The effectiveness of durophagous tooth morphologies when crushing *Mytilus* and *Nucella* shells

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Abstract

Teeth are the interface between an organism and its prey, and can be used to infer an organism's diet. However, little is known the interactions between tooth morphology and prey items in durophagous systems. To begin to investigate these interactions, we tested the force required by two tooth morphologies, one cupped and one pointed, to break model snail and mussel shells. We believed that the cupped tooth would best crush the snail shell, and the pointed tooth would best break the mussel shell. The pointed tooth was found to crush the snail shell at a lower force, while for the mussel shells there was no difference in crushing forces between tooth morphologies. Further study is needed to fully appreciate the interactions between tooth and prey morphologies, especially to be able to accurately predict an organisms' diet based on tooth shape.

Introduction

Durophagous organisms eat hard prey items like mussels and snails by crushing prey with specialized teeth. Teeth are important in food processing for many organisms, and can be specialized to better process specific foods. A more effective tooth requires less force to break a prey item, allowing the predator to eat its prey more easily. Massare (1987) divided marine mammals into feeding guilds based on tooth morphologies, including two guilds with tooth forms specialized for durophagy. The crushing guild had wide, flattened teeth which were specialized to eat hard-shelled prey like clams, while the crunching guild had blunted pointed teeth to eat prey such as crustaceans and thin-shelled ammonites. Based on these classifications it is possible to use tooth morphologies to make guesses about an organism's diet. For instance, the ichthyosaur *Mixosaurus fraasi* was classified in the crushing guild based on tooth form, and this classification was also supported by tooth wear patterns indicating that the teeth met frequently and concentrated force in one area (Motani 2005). However, these are broad categories, and it would be valuable to know more about what specific morphologies correspond to specific prey. This could allow predictions about more specific prey items consumed by an organism based on its tooth morphology, instead of simply placing an organism in a broad guild that consumes a wide range of prey.

Previous studies have investigated the effectiveness of various teeth designs by measuring the energy required by a tooth analogue to process the prey item tested. Evans and Sanson (1998) made punches with different surface areas were used to split beetle exoskeletons. Sharper teeth models required less energy to penetrate and split the beetle exoskeletons. Anderson and LaBarbera (2008) found that notched blades required less force to cut muscle tissue. Anderson (2009) also found that blades with the ability to trap tissue required less force to cut through the tissue. However, these tests are less applicable to durophagous teeth, which crush hard prey instead of cutting or splitting soft, tough prey.

Tests on the durophagous teeth of the monosaur *Carinodens belgicus* showed they could easily crush hard prey (Schlup 2004). Schlup, however, did not focus on the specialization of the mosasaur teeth by investigating the force required to crush prey items. Instead, the study simply indicated if the prey item was crushed within the bite force the mosasaur was predicted to have. These tests indicate potential prey of the mosasaur, but say little about which prey items they (or other durophagous teeth) are specialized to eat. In fact, the study indicated that the mosasaurs may have also eaten soft bodied prey (Schlup 2004).

To begin investigate which durophagous tooth morphologies are specialized for specific prey, we examined two different tooth morphologies; one tooth type was convex (pointed) and one was concave (cupped). We tested which tooth type was more effective at breaking different hard prey items. The hard prey items we tested were a snail shell and a mussel shell. By testing these different shell morphologies and tooth morphologies we can start to understand what tooth shapes might be specialized for specific prey items. We believed that the cupped tooth will break a snail shell better and the pointed tooth will break a mussel shell better.

The cupped tooth more closely resembled Massare's crushing guild morphology, and the pointed tooth was a cruncher, since it has a blunt point. We believed the snail shells were crush prey, since their spiral shell had multiple layers to break and the shell was thicker, while we believe the less complex, thin mussel shell was a crunch prey. Also, mussel shells were slightly curved and had only one thin layer, so they might have responded similarly to the beetle exoskeletons tested by Evans and Sanson (1998). If this was the case, the pointed tooth would be more effective at breaking them. In contrast, the snail shell was thicker and was much more curved on the outside, which was very unlike a beetle, so the tooth morphologies that effectively break beetle exoskeletons would not be expected to be specialized to break snail shells. Therefore the pointed tooth would be more effective at crunching the mussel shell, while the cupped tooth would be more effective at crushing the snail shell.

