INITIAL METHODS FOR AGEING ATLANTIC MUD-PIDDOCK (BARNEA TRUNCATA)

D. Roddick and C.M. Clark

Science Branch, Maritimes Region
Fisheries and Oceans Canada
Bedford Institute of Oceanography
1 Challenger Drive, Dartmouth, NS
B2Y 4A2

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Dale Roddick and Caira M. Clark

Science Branch, Maritimes Region
Fisheries and Oceans Canada
Bedford Institute of Oceanography
1 Challenger Drive
Dartmouth, NS
B2Y 4A2

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ABSTRACT

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The Atlantic Mud-piddock, *Barnea truncata*, is listed as a threatened species in Canada, where the only occurrence is a small disjunct population in the Minas Basin of Nova Scotia. This initial study of age and growth for this population was restricted in sample sizes due to the status of the species, but shows that they can be aged using growth lines in hinge to posterior cuts of the shell. Thin sections can be used for ageing, but etching and staining increases readability of the growth lines. The resulting growth curve is the first recorded for this species, and shows that the Minas Basin population has a lifespan of approximately 11 years. The first ring is deposited at around 6 mm shell length.

RÉSUMÉ

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La pholade tronquée (*Barnea truncata*) est sur la liste des espèces menacées du Canada, où sa seule présence est une petite population isolée dans le bassin des Mines, en Nouvelle-Écosse. La taille des échantillons de cette première étude de l'âge et de la croissance de cette population a été limitée en raison de la situation de l'espèce. Toutefois, elle démontre que l'âge de l'espèce peut être déterminé par des lignes de croissance sur la charnière postérieure de la coquille. De minces sections peuvent servir à déterminer l'âge, mais la gravure et la coloration augmentent la lisibilité des lignes de croissance. La courbe de croissance résultant de cette étude est la première enregistrée pour cette espèce, et démontre que les individus de la population du bassin des Mines vivent environ 11 ans. La première ligne de croissance se forme lorsque la coquille mesure environ 6 mm.

INTRODUCTION

The Atlantic Mud-piddock, *Barnea truncata*, is a species that – in Canada – is found only in the Minas Basin, Nova Scotia (Figure 1). Outside of Canada, its nearest location is 475 km away in southern Maine (DFO in press). Knowledge about its life history is limited. It is an intertidal bivalve mollusk, 3 to 5 cm long, which grows while burrowing conically into the substrate (Figure 2). As a result of this growth pattern, Mud-piddock become entombed in their burrows and are reliant on suspended food and local water quality for food, respiration and other physiological functions (COSEWIC 2009).

Throughout its range, Mud-piddock is found intertidally, with only one sub-tidal occurrence recorded in Florida (COSEWIC 2009). It is always found in soft muds, mudstones, or peats, with one exception: a single report of valves retrieved from a sample of submerged wood (Frank 2009; Jacobsen and Emmerson, 1961). The species is limited in Canada to a specific geological formation: red-mudstone substrate interbedded in sandstone and conglomerate that occurs only within the intertidal zone of the Minas Basin (COSEWIC 2009). The total extent of exposed red mudstone was estimated by Clark *et al.* (2019) as a maximum of 1.86 km^2.

In 2009, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) recommended a threatened status for the Atlantic Mud-piddock (COSEWIC, 2009). Mud-piddock was listed as Threatened on Schedule 1 of the Species at Risk Act (SARA) in 2017. Designation reasons included: its Canadian distribution consisting of a single small population; limited available preferred habitat; and the potential for changes in sediment deposition from increased frequency and severity of storms, erosion from rising sea level, increased rainfall, and coastal development.

Knowledge about Mud-piddock is limited, primarily due to its biology and use of red mudstone habitat. Mud-piddock conically burrow into the red mudstone, and are difficult to remove and replace without causing their mortality. The extent of the species' use of red mudstone is also difficult to determine from the surface, as many more Mud-piddock may inhabit an area of red mudstone than are initially visible. A population estimate, for example, would require disruptive and destructive sampling through removal of red mudstone at selected sites, then cutting cross-sections through bore-hole assemblages. DFO (2010) concludes that a population estimate is therefore currently impossible.

This study was undertaken with existing data limits in mind to increase knowledge about the species in general and the Nova Scotia population in particular. It examines methods of age determination in order to provide initial information about growth rate and to contribute to future studies of population structure, mortality, and other life history parameters. Specifically, this study initially was developed to see if Mud-piddocks could be aged using thin sections of the shell. It was then expanded to both increase the sample size and to examine if acetate peels or etching and staining would increase the ease of interpreting the growth lines. Its goal was to determine the optimum ageing procedures and to produce the first growth curve for the species. As it

relied on small sample sizes and did not include an verification study of the ageing results, it is intended as a first step and can be expanded upon in future years.

LITERATURE SEARCH

A literature search revealed no *Barnea truncata* ageing studies, and only a few within the family Pholadidae. As well as searches for growth and ageing studies on *Barnea* species, and on other genera within the Pholadidae, the search included the database from Moss *et al*. (2016), which reviews "the entire publication run of 30 peer reviewed journals likely to contain articles reporting lifespan and/or growth rate data on marine bivalves." This database encompasses 297 species from 1,148 local populations and represents an exhaustive search of literature up to 2015. Four papers were found that appeared relevant to examining Mud-piddock shells for annual growth lines, including one that looked at a boring Mytillidae.

Evans (1968) looked at *Penitella penita* growth rate in relation to substrate hardness in intertidal areas on the Oregon coast, USA. He used test boards and replant experiments, but also examined the "growth lines," the external ridges on the anterior slope of the shell. Evans hypothesized that each growth band represented a period of active boring followed by a period of shell deposition; this means that his growth bands are not annual marks. He used transplant experiments to examine the number of growth bands per year and found they decreased with age. Evans estimated that sixty growth bands were deposited in the first year and that subsequently, one band was deposited an average every 16.3 days or 22.4 per year. He came up with a formula to reach a mature size: (Total number of bands - 60)/22.4 = n - 1. Evans cites previous work to say that *Penitella penita* exhibits determinate growth, reaching a mature size at which point both boring activity and growth cease, while it can continue to live for another 6 years.

Evans and LeMessurier (1972) looked for evidence of daily growth lines in *Penitella penita* using ground sections through the shell sulcus, acetate peels, and scanning electron microscopy. They found a recognizable but slight slowdown in growth during the winter, but in hard-rock animals they found that slowdowns occur in no particular pattern and for no regular duration. They state it is likely that the slowdowns reflect variations in substrate hardness rather than seasonal temperatures. Evans and LeMessurier conclude that Penitella's boring habit protects it from environmental stresses, and so it's responses are damped down to such an extent that they are almost undetectable in the shell layers.

Bagur et. al (2013) looked at *Lithophaga patagonica* from Argentina, a burrowing Mytilidae. They used acetate peels to age them, but the annual structures they used for aging were "clefts" in the exterior of the shell caused by changes to the rate and direction of shell deposition due to low winter temperatures. They used acetate peels of seasonally sampled shell sections to illustrate the formation of the "clefts," and illustrated how internal lines in the hinge section of the shell did not give accurate age estimates. Although the study used acetate peels, the external clefts that Bagur *et al.* (2013) identified should be visible in thin sections.

The most relevant paper is Pinn et. al (2005) which examined three piddock species *(Pholas dactylus*, *Barnea candida* and *B. parva*) from five locations along the south coast of England. Among their studies they looked at population structure using modal analysis, and age using acetate peels of shell sections. The modal analysis separated the populations of *Pholas dactylus, Barnea candida* and *B. parva* into eight, three and five modes respectively. The acetate peels are described as showing thin dark annual growth lines, but there is little detail, and only one figure showing images with two, two, and one growth line for each species respectively.

METHODS

3.1. SAMPLE COLLECTION

Mud-piddock shells from the Minas Basin are difficult to obtain because Mud-piddock was listed as Threatened on Schedule 1 of the Species at Risk Act (SARA) in 2017. Its listing restricts collections. A compounding factor is that the species burrows conically into red mudstone, so intact extraction of organisms is very difficult. Most larger shells are eroded at their exposed posterior end (see Figure 3 for shell anatomy), probably due to sediment and ice scouring, which makes them less useful for growth and ageing studies as their true total length is unattainable.

