Correlation Between Shell Size and Presence and Size of Apertural Teeth in the Low-Intertidal Zone Snail, *Nucella lamellosa*

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Abstract

The low-intertidal zone marine gastropod, *Nucella lamellosa*, exhibits remarkable phenotypic plasticity between habitats as close as 100 m from one other. Shells that develop in different environments can display variation in color, banding, shape, sculpture, and thickness, and the most documented explanation for this variation is predator-induced resistance. The snails collected from the beach in front of the Friday Harbor Laboratories are of the thick-shelled variety of *N. lamellosa* and are known to exhibit a row of teeth within their apertural openings, presumably as a defense against their predator, *Cancer productus*. I collected 25 of these snails at low tide and took systematic measurements (length, width, weight) to quantify shell size, and I used high resolution images of the apertural openings to measure average tooth height and apertural lengths. I found that, while a majority of the snails (19 out of 25) displayed apertural teeth, there were 5 snails that did not (one snail exhibited drastically different morphology). Of the range of shell lengths I collected (1.03 cm – 5.84 cm), the largest snail without apertural teeth was 1.93 cm. By comparing shell width and weight to shell length, I determined that there was no significant difference between the way snails with and without apertural teeth developed; both groups showed a significant positive correlation between shell width and weight and shell length. I also found that for those snails with apertural teeth, there was a significant positive correlation between apertural length and apertural tooth height.

Introduction

Predator-induced defenses exist among colonial and clonal invertebrates as well as marine algae and plants (Appleton and Palmer 1988; Trussell 1996), yet solitary animals have recently been attracting much scientific attention. Padilla and Savedo (2013) searched the literature for studies on phenotypic plasticity in marine animals and plants and found that 192 of the publications focused on marine invertebrates whereas only 36 focused on marine algae or plants. The most documented occurrence of predator-resistance in solitary invertebrates is within the genus *Nucella* (previously *Thais*), an intertidal zone gastropod. Many of the species within *Nucella* (*N. canaliculata, N. emarginata, N. lamellosa, N. lapillus*; Crothers 1984; Palmer 1990) exhibit phenotypic plasticity; however, *N. lamellosa* exhibits some of the most striking variation in shell morphology among the genus and thus have been studied by marine biologists for decades (Kincaid 1957; Spight 1973; Palmer 1985; Bourdeau 2009). Variation in shell color, banding, shape, sculpture, and thickness can vary drastically among *N. lamellosa* from different habitats as close as 100 m from one another (Kincaid 1957; Palmer 1985). Additionally, *Nucella lamellosa*, a low-intertidal species, exhibits greater predator-induced defense than higher shore species (Bourdeau 2011).

While differences in shell morphology are likely due to a number of environmental factors, it has been shown that thick-shelled snails are found on protected beaches with low energy wave action whereas thin-shelled snails are found in high wave energy environments (Kitching 1966). This pattern can be attributed to the ability of predatory crabs to feed in protected environments, whereas crabs have much more difficulty feeding in the presence of high-energy turbulence (Bourdeau 2012). In addition, Palmer

(1985) showed that thin-shelled *N. lamellosa* are more likely to be eaten by the predatory red-rock crab *Cancer productus* than thick-shelled snails with apertural teeth. Palmer (1992) argues that shell production is energetically expensive, so gastropods that are not exposed to predatory crabs tend not to grow thick shells. However, Bourdeau (2010) showed that for *N. lamellosa*, shell-growth in predator-resistant snails is a passive result of reduced feeding rather than an active defense against predation.

While crabs are the most common predator of *Nucella lamellosa*, a study (Bourdeau 2009) showed that the snails produce an elongate shell with high spires when exposed to chemical cues from sea stars. He also found that snails with this shape were eaten less by seastars, but were more vulnerable to crab predation.

In this study, I examined the predator-resistant shell morphologies in a single population of *Nucella lamellosa* on San Juan Island. I tested for correlations between shell length, width, and weight and presence (and size) of defense-induced apertural teeth. Based on initial observations of the *Nucella lamellosa* from the beach in front of the Friday Harbor Laboratories, I predicted that small animals would have a different relationship between size and mass than larger animals. I also looked at the length (and therefore age) that the snails began to grow thicker shells and apertural teeth.

