Ministry for Primary Industries
Manatū Ahu Matua

Paua ageing using protein layers in the shell

New Zealand Fisheries Assessment Report 2015/63

R. Naylor
H. Neil

ISSN 1179-5352 (online)
ISBN 978-1-77665-082-8 (online)

October 2015

New Zealand Government

Growing and Protecting New Zealand

Requests for further copies should be directed to:

Publications Logistics Officer
Ministry for Primary Industries
PO Box 2526
WELLINGTON 6140

Email: brand@mpi.govt.nz
Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries websites at:
http://www.mpi.govt.nz/news-resources/publications.aspx
http://fs.fish.govt.nz go to Document library/Research reports

Contents

EXECUTIVE SUMMARY

Naylor, R.; Neil, H. (2015) Paua ageing using protein layers in the shell.
New Zealand Fisheries Assessment Report 2015/63. 36 p.

This project aimed to determine whether protein layers in the shells of paua could reliably be used to estimate age in paua. The number of these rings in the shells of abalone has been used to estimated age in many overseas species; however, the literature is variable and conflicting.

Oxygen isotope ratios were used to estimate the number of seasonal temperature cycles along the growing axis of the shell. The basis of this method is that variations in water temperature lead to a measurable change in the $^{18}O/^{16}O$ ratio of shell material (expressed as $\delta^{18}O$), and this relationship is believed to be approximately linear between about 5 and 30^{o}C. These shells were then sectioned, and the number of protein layers within vertical sections of the shell were counted and compared with the number of temperatures cycles estimated by oxygen isotope analyses. The shells examined came from three sites in PAU 5A and three sites in PAU 3.

There appeared to be a relationship between the number of protein layers in the shell and the age indicated by stable oxygen analyses in many of the shells examined; however, there was no evidence that the protein layers were laid down on an annual basis. A major problem is that the protein layers do not appear to be laid down in shells at the same time of year at the same location. If these layers were always laid down at a similar time of year at sites, it may provide some evidence of seasonal or annual deposition. The Shellfish Working Group has suggested that regular monitoring of a local population for a period extending beyond a year may provide evidence about the seasonality of deposition of the protein layer.

OVERALL OBJECTIVES:

1. Determine relationship between age of paua and growth rings on the shell.

SPECIFIC OBJECTIVES:

1. Develop a methodology to age paua using growth checks on the shell.

2. Determine the most appropriate method(s) to validate the growth check methodology as a reliable estimate of age.

3. Collect and age paua from a number of locations using the techniques determined in objectives 1 & 2.

1 METHODS

The proposed methodology for this project was presented to the Shellfish Working Group on the 18th of October 2011. The Working Group was generally comfortable with the proposed methods, but proposed some variation to the methodology outlined in the final tender document.
Specifically, they thought that it was unnecessary to attempt to develop protocols for the estimation of decimal age from shells. This was because the assessment model does not require age estimates at this scale. They also thought that the calculation of a gonad index was not informative, as the time of spawning varies from year to year.

OBJECTIVE 1:
Develop a methodology to age paua using growth checks on the shell.

METHODS
It was proposed that the method for ageing would be similar to that developed by Muñoz-Lopez (1976, cited in Prince et al. 1988) and subsequently adopted by Prince et al. (1988), McShane & Smith (1992), and Shepherd et al. (1995a) to age abalone. Using this method the spire of each shell is ground with abrasive paper until a small hole is visible in the spire. This results in the exposure of concentric rings of nacre surrounding the hole. This method provides a horizontal view of the growth checks. The tender document suggested, however, that the method chosen would be guided by the outcome of a literature review of abalone ageing techniques which was being compiled at the time. The two main methods which have been used to age abalone are the method described above and longitudinal cross sections of the shell (Sinclair 1963, Murray 1986). This method provides a vertical view of the growth checks. In theory, if either method is used on the same part of the shell (i.e., through the spire of the shell) the result should be the same because the growth checks are merely being viewed from different aspects.

