: six *A. vulsa*, two *Bothragonus swanii* and four *Bathyagonus alascanus*. Twelve plates were taken from each fish (a set of three plates from the dorsal, the two lateral, and the ventral row of plates) starting posterior from the dorsal fin. These plates were separated from each other and placed in a well plate according to the individual specimen. The plates were transferred to a well plate, and 1.5 mL of Sigma-Aldrich

0.025% Trypsin solution was pipetted into each well to submerge the plates (Leipertz, S.L.,1988). They were then placed on the stir plate for one hour 2h. In order to remove the tissue, the plates were transferred into a well plate that had several holes drilled into each well using a Dremel, and the entire well plate was placed into a SPT Sonicator for 18min. Next, each plate was cleaned (using tweezers and paper towel) and placed into a new (dry) well plate. These were left uncovered to dry overnight. (prior to this, plates were not allowed to dry out). The following day, six plates from an *A. vulsa* specimen were critical point dried according to protocol using a Tousimis Samdri-790. They were then set on a specimen holder using carbon conductive tabs with three plates facing up and three down, sputter coated using a Cressington Sputter Coater 108 and SEM images were taken using a NeoScope JCM-5000 so that the interlocking ridges of the plates could be seen clearly.

Plate material testing

The cleaned and air-dried *A. vulsa* plates prepared, as described above, were used for material testing. To prepare them for measurements, they were attached to custom built specimen holders using super glue, and once the glue dried, sanded flat using 220 sandpaper. The specimen holders were bolted to the material tester, a Tetra Basalt Must LMS 20, and measurements were taken to test the stiffness of the material. Five measurements were taken per plate, and three plates were used from each specimen of *A. vulsa*. A total of three *A. vulsa* were used. Vertebrae from the same fish were prepared for comparison by placing them in Trypsin for three hours, air drying overnight, and sanding (according to the protocol above for the plates). These pieces were tested in the same way and with the same quantities as the plates. In addition, three fresh plates (that had not been digested) were measured as a reference. The plates were indented with a needle that had a tip radius of 0.088 mm. Once all measurements were finished, Rstudio was used to find the Young's modulus from the force distance curve. Using ANOVA, these values were compared and tested for statistical significance.

Results

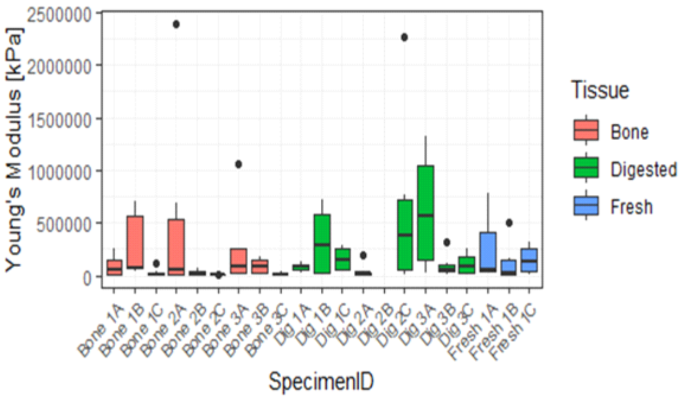

Figure 2.1 Shows the Young's Modulus for each plate measured according to tissue type.

We calculated the mean Young's Modulus to be 722 MPa for the digested plates, 142 MPa for the skeletal bone, and 167 MPa for the fresh plates. Our ANOVA showed that there is no significant difference between the stiffness of the *A. vulsa* plates that had not been digested in Trypsin, plates that had and pieces of the backbone (p=0.35).