When this study was initiated, the shells available for analysis were empty single shells collected after the organisms had died and were washed out of their burrows. These were obtained from collections held by DFO and the Nova Scotia Museum via the Curator of Zoology, Andrew Hebda (Table 1). Many of the shells were broken and the posterior ends were eroded. Although the collection dates were known, the dates that the specimens had died were not.

This study initially proceeded with the previously collected specimens, but shortly after beginning, shells from samples collected live by the Nova Scotia Museum and that were being used for genetic analysis were made available. These samples consisted of 37 Atlantic Mud-piddock collected live from four locations in the Minas Basin. The specimens had both shells, although many were broken, and as they were collected live the date of death was known. Later, additional live Mud-piddock were collected under Species at Risk Act Permit No. DFO-MAR-2018-15a to attempt to fill in the small (<5mm) and large (>25mm) ends of the size distribution to fit a growth curve for ageing. During permitted sampling, additional Atlantic Mud-piddock that were exposed but not retained were also measured. The soft tissues from retained specimens were also used for genetic analysis and preserved for future size- and age-at-maturity analysis.

3.2. LENGTH FREQUENCIES

The shell length frequencies of the samples collected live were examined to see if there was any evidence of modes that might represent year classes. Samples that were collected live from a single location were a sample of 29 collected for DNA analysis and a sample collected under a special permit for this study. The permitted sample consisted of 30 clams collected live and measurements from an additional four that were uncovered during collection but not retained. Other live DNA samples with

six or fewer Mud-piddock were collected at different times. Length frequencies were plotted and examined for evidence of modes in the distribution which could indicate year classes, especially for the youngest ages.

3.3. EMBEDDING AND SECTION PREPARATION

Samples of the single shells were used to examine the direction of cut and thickness for the shell sections to provide the best sections for ageing. Sections from the hinge nucleus towards the posterior end provide longer sections, which sections through the anterior slope go through the tooth ridges, the most pronounced external sculpture in the shell. Cuts were made from the hinge through the anterior slope, along the anterior-posterior transition (where the sulcus occurs in other Pholadidae, Figure 3), through the posterior slope, and through the posterior end of the shell.

Shells were completely embedded in clear polyester resin blocks for sectioning (Figure 4). As the sample size was small, molds were made from strips of modelling clay on a waxed glass plate. Both Easy-Cast polyester resin and Araldite 502 resin were tried. The Ardalite resin was harder and supported the shell more than the Easy-Cast and was used for most of the embedding. The molds were first poured with a thin layer of resin which was allowed to harden. The shells were then coated with resin, placed in the mold on top of the hardened resin and the mold filled to cover the shell. The molds were left to cure for at least four days and then the blocks were removed from the modeling clay molds, trimmed, and cleaned. The resin blocks were initially labelled with paper labels embedded in the blocks, and as they were processed the sample label was transferred to all cut blocks and sections.

Initial sections were cut with a single diamond blade on an low speed Isomet saw. This was switched to using two blades and a 3 mm spacer as it reduced processing time and the parallel sides of the section resulted in less movement in the slide press when mounting the slides. The cut sections were ground (130, 600 grit) and polished (0.3μ alumina) on the side containing the hinge nucleus, using Meta-Serv 3000 grinder-polishers with 8 inch discs. The polished sections were cleaned in running water with a soft bristled brush, air dried, mounted on a slide with resin, and placed in a press to cure for at least four days. The use of a press during mounting ensured the section was level and flush to the slide. This helped prevent parts of the section being ground away during processing for thin sections.

The mounted sections were initially ground to ~0.5 mm on a Petro-Thin thin sectioning system, but this appeared to chip the edges of the shell, even supported in the resin. This step was eliminated and the sections were ground down with the 130 grit disk. Grinding and polishing used the same grits as above. During this process the sections were examined periodically under a microscope, and grinding and polishing continued until lines were evident in the thin section. Final thicknesses ranged from 0.1 to 0.7 mm depending on the shell. To see if additional thinning improved the visibility of the growth lines after the lines were first apparent, some sections were aged and the section thinned and examined again, repeating until the section was essentially ground away.

Mounted sections were examined with a SZX16 Olympus microscope under transmitted light. Growth lines in the section were counted, and these were used as the assumed age of the specimens.

For documentation, photographs were taken using either an Olympus DP-70 camera mounted on the SZX16 microscope using Image-Pro Plus software (Media Cybernetics Inc.), or a Nikon DS-Fi1 camera mounted on an Olympus SZX18 microscope (Nikon Inc.). Images were mosaicked and annotated using Adobe Photoshop CS5 and Adobe Illustrator CS5 (Adobe Systems Inc.).

3.4. GROWTH CURVE

The length at age data was fit to a von Bertalanffy growth curve:

$$L_t = L_\infty (1 - e^{-k(t - to)}) \quad (1)$$

where L_t is the length at age t; L_∞ is the asymptotic length; k is the growth coefficient; and t_o is the theoretical age at zero length. The curve was fit by non-linear least squares using the nls routine in the R statistical computation and graphics system (R Core Team 2018).

3.5. CROSS VALIDATION

Once the sample of shells had been aged they were set aside for a week and then aged again by the same reader. A bias plot was produced and the coefficient of variation was calculated for the two readings. This type of plot displays one readings assigned ages against another reading in reference to an equivalence line where the two readings have assigned the same ages. Specifically, for all animals with a given age, the mean age and 95% confidence intervals of the ages assigned by the second reading are plotted against the original age reading. Precision estimates were calculated by using the Coefficient of Variation (CV) as described by Chang (1982) and Morales-Nin and Panfili (2002):

$$CV_j = 100 * \frac{\sqrt{\sum_{i=1}^{R} \frac{\left(X_{ij} - X_j\right)^2}{R-1}}}{X_j} \quad (2)$$

where X_{ij} is the age estimate of the ith clam with consensus age j, X_j is the consensus age j, and R is the number of clams of consensus age j. CV is then averaged across clams to produce a mean. CV is more flexible and statistically more robust than other measures of precision, such as percent agreement or Average Percent Error (Kimura and Lyons, 1991).

3.6. ADDITIONAL PROCESSING TO INCREASE GROWTH LINE VISIBILITY

A limited sample of thin sections were used to see if two techniques provided better discrimination of the growth lines than thin sections. The two techniques were acetate peels and etching and staining. In both techniques, a polished shell section is

prepared and then the shell is etched by dissolving the calcium carbonate to expose the organic matrix. Acetate peels are made by flooding the etched surface with acetone and applying an acetate sheet to the surface. The acetate melts into the etched surface and after drying is peeled off and examined under a microscope (Ropes 1987, Nolan and Clarke 1993). With staining and etching, the polished shell surface is treated with an etching solution that also contains a stain for the organic matrix. In this case, the etched and stained surface is examined directly under a microscope (Schone *et al*. 2006, McCoy *et al.* 2014). Both of these techniques have been used for long-lived bivalves where the growth lines at older ages are very closely spaced and difficult to discriminate. Usually these techniques are applied to embedded and cut blocks and not thin sections, as the time and resources to prepare both sides of a thin section are not necessary as only one polished surface is required. Applying them to thin sections allows a more direct comparison of the results of the three methods.

A review of papers using acetate peels showed that hydrochloric acid (HCl) was the most common etchant used, with a variation of higher concentrations for short times to low concentrations for longer times. Other etchants were acetic acid, citric acid, and Decal™ (trade name for a histological decalcifying agent that is HCl based).

One mixture used in more recent papers on shell ageing was Mutvei's solution (Schöne *et al*. 2005) which combines acetic acid to etch the shell, glutaraldehyde to preserve the organic matrix and Alcian Blue to stain mucopolysaccharides. Wannamaker *et al.* (2008) used a variation of Mutvei's solution, omitting the alcian blue, and using acetate peels of the etched surface. This procedure is of interest as they were able to successfully age a 510-year-old *Arctica islandica* shell with this technique (Wannamaker *et al.* 2008). The extremely fine lines in the most recent growth of this shell demonstrated the resolution possible with this method.