It was proposed that 10 shells would be sectioned using both methods to determine whether similar numbers of growth checks are visible using each method. Five of the10 counts were the same for both methods. Vertical counts were greater than horizontal counts in three shells, and horizontal counts were greater than vertical counts twice. It was suggested by the Working Group that NIWA examine a further 30 shells using both methods to determine which to use for isotope validation work. A further 30 shells were subsequently sectioned using both methods. The report on this work was posted on the Ministry's Science Working Group's site as horizontal or vertical assmt of growth rings paua.pdf. Of the 30 shells examined 20 counts were the same, but counts were higher in vertical sections than in horizontal sections on 10 occasions. Differences were caused either by a partial check not being counted in the horizontal section, but being very apparent in vertical cross section, the horizontal section not being ground down enough to reveal all growth checks on this plane, or by fungal infection in the spire obscuring or removing checks on this plane. The Ministry and the Shellfish Working Group agreed that vertical sections only should be examined in the 24 shells for which oxygen isotope analyses were subsequently carried out.

OBJECTIVE 2:
Determine the most appropriate method(s) to validate the growth check methodology as a reliable estimate of age.

Following the presentation of a literature review of abalone ageing techniques (Naylor 2011a) the Shellfish Working Group agreed that growth checks under the spire of the shell should be counted and that stable oxygen isotope analyses should be done on the shells to determine whether these growth checks are annual or otherwise.

OBJECTIVE 3:
Collect and age paua from a number of locations using the techniques determined in objectives 1 & 2.

METHODS
Four shells were collected from each of three sites in PAU 5A and three sites in PAU 3 (Figures 1, 2, and 3, Table 1). Archived shells were sampled from PAU 5A because weather had delayed the collection of fresh shells from PAU 5. Shells were analysed for oxygen isotope ratios to estimate the number of temperature cycles along the growing axis of the shell, then later sectioned to reveal the number of protein layers within vertical sections of the shell. The numbers of both were compared.

Table 1: Dates of shell capture for sites in PAU 5A and PAU 3.

Fishstock	Site	Date of capture
PAU 5A	George	25-3-2006
	South coast	6-12-2005
	Chalky	7-12-2005
PAU 3	Akaroa (Squally Bay)	8-2-2012
	Motunau	15-3-2012
	Okiwi Bay	26-4-2012

OXYGEN ISOTOPE ANALYSES
The use of stable oxygen isotopes to estimate growth was developed in the 1950s when it was found that the proportion of different isotopes of oxygen present in shell carbonate reflected the ambient water temperature in which the shell was deposited (Epstein et al. 1951; Urey et al. 1951). Variations in water temperature lead to a measurable change in the $^{18}O/^{16}O$ ratio of shell material (expressed as $\delta^{18}O$), and this relationship is believed to be approximately linear between about 5 and 30°C (Epstein et al., 1951, 1953). The equation reflecting this relationship was determined primarily from the $^{18}O/^{16}O$ ratios at the growing edge and preceding growth layers of the black abalone *H. cracherodii*, grown in temperature-controlled aquaria, or sampled from areas where the seasonal temperature variation was known (Epstein et al. 1953). The basis of the relationship is the differential kinetic and vibrational energies of ^{18}O and ^{16}O, where in cold water, the crystallisation kinetics of shell formation favour the precipitation of the heavier ^{18}O isotope. At elevated temperatures, this effect is less pronounced, and relatively more of the lighter ^{16}O isotope is precipitated. A shelled organism therefore accumulates a record of combined water temperature variation throughout its growing life.

Epibiota was removed from the surface of the 24 shells with a wire brush, and calcite samples were removed from the outer shell with a scalpel at about 2 mm intervals along the growing axis of the shell. Carbonate samples from shells were analysed for oxygen isotopes using a mass spectrometer. Shell material was reacted with phosphoric acid at 75°C in an automated carbonate reaction device (Kiel III) attached to a Finnigan-MAT 252 mass spectrometer. The evolved CO_2 gas was purified, ionized and fractionated according to atomic weight by means of a powerful magnetic field. Results are in the form of relative abundances of different isotopes compared to a known standard. Values are reported in standard delta (δ) per mil (‰) notation relative to the vPee Dee Belemnite (PDB) international standard (Epstein et al. 1953) where $\delta^{18}O$ has a value of -2.20‰ for NBS19 calcite. External reproducibility is

ensured via calibration to National Bureau of Standards NBS-19 and internal standards. Concurrently run carbonate standards (NBS-19) had an internal precision of measurements of 0.02–0.08‰ for $\delta^{18}O$ and 0.01–0.06‰