Study System

The species used in this study was *Nucella lamellosa*, a low-intertidal gastropod found in most intertidal zone habitats on San Juan Island. *Nucella lamellosa* can exhibit phenotypic plasticity even along a single beach (Palmer 1985), so I chose to collect my snails from a 10 m stretch along the beach in front of Friday Harbor Laboratories to ensure that each of the snails was exposed to the same predatory environment. Initial observations indicated the presence of apertural teeth and thick shells, allowing me to conclude that *N. lamellosa* had induced predator-resistant defenses in this habitat. Yamada and Boulding (1996) studied the role of crab predators and zonation in their gastropod prey in front of the Friday Harbor Laboratories and found that *Cancer productus*, the major *N. lamellosa* predator, is abundant along the beach. In order to constrain a relationship between shell size and predator-resistance, I collected 25 snails of the greatest range in size that I could find. The largest snail I collected exhibited significant shell ornamentation and had a thinner shell whereas the rest were smooth, so I concluded that this snail had been collected from a different location and returned to the Friday Harbor Laboratories beach. I included it in my study in order to compare it to the thick shelled *N. lamellosa* that I focused on.

Methods

Nucella lamellosa of varying shell lengths were collected in early July from the Friday Harbor Laboratories beach. The 25 snails were collected at low tide as *Nucella lamellosa* is a low-intertidal zone gastropod. Each snail was numbered individually, and shell lengths (from apex to tip of siphonal canal) were measured using outside-measuring digital calipers (± 0.01 mm). Shell widths were measured using the same calipers (± 0.01 mm) by measuring the greatest width of the first whorl along the shell perpendicular to the length (figure 1). Shell masses were estimated by submerging the snail in seawater, probing the operculum to remove any potential air bubbles, and weighing the snail using a bottom-hanging electric balance (± 0.001 g). This method of obtaining the weight of the shell only, not including the soft tissue, assumes that the animal tissue has the same specific gravity as seawater, and is a method used extensively in studies of molluscs (Palmer 1985; Appleton and Palmer 1988; Bourdeau 2010; Edgell 2010; Nienhuis et al. 2010).

Each snail was held in place on a glass slide with molding clay and viewed with a dissecting microscope. The shell was oriented so that the greatest height of apertural teeth was perpendicular to the optical axis of the microscope (Figure 2). High resolution, high magnification photos were taken of the apertural openings of each snail at the same orientation as the microscope so that the greatest height of each row of teeth was visible in the photos. Using image analysis software (ImageJ) the height of each tooth was measured by defining an estimated base (where the shell would be if there were no teeth) and measuring the distance between this base and the tip of each tooth (Figure 3). The number of teeth in the aperture of each snail was noted so that the average tooth length was calculated. The lengths of the apertural openings were also recorded.