3.6.1. Etching and Acetate Peels

A sample of the sections was etched and acetate peels produced using the procedure in Wannamaker *et al*. (2008). An initial test was done to determine the immersion time, as there were no papers giving an etching time for this procedure on shells from this family. A solution of 250 ml 1% Acetic acid and 250 ml 25% glutaraldehyde was used to etch two sections each for 5, 10, 15 and 20 minutes. Sections were then rinsed in reverse osmosis water and air dried. Etched sections were examined under the microscope directly, photographed, and then acetate peels of the etched sections were produced. The acetate peels were examined under the microscope to see if growth lines were more apparent in the peels than in the thin sections or the etched sections. Once an etching time was selected an additional eight sections were processed for acetate peels.

3.6.2. Etching and Staining

A literature search showed the stains Alcian Blue (Schone *et al.* 2005), Coomassie Blue (McCoy Loria and Hauato-Soberanis 2014) and Amido Black 10B (Evans and LeMessurier 1972), have been used to bring out growth lines in bivalve shells. Coomassie Blue and Amido Black are nonspecific protein stains, while Alcein Blue

stains mucopolysaccharides. It was decided to try Amido Black 10B as a stain to use with the acetic acid and glutaraldehyde etching solution as it has been referred to as a scleroprotein stain (Evans and LeMessurier 1972). To test the concentration of stain to use, two slides were processed with 250 ml of the etching solution with the addition of 0.5 g of Amido Black 10B, and two slides were processed in 250 ml of a 50% v/v dilution of the etching solution with 1.0 g of Amido Black 10B. Once a solution was selected, an addition six slides were processed and examined to compare the ease of discrimination of stained rings versus those in thin sections, etched sections and acetate peels.

RESULTS

4.1. SAMPLE COLLECTIONS

The numbers of shells and details of the collections are given in Table 1. The length frequencies of available shells and those selected for ageing is shown in Figure 5 and the ageing results in Table 2. Many of the shells that were collected as dead single shells were too damaged to get an accurate length or to be used for ageing.

4.2. LENGTH FREQUENCIES

Length frequencies for all shells for which a shell length could be measured and those that were aged is shown in Figure 5. There were an additional 52 shells that were considered too damaged to accurately measure shell length.

Length frequencies for the two samples that were collected live from a single location and contained more than six clams are shown in Figures 6 and 7. There is some evidence of a mode of small clams less than 10 mm in length in the Nov. 2018 collection from Burntcoat Head. There are also possible modes at 16 and 22 mm, after which the number of shells is too low to suggest separate modes. The September 2007 sample from Sloop Rocks has a central mode around 20 mm shell length, but on either side of that there are only single measurements.

The first growth line is often difficult to locate in bivalves, as it is often weak, very near the hinge and the shell surface, and subject to erosion as the bivalve grows (Ansell 1961, Oshima *et al.* 2004, Prusina *et al.* 2015). Atlantic Mud-piddock in the Minas Basin have been observed to be spawning in late July to early August (Andrew Hebda, personal communication, Jan 21, 2019). This means that the small mode in the November sample is the first winter size. If the growth rings are laid down during the period of slow winter growth, the ratio of total shell length to the hinge-posterior length indicates that the first growth ring in the sections should occur approximately 2-3 mm from the hinge.

4.3. EMBEDDING AND SECTION PREPARATION

Initial sections showed that cuts through the anterior slope provided clear growth lines, but they were too numerous to be considered annual. They appear to be the result of cycles of shell deposition and burrowing activity as described by Evans (1968), and do not appear to be annual (Figure 8). Sections through the anterior-posterior transition area, and the posterior slope, although longer, did not give as much internal detail and

presumed annual growth lines were difficult to distinguish. Sections from the hinge through the posterior end produced the best sections for ageing, giving what appear to be distinguishable annual lines. Not all thin sections provided good results. Problems encountered during the process were: sections not mounted perfectly flat to the slide, resulting in varied thickness through the section or even one end being ground off; sections being over-ground and ending up too thin to provide good readings; sections chipping or fracturing during grinding/polishing, obscuring detail; and as the section was ground to 0.1 mm or less “plucking” would be seen where small pieces of the shell were removed from the section by the grinding and polishing process.

There did not appear to be an overall optimum thickness for the thin sections, as there was a large variation between shells for the thickness where growth lines were apparent. Sections that were thinned further after growth lines were evident tended to initially improve, but then degrade rapidly with further thinning. With the variation between shells, gradual thinning and polishing with frequent examination under the microscope produced the best results.

4.4. GROWTH CURVE

The ages determined and the fit of a von Bertalanffy growth curve are shown in Figure 9 along with the parameters for the fit. The von Bertalanffy curve is a better fit than a linear model (Likelihood ratio test $p = 0.002$, AIC = 294.45 versus 301.77), indicating that growth is asymptotic. The shell lengths however, are all below the asymptotic size, indicating that growth is still relatively rapid for the oldest Mud-piddock examined. The largest Mud-piddock in the sample was 45 mm while the largest reported in the literature was a 54 mm shell from Argentina (Fiori *et al.* 2012), so a larger aged sample might produce older clams and show more of a reduction in growth around the asymptotic size.

4.5. CROSS VALIDATION

The cross validation results are shown in Figure 10. There did appear to trend of assigning older ages to the younger Mud-piddock and under ageing the oldest clams when comparing the first reading to the second by the same reader. All age groups contain few Mud-piddock, so a larger sample size would be preferable before drawing definite conclusions. The resulting CV of 7.84% is close to the 7.6% reported as the median CV for 117 papers reporting precision values by Campana (2001). It is higher than the 5% cited as a target reported by some laboratories for organism of moderate longevity and reading complexity in the same paper. It is probable that training and a reference set would reduce the CV, but there will never be studies ageing a large number of shells from this population while it retains its Threatened status.

4.6. ADDITIONAL PROCESSING TO INCREASE GROWTH LINE VISIBILITY

4.6.1. Etching and Acetate Peels

The slides etched for five minutes did not look different than non-etched sections and the peels did not appear to have better resolution than the thin sections. At ten minutes there was visible etching but the detail was not increased. There was some

additional detail in the peels over the thin section, but the growth lines were not more apparent. The slides etched for fifteen minutes had a significant increase in the detail visible, and the peels showed more fine detail than the thin sections. Growth lines were more apparent in the peels than in the thin sections (Figure 11). The sections etched for twenty minutes were etched very deeply and the remaining section was so far below the surface of the resin that it was difficult to see details along the edge of the section. The peels had many bubbles where the acetate did not melt down into the etched section. This is a result of the thin Barnea shell producing a very narrow groove in the etched section, and would be less of a problem for species with a thicker shell. Two thin sections that had been ground very thin were lifted completely off the slide by the process, the sections have to be thick enough to resist being torn off the slide. This means that for many shells it would not be possible to make acetate peels from thin sections once they are thin enough to see the growth lines, but usually both methods are not being done on the same shells.

4.6.2. Etching and Staining

For the two test slides processed with the Amido Black 10B etching/staining solution for 15 minutes the lines were more apparent but there was not a lot of staining. The slides processed with the diluted etchant and increased stain showed clear lines in the shell that were much more apparent than they had been in thin section (Figure 12). This method produced sections with the clearest growth lines of the methods tried in this study.

DISCUSSION

This study demonstrates that Atlantic Mud-piddock can be aged using internal lines in the shell. It is cautioned that although these lines have been used as annual markers on other bivalves (Ropes 1985, Pinn *et al.* 2005, Wannamaker *et al.* 2008, Mirzaei *et al.* 2014) and have been verified as such (Neves and Moyer 1988, Kilada *et al.* 2007, 2009, Vadopalas *et al.* 2011), they have not yet been verified for Atlantic Mud-piddock. Verification of the annual structures used is an important part of ageing studies and is sometimes overlooked (Campana 2001). Verification using the Minas Basin population would be difficult due to its SARA status, but could be done using another, more abundant, population.