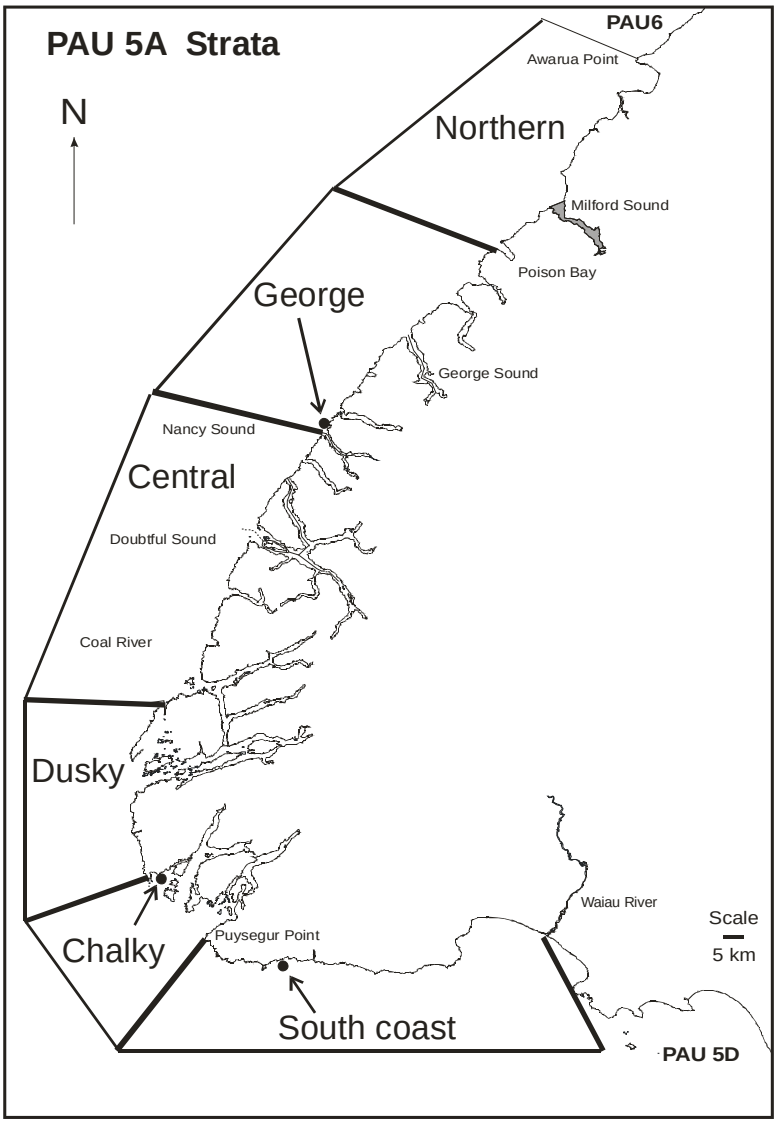


Figure 1: Location of sites that shells were collected from in PAU 5A.

for $\delta^{13}C$, and an external precision (between runs) of 0.03‰ for $\delta^{18}O$ and 0.02‰ for $\delta^{13}C$, relative to vPDB.

The isotopic temperature of paua shell calcite was estimated by solving the carbonate paleotemperature equation of Epstein et al. (1953) in the form:

$$T\,(^{o}\mathrm{C}) = 16.5 - 4.3\,(\delta^{18}O_s - \delta^{18}O_w) + 0.14\,(\delta^{18}O_s - \delta^{18}O_w)^2\,,$$

where $\delta^{18}O_s$ is the oxygen isotopic value of the sample and $\delta^{18}O_w$ is the oxygen isotopic value of the water. The equation of Savin et al. (1985), which relates salinity in surface waters to $\delta^{18}O_w$ according to the relationship $\delta^{18}O_w = (0.687 \times \text{salinity}) - 23.74$, was used to estimate mean seawater isotopic composition.

Because it was not practical to derive salinity estimates from ten separate sites retrospectively, salinity estimates for sites were derived from the literature (Ridgway et al. 1979), or from NIWA's CTD database. The range of salinities between sites was between 34.7 and 35.1 parts per thousand.

