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Written in Bone: Damage Patterns in Agonopsis vulsa Armor

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Written in Bone: Damage Patterns in *Agonopsis vulsa* Armor

A Thesis Presented
by
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To the Keck Science Department
Of Claremont McKenna, Pitzer, and Scripps College
in partial fulfillment of
the degree of Bachelor of Arts

Senior Thesis in Art and Biology
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Abstract

Naturally occurring armor has evolved in many different classes of organisms, often in response to predation, although other factors may play a part. In this study, the scales of the benthic armored fish *Agonopsis vulsa* were examined for damage patterns in order to illuminate the life history and environmental interactions of the fish. Scales from the fish were systematically destroyed in the lab, and observations made from the damaged scales were used to create a categorical damage rating, which was applied to 34 specimens ranging in trunk length from 2.3 cm to 14.2 cm. The specimens were rendered as three-dimensional digital models after being scanned with a micro-CT scanner. During data analysis, the damage categorization was simplified to a binary of damaged vs. undamaged and statistical significance of damage was calculated using probability loop simulations. Statistically significant damage was found in a few small clusters across the ontogeny, consistent with predation and intraspecies competition. This study is also suggests that the flattening of ventral scales in *A. vulsa* is not congenital. The scales are most likely ground down by constant friction against the sea floor over the lifespan of the organism.

Introduction

Natural armor has evolved many times, in a multitude of species, in very diverse environments. In vertebrates alone, armor is a trait that has appeared in many different forms, including thickened and keratinized scales, spines (Broeckhoven et al., 2018), plates of dermal bone, and carapaces (Broeckhoven et al., 2015). These morphologies appear in mammals, reptiles, and fish in a variety of ecological niches.
Evolutionary thinking generally agrees that predation is the primary evolutionary force on the presence of natural armor (Broeckhoven et al., 2015; Broeckhoven et al., 2018; Marchinko, 2009; Song et al., 2011; Young et al., 2004), though recent studies have brought some new theories to the discussion.

There is evidence of direct selection informing armor in animals. It has been demonstrated that predation by shrikes is a clear evolutionary influence on horn length in flat-tailed horned lizards (Young et al., 2004). Different types of predators have also been shown to cause different expressions of armor—in threespine sticklebacks, research indicates that populations in areas with high concentrations of piscivorous birds and predatory fish (both of which are gape-constrained) evolve heavier armor and longer spikes (Marchinko, 2009). Populations of sticklebacks that are preyed on as juveniles by aquatic insects, such as dragonfly naiads (Foster et al., 1988), have lighter armor and smaller spikes, as longer spikes allow insects to more effectively hold onto and consume prey (Marchinko, 2009).

However, this hypothesis of a direct correlation between predatory selection and armor has also been explored in other species with interesting results. Comparing ecological surroundings and predator guilds in Cordylinae, a family of armored South African lizards, suggest that predation may not be the driving force it is generally believed to be (Broeckhoven et al., 2018), and instead that armor may be more correlated to environment (Broeckhoven et al., 2018), either through the exploitation of niches that favor certain types of defense, or through non-predatory influences (Broeckhoven et al., 2015). A study comparing bite forces of mammalian predators against cordyloid armor from the same habitats demonstrated that in many environments, the armor was not tough enough to withstand predation, implying that other forces are at work in the evolution of armor durability and
shape (Broekhoven et al., 2015). For example, it may be more difficult for a predator to remove an individual with heavy armor and large spines from a crevice, or for a snake to constrict (Broekhoven et al., 2015). In mammals, body armor evolution can be facilitated by an increase in body size and arid or exposed habitats, rendering the animal more visible to predators (Stankowich and Campbell, 2016). Other factors for armor development include intra-specific conflict. *Polypterus senegalus*, a predatory armored fish (Song et al., 2011), is primarily threatened by attack from members of its species during cannibalistic attacks or territorial disputes (Song et al., 2011). Armor may also play a role in thermoregulation (Broekhoven et al., 2018) in cordylid lizards. These studies suggest that there are more factors affecting armor selection than simple predator-prey interactions.