The *C. belgicus* teeth tested by Schlup (2004) were similar to our pointed tooth, and effectively broke *Mytilus edulis* shells, further suggesting our pointed tooth would better crush our mussel shells. However, *Littorina littorea* shells were broken at a much higher force, and jumped out from between the teeth (Schlup 2004). This suggests the *C. belgicus* teeth were not specialized to crush spiraled shells like those of the *L. littorea*, and therefore our pointed tooth would not be specialized to crush our *Nucella* shell. The snail shell fit into the cupped tooth very well, so it could have been effective at trapping the shell during food processing. This would make the cupped tooth more effective at breaking the snail shell than the pointed tooth. However, the mussel was flatter and wider than the snail shell and did not fit into the cupped tooth's indentation, so the cupped tooth's advantage at trapping prey would not be relevant for the mussel.

Method

Tooth shapes were generated with Gaussian curves; the "pointed" tooth was created with one Gaussian curve stacked on top of a second curve, while the "cupped" tooth was created with the second curve subtracted from the first curve. The equation used to generate the tooth shapes is:

$$y = x^{32} + h * e^{\left(-\left(\frac{x^2}{0.4}\right)\right)}.$$

The variable h is the height of the dome; for our pointed tooth h was 0.5, and for our cupped tooth h was -0.5. The model teeth (Figure 1 A and B) were made out of 1 inch diameter aluminum rod carved on an automated mill Tormach PCNC1100 (Tormach, Waunakee, WI, USA) that was programmed with SprutCAM 7 (SPRUT Technology Inc., Naberezhnye Chelny, Russia). The cupped tooth stood 3.1cm tall, and the pointed tooth was 4cm tall. The diameter of the cup and the point was 1.1cm at their widest parts.

The model shells were generated from CT scans of real shells. The mussel shell was a shell from a *Mytilus sp.* and the snail shell was a shell from a *Nucella sp.* These were rendered in Amira version 5.2.2 (Visage Imaging, Richmond, Australia) and printed on a Z-Printer 310 3-D printer that runs Z-Print software version 7.10.3.7 (Z Corporation, Burlington, MA, USA). The printed shells were made from plaster based powder (zp150 high performance composite powder, Z Corporation) that was set with a solution of Epsom salts that must be applied manually after the shells have been removed from the printer. The snail shells were sprayed with the Epsom solution, which allowed the solution to fully penetrate the shell model. The mussel shells were placed on wire screen and the Epsom salt solution was applied dropwise with a thin syringe needle starting from the outer lip of the shell and working in, to avoid applying too much solution and deforming the shell. After the plaster was treated with the Epsom salt solution the shells were placed in an 80°C oven to dry for 12 hours.

The testing was done using the materials testing system MTS Synergie 100 (MTS Systems Corporation, Eden Prairie, MN, USA), and data output was measured by TestWorks 4 (MTS Systems Corporation, Eden Prairie, MN, USA). For testing, a model tooth was screwed onto a moveable crosshead with a 500N force transducer. Each model shell was placed below the MTS crosshead on a silicone pad. To keep placement constant for each test the pad had marks drawn to indicate where the shell should be placed. The pad was cleaned off between each test to remove excess plaster from previously broken shells. Each test was performed at a speed of 1.27mm/second. Tests automatically shut off at a break sensitivity of 75% for the snail shells and 60% for the mussel shells. This meant that, in the case of the mussel shells, when the force applied at any time was less than 60% of the maximum force that had been applied during the test, the MTS would end the test. Thirty six mussel and snail shells were tested for each tooth type, creating a total of 144 shells tested.

During testing both the load at yield (N) and the peak load (N) were recorded. The load at yield was the force on shell models when they first crack. The peak load was the maximum force that is applied to the shells. Usually the load at yield and peak load were the same, but occasionally the test would not stop, and the tooth would continue pushing on the shell after it had been fully broken (Fig. 2). This would lead to falsely high values for peak load. Additionally, after initial failure, any organism inside the shell would vulnerable to a predator. Therefore only the load at yield was considered in data analysis.

