

**Assessing the potential use of microtraces of gastropod
predation as a diagnostic for predator-prey interactions in the
fossil record**

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Abstract

Predatory gastropods have left signs of boring predation in the fossil record as early as the Cambrian (McMenamin and Schulte McMenamin, 1990; putative Proterozoic examples [e.g., Bengtson and Zhao, 1992] remain controversial). Through studying drill holes in prey, we can better understand predator-prey interactions in marine communities. It has been proposed that further study of the interior of drill holes yields microtraces left by the radular teeth during the drilling process (Schiffbauer et al., 2008; Tyler and Schiffbauer, 2012). The pattern and orientation of the traces can be used to associate predator with prey and act as a diagnostic feature to identify the predator.

Trace fossils can provide great insight into past environments, but only when they are well preserved. Through assessing one hundred and eighty drill holes using scanning electron microscopy, I provide here evidence suggesting the limited presence of predatory microtraces. Interpreting shell deterioration and extrapolating the observed degradation of modern specimens to hypothetical paleoenvironments suggests that preservation of such minute traces would be poor and would thus negate the purpose of creating such a diagnostic. Additionally, the current understanding of the drilling process suggests that the preservation of microtraces within the drill hole margins is an infrequent occurrence. This may be due to the fact that before utilizing their radular teeth, predatory gastropods deploy secretions from the accessory boring organ (ABO) to break down the shell surface, lessening the preservation potential of predatory microtraces (Carriker, 1969).

This study has discovered porcellaneous rims surrounding the drill holes in the Miocene *Saxolucina*. Further study into these rims may provide additional insight into the drilling strategy of naticid gastropods.

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II. INTRODUCTION

a. Gastropod Morphology

The class Gastropoda is highly diverse, containing snails, limpets, nudibranchs and slugs. The fundamental characteristics of gastropods are: 1) having an operculum, and; 2) undergoing torsion during the larval stage (Brusca et al., 2016). Gastropoda is further classified into to five clades: patellogastropods, vetigastropods, neritomorphs, caenogastropods, and heterobranchians. The five clades are established on morphological characteristics (Zapata et al., 2014). Gastropod lineages can be distinguished by their radulae, a highly intricate structure made of chitin and tanned protein. Collectively, the class Gastropoda comprises nearly 80% of the phylum Mollusca (Brusca et al., 2016).

Morphological features of the mouth parts in Caenogastropoda were considered in an effort to understand the process of drilling predation. The complex process of drilling in predatory gastropods involves the radula, odontophore, proboscis, and accessory boring organ (ABO), which together weaken the prey's shell, scrape away material, form the drill hole, and consume the flesh of the prey. The radula, a chitinous ribbon of serrations and pseudo-teeth, is located on the odontophore (Figure 1) and is used to rasp at the shell material, drilling into the shell. The radula is composed of tanned protein and is designed to withstand the wear and tear of the rasping (Brusca et al., 2016). Thus, it is plausible for the radula to leave signs of the drilling process on the shell of the prey.

As examples of the genera of the families Naticidae and Muricidae can be distinguished by differences in their respective radulae, observation of traces on prey shells can aid in the understanding of predator-prey interactions in both modern and fossil communities. Variations in radulae (Figure 2) would theoretically result in varying traces or rasp marks inside the drill hole. For instance, recent caenogastropods have two marginal teeth per row in addition to two lateral and a central radular cusp. This configuration is characteristic for taenioglossate radula (Brusca et al., 2016). In contrast, the derived Neogastropoda have rachiglossate radula, that is, radulae without marginal teeth. Occasionally, gastropods may reinforce the radula with minerals to strengthen them against the surface which they are rasping. These are docoglossate radula, used by Patellogastropoda (Brusca et al., 2016). For example, some deep sea limpets are known to use opal or iron to reinforce radulae, the later state being homoplastic in chitons.

As a result of these variations, one could conceivably link the radular traces to a particular predatory gastropod, forming a diagnostic to apply to the fossil record to help understand paleocommunity composition and to analyse the extent of predation. However, due to a structure called the accessory boring organ, named by Carriker (1969), the preservation of the potential microtraces may be limited.

The location of the ABO differs between muricids and naticids. The accessory boring organ can be found within the pedal foot of a muricid. In contrast, naticid gastropods have the accessory boring organ located on the tip of the proboscis adjacent to the radula (Carriker and Yochelson, 1968).

b. Drilling Predation

i. The Drilling Process

Predatory gastropods use a combination of chemical and mechanical methods in the drilling of prey. Mechanical mechanisms include both the rasping of the radula as well as the orientation of the gastropod itself. Figure 3 shows observed orientation of the predator *Lunatia* relative to the prey (Ziegelmeier, 1954). The lines within the circle representing the drill hole symbolize the radular traces while the inner circle shows the placement of *Lunatia* during the drilling process. Ziegelmeier (1954) notes that there is a change in direction during the drilling process that contributes to the symmetry seen in naticid drilled prey. He describes the process in which the odontophore places the radula on the prey and begins scraping. The direction that the predator turns to continue rasping is counteracted by the turning of the odontophore during the rasping period. In Figure 3, the direction that the gastropod turns is indicated by the arrows outside of the circles. The large circle represents, not the drill hole, but the gastropod. The circle centered at the base (outlined in red) is the drill hole forming. Rasp mark orientation is provided by the striations within the forming drill hole.

A chemical component is added to the drilling process to weaken the shell microstructure before rasping begins. Before Ziegelmeier's 1954 publication, Troschel (as cited by Zeigelmeier, 1954) had discovered a gland in naticids that he suggested somehow aided in the drilling process. Simroth (as cited by Zeigelmeier, 1954) later described it secreting a substance during the drilling process. There was no conclusive evidence at the time to state that an acid or

chelating agent was secreted (Zeigelmeier, 1954). Carriker (1969) later described what is now known as the accessory boring organ. Still not fully understood, the accessory boring organ is thought to be a gland or gland-like structure that secretes a combination of acid, chelating agents, and enzymes that are applied to the site of the drill hole (Carriker, 1969, 1978, 1981).

ii. Drill Hole Morphology

The Naticidae and Muricidae leave different signs of drilling predation in drill hole morphology (Carriker, 1969). Generally speaking, drill holes will either appear with straight sides and are narrow or they will have a sloped or beveled wall. The beveled drill holes are often (but not always) larger. While not definitively diagnostic, straight sided drill holes are often formed by muricids and shallow sloping drill holes are formed by naticids (Figure 4). Carriker (1969) notes the importance of rasp mark orientation as described by Zeigelmeier (1954). Specifically, he states that Zeigelmeier (1954) describes the traces created by muricids to be asymmetrical while naticids form symmetrical traces.

Interruption of the drilling process can result in occasionally finding an incomplete or failed drill hole. The predator may have ceased its attempt at feeding or was interrupted by another predator. The incomplete drill holes that remain are not ideal cylindrical borings, in the case of muricids, nor gently sloping and rounded drill holes, in the case of naticids. As seen in Figure 4I, a central boss can be left at the base of the failed drill hole. This region is described by Carriker (1969, 1978) as being a likely location for finding radular microtraces. Zeigelmeier (1954) describes the initial perforation into the interior

of the shell as taking place on the edge of the central boss. Zeigelmeier (1954) states that *Lunatia* creates a kidney shaped perforation through the deepest part of the drill. The drilling process is not perfectly symmetrical as one radular pass does not span the entire circular drill hole. Penetration will thus occur on one side at a time. However, a kidney shaped perforation could impair feeding. As the proboscis may not comfortably fit through the hole, the drilling process continues, leading to a completed drill hole.

iii. Identifying Predatory Microtraces

Tyler and Schiffbauer (2012) imply that number and orientation are the most important characteristics when identifying microtraces. Orientation being parallel, subparallel or crosscutting with respect to the shell microstructure and number referring to the number of traces found parallel to each other. Tyler and Schiffbauer (2012) also note the number of radular cusps from dissected radulae in the study and compare them to artificially produced traces on wax.

When determining the presence of predatory microtraces or inferring their potential presence, I referred to Carriker (1969) and to other published images of known traces (Figures 5-7). I would like to make note of the observed differences between the predatory microtraces in Tyler and Schiffbauer (2012; my Figure 8) and in in Carriker's (1969) study (Figure 9). Keeping in mind that although these studies all used genera within family Muricidae, the alleged predatory microtraces are still remarkably different. Those included in Carriker's (1969) study (Figure 9) are significantly larger than those created by *Nucella* (Figure 8). Crosscutting of microtraces are clearly defined in Figure 9, yet these are not readily identifiable

in Figure 8. I suggest that there is high probability that those pictured in Figure 8 expose the cross lamellar fabric of the prey shell, such as seen in Figure 10.

The traces observed by Carriker (Figure 9) were left by *Urosalpinx cinerea* during multiple drilling processes which Carriker (1969) interrupted. Carriker (1969) proposed that in order to leave the radular marks, the gastropod must have been interrupted before deploying the accessory boring organ and its secretions. Thus, Carriker (1969) gained experimental insight into which rasping mechanisms are used in drilling prey.

Carriker (1969) observed *U. cinerea*, through a transparent surface, consuming its prey, *Mya arenia*. Carriker (1969) counted the number of times that the radula scraped the prey's shell. After a predetermined number of rasps made by the radula, he interrupted the muricid and prepared the prey's shell for imaging under a scanning electron microscope. Figure 9 below shows scanning electron micrographs taken by Carriker (1969) after intervals of rasping done by *U. cinerea*. Carriker (1969) concluded that the instances where radular marks or microtraces were discovered were instances when the accessory boring organ had not yet secreted an acid to dissolve the previously present microtraces.

Several defining characteristics of potential radular marks were used to classify a specimen as showing predatory microtraces. As seen in images published by Carriker (1969), traces do not appear randomly, but form a pattern. They often span from one end of the drill hole to the next and not in concentric passes which can often be confused with concentric lamellar structures in the prey shell.