Armor in fish generally comes in the form of scales. However, scales themselves are extremely diverse in composition among the various lineages of ancient and modern fish. Chondrichthyes have placoid scales, composed of dentine and enamel (Liem et al., 2001); basal antinopterygians have ganoid scales with multiple layers of ganoine, a substance similar to enamel (Bräger and Moritz, 2016; Vickaryous and Sire, 2009); while teleost fish have elasmoid scales, thin plates of collagenous lamellar bone (Bräger and Moritz, 2016), which may have been derived from placoid-like dermal denticles (Sire and Huysseune, 2003). Teleost scales have been found to be remarkably durable and functional, capable of withstanding severe puncture trauma (Khayer Dastjerdi and Barthelat, 2015), while also allowing fish extreme flexibility and mobility (Vernerey and Barthelat, 2014). A well-known example is the armored alligator gar, with ganoid scales arranged in a hierarchical structure with a thin, stiffer outer layer and a thick, pliable inner layer offering excellent energy dissipation (Allison et al., 2013). There are some species of fish with bony scutes, such as the
armored catfish *Corydoras arcuatus*, which are characterized by a layer of hyaloine, an enamel-like substance, on the surface of the scute (Sire, 1993). Scutes are thick keratinized scales which act as heavy armor (Broeckhoven et al., 2015; Sire, 1993).

The subject of this study, *Agonopsis vulsa*, of the Agonidae family within the Scorpaeniformes lineage, has traded mobility for heavy armor to an unusual extent. *A. vulsa*, like all fish in the Agonid (Poacher) family, has no swim bladder (Eschmeyer et al., 1983). It is also an extremely stiff fish, with very little undulatory movement, probably due to its armor plating and the properties of its vertebral column (Podell-Eberhardt, 2012). These thick, bony plates are so heavy and constricting that the fish swims almost exclusively with its pectoral fins, only using caudal movement during escape reactions (Nowroozi et al., 2009). This armor has clearly evolved in response to strong selection, considering the drawbacks that the fish faces due to its weight and stiffness. However, modern research is not clear on what environmental factors have driven this particular adaptation. The armor itself may be able to tell us, as it does not regrow over time, retaining all of the damage inflicted during a fish’s lifetime. These damage patterns have the potential to inform us about how these fishes live—what predator-prey interactions they are involved in, what types of intra-species aggression they take part in (if any) and how their environments physically alter them over the course of their lifetimes.

Theoretically, the differences in damage over the lifespan of the fish will offer insight into predatory damage versus damage sustained from intra-species combat. Damage in larger, more mature fish that is not present in juveniles is likely to be related to sexual competition or cumulative damage from the wear and tear of life on the ocean floor—for example, abrasion from locomotion through or over rough habitats. Damage that is consistent
throughout the ontogenetic line indicates predatory influence. Location of damage will provide information about predators. Minor damage all over the body may correspond to attacks by small arthropods, while major groupings of damage along the tail could indicate a narrow escape from a larger animal. Damage sustained by intra-specific competition would likely leave patches of lateral damage along the anterior of the fish. Additionally, damage and scale shape along the underside of the fish may give us information about the effects of the environment on *A. vulsa*.

I expected that the smaller the fish, the less overall damage it would show—larger, more mature fishes would accumulate more damage over their lifetimes, due to predation, competition, or environmental interaction. I also expected that larger fish would show large patches of damage on their posteriors as evidence of attempted predation, as well as anterior lateral damage consistent with competitive wrestling.

**Materials and Methods**

**Study Organism**

*Agonopsis vulsa* is a heavily armored benthic marine fish which is found all along the Pacific coast of North America, from Alaska to southern California (Eschmeyer et al., 1983). Found in cold waters, *A. vulsa* (also known at the Northern Spearnose Poacher) prefers soft or mixed seafloors (Kells et al., 2016), generally at depths of about 60 to 1,300 feet (Eschmeyer et al., 1983; Kells et al., 2016). *Agonopsis vulsa* is lacking a swim bladder, and due to its protective bony plating, generally rests on or swims near the sea floor, moving primarily using its pectoral fins (Nowroozi et al., 2009). It feeds on marine invertebrates, mainly crustaceans (Eschmeyer et al., 1983), and may be preyed on by larger fish, seals, and...
marine birds (Zier and Gaydos, 2014).