One of the issues that comes up in ageing studies is the determination of the first ring. Since it is deposited at a small size and the area near the hinge is often eroded through time, it is sometimes difficult to discriminate the first ring. The length frequency plots (Figure 6) showed a mode of small Mud-piddock near 6 mm for the November collection from Burntcoat Head. The question then is if this mode represents the current years recruits and their growth from a late July-early August spawning.

Wei *et al.* (1997) found *Barnea davidi* grew to 7.6 mm from June to August in the laboratory, while Evans (1968) found that *Penitella penita* growing on the Oregon coast grew to over 8 mm from August to April. *Pholas orientalis*, a large Pholadidae, was found to grow to 9.9 mm in 4 months (Ng *et al.* 2009). Comparison to these closely related species shows that it is reasonable that Atlantic Mud-piddock grows to 6 mm by November of its first year of life. If the first ring is deposited during the slow

winter growth period it would be expected to be deposited 2-3 mm from the hinge. That corresponds to the first ring identified in the sections examined in this study. It is noted that if heavily eroded shells are aged the first ring may be missing, as it will be deposited close to the surface. This can be taken into account when assigning ages to identified rings as juvenile growth is fairly rapid and size has more than doubled by the time the second ring is deposited.

The ages produced in this study are shown to be fairly precise, with a CV of 7.8%, and their accuracy for the first few ages is supported by the length frequencies. The precision of the readings would probably be improved with practice and the use of reference sets. The sections from this study can form the start of a reference set for the Minas Basin population.

The ageing and growth curve indicate that *Barnea truncata* from this population has a life span of around 11 years, and that growth is fairly continuous over this period. This is based on limited sampling and may change if there is an increased sample size. It may be worthwhile to conduct more extensive sampling from another population that does not have a threatened status. Growth at the oldest ages observed was still rapid, but the largest Barnea sampled in this study are still well below the 54 mm maximum length recorded for the species (Fiori *et al.* 2012), and if there are older Barnea in the population, growth may be shown to reach an asymptote.

LIMITATIONS

This study is based on small sample sizes both for the ageing and for the testing of different cuts and methods. This was a necessity due to this populations Threated status. *Barnea truncata* is more common in other areas, and future studies could use samples from other populations to determine protocols and for comparison of basic life history parameters. Life history parameters that require live collected samples will probably always be based on small samples for the Minas Basin population. 7. CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

This study shows that *Barnea truncata* can be aged using thin sections, but etching and staining is recommended to increase ease in interpretation of growth lines. The growth curve fit to the Minas Basin population is the first recorded for this species. The Minas Basin population is shown to have a lifespan of approximately 11 years and shows non-asymptotic growth using a limited sample size. The first ring is deposited at around 6 mm shell length.

7.2. RECOMMENDATIONS

7.2.1.Readability and Staining

This study has shown that staining increases readability and should be incorporated into future Atlantic Mud-piddock ageing studies. There should be further experimentation on the staining protocols, however, as the methods in this study are initial. Removing the periostacum before processing, probably with a bleach solution,

may further increase readability, as the periostacum tends to roll down over the etched sections, obscuring the edge. The Amido Black stain is stable for at least six months, but it may fade during longer storage. It should be possible to restain sections as the protein matrix should be preserved.

7.2.2.Hinge-anterior Measurement Analysis

A hinge-anterior measurement analysis could be developed to accompany this ageing study. This type of analysis could provide an estimate of undamaged shell length based on dead eroded shells and may prove useful if ageing these types of shells is deemed to be better logistically than harvesting live organisms. Hinge-anterior measurement analysis was started initially in this study when the only samples available were single dead eroded shells, but discontinued when live samples became available.

7.2.3.Ageing Validation Study

The ageing methods described in this study and that may be developed in the future for Atlantic Mud-piddock should only be used for population analysis if an ageing validation study takes place. This type of study would verify that the ages assigned to each individual correspond to the development of their “growth rings.” One approach tracks settlement and growth in man-made or freshly exposed substrate in the Minas Basin to provide known age samples. This approach could also gather independent growth rate data, and data on the mortality rate and age or size at maturity. Another approach is to use similar methods but with another, more abundant population of Atlantic Mud-piddock that does not have a threatened status and can be sampled more extensively.

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TABLES

Table 1. Details of Atlantic Mud-piddock samples used in this study, including number of Mud-piddock shells or individuals (n), status, location gathered, date collected, and provenance.

Sample	n	Status	Location	Date	Provenance
1	31	Dead (shell)	Burntcoat Head	August 2006	Nova Scotia Museum
2	27	Dead (shell)	Port Williams	Fall 2017	Fisheries and Oceans Canada
3	9	Dead (shell)	Sloop Rocks	May 4, 2008	Andrew Hebda, private collection
4	1	Live (whole)	Spencer Point	Feb. 2, 2018	Nova Scotia Museum
5	1	Live (whole)	Starr's Point	Feb. 6, 2018	Nova Scotia Museum
6	6	Live (whole)	Sloop Rocks	Feb. 6, 2018	Nova Scotia Museum
7	29	Live (whole)	Sloop Rocks	Sept. 16, 2007	Nova Scotia Museum
8	34	Live (whole)	Burntcoat Head	Nov. 9, 2018	Fisheries and Oceans Canada, SARA Permit MAR-2018-15a
9	18	Dead (shell)	Burntcoat Head	Nov. 9, 2018	Fisheries and Oceans Canada, SARA Permit MAR-2018-15a
Total	156				

Table 2. Details of Atlantic Mud-piddock shells and resulting age readings. Sample numbers correspond to Table 1.

Sample	Shell Catalog No.	Shell Length (mm)	Section Thickness (mm)	Age 1	Age 2
1	1	34.5	0.1	7	7
	2	45.0	0.4	9	9
	3	35.7	0.5	9	9
	4	37.2	0.6	7	8
	5	36.8	0.7	6	5
	9	34.3	0.5	9	8
	10	31.8	0.7	8	7
	11	29.9	0.4	6	7
	12	30.6	0.4	6	7
	14	27.7	0.2	4	5
	23	19.1	0.1	2	3
	26	25.8	0.1	5	6
2	2	44.7	0.2	11	10
3	1	34.5	0.2	5	5
	2	35.2	0.5	5	4
	6	35.3	0.7	5	6
	9	41.1	0.7	11	9
7	33402	28.0	0.2	5	6
	33406	23.5	0.1	4	5
	33407	22.0	0.1	3	3
	33408	15.3	0.2	2	3
	33409	15.8	0.3	3	3
	33410	25.4	0.1	7	6
	33412	24.9	0.2	3	3
	33414	21.4	0.5	3	3
	33415	29.4	0.3	7	6
	33420	21.3	0.3	4	4
	33422	20.0	0.3	3	4
	33425	22.0	0.2	3	4
	33426	18.9	0.2	3	4
	33427	17.7	0.3	3	4
	33433	17.6	0.2	3	3
	33435	19.4	0.4	4	4
	33436	14.6	0.5	3	3
	33437	11.7	0.3	2	2
	33438	10.0	0.4	2	2
	33438b	10.0	0.3	2	2

Sample	Shell Catalog No.	Shell Length (mm)	Section Thickness (mm)	Age 1	Age 2
8	36322	27.1	0.5	7	6
	36324	24.4	0.4	5	5
	36326	27.2	0.3	7	8
	36327	29.5	0.5	7	6
	36329	16.3	0.3	3	3
	36330	21.0	0.4	4	4
	36343	21.8	0.3	5	5
	36346	23.2	0.2	5	6
	36347	22.6	0.3	6	6
	14	6.1	0.15	1	1
	15	8.1	0.2	1	1
	16	6.3	0.2	1	1
	21	5.6	0.3	1	1
9	4	11.6	0.1	2	3
	20	30.5	0.3	6	6