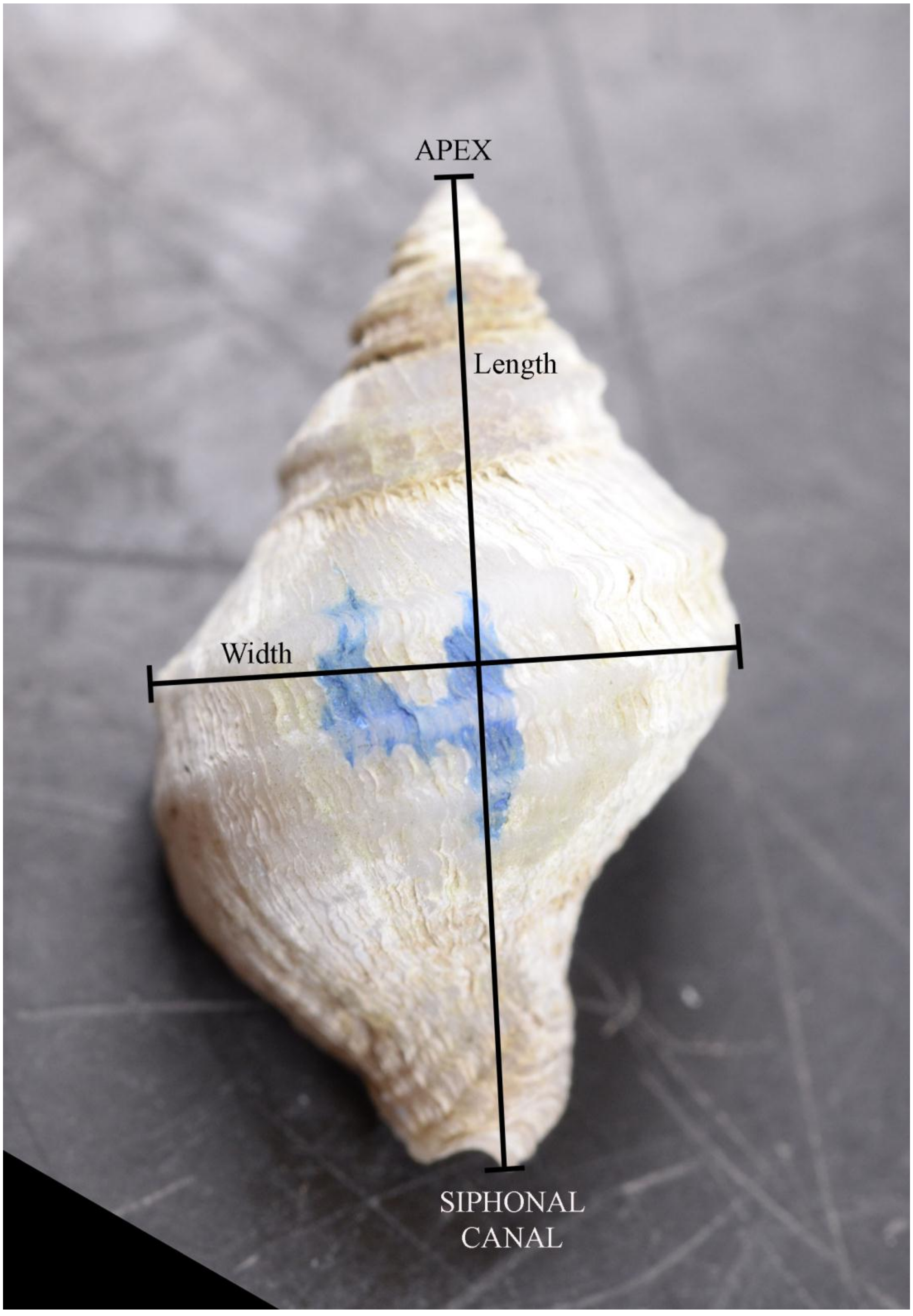

Figure 1: Length of each shell was measured from the apex to the tip of the siphonal canal. Width of each shell was measured at the widest part perpendicular to the length measurement.

Figure 2: Experimental setup for taking photos of apertural teeth. Each snail was photographed individually at high magnification.