The traces additionally appear in multiples or in sets. This is supported by the arrangement of radular cusps in the predator. Gastropods do not use a single radular tooth row to rasp away material. The radula can contain sets of two to five teeth. Specifically, Caenogastropoda has taenioglossate radulae (Brusca et al., 2016). Thus, it is reasonable to expect the microtrace groove number to correspond to the number of radular cusps. Figure 9, image B by Carriker (1969) shows incomplete drill holes where radular marks appear in sets of three.

c. Gastropod Evolution and Diversification

i. Proterozoic and Cambrian

While the origins of the earliest reported drill holes remain controversial, they have been found in a variety of organisms including foraminifera, echinoids, and brachiopods (Sohl, 1969; Zappata et al., 2014) and appeared at least as early as 550 million years ago (Sohl, 1969). Notably, *Hyolithellus*, from the Lower Cambrian Puerto Blanca Formation from Sonora, Mexico, is an early example of boring predation. Figure 5 depicts the drill hole found within *Hyolithellus*. *Hyolithellus* is an Early Cambrian small shelly fossil that was extracted from limestone by acetic acid maceration. While not a definitive example of a gastropod drill, Figure 5 provides an example of predation through drilling into a prey's mineralized exterior to reach the prey's soft tissues.

Hyolithellus is believed to be an ancestor to annelid worms that lived during the Cambrian. The presumed stem-group annelid lives in a self-created phosphatic (hydroxyapatite) tube. The tube is anchored within the substrate with a widened base supporting it. Skovsted and Peel (2011) evaluate the positioning and growth

of *Hyolithellus*, suggesting that the organism lived anchored in the substrate with the growing portion facing upwards into the water column. As a result, the soft body of the worm was protected by the secreted phosphatic casing.

To reach the fleshy part of the organism, a predator must either open the casing through crushing it or enter the apatitic tube. The predator that reached the organism within the tube as shown in Figure 5 managed to drill into and through the apatitic shell. We do not know the identity of the predator of the *Hyolithellus* in Figure 5.

Using the potential diagnostic suggested by Tyler and Schiffbauer (2012), one could observe the borehole using scanning electron microscopy. While preservation of Lower Cambrian fossils is likely poorer than the Plio-Pleistocene and Miocene specimens observed in the proceeding study, the idea behind the diagnostic is that families of predatory gastropods could be distinguished by the microtraces left behind by their varying radulae. While the diagnostic has not been developed to identify predators to family, the mere presence of predatory microtraces is diagnostic for molluscan origin. Sponges, some of which are often the cause of early borings, do not leave microtraces, as members of the phylum Porifera do not have radulae.

ii. Paleozoic

Fischer (1964) summarizes key factors in determining if a drill hole has molluscan origin. Included in these considerations are the number of drill holes, the orientation relative to each other, and the size and the shape of the drill holes.

For example, potential drill holes appearing in close proximity to each other, with small average diameter, oriented at various angles, are unlikely to be formed by predatory gastropods. These characteristics are characteristic for sponge boreholes (Fischer, 1964; Carriker and Yochelson, 1968).

Carriker and Yochelson (1981) note the first appearance of cylindrical drill holes in the fossil record. *Taphrorthis peculiaris* from the Pratt Ferry Formation is believed to be the oldest known cylindrical drill hole. The drill hole found in *T. peculiaris* is both cylindrical and slightly asymmetrical, two features that are common in drill holes in the Middle Ordovician (Carriker and Yochelson, 1981).

Yakovlev (1926) described Permian brachiopods with perforations. Yakovlev (1926) suggested that *Naticopsis* may have been the predator. Additionally, Brunton (1966) noted the presence of cylindrical perforations within a Carboniferous brachiopod. Brunton (1966) described the isolated brachiopod as having as many as 44 boreholes, which suggests that the holes were not of gastropod origin.

In 1968, Carriker and Yochelson published a study on Ordovician boreholes from localities in Kentucky and Ohio. Carriker and Yochelson (1968) worked under the assumption that the drill holes were produced by predatory gastropods. Gastropod predation is known to have appeared by the Ordovician (Fischer, 1962) so it is reasonable to infer that the boreholes represent signs of gastropod predation. However, after careful analysis by Carriker (1968), a small number of the drill holes present in various bivalves from the Ordovician do not appear to match the typical muricid drill hole. The cylindrical borings occur with straight

sides and incompletes show no evidence for beveling or presence of a central boss. By measuring the ratio of outer to inner borehole diameter, Carriker and Yochelson (1968) noted that the drill holes lacked the sharp angle from the surface to the interior of the borehole.

Upon further investigation, and through careful measurements of diameters and depth, Carriker (1968) determined that some of the drill holes were in fact boreholes formed by members of the family Clionidae, a family of boring sponges. Gastropods are not the only boring or drilling predators known from the Paleozoic. Cirripedes, sponges, and even some polychaetes adapted to drilling and boring (Carriker and Yochelson, 1968; Fischer, 1962). With multiple potential predators, an ability to examine a borehole and determine whether or not it is molluscan in origin would expedite paleobiological investigations.

The presence of radular marks within the drill hole surface would indicate a gastropod as the predator. While the ideal diagnostic would narrow down the predatory gastropod to family or genus, there are some caveats to this approach. Diagnostics can be successfully applied only when there are in fact predatory microtraces. If microtraces are not preserved, the specimen could have been preyed upon by a gastropod or the borehole could have been formed by other types of boring organisms.

iii. Mesozoic

The Mesozoic saw the appearance of numerous new naticid genera and species. Since the Cretaceous, members of the family Naticidae have been

important predators of molluscan communities (Sohl, 1969). Despite their presence in the Cretaceous, it is not their first appearance. Huddleston (1887, 1896) and Morris and Lycett (1854), cited by Sohl (1969), have identified naticid genera from Jurassic assemblages.

It was originally thought that naticids evolved in the Triassic. The classification of these specimens has been questioned due to poor preservation and a lack of defining characteristics (Sohl, 1969). A subsequent surge in naticid biodiversity is reflected by an increase in drill holes among molluscan prey in Late Cretaceous fossil assemblages. This suggests that the adaptation of drilling predation did not exist in early naticids and in fact evolved during the Late Cretaceous (Sohl, 1969).

Unlike naticids, muricids did not evolve until the Cretaceous. Much is known about their evolution and origin (Stephenson, 1952). *Hillites*, the earliest muricid known, is found in the Aptian-age PawPaw formation of Texas. The range of the genus extends to Kansas during the Cenomanian. Diversity of the Muricidae remains relatively constant until the late Paleocene (Figure 6).

The family Cassidae is thought to have appeared in the Late Cretaceous during the adaptive radiation of naticids, despite Cassidae having adapted to preying upon echinoderms through drilling predation (Hughes and Hughes, 1981).

iv. Tertiary

The Eocene saw significant increases in biodiversity for the most abundant families of drilling gastropods. These include the families: Naticidae, Muricidae,

Cassidae, and Cymatiidae. Additionally, during the Tertiary, the two families Buccinidae and Nassidae first appeared (Fischer, 1962). However, according to Fischer (1964), the appearance of cylindrical drill holes does not occur until the Eocene. Straight sided or cylindrical drill holes are often associated with the family Muricidae. Fischer (1964) showed that straight sided drill holes appear during the Eocene.

Cylindrical versus beveled forms a spectrum for gastropod drill holes (Figure 4). Some are much more obviously cylindrical than others, which then would contrast with the beveled sides of the holes of naticid drilled prey. This perhaps correlates with Fischer's (1962) statement that cylindrical drill holes did not appear until the Eocene. It is unlikely that muricids adapted new radular cusps that would alter drill hole shape between the Lower Cretaceous and the Eocene. While species to species variations would surely be present, the overall rachiglossate form for derived caenogastropods has an underlying consistency (Brusca et al., 2016).

III. PREVIOUS STUDIES

Perhaps the most extensive characterization of gastropod predation was done by M. Carriker (1969). Carriker's (1969) previous work has spanned from coining the phrase "accessory boring organ" to carrying out experiments to quantify the act of drilling predation and the extent of the role of the radula and the ABO.

a. Radular traces as a diagnostic feature

Schiffbauer et al. (2008) and Tyler and Schiffbauer (2012) suggest that predatory microtraces, or rasp marks, left by the radula of a drilling gastropod, could be used as diagnostic features in the paleontological record. Tyler and Schiffbauer (2012) focused on a single modern species of prey, *Mytilus edulis*, drilled by the muricid, *Nucella lamellosa*. Tyler and Schiffbauer (2012) reported twenty-six of thirty-five specimens to express potentially diagnostic microtraces.

Tyler and Schiffbauer (2012) identified, in approximately 74% of specimens, the presence of predatory microtraces without explicitly stating the definition of a predatory microtrace. Images were taken of the specimens using environmental scanning electron microscopy (Figure 8). These provided limited information regarding the image's orientation relative to drill hole margins, shell hinge, anterior or posterior edge of the shell and other identifying points and features. As a result, Tyler and Schiffbauer (2012) are not explicit about the location of the drill hole, the angle at which the alleged traces are oriented with respect to the drill hole, and other potentially useful information.

However, the study provided an extensive quantitative assessment of the radular cusps and their comparison to the potential traces found within the drill hole margins. Tyler dissected *Nucella* specimens and sputter coated their radulae with an Au-Pd coating. Spacing between radular cusps was found to have a range of 12.5 to 50.4 μm . The range for the width of potential radular traces is 2.3 to 30.6 μm pooled from the 35 specimens.

Tyler and Schiffbauer (2012) included in their study the artificial production of radular traces. C. Tyler dissected the radulae from the *Nucella lamellosa* used in the study to artificially create predatory microtraces on a wax surface (Tyler and Schiffbauer, 2012). These traces within the wax mimic both single- and multiple-pass radular traces in order to more accurately represent their naturally-occurring counterparts.