Data was analyzed using SigmaPlot 11 (Systat Software Inc., San Jose, CA, USA). The data was ranked manually, and then a two way ANOVA was calculated by SigmaPlot on the ranked data. The ranked data failed the equal variance test but, since ANOVAs are robust to assumption violations, the test was run anyway (Box 1954). SigmaPlot was also used to calculate pairwise comparisons using the Holm-Sidak method.

Results

The mussel shells initially broke at lower mean forces the than the snail shells, regardless of the tooth type tested (Fig 3). The pointed tooth required a lower force to break the snail shells than the cupped tooth (t=9.277, P<0.001). The pointed tooth required less force to break the mussel shells also, however, this was not found to be significant (t=1.041, P=0.3). The mean force required by the pointed tooth to break a snail shell was 105.23 N, while the cupped tooth required an average of 223.13 N to initially break a snail shell. Similarly, the pointed tooth required 29.91 N on average to break a mussel shell, while the cupped tooth required 31.33 N. The interaction between tooth and shell types was also found to be statistically significant (analysis on ranked data, $F_{1,\ 139}$=33.615, P<0.001), so the effect of a tooth morphology on breaking a shell depends on the shell type.

Discussion

Our hypothesis that the cupped tooth would require less force than the pointed tooth to break the snail shell was not supported. The pointed tooth was found to require less force to break the snail shells than the cupped tooth. We believe this may be because the snail shell should have actually been considered “crunching” prey (Massare 1987) instead of the “crush” prey we believed them to be. Their shells may not have been thick enough to be classified as “crush” prey. When the *Littorina littorea* were tested by Schulp (2004), they were described as large and smooth, and this was believed to be why they tended to jump out of the teeth during testing. We believed a cupped tooth could help solve this problem, but since the shell was sitting on a flat silicone pad instead of another tooth, it is possible that any trapping abilities the cupped tooth may have had were rendered unnecessary.

The pointed tooth may have been more effective because it had a smaller surface area in contact with the shells than the cupped tooth. This is true for both the snail and mussel shells. In biomechanics, stress is a measure of the internal forces acting within a body as a reaction to external forces applied to the object. Stress is equal to force divided by surface area. Therefore, if area decreases force must also decrease to keep stress constant. We would expect stress to remain constant for all of the snail shell models, since stress is and internal property and the shells are (in theory) identical. Therefore, it is logical that a pointed tooth would be able to break the snail shells at a lower force than the cupped tooth. The same logic can apply to the mussel shell models, since the stress the mussel shells can take should also be constant for every model tested.

Our second hypothesis was that the pointed tooth would require less force to break the mussel shells than the cupped tooth. The pointed tooth required less force to break the mussel shells than the cupped tooth, but the difference was not found to be significant. Therefore, we were unable to reject our null hypothesis that our tooth morphologies had an effect on the breaking force of mussel shells. It is possible that neither tooth we tested is particularly specialized for processing mussel shells. However, one of our concerns with this study was some difficulty in making our test shells. The mussel shells are very thin, and when they were sprayed with the Epsom salt solution to cure the plaster they would tend to deform, becoming flatter on the top. There was also a problem with the edges flaking away on some shells when they were removed from the printer, and this would sometimes prevent them from sitting flat on the silicone pad before the tooth models crushed them. The flattening of the mussel may have changed how the teeth interacted with them, and prevented us from accurately gauging if either tooth morphology is more specialized for crushing these mussel shells.

We would like to continue working on our method of curing the shells to see if there is a way to prevent shell deformation during the process, and then retest the shells to see if the flattening had an impact on the tests. It is also worth noting that we tested only one mussel shell, while in reality a mussel is a bivalve, so an organism hoping to eat a mussel would have to break into two shells held together by ligaments. This means an organism may break through two layers of shell, instead of the one we tested. It is also possible that mussel shells crack along their hinge instead of on the top of the shell when force is applied a complete shell. Our test did not reflect this, and further tests would be beneficial to truly understand the interactions between tooth morphologies and the mussel shell.