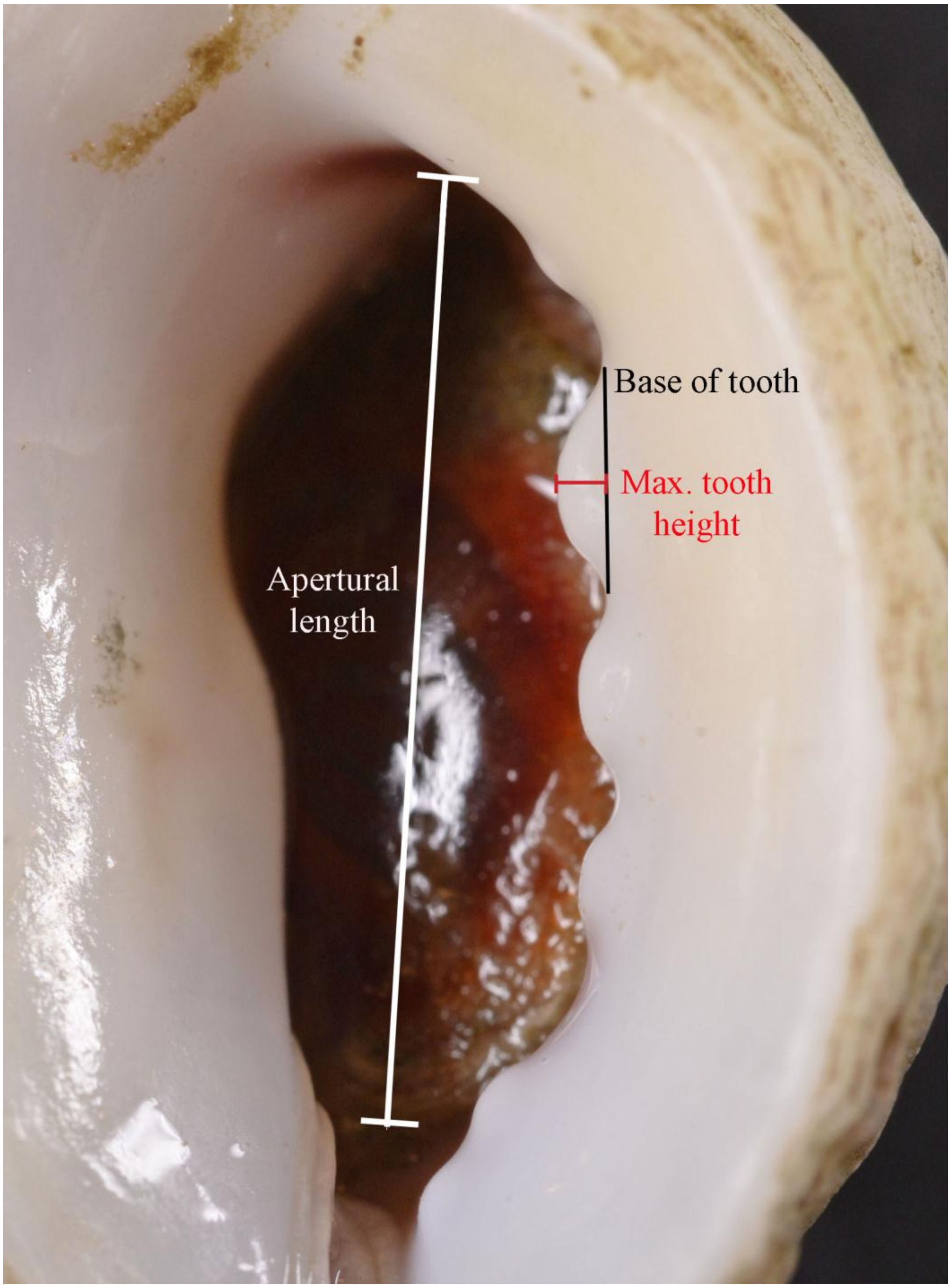

Figure 3: Maximum tooth heights were measured by defining a base and measuring the maximum length of the tooth to that base. Length of the apertural openings were measured by estimating the length of the aperture that the operculum takes up.

Results

Snails with apertural teeth were separated into a different data set from those without apertural teeth and the two data sets were graphed together. Three graphs were made comparing the data; shell width vs. shell length (Figure 4), ln submersed weight vs. ln shell length (Figure 5), and average apertural tooth length vs. apertural opening length (Figure 6). Each data set in each graph was analyzed using a Standard Major Axis (SMA, model II) regression.

For those snails with and without apertural teeth, there was a significant positive correlation between the shell length and shell width (Figure 4). The Pearson product-moment correlations (Table 1) for both groups of snails were high (with teeth: $r = 0.9963$; $P < 0.00001$, without teeth: $r = 0.9994$; $P = 0.000018$). The slope of the line for those snails without teeth was 12.6% steeper than the slope of the line for those snails with teeth; however, the two data sets merge almost seamlessly (with teeth: $y = 0.587x + 0.0386$; without teeth: $y = 0.6661x – 0.0972$). The outlier (green X) in this graph represents the one *N. lamellosa* that had very different morphology than the other snails. The data point for this snail lies below the regression line; its width is less than expected relative to its length when compared to the other snails.

There was also a significant positive correlation between the natural log of shell length and the natural log of submersed weight (Figure 5) for those snails with and without apertural teeth. The Pearson product-moment correlations (Table 1) for both groups of snails were, again, high (with teeth: $r = 0.9947$; $P < 0.00001$, without teeth: $r = 0.9997$; $P < 0.00001$). The slope of both regression lines was 3.14, slightly higher than the expected value of 3 for a weight vs. length regression line with geometric similarity (with teeth: y = 3.1397x – 2.4344; without teeth: y = 3.1455x – 2.4528). Similar to Figure 4, the outlier snail was lighter for its length than would be expected in a snail from the Friday Harbor Labs habitat of the same size.

For those snails with apertural teeth, there was a significant positive correlation between the size of the aperture and the height of the tooth. While the points in this data set lie much farther from the regression line than in the other two graphs, the data are still significant, with an R value of 0.8927 and a P value of less than 0.00001 (y = 0.0299x – 0.0061; Table 1).