FIGURES

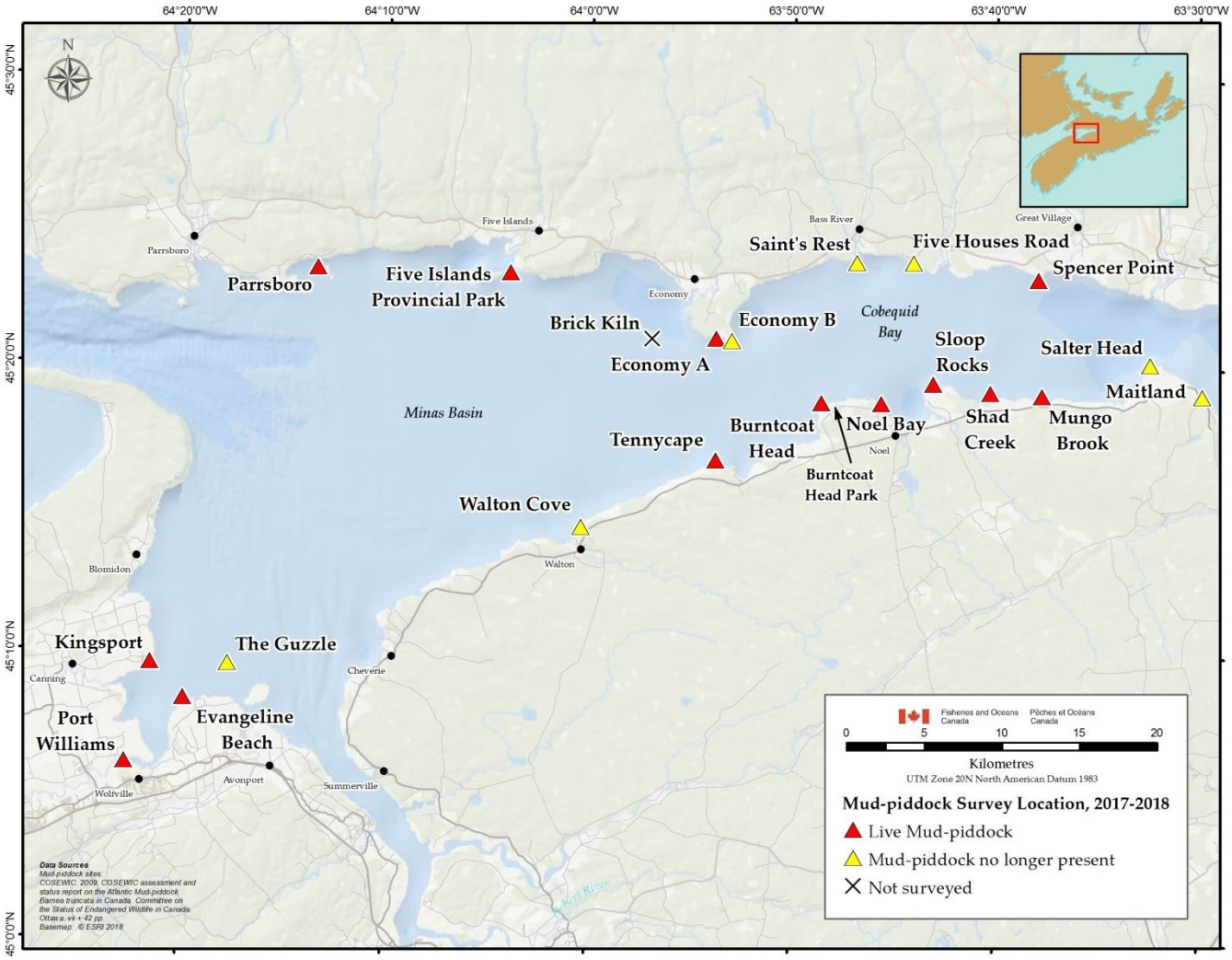

Figure 1. Extant and extirpated sites of Atlantic Mud-piddock habitat on the Minas Basin.

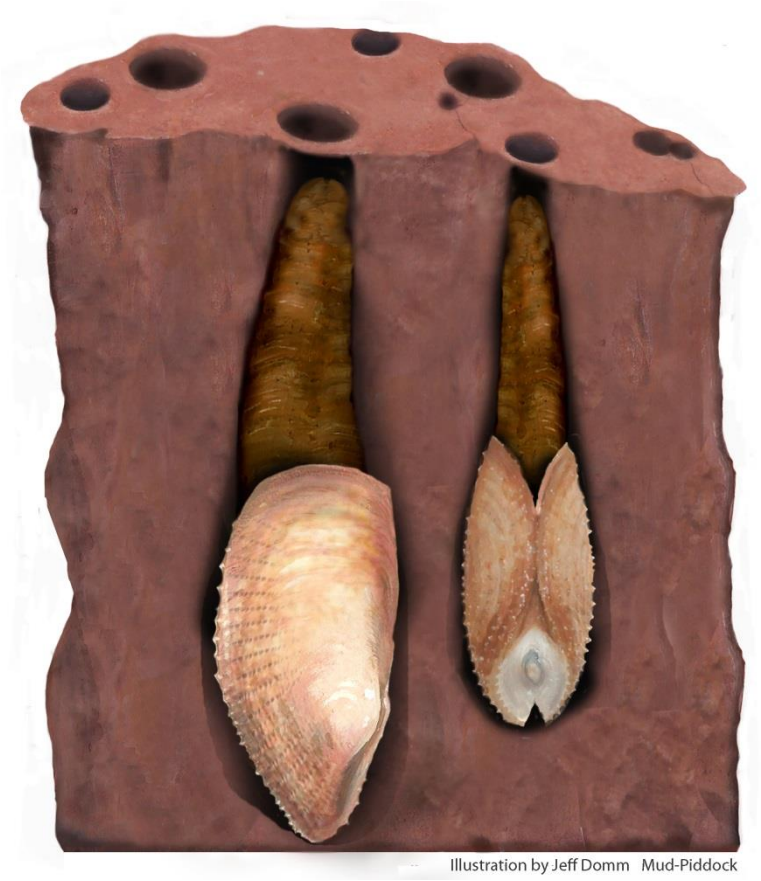

Figure 2. Atlantic Mud-piddock in-situ in conical burrows in red-mudstone substrate.

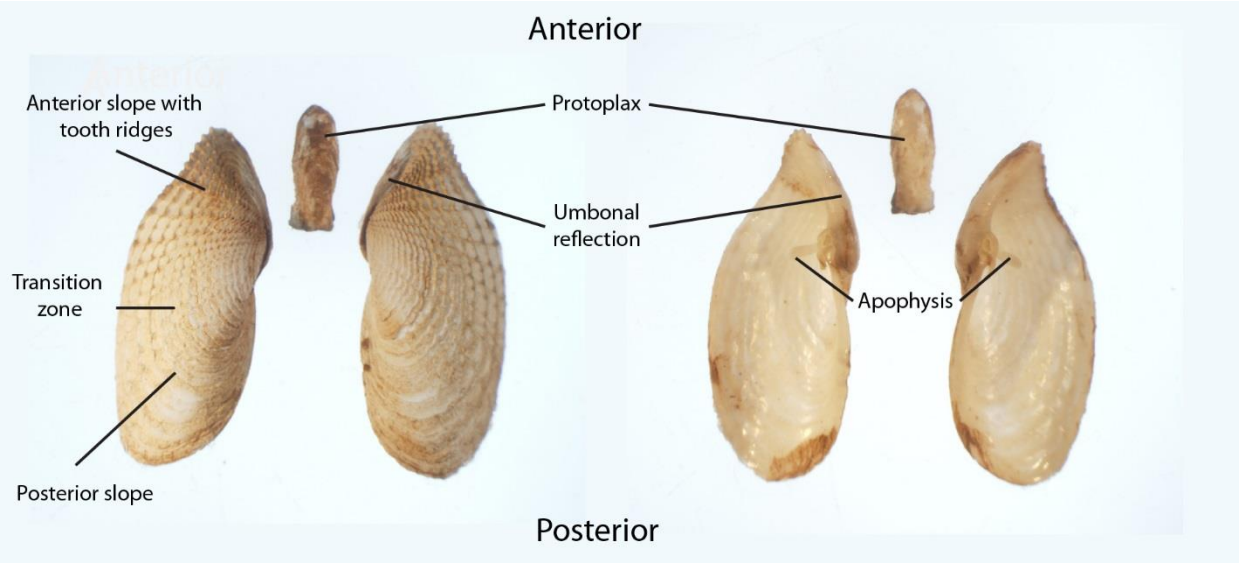

Figure 3. Exterior and interior views of an Atlantic Mud-piddock shell.