IV. GOALS OF THE STUDY

Specimens from Tyler and Schiffbauer (2012) were reimaged after they were drilled five or more years ago. The specimens were exposed to air after drilling occurred, set to dry, imaged, and stored individually. I hypothesize here that during the time in which the specimens were stored, a degree of shell deterioration occurred and resulted in the current lack of microtraces. Expanding on this inference, it is not that the *Mytilus edulis* lacked radular marks in Tyler and Schiffbauer's (2012) study, but rather that the already unstable shell microstructure flaked, warped, and deteriorated over time, ultimately losing most if not all of the microtraces.

It appears as if it is not purely environmental agencies such as storage conditions and passage of time that can alter trace preservation, but also aspects of the gastropods' drilling structures. The role and extent of the accessory boring organ has been debated (Beesely et al., 1998; Carriker, 1981; Zeigelmeier, 1954).

The accessory boring organ is an acid-secreting structure which is believed to aid in softening the shell before the radula is deployed to scrape away the shell layer. Following this and previous studies on the role of the accessory boring organ (Carriker 1969, 1978, 1981), I believe that there exists a narrow window of opportunity for the preservation of predatory microtraces.

The accessory boring organ weakens part of the shell so that the radula can rasp or scrape it away. The gastropod repeats this process for an indeterminate

amount of time; lasting hours and sometimes over a day (Carriker, 1978). Carriker carried out extensive studies interrupting muricids in their drilling process after a set number of rasps against prey. Carriker (1978) believed that the only place one would find microtraces of the radula would be in incomplete or failed drill holes where the gastropod predator had been interrupted, due to, say, interference by another predator, or when the gastropod relocated to a new portion of the shell (Carriker, 1978). I further hypothesize that predatory traces will be found within incomplete drill holes and/or on the inner margins of complete drills where the acid was not applied and the material scraped away.

V. METHODS

a. Specimens

A total of 180 drill holes were examined from the Paleontological Research Institution and Mount Holyoke College, including both modern and fossil specimens. In preparatory work conducted with Gregory Dietl and Jansen Smith at the Paleontological Research Institution, I studied and photographed 135 specimens, yielding 137 drill holes. Along with specimens from Mount Holyoke College, 176 specimens were imaged in total, including 180 drill holes.

The fifteen specimens from Mount Holyoke College are fossils from the Miocene or Plio-Pleistocene having 16 drill holes, while 164 are drill holes from modern specimens that were raised in sea table tanks at Mount Holyoke, ensuring that predator-prey interactions were known. Of the 164 modern specimens I observed, 94 were known to be naticid drilled prey and the remaining 70 were victims of muricid predation.

b. Paleontological Research Institution

Gregory Dietl and Jansen Smith acquired specimens from Tyler and Schiffbauer's 2012 study which I imaged using a JEOL Neoscope JCM 5000 benchtop scanning electron microscope. After thorough exploration of the original specimens of Tyler and Schiffbauer (2012), the prevalence of these predatory microtraces and their utility as diagnostic features is less certain.

I expanded my study to include following naticids: *Neverita duplicata*, *Euspira heros*, and *Sinum*. During preliminary data collection, I took nearly 2500 SEM images that covered fifteen different predator-prey interactions, and nevertheless encountered only a few potential predatory microtraces. I developed a series of preliminary guidelines to act as a form of diagnostic tool to determine if a feature was in fact a trace mark or whether it was a damaged layer of microstructure or other feature warped by the electron beam, or was due to deterioration over time.

c. Live Specimens

Live specimens were housed in a series of three sea table tanks, holding a total of 200 gallons, at Mount Holyoke College. I divided each tank into two, using perforated, plastic dividers. Five of six subdivisions housed actively occurring predator-prey interactions. The sixth and final subdivision acted as a holding tank for live specimens that were not actively involved in an observed predator-prey interaction. The specimens held at Mount Holyoke College included: *Euspira heros* (formerly known as *Lunatia*), mytilids, *Littorina littorea*, *Mercenaria mercenaria* and *Nucella lapillus*.

I introduced *L. littorea* to one of the subdivided tanks housing the predator *Euspira*. Here, *Euspira* captured its prey, a large *Littorina littorea*, and failed to drill through the shell after three and a half days. The prey was released and the failed drill observed under the scanning electron microscope. *E. heros* continued to complete drill holes in three other *L. littorea* which were imaged using a scanning electron microscope.

The remaining subdivisions of the open sea tables held *Nucella lapillus* and *Mytilus edulis*. Each subdivision held two *N. lapillus* and three to four *M. edulis*. These interactions were meant to recreate Tyler and Schiffbauer's (2012) study and yield fresh material while limiting the chance of damage to the specimens and deterioration of the shell microstructure.

d. Specimen Preparation

Care was taken to eliminate any damage or degradation to shells during storage. Live specimens from Mount Holyoke College were removed from the experimental tanks after predation was completed. I imaged each specimen within seven days of being drilled. During this time, I set each specimen on a clean and dry surface to air dry for no more than twelve hours. The surface on which they were drying was changed between specimens to prevent any fragments of a specimen from being associated with another.

Once the specimens were dry, I placed each individually in their own specimen bag. I housed the dried specimens in a box in a controlled environment until I imaged them. I took care to ensure that the weight of the specimens would not cause damage to the ones below in the box. Plastic packaging and bubble wrap were occasionally used to separate different groups of interactions and to ensure that fragile specimens were secure in the storage box.

The Paleontological Research Institution provided me with two sputter-coated specimens of *Mya arenia*. Excluding these, I refrained from coating any other specimens to prevent the possibility of obscuring faint predatory microtraces.

e. Imaging Process

All specimens from the Paleontological Research Institution were imaged using a Jeol Neoscope JCM 5000 benchtop Scanning Electron Microscope. Specimens at Mount Holyoke were imaged on the FEI Quanta 200 Scanning Electron Microscope. Each specimen was numbered and its drill hole imaged in a grid pattern, moving clockwise to ensure an established orientation relative to a drill hole margin at all times.

Depending on the size of the specimen and the location of the drill hole, I placed all bivalves on the stage with either the hinge facing the back of the machine or facing front to the stage door. Gastropods were placed with the protoconch facing the back of the microscope. One exception to this is the interaction of *Sinum x Donax*, where twelve *Donax* valves were placed with the umbo facing the center of the stage due to the length of the specimens. I imaged the specimens viewing them as close to perpendicular with the drill hole as possible.

VI. RESULTS

a. Microtrace Abundance

Of 180 drill holes imaged, three are believed to bear potential radular microtraces. Of the three specimens with potential microtraces, one occurs on an incomplete drill hole and two are found on complete drill holes. The data and distribution of potential microtraces are described in Table 1.

All predator-prey interactions are shown in Figure 11. The histogram shows the abundance of potential microtraces in each predator-prey interaction in both modern and fossil specimens. The histogram shows the presence of interactions yielding potential microtraces: *Mercenaria mercenaria* and *Littorina littorea* drilled by *Euspira heros* and *M. edulis* drilled by *Nucella lapillus*. Figure 12 shows the prey species abundance and of predatory microtraces in fossil specimens.

One of the completed drill holes with predatory microtraces is depicted in Figure 13. The location of the microtraces is in accord with Carriker's (1969) predictions. Figure 13 shows a simple line sketch of the outer and inner drill hole margins. The red circle denotes the location of the microtraces observed where the bottom of the beveled drill hole margin had not been removed by the radula. The specimen, a *Littorina littorea* drilled by *Euspira heros* under experimental conditions at Mount Holyoke College (#138, Figure 14), holds a small series of microtraces that are highly likely to be both predatory in nature and caused by

radular scraping. The microtraces appear as three separate sets, each consisting of at least three grooves, and are each approximately eight to thirteen microns in width (Figure 14).

b. Shell Deterioration

I had expected to note signs of shell deterioration in fossil specimens and, to a certain extent in modern specimens as well. I found signs of shell deterioration in specimens imaged within one week of the death of the prey. Figure 15A shows a mytilid, from the tank at Mount Holyoke College, less than seven days after drilling by *Nucella lapillus*. Arrows on the image indicate signs where the shell surface within the drill hole appears to have detached from the rest of the shell.

The same specimen was imaged exactly three weeks later. Figure 15B shows where portions of the drill hole surface that have detached from the shell. Some of the detached shell was able to detach between instances of imaging under scanning electron microscope. For comparison, Figure 16 is an example of *M. edulis* preyed upon by *N. lamellosa* provided by C. Tyler. The specimen loaned for the study was drilled five to six years ago and stored in a climate controlled environment and proper packaging.

Figure 17, *M. edulis* specimen #053, which was drilled under experimental conditions at the Paleontological Research Institution, shows presumed radular microtraces on periostracum and is included here as a comparison to the microtraces shown in Figure 8.

c. Drill Hole Margins

Six drill holes in five fossil specimens, *Saxolucina (Megaxinus) anodonta* (Say), were included in the fossil sample (Figures 18-19). Recovered from the Choptank Formation in Maryland, the Miocene specimens are part of a larger collection from the United States Natural History Museum (USNHM). Upon observation of the drill holes, I discovered that three of the six have an apparent nacreous or porcellaneous rim. I imaged the five complete and one incomplete drill holes under the scanning electron microscope. The three *S. anodonta* that showed development of the potential nacreous or porcellaneous rim showed a difference in composition from the original surface shell layer as noted by the arrows in Figures 18 and 19. Additionally, the drill hole margins appear to have higher relief from the rest of the shell surface.

VII. DISCUSSION

a. Presence of Potential Traces

The limited number of potential predatory microtraces preclude the evaluation of my hypotheses regarding location and abundance of predatory microtraces. I hypothesized that microtraces, if found, would be located in incomplete drill holes or on the deepest parts of complete drill holes. Specimen 138, *Littorina littorea*, (Figure 14) shows the most likely case of predatory microtraces found during the study. The location of these microtraces are depicted in the sketch in Figure 13.

I found the potential microtraces on the edge of the inner drill hole margin on a small portion of shell that extended into the drill hole. The deepest part of the interior of the drill hole would theoretically be the last of the shell to be removed during the drilling process.