_Agonopsis vulsa_ belongs to the family Agonidae, the Poachers, which are found throughout the northern Atlantic and Pacific oceans. The Agonidae belong to the order Scorpaeniformes, which includes sculpins, snailfishes, and lionfish. Scorpaeniformes, in turn, belong to the class of Actinopterygii, or ray-finned fishes (Froese and Pauly, 2017).

_Figure 1._ _A. vulsa_ has an elongate body pattern with rows of bony plates equipped with a spine.

The Northern Spearnose Poacher, along with many of the Poachers, is an elongate fish with a spiny head and two forward-facing spines at the tip of its snout (Eschmeyer et al., 1983). The pectoral fins are large and fanlike, with two dorsal fins (Figure 1). Almost the entire body, with the exception of a ventral area between the pelvic fins (Peixoto et al., 2018), is covered in ossified dermal plates in eight rows of approximately 40 scales, which are constant throughout its lifespan (Peixoto et al., 2018). The scales are covered in trabeculae (Bouilliart et al., 2014), microscopic structural support elements, creating a pitted web-like structure which can be seen in Figure 2.
Figure 2. Scanning electron microscope image and illustration of a lateral *A. vulsa* scale, taken after cleaning with papain and sonication. Note the intricate structure of the trabeculae. The scale is about 3.5 mm long.

Specimens of *A. vulsa* were sourced from multiple locations. Some specimens were purely digital models saved in lab archives, while some specimens were scanned for this specific experiment. The specimens that were scanned were drawn from two sources—old specimens preserved in lab freezer storage, and preserved specimens captured via trawling of the San Juan Channel off of Friday Harbor, Washington. Trawling was conducted by researchers on the Centennial, a research vessel sponsored and owned by the University of Washington.

**Specimen Preparation and Experimental Procedure**

Specimens were preserved in 70% ethanol, then scanned using a Bruker SkyScan 1173 MicroCT scanner. In preparation for scanning, the fish (or multiple fish) was wrapped in dampened cheesecloth, inserted into a 3-D printed can specially designed for use in the scanner, and wrapped in cling-film to prevent any water from escaping. The specimens were scanned using an aluminum 1.0 mm filter, with a voltage of 65 kV and an amperage of 123 µA, at a resolution of 2240x2240 µm. After the scans were completed, the data was...
reconstructed in NRecon. Specimens were then isolated in Dataviewer and checked in CTVox. After being cut down in Dataviewer into smaller files, these were converted into DICOM format using DICOMCT. All of these programs are developed and offered by Bruker, as supplements to the CT scanner. These DICOMs were opened in 3D Slicer, an open-source program designed for medical research. They were then segmented and visualized as three-dimensional surfaces within the program.

Damage to *A. vulsa* scales was investigated by damaging single scales in a controlled environment and observing the damage patterns. Scales were harvested from a single specimen that was obtained from the lab’s storage freezers. The specimen was dissected, and large strips of lateral, dorsal, and ventral scales were removed, along with flesh and skin. These samples were submerged in a papain solution. Papain is an enzyme found in unripe papaya fruit, which attacks peptide bonds and digests proteins, leaving mineralized tissues—the bony scales themselves—intact. This solution was sonicated, left overnight, then sonicated again. Sonication, a technique in which particles in a bath are exposed to high-frequency sound waves, is used for many applications, from speeding dissolution to producing emulsions to cleaning jewelry. The scales were then removed and sonicated in a detergent solution and left to dry. These scales were examined and photographed with a JEOL JCM-5000 NeoScope Table Top Scanning Electron Microscope.