Figure 4. Shell embedded in resin block ready for sectioning.

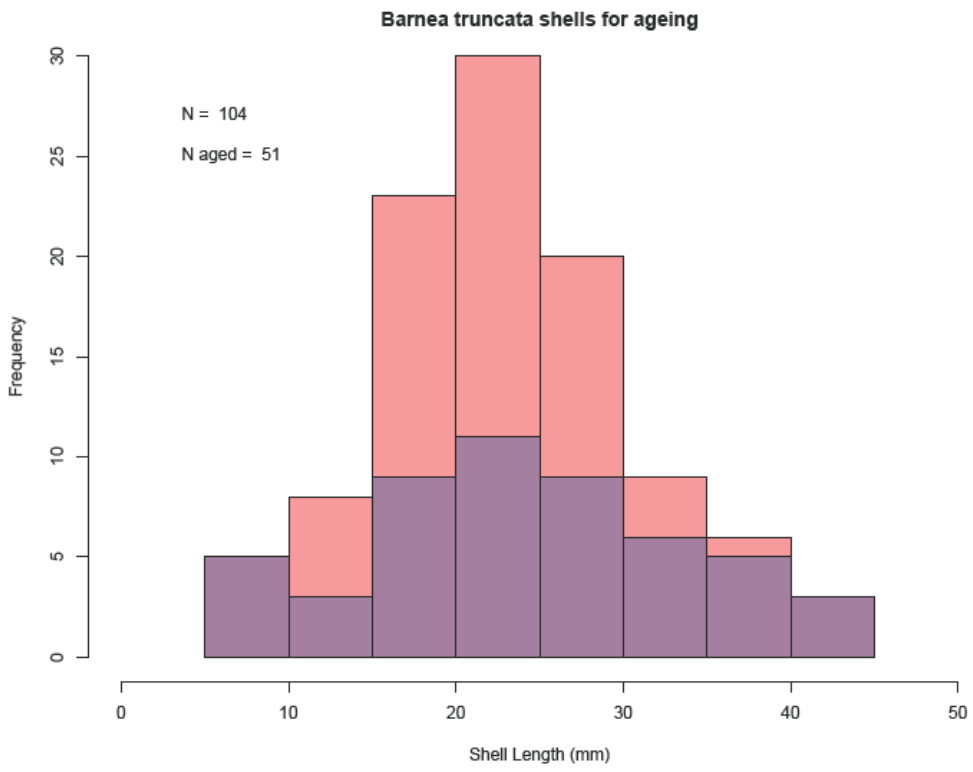

Figure 5. Length frequency of all Barnea truncata shells available from the various collections (purple and pink) and those selected for ageing (purple). Note that shells that were broken or too eroded to obtain a reasonable length estimate are not shown.

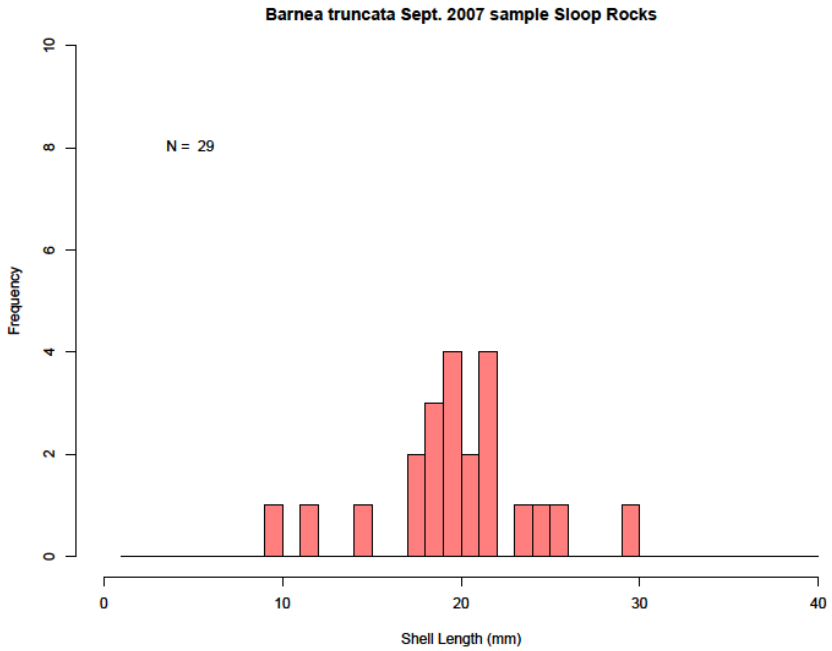

Figure 6. Shell length frequency for live Barnea truncata from Sloop Rocks in Sept. 2007.

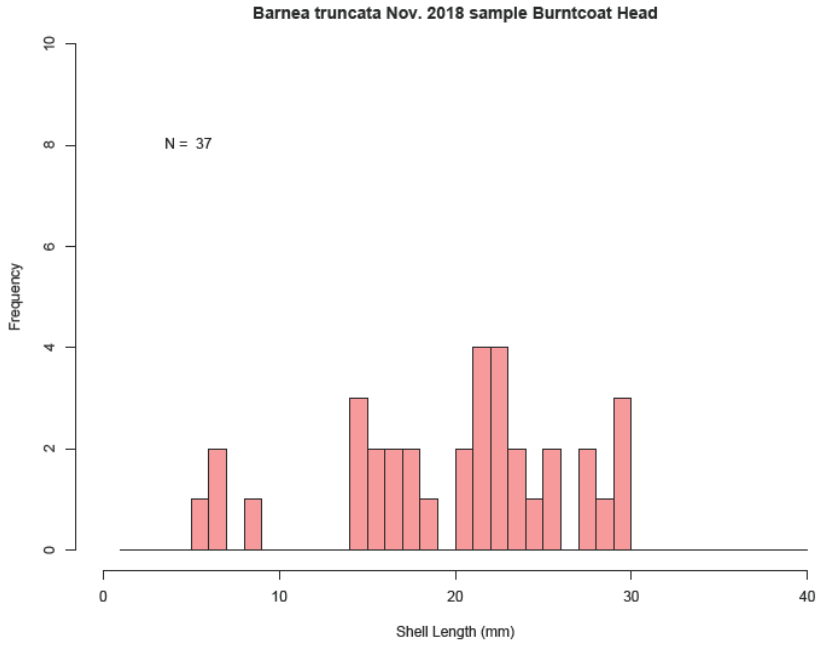

Figure 7. Shell length frequency histogram of live Barnea truncata from Burntcoat Head in Nov. 2018.