The drill hole size is strongly correlated with the size of the accessory boring organ and not the proboscis (Carriker and Yochelson, 1968; Carriker 1969, 1978), both of which are larger than the radula. Thus, the proboscis enters through a hole whose edges may have been weakened by the accessory boring organ or partially rasped away by the radula.

Fossil specimens, as summarized in Figure 12, are not extensive enough to yield statistically significant results. Yet, with the limited number of specimens, and from varying times, the data do support the hypothesis that fossil specimens are unlikely to bear predatory microtraces. Potentially poor shell preservation

suggests that the delicate microtraces would have a limited chance of being preserved.

b. Nacreous or Porcelaneous Drill Hole Margins

Three *Saxolucina (Megaxinus) anodonta*, were imaged showing a compositional contrast on the shell surface. Located along the exterior margin of the drill holes, the nacreous/porcelaneous rims, in this study, are unique to *S. anodonta*. As previously mentioned in chapter II, section b. i., the accessory boring organ secretes a combination of acid, enzymes, and chelating agents. The known composition and combination of secretions has been debated (Zeigelmeier, 1954 and Carriker, 1978), but is ultimately unknown. Knowing that an undetermined combination of secretions is used by the accessory boring organ (Carriker, 1978), Mark McMenemy and I suggest that new shell material may be secreted by the predator during the drilling process (Lapic and McMenemy, in review). Whether this somehow involves secretions from the ABO is currently unknown but is certainly an intriguing possibility.

During drilling, the accessory boring organ deploys a series of secretions, presumably including an acid of unknown composition. When the prey's shell has been weakened, the radula is used to scrap away the shell, where it is consumed by the gastropod. After repeating this process, the gastropod perforates the shell of its prey. At this time, the predator inserts its proboscis into the prey and consumes the prey. The drill holes created by the gastropod are often rough along both the exterior and interior margins. Inserting much of its mouthparts through a rough opening may cause damage. The predatory gastropod is at high risk during

this time. Tides and other predators may increase the chance of damaging its proboscis. We hypothesize that the predator secretes a shell layer in addition to the combination of acids and chelating agents. The shell layer acts as a buffer between the rough drill hole margins and the gastropod's fleshy mouth parts (Lapic and McMenamin, in review).

c. Shell Deterioration

I expected to encounter shell deterioration in the hole region despite imaging predominantly modern specimens. Specimens observed at the Paleontological Research Institution were not very recently drilled, and some remained in storage from the experiments in 2012 and before until the summer of 2017.

Recent or live specimens were those that I imaged within seven days of drilling. Included in the specimens provided by the Paleontological Research Institution were thirty-three *Mytilus edulis* provided by C. Tyler. Tyler's specimens from Tyler and Schiffbauer (2012) are were imaged by me between five and six years after the initial imaging by Tyler. Tyler and Schiffbauer (2012) observed microtraces in 74% of *Mytilus edulis* drilled by *Nucella*. While obvious microtraces were scarce, I imaged the same specimens on more than one occasion to see if deterioration occurred, involving loss of the shell material that would have held the microtraces. One such case is shown in Figure 15.

While microtraces were not abundant in specimens produced at Mount Holyoke College, we must consider the obvious difference in the preservation of the shell. Figure 15 shows a comparison of mytilid specimen 143 which was

drilled under experimental conditions and imaged at Mount Holyoke College. Figure 16 provides an image of a typical *Mytilus edulis* provided by C. Tyler from the Tyler and Schiffbauer (2012) study. Specimen 143, when originally imaged, provided a view of the concentric lamellae shown on the surface of the interior part of the drill hole (Figure 15). When I imaged the specimen three weeks later, the lamellae were not seen and it appeared as if the surface shell within the drill hole had pulled away from the remaining shell.

This created the appearance and texture of crumpled tissue paper within the drill hole. The change in surface texture not only shows that taphonomic change occurred in the span of only three weeks, but that the change was also potentially hazardous for the operation of the scanning electron microscope. Stray material that is not thoroughly attached to the specimen can cause damage to the vacuum chamber of scanning electron microscopes. Additionally, loose fragments can obscure desired features on a specimen. Unanchored particles may be accidentally removed during transportation or in the process of the microscope reaching its target pressure and vacuum.

I purposely sought out finding a view of a drill hole that matched those published by Tyler and Schiffbauer (2012). Figure 10 shows the view closest to Figure 8. Located in a drill hole from *Mytilus edulis* drilled by *Neverita duplicata*, Figure 17 is not the interior of the drill hole, but the periostracum which adhered to the interior during storage.

Figure 15A identifies areas where material within the drill hole has begun separating from the rest of the shell. This makes a particularly good example for a

specimen where there could have been potential microtraces, but as seen in the taphonomic change from Figure 15A to Figure 15B, it would be unlikely that the predatory microtraces would be preserved.

With that being said, some specimens within the 164 modern specimens bear what I could consider potential predatory microtraces. Although, as seen in Figure 15, shell deterioration appears to occur more quickly than I originally considered. In the case that predatory microtraces were present, the chances of observing them a year after predation occurred are limited.

The original concept of identifying predatory microtraces and the gastropod which created them is to help to identify the appearances of particular predatory taxa in the fossil record (Schiffbauer et al 2008). By doing so, we equip ourselves with a tool necessary to better understand the predatory-prey interactions of paleocommunities. However, unless preservation is excellent in a particular rock unit, the specimens from said formation will be unlikely to preserve such minute features.

Modern specimens show limited preservation of microtraces even under controlled conditions. Preservation in the fossil record is comparatively worse in most cases. Exceptional preservation may be encountered at some localities. In these uncommon lagerstätten, it is more likely to find the preserved microtraces and other micro-scale characteristics of predation. The selected Plio-Pleistocene specimens from Florida provide well preserved drill holes with limited degradation (i.e. limited matrix debris and crystallization). This is likely since they were taken as nesting from within larger fossil shells where they were

protected during tides, storms, and from severe diagenic alterations, the nested shells providing sheltered microenvironmental spaces. Nevertheless, no original shell surface remained.

In comparison to the *S. anodonta* from Maryland, the small shells of the Plio-Pleistocene in Florida are better preserved in a taphonomic context. The preservation difference is possibly caused by the size differences between the samples. The Plio-Pleistocene shells range from approximately six to twenty-five millimeters while the drill hole alone of *S. anodonta* has a diameter of nearly four millimeters.

VIII. CONCLUSIONS

In order to maximize my observational scope, I incorporated in the use of recently living specimens to limit the possibility of shell deterioration and to increase the likelihood of finding predatory microtraces. Even among the recent specimens that were housed at Mount Holyoke College, it is clear that rapid taphonomic changes are rife. In considering the taphonomic changes, it is crucial to understand that preservation falls on a spectrum.

I found numerous signs of degradation within the drill holes along with both good and poor overall shell preservation. Preservation was, overall, poorer than I had expected, particularly in the recent specimens. By re-imaging a *M. edulis* specimen, I was able to view the extent of deterioration within the drill hole. While specimen 143 appeared intact and with limited shell breakage, the interior of the drill hole contrasted greatly. Potential causes of the interior shell deterioration include the preservational capacity of the shell material.

Consider the nacreous or porcellaneous rims surrounding three of six *S. anodonta* drill holes. Surface shell on the exterior of a bivalve is not composed of nacre. Nacre is secreted inside the interior of the shell, from the mantle.

The majority of the recent shells imaged had periostracum remaining. Periostracum would begin to break down and fall off of the shells by the time that the drying period had ended. By the time that the shells were to be imaged under the scanning electron microscope, much of the periostracum had been removed or

was at risk of breaking off during the imaging process. As a result, loose periostracum was removed before each imaging session.

A clear limitation of this line of investigation is that in order to determine the origin of gastropod microtraces, the microtraces must actually be preserved. Without them, predation could be caused by a gastropod, sponge, cirripede, or polychaete. Exceptional preservation will be required to utilize predatory microtraces for taxonomic and paleoecological study. Even under conditions of higher quality preservation such as shell nesting, early diagenesis fossil specimens will ordinarily not preserve the microtraces needed for precise determination of predator-prey interactions.

IX. Future Work

Following the work of Carriker (1968, 1978, 1981) and Zeigelmeier (1954), my research was directed towards observing incomplete drill holes and those in which the gastropod had just perforated the inner layers of the shell. Future work must focus on such incomplete drill holes, as current data is strongly biased towards complete drill holes (Table 1).

M. edulis provided seventy of the one hundred and eighty observed drill holes, and this represents a potential bias in the data set. There is the possibility that *M. edulis* has a less stable shell microstructure that has contributed to the shell degradation that can be seen in Figure 15. A number of *M. edulis* show unstable shell parts when imaging with the scanning electron microscope. The unstable shell is identified by separations in the interior drill hole (Figure 15A) or crumpling of the surface of the drill hole (Figure 15B).

Thus, future work would entail continuing a focus on incomplete drill holes in search of predatory microtraces. In the portion of this study dedicated to replicating Tyler and Schiffbauer's 2012 research, additional specimens of *M. edulis* drilled by *N. lapillus* were interrupted, as per Carriker (1969, 1981), to increase the likelihood of observing predatory microtraces. This yielded only one incomplete drill hole with microtraces.

Predatory gastropods do not feed frequently and limited time was available to interrupt the predation process during my study. Acquiring additional incomplete

specimens would provide additional data to test the concept of using microtraces as tool for studying gastropod predation. As previously mentioned, there may exist a bias with regard to mytilid shell microstructure. Thus, it may be worthwhile to explore additional predator-prey interactions where prey shells are well preserved in the fossil record. For example, rapid silicification can sometimes lead to ultrastructural preservation in fossils. Greater quality of preservation may also be associated with a more stable shell material, leading to better short-term and long-term preservation of shell surface features.

L. littorea, despite serving as a live specimen in the study, was only represented by four of one hundred and eighty drill holes. The limited number of interactions was due to the death of *Euspira heros*, the predator species of multiple interactions in this study. Additionally, *E. heros* may have been feeding more infrequently during the time of the study. *E. heros* died after extracting itself from its shell and patrolling the sea table shell-less for fourteen days.