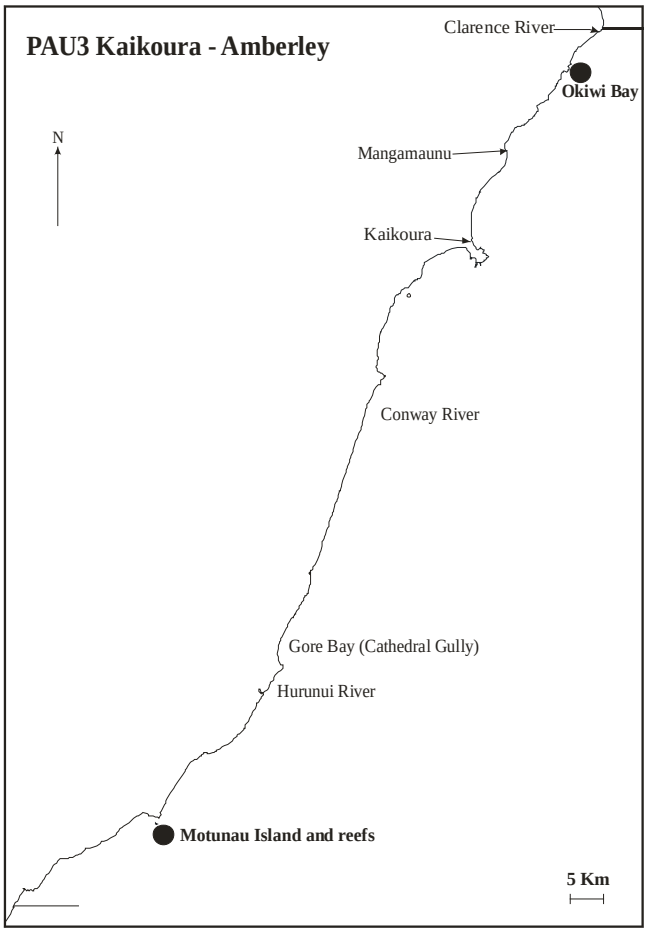


Figure 2: Location of sites that shells were collected from in the northern part of PAU 3.

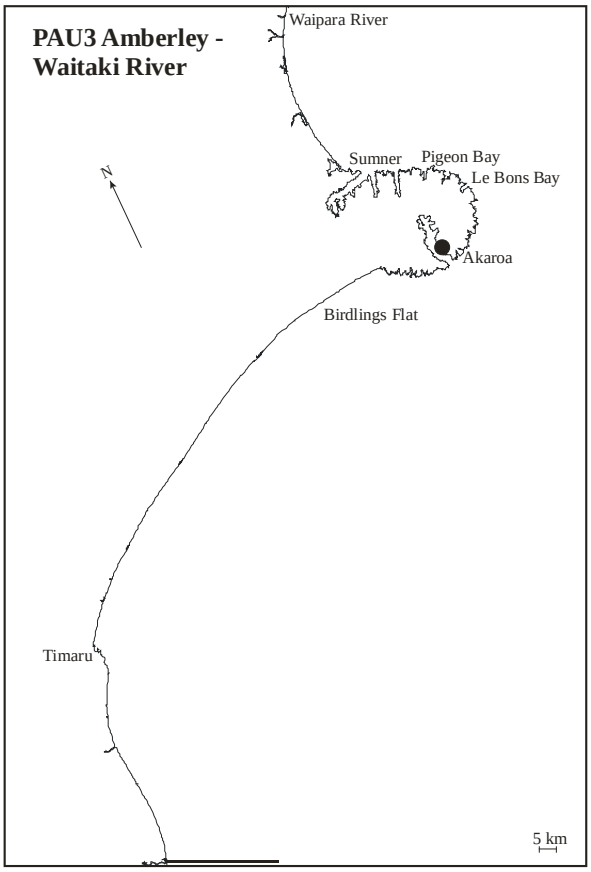


Figure 3: Location of site that shells were collected from in the southern part of PAU 3.

All 24 shells were analysed for oxygen isotopes; a total of 968 samples were analysed in the mass spectrometer. Shell lengths ranged between 98 mm and 152 mm. The shell lengths at each sampling site along the shell were measured and recorded, and the water temperatures at the time of shell precipitation were estimated.

Shells were sampled from the outside edge of the shell and incrementally towards the spire of the shell (Figure 4).

Figure 4: Sample sites along the outside of a shell, marked either side of excavation.