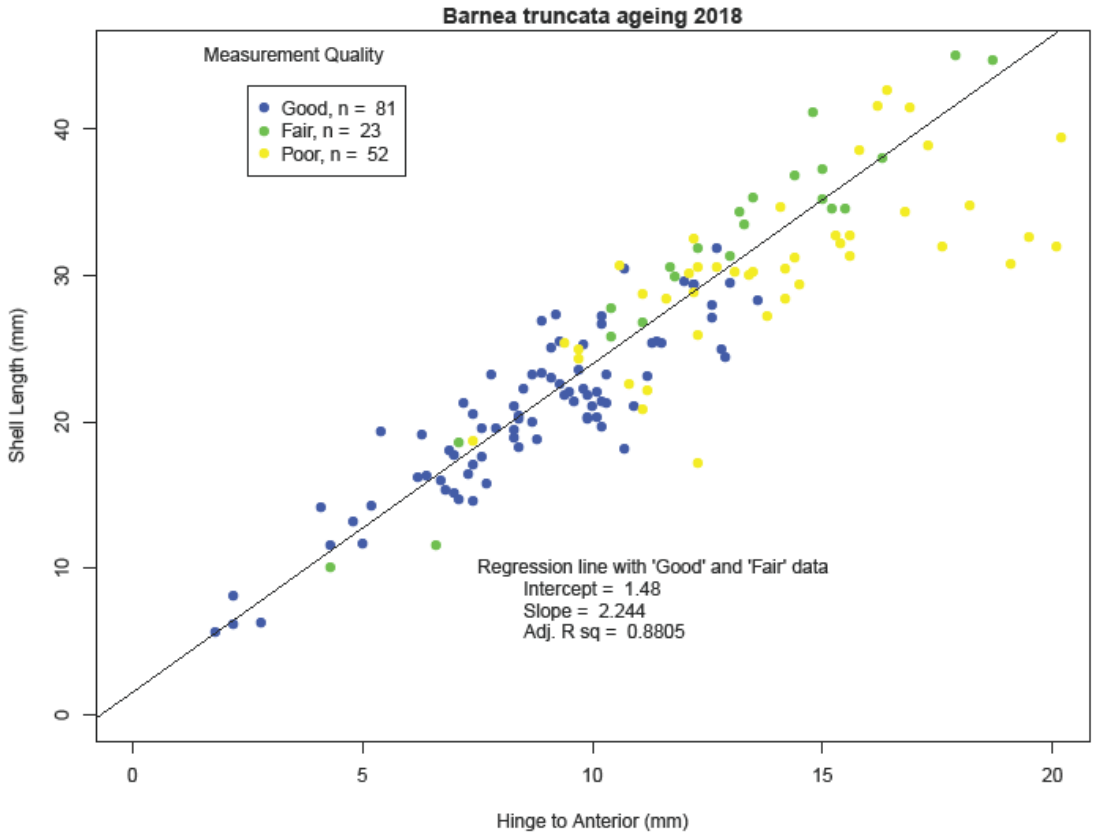

Figure 8. Regression of shell length on hinge to anterior end measurement. Regression line and results are for shells that were classed as having a good or fair length measurement. Shells classed as poor had shells that were too chipped, broken or eroded to obtain a reasonable length estimate.

Figure 9. Shell section cut from hinge down anterior slope showing internal lines associated with tooth ridges, presumably formed by alternating periods of active burrowing and shell growth.

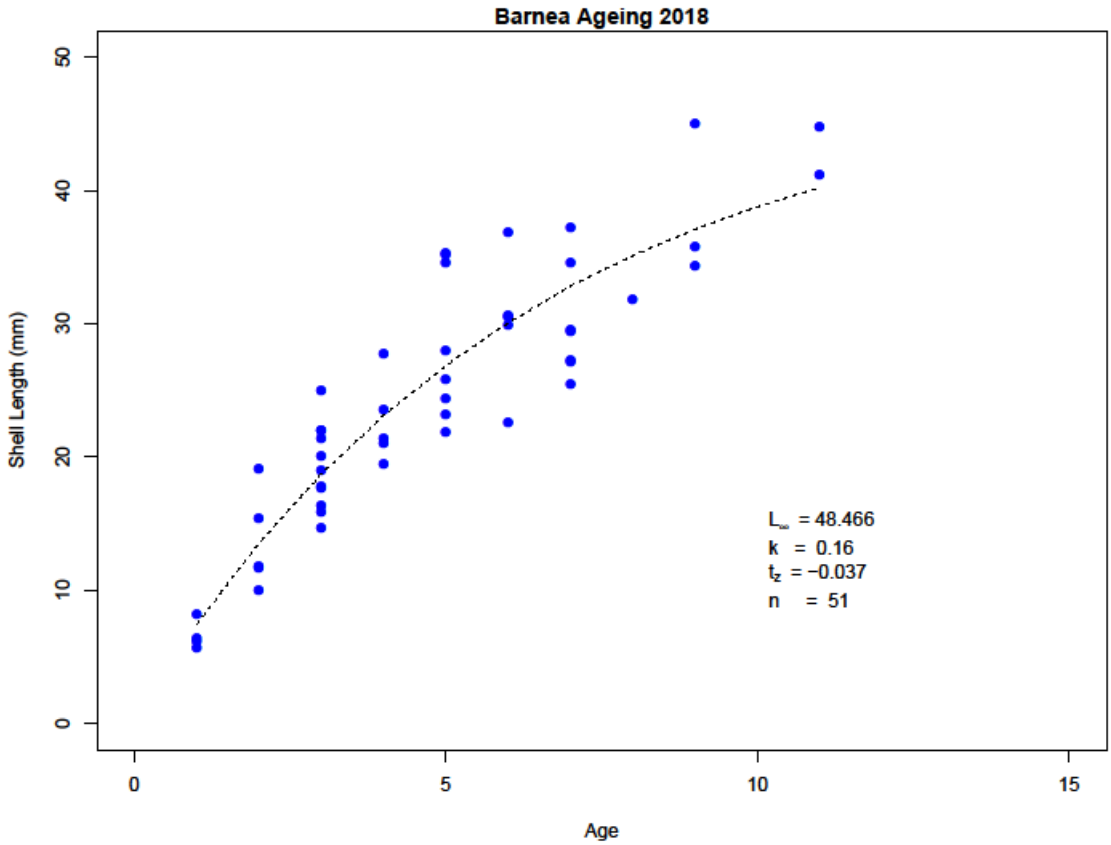

Figure 10. Growth curve from aged shell sections. Curve is a von Bertalanffy curve fit to the data using the non-linear least squares routine in the R statistical package. Fitted parameters are shown.

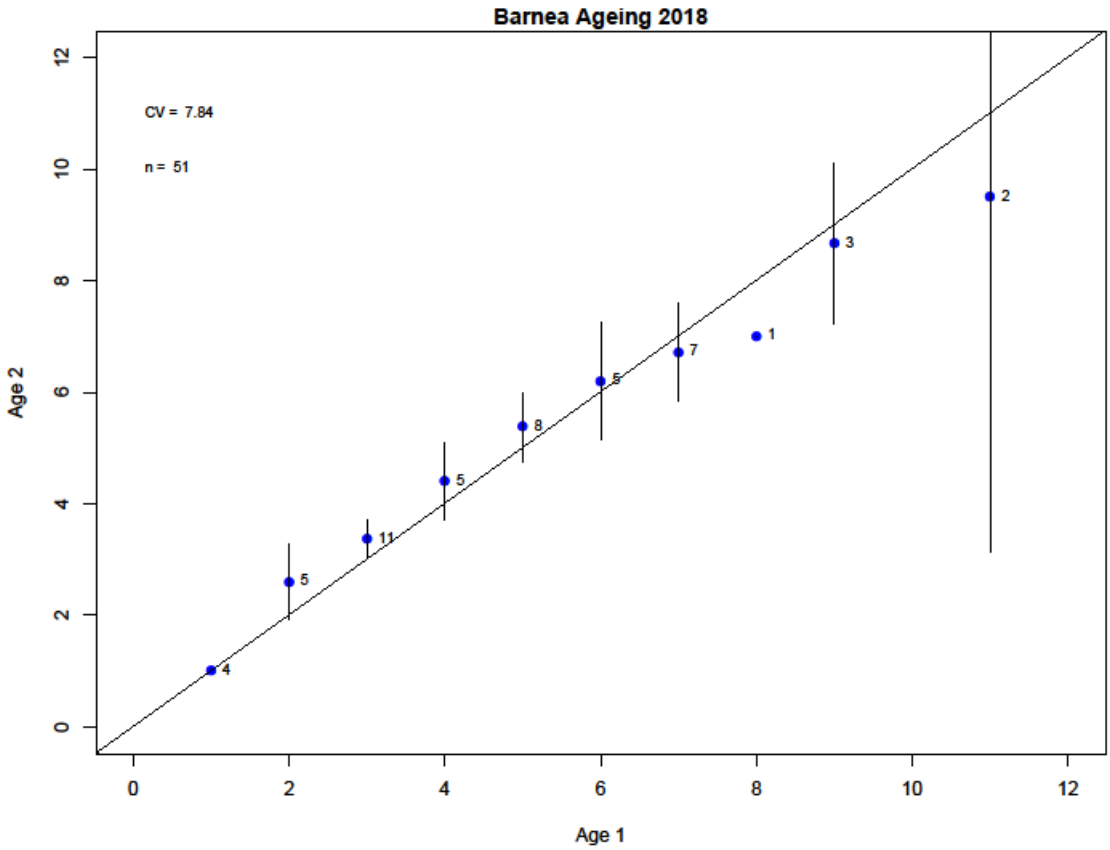

Figure 11. Cross validation of two separate readings of presumed annual lines in thin sections of Barnea truncata shells. Dots and vertical bars show mean and 95% confidence interval of ages assigned by the reader the second time for clams of each age from the first reading. Numbers are the number of clams of that age in the sample from the first reading. The Coefficient of Variation for the two readings is shown in the upper left.