Another limitation of our study is the fact that we tested only individual tooth morphologies. We did not investigate other aspects of teeth, such as the material properties of teeth, nor did we investigate an accurate representation of teeth in a jaw. We had only one tooth, instead of many teeth in a row, and force was applied from one model tooth onto a shell on a silicone pad, instead of another tooth that could also apply force. The teeth models were also aluminum, which is much stronger than the plaster the shell models were constructed from. These teeth did not break or even wear appreciably during the testing, which is not accurate to real life. Further tests could later be done on the material properties of teeth and their wear patterns after eating hard prey, to investigate factors other than just tooth and shell morphologies that might affect tooth designs in nature.

This study is valuable because it adds more data to our knowledge about the interactions of tooth morphologies and prey items for durophagous animals. It seems that snail shells fall into the “crush” guild prey, and are more effectively processed by teeth with a rounded point than teeth with a cupped morphology. Other prey items can also be effectively processed by pointed teeth, including bivalves, some gastropods, sea urchins, small squid, and shrimp (Schulp 2004), so it is important to continue testing variations on tooth morphologies to identify the most effective tooth morphologies for a given prey item.

This study is a preliminary step into discovering the tooth morphologies that are most specialized at crushing hard prey. Other factors, such as material properties of teeth, wear patterns on teeth, interactions between multiple teeth, material properties of shells, and other properties of prey items (such as the ligaments in bivalve shells) should be studied to truly be able to identify an animal’s diet based on its teeth.

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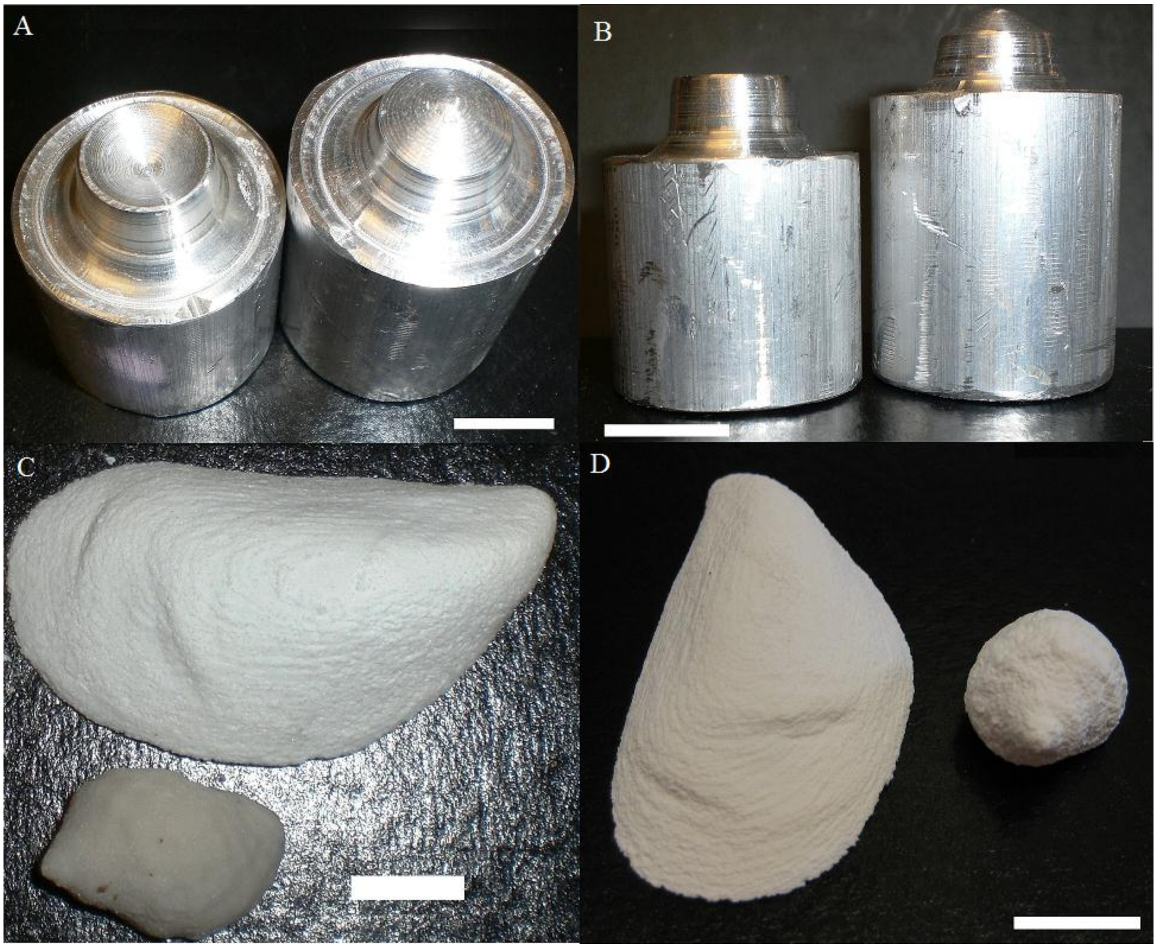