They were then systematically damaged—scales were smashed, abraded against rocks and other scales, crushed, subjected to sharp and slow impacts, and the scale spines were snapped. Twenty-two scales were observed in this phase. A single scale was smashed with a hammer. Scales were abraded against a stone gathered off a beach at Friday Harbor, a location which *A. vulsa* is native to. Scales were also abraded against other scales, which
were fashioned into a specialized tool constructed from a tongue depressor, which allowed the small scales to be handled. Abrasion was also performed using sandpaper. Different crushing damages were inflicted with a pair of lab forceps and with a palate knife against a tray. Sharp impacts were caused by short, forceful strikes to the scale with the back of dissection tweezers. Slow impacts were caused by the same instrument on a tray, with a slow and forceful downward pressure. Spine scales were snapped by the simple expedient of gripping with tweezers and pulling. Snapping damage was conducted with the grain of the scale, against the grain, across the grain, and through direct upward force.

These types of damage were logged, the scales were identified, and the damage patterns were examined and photographed again with the SEM. However, it must be noted that these scales had already sustained damage during the lifespan of the fish they were removed from, making the pre-damage images invaluable. These ‘before and after’ photos were used to determine the extent of and patterns left by these deliberate damages.

Ultimately, these photos helped influence the calibration of a scale for macroscopic damage. It was determined that damage to the spine of the scale would be most noticeable, and most likely according to the photos of scales that had not yet been damaged in the lab. After observing the digital three-dimensional renderings of several A. vulsa specimens in the digital archives, a categorical classification was devised. There are two major types of damage inflicted on these animals—friction damage, caused by long term wear, primarily (in the ventral scales) against the sea floor, and impact damage, caused by short-term, traumatic events. It was determined that there would be two sub-classes of each type—minor and major. Minor friction damage constituted the loss of the fine tip of the spine, while major friction damage was denoted as the almost complete loss of a visible spine—an almost
flattened scale. Minor impact damage meant a sharp break in the spine, with a visible shortening. Major impact damage described a severe shortening of the spine with a sharply broken base. These categories were given numerical designations—an undamaged spine was 0, minor friction damage was 1, major friction damage was 2, minor impact damage was 3, and major impact damage was 4. Examples of each designation can be seen in Figure 3. The scale shown in Figure 2 would be designated a 1.

Figure 3. Examples of spine damage. 0—undamaged. Note fine point on spine tip. 1—minor friction wear. Note rounded spine. 2—major friction damage. Note dramatic flattening of spine and scale. 3—minor impact damage. Note abrupt breakage and shortened spine. 4—major impact damage. Note extreme abrupt breakage.

These categories were applied to the scans of fish that had been visualized as three-dimensional surfaces in 3D Slicer. Beginning at the anterior of the scale rows, each scale was surveyed and the state of its spine was described by the numerical categories and entered into a datasheet. The scales were organized by position and scale row within the sheet, along with the trunk length of the fish. 34 specimens were examined, each of which had eight rows of 40 scales each. In total, 10,880 scales were surveyed for this experiment. The length of the fish was measured from the first ventral scale to the end of the last ventral scale. This measurement was due to the available fish models—some were missing portions of their heads, making a more standardized length measurement impossible. The measurements were performed in 3D Slicer using the Ruler and Fiducial tools.
Data Analysis

For data analysis, the categories of damage were set aside, and instead a binary system of categorization was instituted. This simplified the process of analysis while also allowing study to focus primarily on the position of the damage, rather than the severity. Scales were either damaged (categories 1, 2, 3, or 4) or they were undamaged (category 0). The fish were also subdivided into five subsets according to trunk length. The subsets were as follows: smallest (fish under 4 centimeters), small (fish 4 to 8 centimeters), medium (fish 8 to 10 centimeters), large (fish 10 to 12 centimeters), and largest (fish over 12 centimeters). With a total of 34 fish, 8 were in the smallest subset, 10 in small, 6 in medium, 5 in large, and 5 in largest.

Figure 4. The naming convention of the rows of scales, anterior view of *A. vulsa*. The outer ring is a cross section through the trunk of the fish. This figure is not to scale.

Commented [LS5]: Not entirely sure if I understand this figure correctly. The outer ring is a cross section through the body?
Every fish has eight rows of scales, four on each side. These are, in order descending from dorsal to ventral, Rows A, B, C, and D. A is the most dorsal row of scales. D is the ventral row. The two sides of the fish are differentiated as Left and Right, as can be seen in Figure 4. For example, the second-most dorsal row on the right side of the fish is designated as RB. There are 40 scales in every row, set in a straight line along the fish from anterior to posterior. This scale pattern is shared by every fish in the dataset. Thus, every fish has, for example, a 32nd scale in the most ventral row on their left side (32LD).