Table 1: Results from Pearson Correlation Coefficient tests for all regressions

	R	N	P
Width vs. Length (teeth)	0.9963	19	<0.00001
Width vs. Length (no teeth)	0.9994	5	0.000018
Weight vs. Length (teeth)	0.9947	19	<0.00001
Weight vs. Length (no teeth)	0.9997	5	<0.00001
Tooth length vs. Aperture	0.8927	19	<0.00001

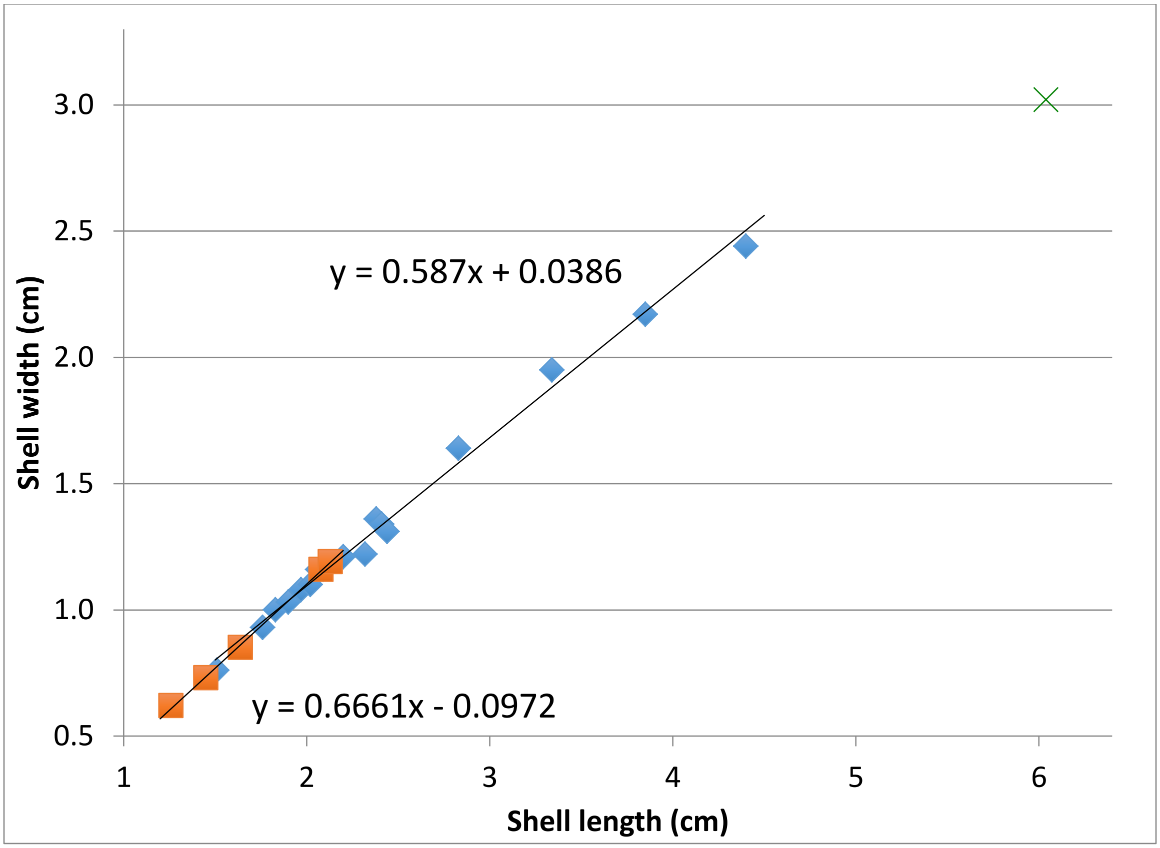

Figure 4: Shell width (cm) vs. shell length (cm) for snails with apertural teeth (blue diamonds) and snails without apertural teeth (red squares). Model II regression lines and equations are displayed for both data sets.