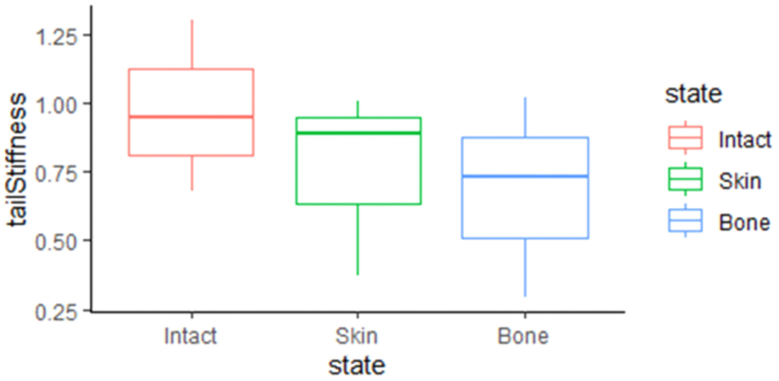

Figure 2.3 Shows the change in tail stiffness [N/mm] for the three different ways *A. vulsa* was measured.

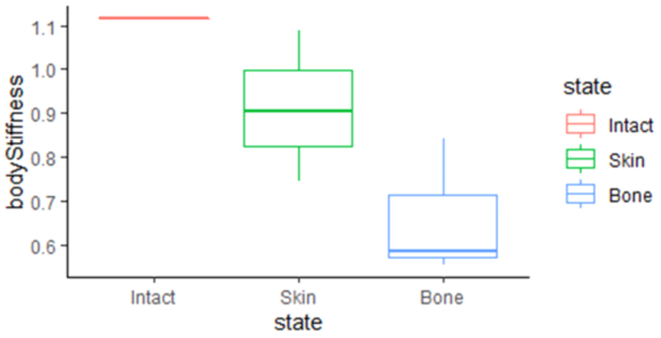

Figure 2.2 Shows the change in body stiffness [N/mm] for the three different ways *A. vulsa* was measured.

In our bending experiment we found that the body was stiffer than the tail. Furthermore, our bending experiment with the whole fish showed the highest stiffness, followed by the same experiment with the same but skinned fish, and the fish after the muscle was removed showed the lowest stiffness. However, in the tail the difference between the three states was much less pronounced than in the body region. In the tail, there was almost no change from the intact fish to the skinned one, and not much of a change from the whole fish to the bone. In the body region, the changes seen were more pronounced. The body of the whole fish required more force to bend than any other measurement taken (when tail or body was being bent). In contrast, the bone of the body region required the least amount of force for any experiment.

Discussion

There was no significant difference between the Young's modulus of the fresh plates, digested plates and bone. This is interesting because it strongly suggests that the plates of *A. vulsa* are made of bone, as expected from other studies looking at protective armor (Porter et al., 2013). The lack of variation between the fresh plates and the rest also validates that the Trypsin used to clean the samples did not influence the sample's stiffness.

When looking at the body stiffness of *A. vulsa* (measured in N/mm), we found that the intact fish was the stiffest, followed by the fish with muscle still attached, and the bone was the most flexible (Hale, M.E., 1996). However, the extent to which this is true varied across the body and tail regions. The body region had a lot more variation in flexibility than in the tail and lost significantly more integrity than the tail region when being dissected. The body region also had larger drops in force between each state, showing that the skin (or plates), muscle and bone are all major contributors to the stiffness of the body region.

Meanwhile, in the tail, the differences in force needed to bend the fish for each state was much smaller. In addition, even when the fish tail was dissected down to the bone, there was still a lot of integrity. This indicates that the bone is contributing heavily to the stiffness of the fish in the tail. It appears obvious that the muscle is not contributing equally since there is less muscle tissue to contribute. However, it was surprising that the plates play a minor role in the tail's stiffness. This may be due to the fact that vertebrae are evolved to offer structural support to the body. Armor, on the other hand, is built to protect the fish from external threats. It is interesting that these structural roles are mirrored by our readings in the tail region, but there appears to be more to the role of poacher body armor in the body region, and that is structural support.

Conclusions

Poachers are heavily armored fish, and as seen in our study of *A. vulsa*, the plates covering their body offer both protection and structural support. We found the plates to be as hard as skeletal bones, supporting the intuitional perception of these fish being well defended. However, despite the plates being pretty hard, the fish are still somewhat flexible due to the way the plates are attached to each other (Martini et al., 2017). This is especially evident in the tail, as seen above. The body region is much stiffer than the tail region and loses more of its integrity when the plates and muscle are removed when compared to the tail.

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