Using predatory microtraces as a means for interpreting past predator-prey interactions is an inviting concept with severe practical limitations. Before putting further effort into research regarding the predatory microtraces, we must better understand the drilling process and, more importantly, the role of the accessory boring organ and its effects on the shell microstructure (and, possibly, on reprecipitation of mobilized material from the prey shell).

The drilling process is not as straightforward as it may first appear. It is a process with many working parts; the radula, accessory boring organ, and the microstructure of the prey's shell all play important roles in the expression of

predatory microtraces. Microtraces still hold great promise for the possibility of developing paleobiological diagnostics, but their promise is more dependent on exceptional preservation (especially early diagenetic processes that forestall degradation) than originally thought.

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XI. Appendix

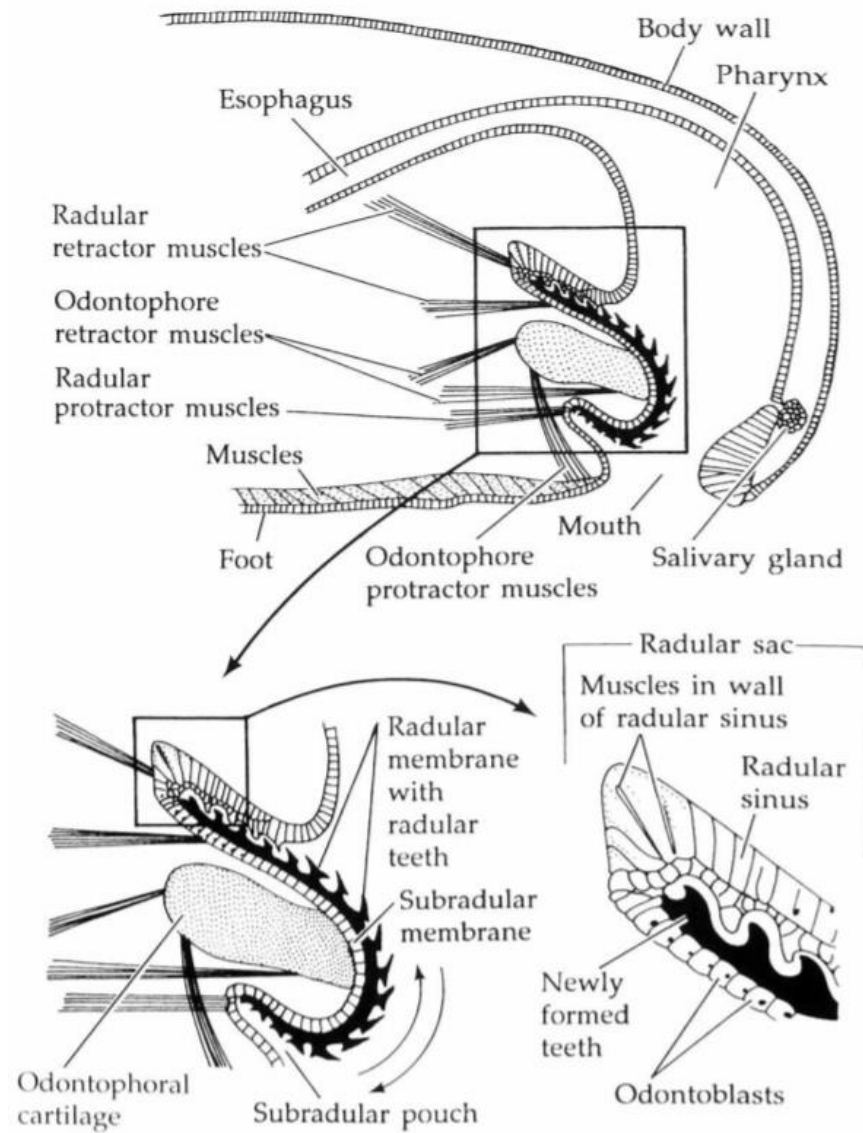


Figure 1: From Brusca et al. (2016); A) a sketch diagram of typical Caenogastropod mouth and feeding parts showing the location and organization of the radula and radular membranes. [Figure 13.23]

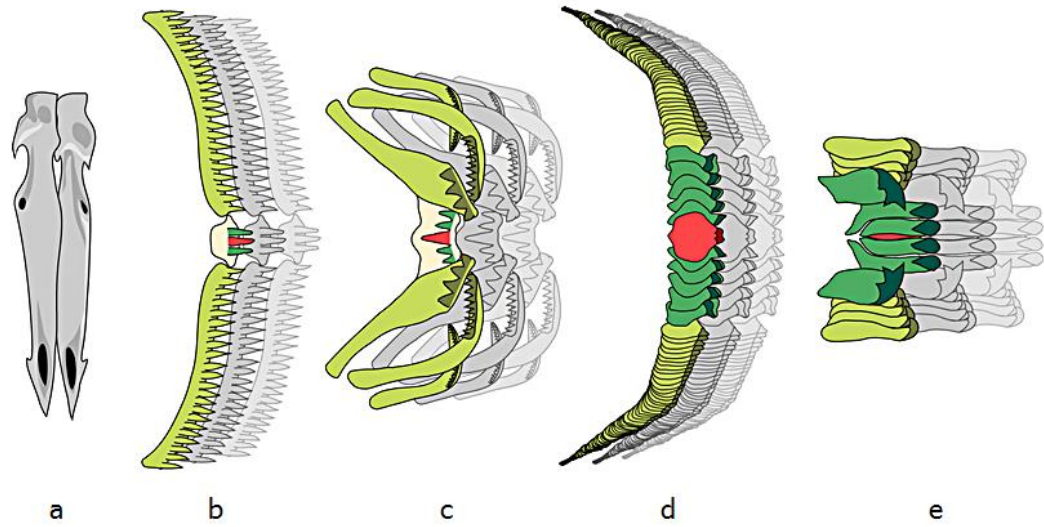


Figure 2: A comparison of radula classification including: c) Taenioglossan, the radula considered in this study. From “The Living World of Molluscs” (Nordseick, R.).

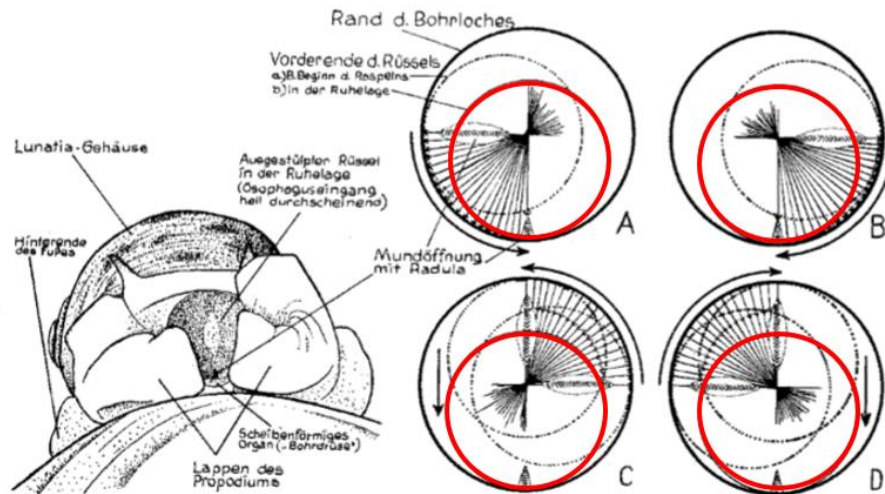


Figure 3: Zeigelmeier provides a depiction of the orientation of *Lunatia* (now classified as *Euspira heros*) while drilling into its prey. As translated from Zeigelmeier (1954): The Schematic representation of the sector-wise formation of the borehole with the help of the radula. The arrows give the direction of the predator during the 18-20 rasps. A) The snail turns the proboscis 90° to the right. B) the proboscis is turned to the left to reach the initial rasp from A. C) The snail turns left and rotated the proboscis. D) The proboscis turned to the left and the snail turns right.

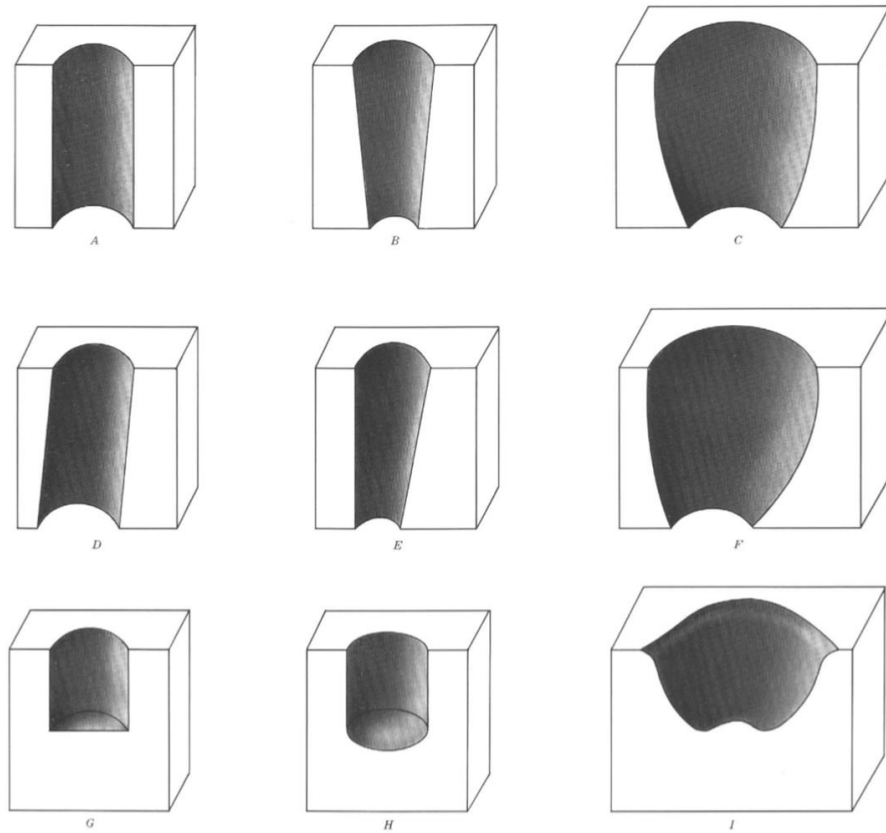


Figure 4: Morphology of gastropod drill holes as seen in cross section; From Carriker and Yochelson (1968). Cylindrical drill holes are depicted in A, D, G, and H while drill holes characterized as beveled are shown in B, C, E, F, and I. Incomplete drill holes can exhibit a central boss on the bottom as seen in I.