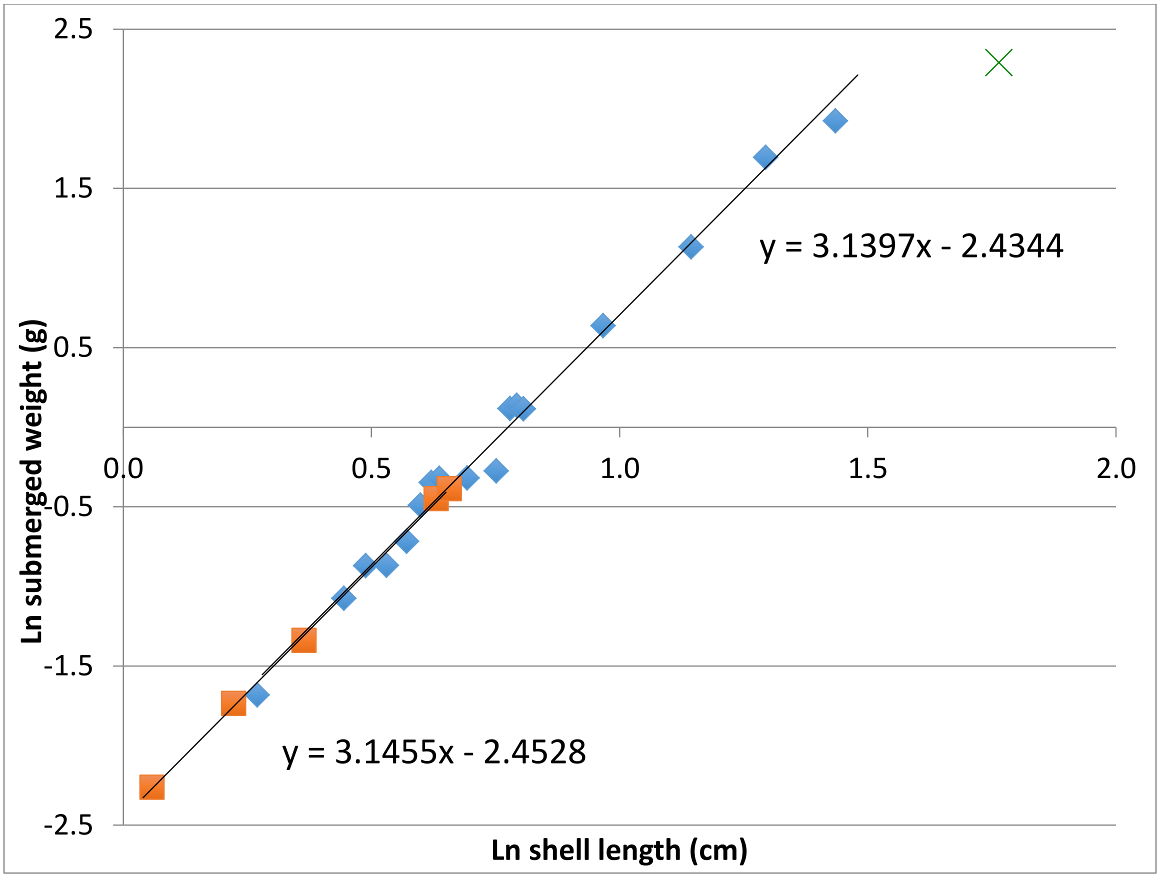

Figure 5: Natural log (ln) of the submerged shell weight (g) vs. ln of the shell length (cm) for snails with apertural teeth (blue diamonds) and snails without apertural teeth (red squares). Model II regression lines and equations are displayed for both data sets.