Figure 1. A. Top view of model teeth tested in our experiment. The tooth on the left is the "cupped" tooth, and the tooth on the right is the "pointed" tooth. B. Side view of model teeth. C. Top view of the model shells tested. The mussel shell is on the left and the snail shell is on the right. C. Side view of the model shells. The scale bar is 1cm long in all images.

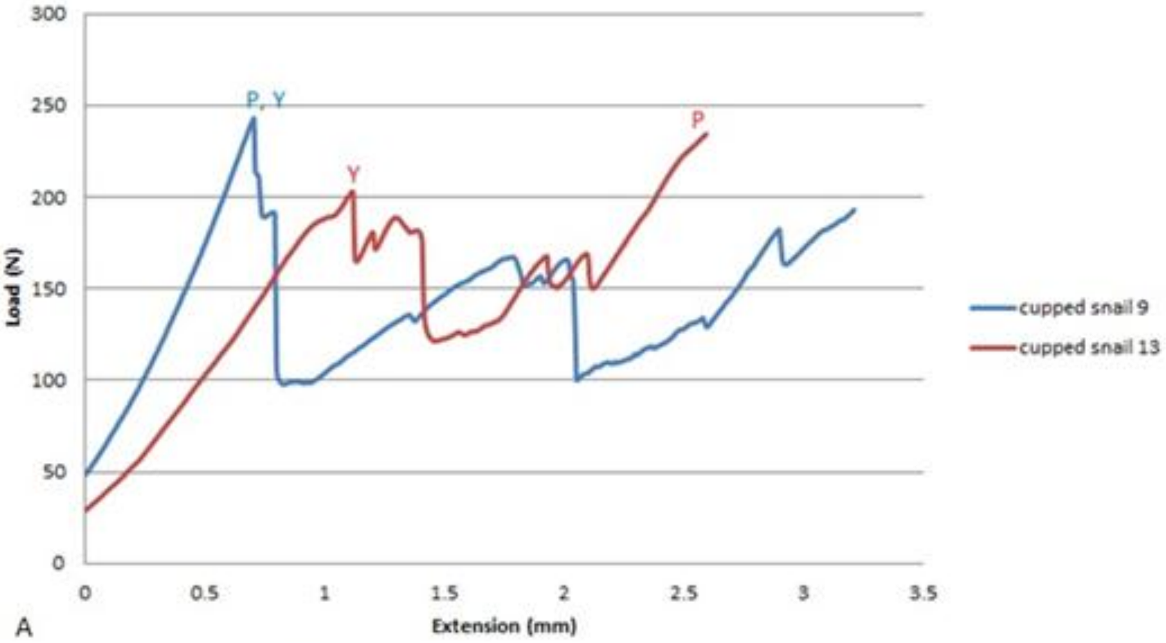

Figure 2 A. Example of an extension vs. load curve. This graph shows the distance moved by the crosshead (extension), and the force applied to the test shell. These data are from two snail shells broken by the cupped tooth. Cupped snail shell 9 (blue) demonstrates a representative curve; the peak load and load at yield (marked on the graph as P and Y) are equal and are much higher than other peaks in the graph. Cupped snail shell 13 (red) broke at 203 N (load at yield) but the peak load recorded was 234N.