Figure 5: From McMenamin and Schulte McMenamin (1990), the drill hole found within the tubular *Hyolithellus* from the Lower Cambrian. Scale bar is 50 μm . [Figure 7.3].

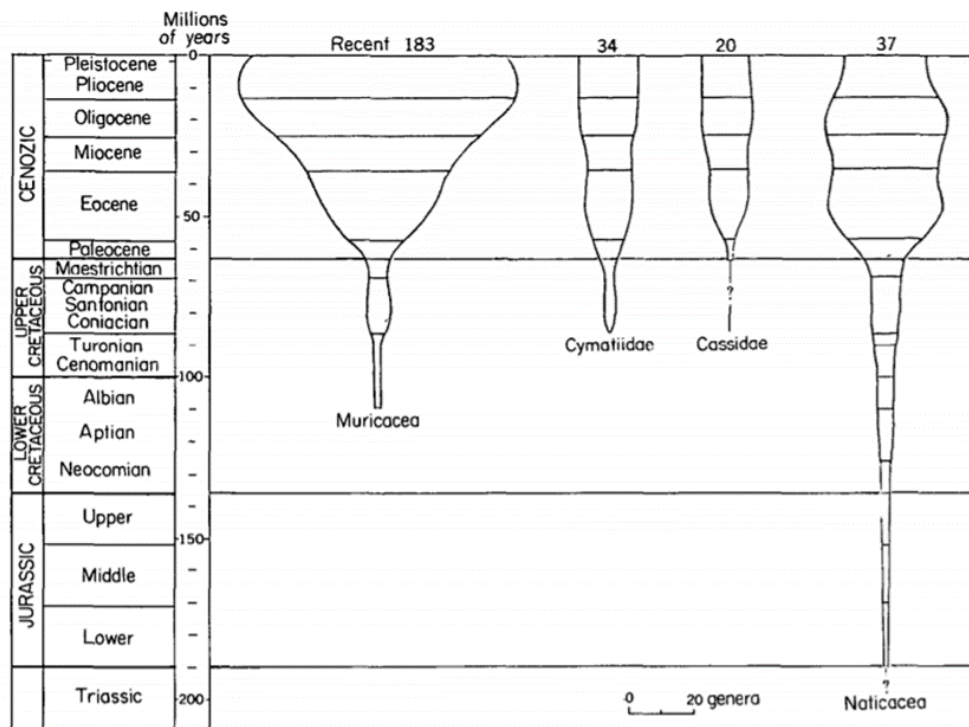


Figure 6: From Sohl (1969); Spindle diagrams showing the diversification of drilling gastropods through time. The unknown initial appearances of Cassidae and Naticidae are denoted with “?” as only a time frame for appearance is generally agreed upon.

	Holocene	
	Pleistocene	Fossil specimens used in the study range from Pleistocene to Miocene
Neogene	Pliocene	
	Miocene	Appearance of two new families of drilling gastropods: Buccinidae and Nassidae (Fischer, 1964).
Paleogene	Oligocene	Appearance of three new drilling gastropods (Fischer, 1964)
	Eocene	Fischer (1964) claims that cylindrical borings (which are not entirely mutually exclusive to muricids) do not appear until the Eocene. Diversification of Naticidae, Muricidae, Cassidae, and Cymatiidae during the Eocene.
	Paleocene	
Cretaceous	Late	Possible appearance of Cassidae during the Late Cretaceous Speciation of naticids corresponds with increase of drill holes in fossil assemblages (Fischer, 1964).
	Early	The first muricid, <i>Hillites</i> , appears (Stephenson, 1952).
Jurassic	Late	Fischer (1964) notes the rarity of drill holes in both mollusks and brachiopods during the Jurassic.
	Middle	
	Early	A limited number of naticid species are described by Morris and Lycett (1854) from Jurassic strata.
Triassic	Late	
	Middle	
	Early	The presence of naticids during the early Triassic is debated, as noted by Sohl (1969) and dismissed by Carriker (1978).
Permian	Late	
	Middle	
	Early	Yakovlev (1926) describes brachiopods with circular perforations.
Carboniferous	Pennsylvanian	
	Mississippian	Brunton (1966) describes a brachiopod of Northern Ireland with 44 cylindrical perforations. It is unlikely that this is gastropod in origin due to the high

		numbers of drill holes (Carriker and Yochelson, 1981).
Devonian	Late Middle Early	
Silurian	Late Middle Early	
Ordovician	Late	Drilling predation aided by chemical component is discovered and debated by Carriker and Yochelson (1968); ultimately determined to be not caused by gastropods; Possibly the earliest case of chemical aid in drilling predation.
	Middle	1 st appearance of cylindrical boreholes (Carriker and Yochelson, 1968).
	Early	Oldest identifiable boreholes are comparable to those of modern gastropods (Carriker, 1958 as discussed by Fischer, 1964).
Cambrian		<i>Hyolithellus</i> contains a predatory drill hole (McMenamin and Schulte McMenamin, 1990); the holes had been noted previously, but were thought to be an original feature of <i>Hyolithellus</i> shell morphology
Pre-Cambrian		

Figure 7: A summary diagram of drilling predation in gastropods. The geologic time scale includes discoveries of processes, adaptations, and radiations of various groups of drilling, predatory gastropods.

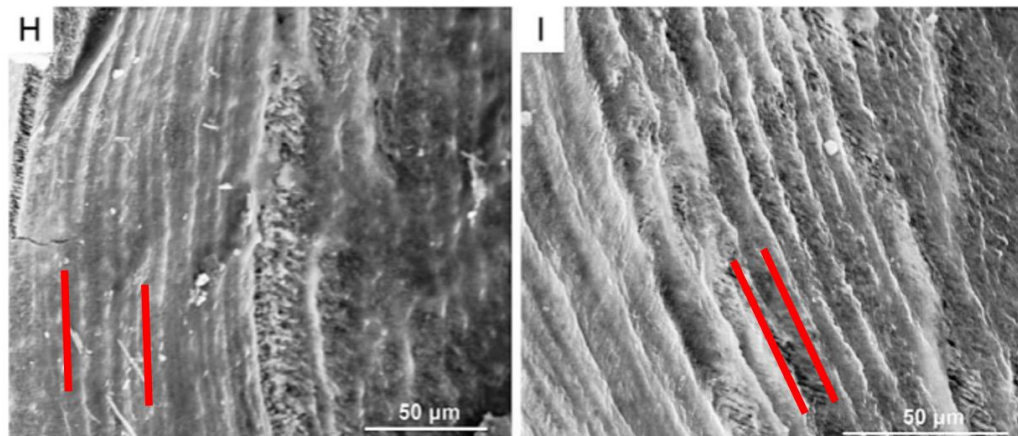


Figure 8: Adapted from Tyler and Schiffbauer (2012); Two examples of *Mytilus edulis* that were identified by Tyler and Schiffbauer as having predatory microtraces present. The lines are running parallel to the identified microtraces.

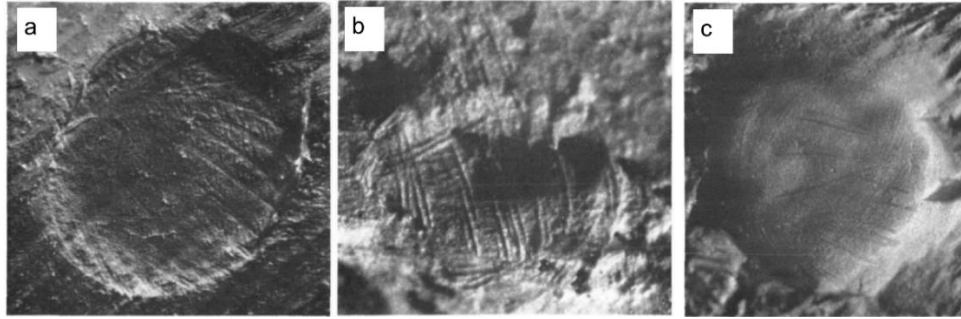


Figure 9: Predatory microtraces left in incomplete drill holes within *Mya* by *Urosalpinx cinerea*. See text for more details. From Carriker (1969).

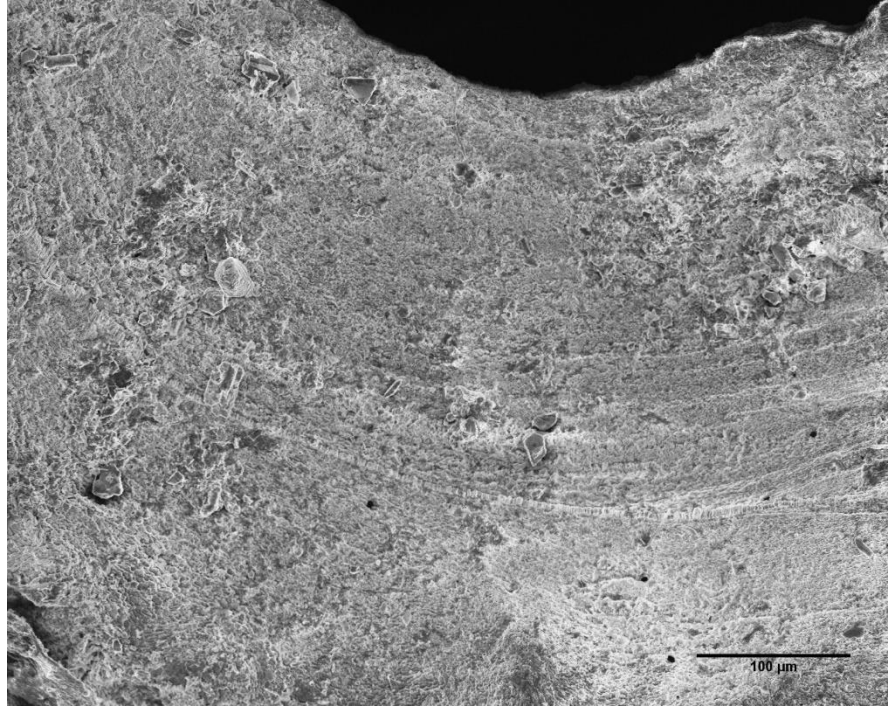


Figure 10: Specimen #040 showing the cross lamellar fabric within the drill hole of *Mytilus edulis* drilled by *Nucella lapillus* at the PRI.