Next, because bilaterally symmetric damage was not hypothesized to be of experimental importance, the left and right sides of the fish were compiled. Rows RA and LA became simply Row A, and so forth.

The total number of damaged and undamaged scales across all fish in a subset at each specific position was counted. As the only possible values were 0 (undamaged) and 1 (damaged), sums and means of the numbers were an efficient way to gauge the total damage to fish. Each fish represented a 320 data points (scales), which could be either damaged or undamaged. The sum of the damaged and undamaged scales was the total number of scales at that position, in that row, on the fish in that subset. The number of damaged scales divided by the total number of scales produced the mean damage at that position—the probability that a scale at that position in that subset was damaged. The total number of damaged scales in all the fish in all the subsets was divided by the total number of scales in the entire experimental sample to find the global mean of damage. These values were used in the probability loop simulation in RStudio.

RStudio was used to run a simulation of the numbers to determine statistical significance of damage patterns. RStudio is a opensource computer program developed by
RStudio Inc. as an integrative development environment for the statistical programming language R. The simulation produced a thousand random numbers with identical probabilities of damage as a given position on the fish. The distribution of these randomly generated values of damage were compared with the true damage rates. If less than fifty of the randomly generated numbers were higher than the observed damage rates, than the P-Value was determined to be less than 0.05 and the scale location was significantly damaged.

There are very high rates of damage in the ventral rows of scales, which I worried would be skewing the overall mean damage of the fish towards a higher value, obscuring damage in the more dorsal scales. To determine if there were significantly damaged scales that were being hidden by the high rates of damage in Row D, the data analysis simulation in RStudio was performed again, excluding the data from the ventral scales.
Finally, the original categorization of scale damage was used. The impact damage (categories 3 and 4) was reexamined in terms of position along the fish in the different subsets. The number of damaged scales present at each scale position along the fish in each subset was tallied, as was the total number of impact-damaged scales.

**Results**

The global mean damage of the sample was 0.807—about 80.7% of the scales sampled were damaged. On average, this means 258 of the 320 scales on a single fish would...
be damaged. The smallest fish showed lower rates of damage than this, while almost all other subgroups of fish showed higher rates.

The ventral scales (Row D), were more damaged than any other row. The mean damage of Row D across all fish in the sample was 0.945—the next highest rate of damage in a scale row, Row A, showed a mean damage of only 0.776 (Table 1). There were definite patterns of damage in the scale rows on the fish.

The total mean damage of the largest subset is 0.850, which is almost a quarter again as much as the total mean damage of the smallest subset, at 0.665 (Table 1). Generally, the larger the fish, the more damage it accrued. However, the medium subset of fish had very high levels of damage, greater than or equal to the damage levels in the largest fish subset. This can be seen in Table 1, as the total mean damage of the medium subset (0.874) is actually higher than that of the large and largest subsets (0.807 and 0.850).

**Table 1.** The mean damage of the of the rows in each subset, as well as the mean damage of the subsets themselves and the mean damage of all rows across all subsets.
In order to look for patterns of heavy damage, the significance of damage in scales was calculated and mapped onto diagrams of the fish. Scales that were significantly more damaged than the global mean of damage (p≤ 0.05) are highlighted in Figure 6a. Note that many more scales are significantly damaged in large fish than in smaller fish. When the significance of damage was calculated using the mean of damage of only the three most dorsal rows of scales, more scales were found to be significantly damaged (Figure 6b), showing that the heavy damage accrued by Row D was skewing the statistics. It also shows that there are some patterns of heavy damage, particularly in Row D and at the front of the medium-sized fish and the tail of the largest fish.