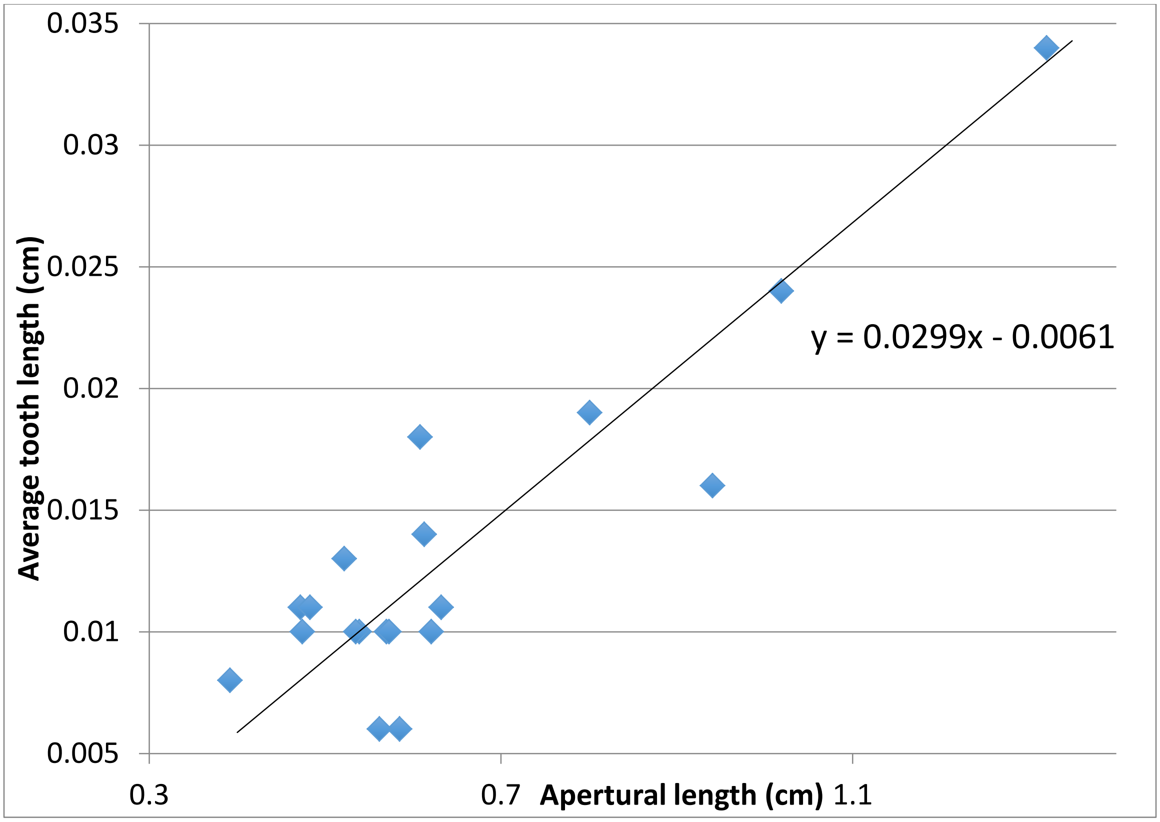

Figure 5: Average tooth height (cm) vs. length of apertural opening (cm) for only those snails with apertural teeth. The model II regression line and equation are displayed.

Discussion:

Graphical analyses of the data collected on *Nucella lamellosa* shell widths, lengths, submersed weights, presence and size of apertural teeth, and apertural lengths showed unexpected correlations between these data. Figure 4 shows that, although there is a slight difference in slope between those snails with and without apertural teeth, all of the shells grow wider and longer in similar ways. Figure 5 shows that there is no difference in how the shells gain mass compared to length in those snails with and without apertural teeth. This might imply that the animals grow thick shells and apertural teeth in response to different stimulations.

Appleton and Palmer (1988) and Boudreau (2012) studied the response of *Nucella lamellosa* to varying predatory influences; the snails will grow thick shells in high risk environments or in the presence of non-predatory crabs, but they will grow larger apertural teeth in the presence of *Cancer productus*, a major predator of these snails. The larger *Nucella lamellosa* in this study that do not display apertural teeth might have inhabited a slightly different micro-environment than the others. It is possible that they have been exposed to different crabs, but not *Cancer productus*, and therefore did not produce apertural teeth, which can be a defense against this predator. I also suspect that the difference between the small snails that do not display apertural teeth (1.06-1.44 cm in length) and the slightly larger snails that do display apertural teeth (1.31-1.77 cm in length) marks the length in which most snails begin to develop apertural teeth when exposed to *C. productus*.

The outlier point in Figures 4 and 5 shows the width and weight compared to length of a shell that appears to have been from a different environment during most of its life.

This snail has a thinner shell and does not have apertural teeth, indicating that it inhabited a low-risk environment rather than a predator-rich environment. If the small snails without apertural teeth were to represent this same lack-of-risk morphology, I would expect that their shells would be thinner and weigh less than observed.

While the relationship between apertural length and average apertural tooth height is more scattered than the relationships between shell width and length and shell weight and length, it does show a significant positive correlation between size of the apertures and average height of the teeth. Shells with larger apertural openings are considered more vulnerable to predation than those with smaller openings, and apertural teeth can provide a mechanism for making the aperture effectively smaller (Vermeij 1982).

Although I did not find any significant difference between shell growth (width and weight vs. height) in small snails (without teeth) and shell growth in large snails (with teeth), I did find that all snails larger than a certain length (1.93 cm) displayed apertural teeth. Further research might try to define a relationship between *Nucella lamellosa* shell length and age, which would then allow me to assign ages to the different shells. This as well as more research comparing shell length, width, weight, apertural lengths, and size of apertural teeth will help to better define the age (or range of ages) when the snails begin to develop predator-resistant defenses.

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