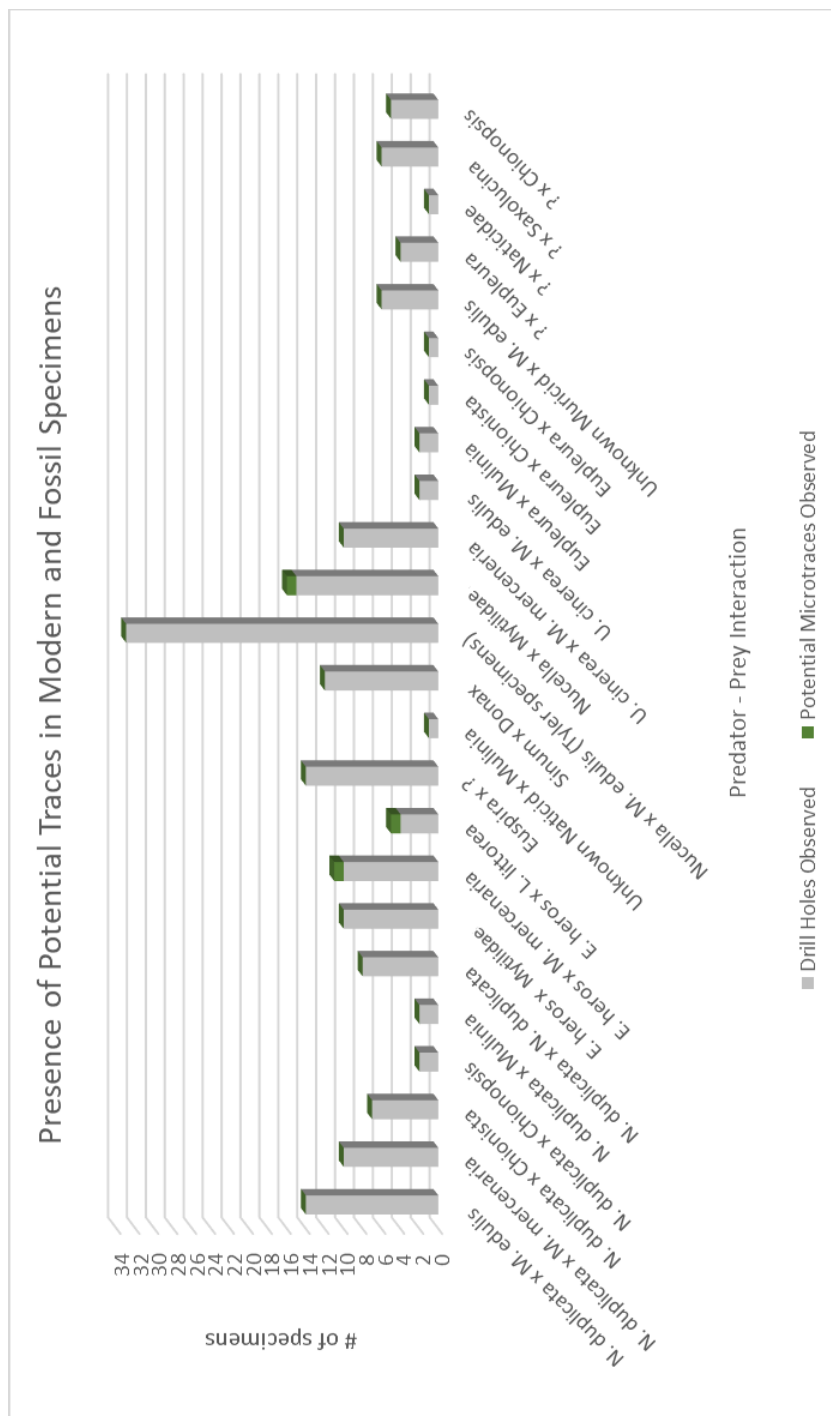


Figure 11: A comparative histogram of all predator – prey interactions observed including fossil and modern specimens. The histogram includes all specimens imaged at both the PRI and Mount Holyoke College.

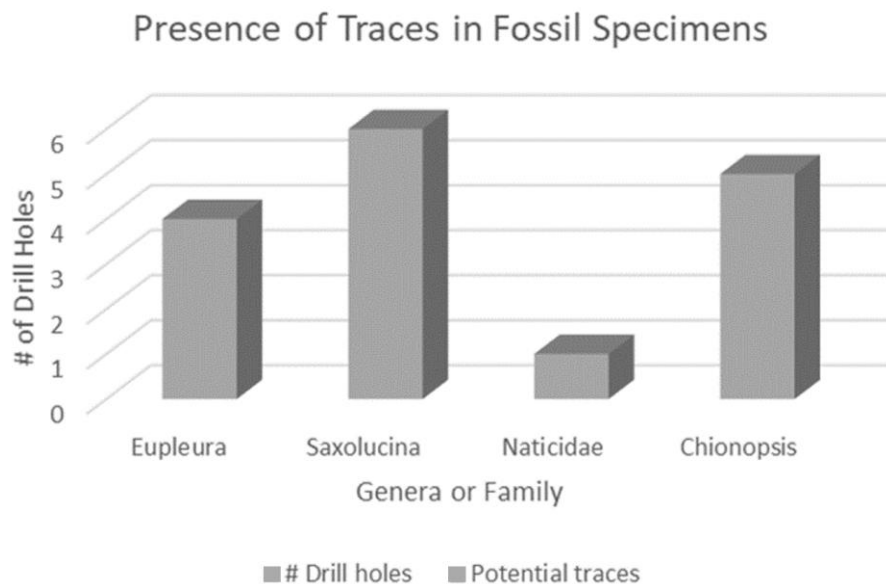


Figure 12: A histogram of the sixteen fossil prey observed and the presence of microtraces within them. No potential predatory microtraces were found in fossil specimens.

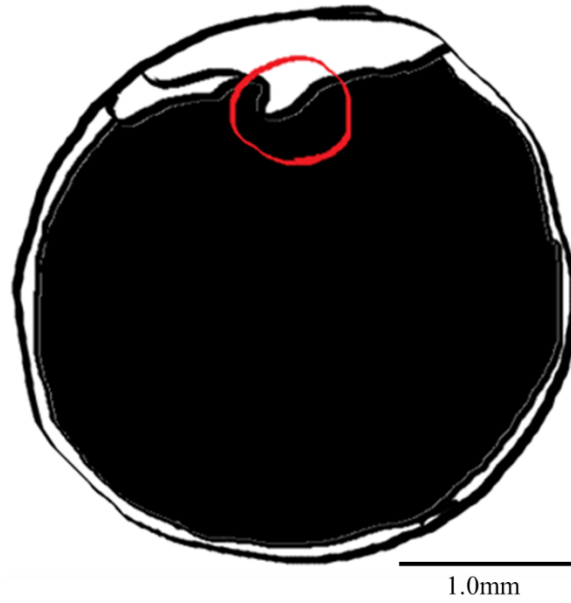


Figure 13: A sketch of a drill hole in *L. littorea*. The red circle shows the location of the microtraces. Scale bar is 1.0mm.

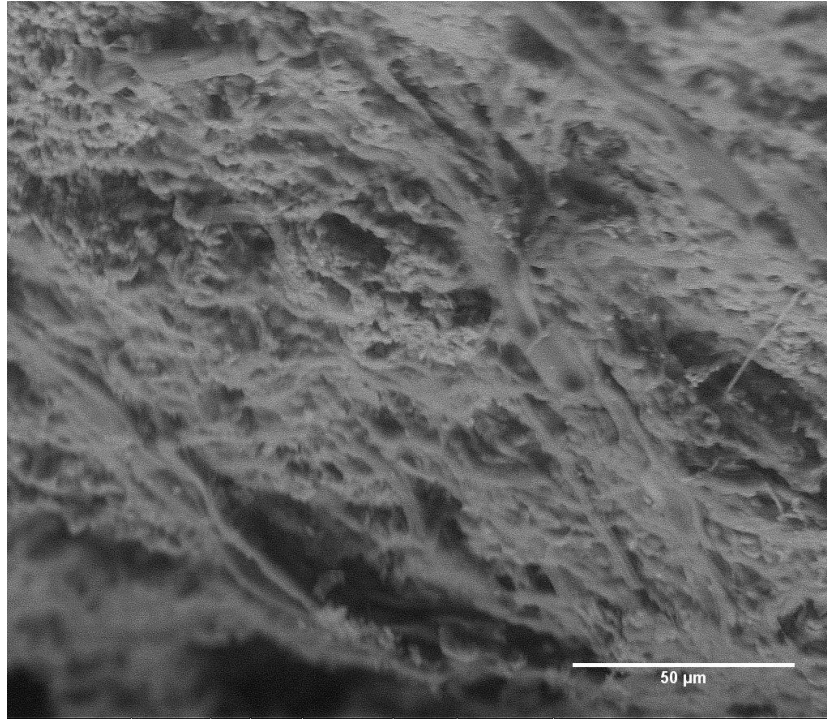


Figure 14: *L. littorea* preyed upon by *E. heros* showing the single most likely case of predatory microtraces.