<table>
<thead>
<tr>
<th>Subset</th>
<th>Row A Mean Damage</th>
<th>Row B Mean Damage</th>
<th>Row C Mean Damage</th>
<th>Row D Mean Damage</th>
<th>Mean Damage of Subset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallest</td>
<td>0.657</td>
<td>0.620</td>
<td>0.587</td>
<td>0.795</td>
<td>0.665</td>
</tr>
<tr>
<td>Small</td>
<td>0.799</td>
<td>0.773</td>
<td>0.756</td>
<td>0.983</td>
<td>0.828</td>
</tr>
<tr>
<td>Medium</td>
<td>0.863</td>
<td>0.829</td>
<td>0.823</td>
<td>0.981</td>
<td>0.874</td>
</tr>
<tr>
<td>Large</td>
<td>0.732</td>
<td>0.762</td>
<td>0.759</td>
<td>0.975</td>
<td>0.807</td>
</tr>
<tr>
<td>Largest</td>
<td>0.8290</td>
<td>0.790</td>
<td>0.788</td>
<td>0.992</td>
<td>0.850</td>
</tr>
</tbody>
</table>

Mean Damage of Row

| Mean Damage of Row | 0.776 | 0.755 | 0.742 | 0.945 |
When examining the impact damage in scales, some interesting patterns were found. In almost all size subsets, there were spikes of mild and severe impact damage between scale positions 28 to 35. Additionally, the smallest and small subsets of fish show higher rates of impact damage than the larger fish, as can be seen in Table 2.
Table 2. The number of scales categorized as mildly damaged by impact, the number of scales categorized as severely damaged by impact, and the total number of scales damaged by impact in the different size-based subsets of fish.

<table>
<thead>
<tr>
<th>Subset</th>
<th>Mild Impact Damage</th>
<th>Severe Impact Damage</th>
<th>Total Impact Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallest</td>
<td>992</td>
<td>296</td>
<td>1288</td>
</tr>
<tr>
<td>Small</td>
<td>1102</td>
<td>645</td>
<td>1747</td>
</tr>
<tr>
<td>Medium</td>
<td>846</td>
<td>431</td>
<td>1277</td>
</tr>
<tr>
<td>Large</td>
<td>866</td>
<td>441</td>
<td>1307</td>
</tr>
<tr>
<td>Largest</td>
<td>984</td>
<td>266</td>
<td>1250</td>
</tr>
</tbody>
</table>

Discussion

It had been hypothesized that smaller fish would show lower rates of damage, due to less time accumulating damage and fewer incidents of attempted predation, and that large fish would show anterior lateral damage from intraspecific competitive wrestling and sizable patches of posterior damage.

If my hypotheses were correct, I would expect smaller fish to show less damage and larger fish to demonstrate specific and common patches of posterior and lateral damage.

There are heavy rates of damaged ventral scales, rates which increase along with the size and age of the fish. The flattened shape of the scales in the ventral rows in *Agonopsis vulsa* is not congenital. These scales are worn flat by the animal’s movement against the sea floor, as these fish rarely move up into the pelagic zone. Very small (very young) fish have defined spines on these scales, and show almost no significant damage in the ventral rows.
However, the next largest subset, a size jump from under 4 centimeters to 4 to 8 centimeters, has much more damage, with both a higher incident of damage and many more significantly damaged scales. This increases over time until the largest subset of fish has almost entirely significantly damaged ventral scales. This is interesting, because *A. vulsa* is described as preferring soft bottom habitats (Kells et al., 2016), composed of smaller sediments. The sandpaper-like effect that these conditions have on the fish raises questions about how other demersal fishes deal with rocky bottoms. There has been evidence showing that gross anatomy in closely-related benthic and pelagic fish is different (Erickson et al., 2016), perhaps due in some part to this effect.