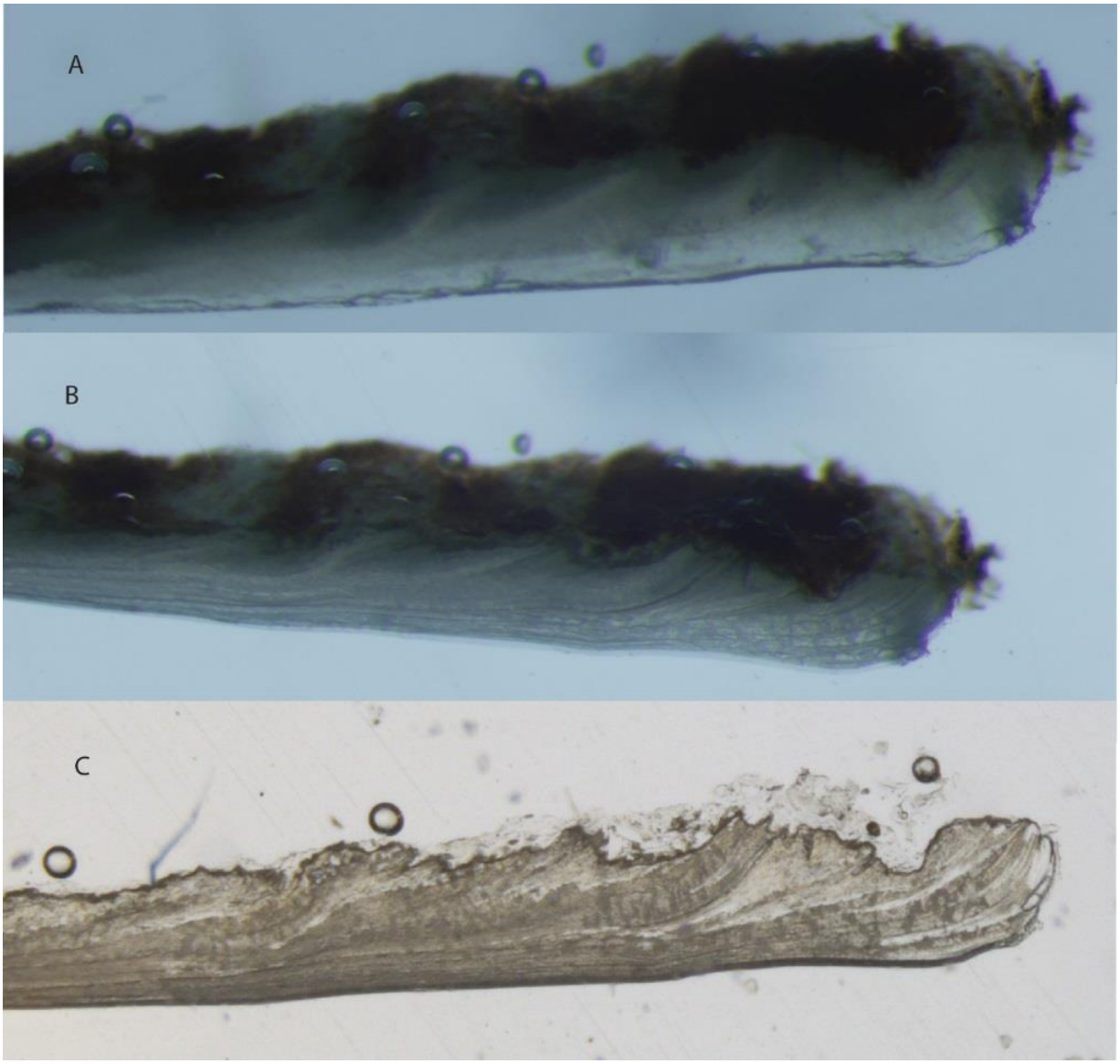

Figure 12. Thin section (A) after etching (B) and acetate peel (C) from the tip of a shell section.

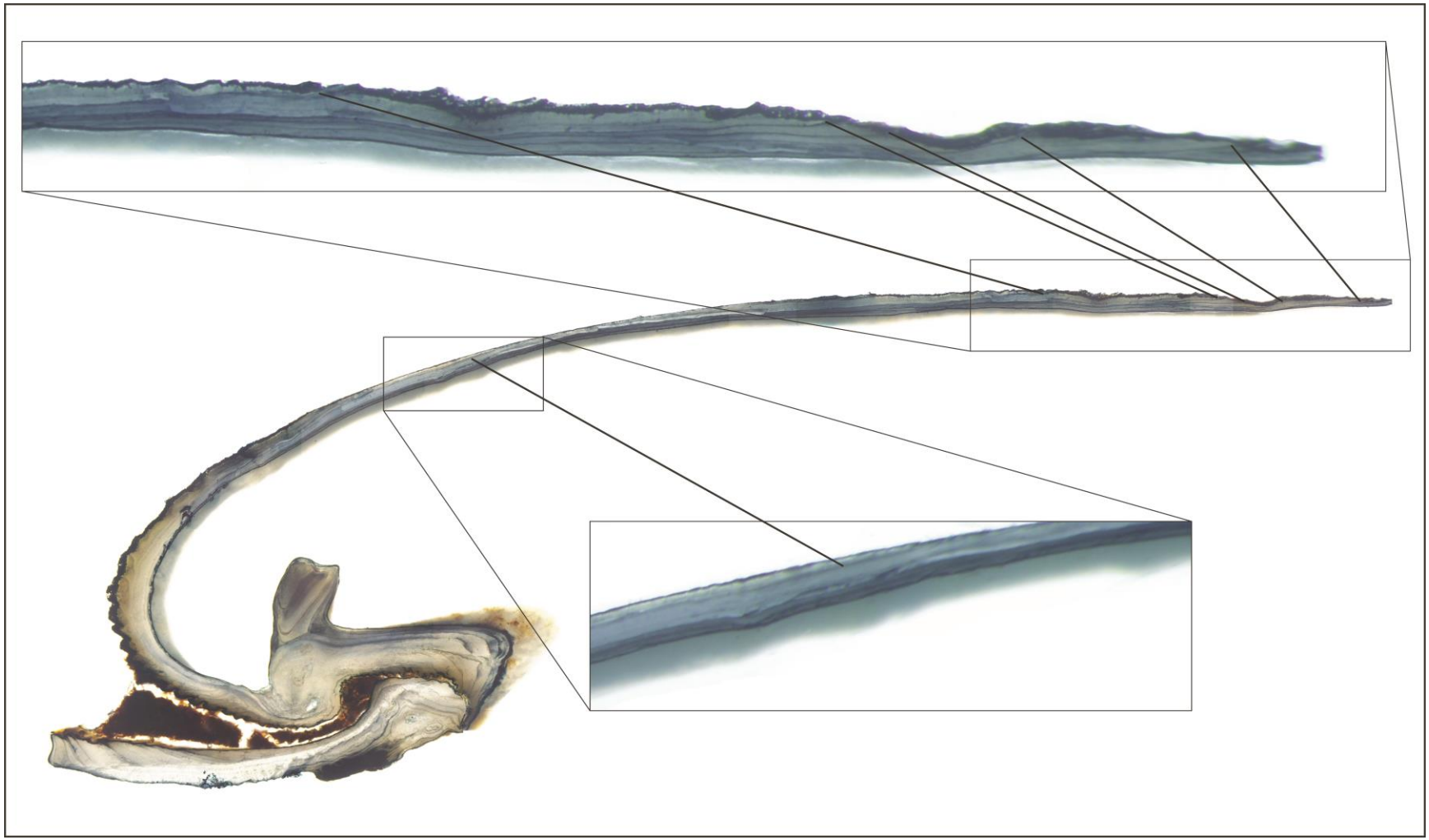

Figure 13. Mosaic of etched and stained shell section showing “annual” growth lines in the shell.