Table 1: A summary of the presence of potential microtraces in both complete and incomplete drill holes. Additionally, the presence in known naticid – drilled holes is compared to known muricid – drilled holes. Fossil specimens are not included in these totals as the identity of the predator is not known. *43 drill holes were analyzed at Mount Holyoke College. Sixteen of these were in fossil specimens and the remaining 27 were in modern specimens.

Type of Drill	No Predatory Traces Present	Potential Traces Present	Total Drill Holes
Complete	160	2	162
Incomplete	17	1	18
Naticid – Drilled	92	2	94
Muricid – Drilled	70	1	86
Drilled at the PRI	137	0	137
Drilled at Mount Holyoke College	40	3	43*

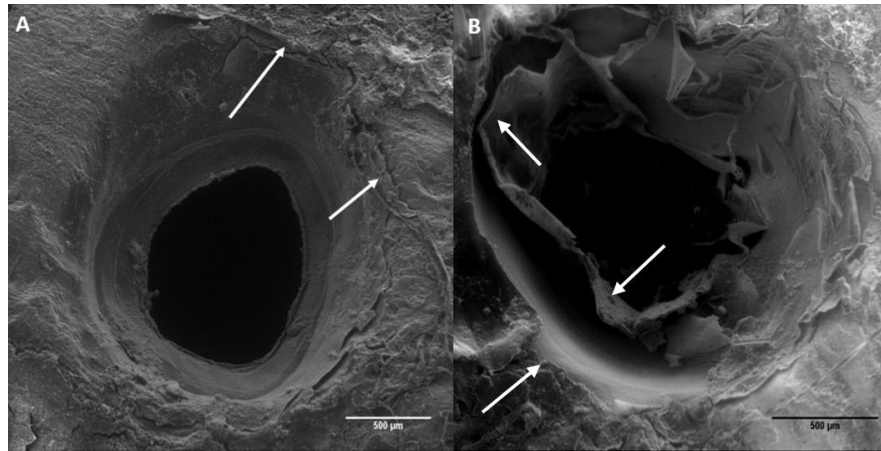


Figure 15: A comparison of specimen 143, *Mytilus* preyed upon by *Nucella lapillus*. Image A is specimen 143 at the time of original imaging for predatory microtraces. The arrow indicates the boundary where the interior of the drill holes has begun separating from the shell surface. Image B shows specimen 143 imaged under the same conditions exactly three weeks later. Image B represents the crumpling or tissue paper like drill hole that I have seen after the initial imaging. Arrows indicate distinct areas of separation of the drill hole surface from the shell.

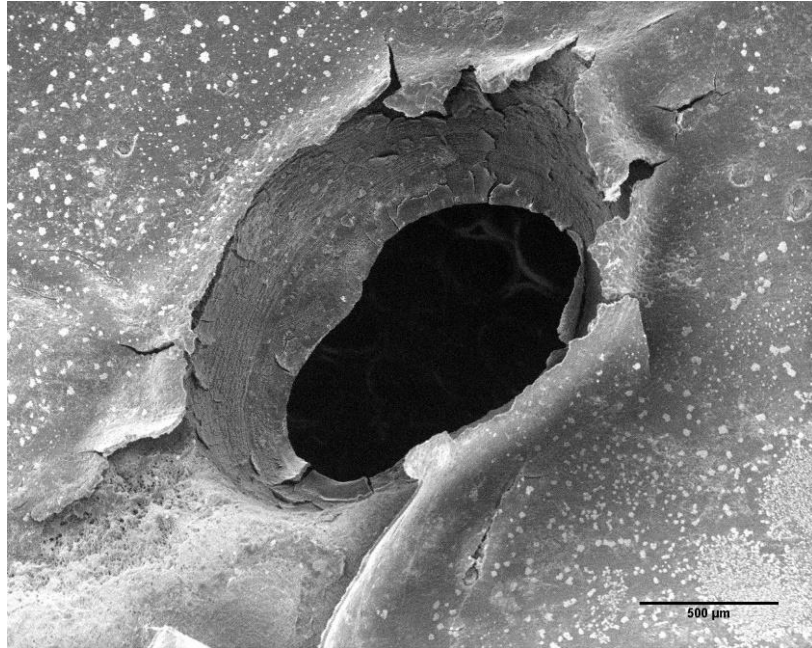


Figure 16: Specimen 131 loaned by C. Tyler. *M. edulis* was used in the 2012 Tyler and Schiffbauer study and reimaged at the Paleontological Research Institution.

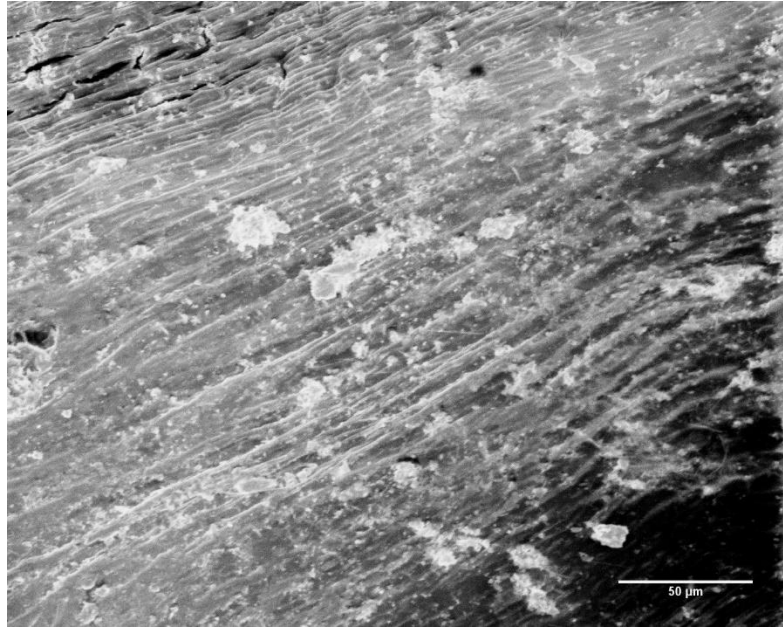


Figure 17: Taken to scale of Tyler and Schiffbauer (2012), specimen #053, a *M. edulis* drilled by *N. duplicata* appears to depict predatory microtraces. These traces are indicated by the arrows, and occur on the surface periostracum.

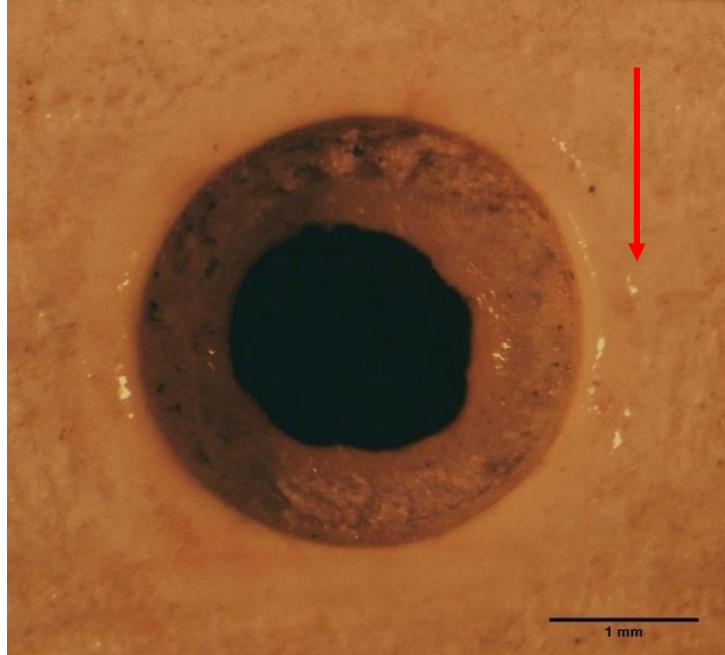


Figure 18: An image of the potentially nacreous or porcellaneous rim surrounding the outer drill hole margin in *Saxolucina*. The red arrow indicated the boundary between the shell surface and the nacreous or porcellaneous rim. This specimen is the same as the specimen imaged in Figure 19 (below).

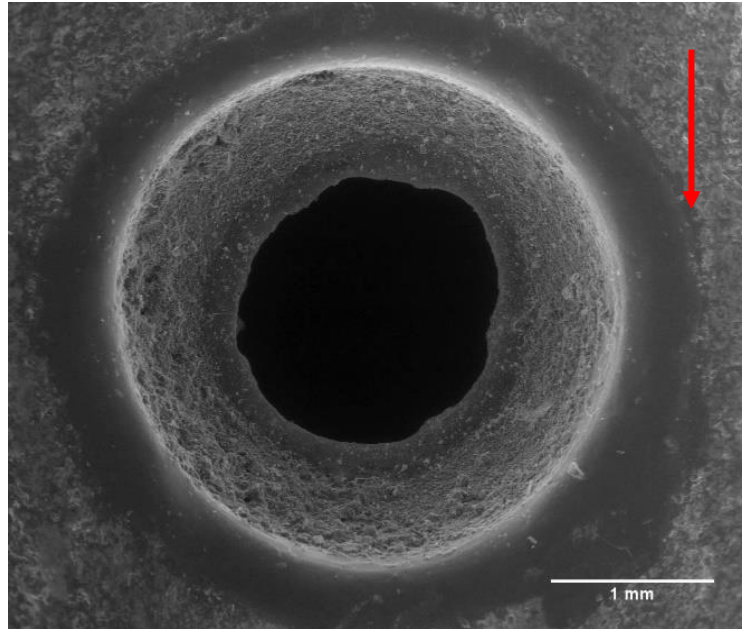


Figure 19 : A scanning electron micrograph of a drill hole left in *Saxolucina* showing a compositional contrast between the surface shell and the area surrounding the exterior of the drill hole. The red arrow denotes the boundary between the surface shell and the nacreous or porcellaneous rim.