There do not appear to be any large patches on the fish with heavy or significant damage that are constant across the ontogeny, from smallest subset to largest subset. Medium fish show relatively heavy damage at the anterior of the trunk, while the largest fish show damage groups around the mid-posterior of the tail. The other subsets do not show any particular grouping of significant damage. However, there are small close groupings of scales which show damage across multiple subgroups. C7 and C8 show significant damage in every subgroup except the smallest. This pattern of shared damage is repeated at scale A20. There are incidences of damage in the anterior dorsal scales (A1) of both the small and medium size subsets, though this does not carry on into the larger subsets. Large and larger fish, on the other hand, both show patches of damage at around B and C28 to 35. It appears that smaller fish have higher incidences of anterior damage, while larger fish have more posterior damage. There is also an interesting spike in damage for the medium sized fish, at 8 to 10 centimeters.
There, as far as I can see, two explanations for the shared scale damage across ontogeny. First of all, it may be due to male-on-male competition—the fish’s bony heads were not examined for damage, but if they locked heads and wrestled, it could account for the damage on the anterior lateral scales. The other option is predation, at least in the smaller fish. I lean more towards predation, as the small fish subset seems too young to engage in combative sexual behaviors, being only slightly larger than the juveniles. Predators could be arthropods or larger piscivorous birds or fishes. I would expect that smaller arthropod predators are responsible for the highly localized damage. Both locations are adjacent to fins, perhaps making them easy to catch and attack, as other studies have found the spines aid insect predation (Marchinko, 2009). The larger and more generalized posterior areas of damage, particularly on the largest fish, could be caused by attacks by bigger predators. This is congruent with the additional investigation into the type of damage. Spikes of impact damage, which would likely be the type of damage inflicted by an attempted predation, are present in the same location as the patches of posterior damage hypothesized to associated with evidence of predation.

The size of the affected fish could be a factor in these attacks—smaller fish are not worth the expenditure of effort and are more armor than food. Once *A. vulsa* reach larger sizes, they could enter the pool of potential prey animals for larger predators, introducing new damage patterns. As a simple illustration of this concept, we can think about the volume versus surface area. We assume volume is flesh, nutritionally useful to a predator, while surface area can be regarded as armor. The scaling effect means surface area is proportionally much greater in smaller fish—the very high ratio of inedible armor to edible flesh makes small armored fish unappetizing. Calculating a simplified volume and surface
area of fish using the formula for cones gives the ratio of 18.62:1 surface area to volume in one of the smallest fish (2.4 cm long), while one of the largest fish (14.2 cm long) shows a more appetizing ratio, at 3.18:1.

The high levels of damage in medium fish is unexpected, as are the high rates of impact damage in smaller and small fish. Two possible explanations are that either fish regenerate their scales over their lifetimes, leading to less damage in larger fish, or that these small and medium subsets of fish simply incurred an unusual amount of damage in their lifetimes. The second possibility seems more likely. These fish could have been heavily damaged during their capture by Friday Harbor Labs in a trawl net. It’s also possible that specimens that survive to large sizes simply have not run into as many damaging situations. A fish that incurs a great deal of damage early in its life is likely injured and easier to capture or eat, meaning that fish used in this experiment may be skewed towards higher levels of small incapacitated fish and large undamaged fish, not reflecting accurate levels of damage in wild populations of A. vulsa.

The data analysis done in this thesis does not take into account all of the categorization of data preformed during the experiment. A continuation of the study would use that additional type and degree data to look more closely into the cause of damage. This binarization of the data was a simplification for time and comprehension, but including additional data will produce more information about what caused this damage. Minor wear damage makes up most of the quantified data, changing interpretations of predatory interactions. An attack would not be expected to cause minor wear, but instead mild to heavy impact damage, with the sharp impact of a gripping predator. More comparisons should be done, taking into account abrasion damage as well.
Additionally, while the total sample size was reasonable, the subdivision by trunk length broke the sample into many small parts, which I feel did not necessarily provide enough data points. Given the opportunity I would be interested in adding more fish of a variety of sizes to the experiment, so as to include more data points to the subsets, which are an important part of the data analysis.

This thesis would benefit from further study, and does not provide any concrete answers about how *A. vulsa* lives, reproduces, or is preyed on. However, it proves something interesting about armored benthic fish. Specifically, ventral armor plating is worn down over time through abrasion against sediment on the seafloor. Thus, not all fish with flattened ventral scales can be assumed to be born with this arrangement and shape of scales. This may have some impact on the study of structure and histological composition of ventral scales, which would theoretically have to be more resilient to wear and/or thick, so as to withstand abrasion over the organism’s lifespan.
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