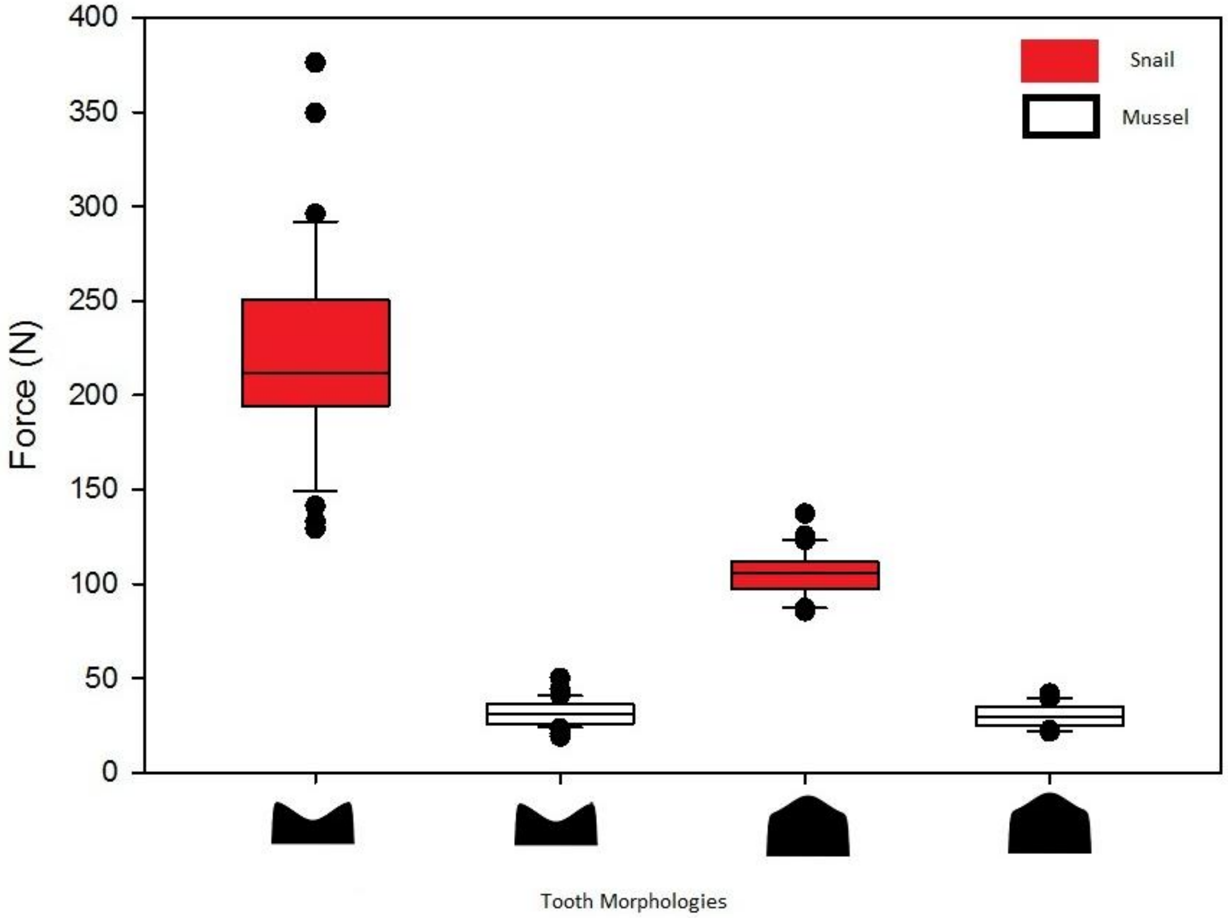

Figure 3. Box and whisker plots showing the force required by a tooth model to make an initial crack in a test shell. The tooth morphologies (cupped or pointed) are shown by the symbols on the bottom. The boxes show the lower quartile, median, and upper quartile for each test. The whiskers show the range of the data. The difference between the mussel and snail shells is significant (analysis on ranked data, $F_{1,\ 139}$=678.938, P<0.001). The difference between the cupped and pointed tooth morphologies is also significant (analysis on ranked data, $F_{1,\ 139}$=52.937, P<0.001).

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