Because the spire is the oldest part of the shell, as well as the highest part, the calcite in that region was often eroded, which prevented sampling. Because paua generally grow between about 20 mm and 30 mm in their first year (Poore 1972, Sainsbury 1982, Naylor et al. 2006) if sampling was not possible within 20 mm to 30 mm of the spire, another isotopic temperature cycle was added to the count of cycles revealed by the isotopes.

GROWTH CHECKS

Shells were then sectioned vertically though the spire (Figure 5) with a cut off wheel attached to a Dremel power tool. This method was chosen after discussion of a literature review of paua ageing methods (Naylor 2011a) by the Shellfish Working Group and after preliminary analyses indicated that horizontal sections frequently underestimated the number of protein layers present.

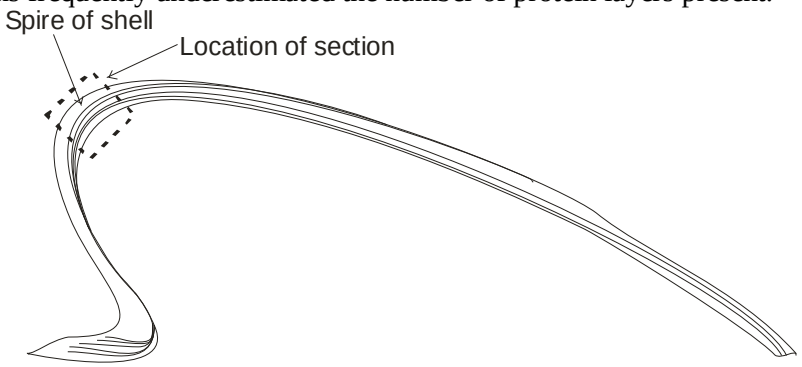


Figure 5: Vertical cross section of the shell showing the location of the spire and the section.

Protein layers were counted within the spire region or immediately anterior of the spire if checks within the spire were convoluted, compressed, or unclear. Protein layers posterior of the spire were frequently compressed.

As well as counting the number of protein layers in the shell, the amount of calcium carbonate deposited since the last protein layer was also measured relative to the thickness of previous calcium carbonate layer. In this way, the number of protein layers may be expressed as layers plus a proportion of a layer.

When the results of this project were presented to the Shellfish Working Group on the 23rd of October 2012, the Working Group noted that the number of protein layers in the shells examined ranged between 4 and 9, and that no very large or very small shells had been examined. A further 10 smaller shells and four larger shells were subsequently sectioned. These shells were from Wellington, the Fiordland south coast, Dusky Sound, and Port Pegasus.

2 RESULTS

OXYGEN ISOTOPE ANALYSES

Oscillations in estimated isotopic temperature were observed in all shells. Age was estimated as the number of seasonal temperature cycles between the lowest points of the isotopic temperature oscillations. The inclusion of high and low points was dependant on a simple decision rule whereby either highs or lows had to be preceded by at least two data points following the same trend (Naylor & Breen 2008). In the last temperature cycle, age is determined as the proportion of the year which had elapsed between the last winter and capture. Oxygen isotope ratios and the estimated water temperatures at the time of shell precipitation, and the number of annual temperature cycles are shown in Appendix 3. Apart from Shells A and B, the temperature trend at the outside towards the outer edge of the shell reflects the expected water temperature trend at the time of sampling, i.e. it is consistent with the expected seasonal water temperature at the time of capture. The number of temperature cycles along the growing axis of the shell is shown in Table 2. The number of temperature cycles ranged from 3.8 to

8.3. Cycles assume a birthdate at the beginning of August and are terminated at the time of capture to provide a decimal estimate of age. The dates of shell capture for sites are shown in Table 1.

GROWTH CHECKS

Protein layers were generally very distinctive and unambiguous. Images of the vertical cross sections of shell are shown in Appendix 1. Additional shells sectioned are shown in Appendix 2. The number of protein layers apparent in each section, the shell length, and the number of temperature cycles in the shell indicated by stable oxygen isotope analyses are shown in Table 2. One section (I) was not readable. There were consistent counts between two readers on 20 of the 23 remaining sections. Agreement was reached between the two readers after examination under the microscope. Disagreement between the two readers arose in two cases where layers were paired and in one case where a layer was faint.

The shell sections were presented at a Shellfish Working Group meeting held on the 23rd of October 2012. The Working Group agreed with most of the counts of protein layers made within shells, however, they questioned three counts, shells A, J, and H. In shell A, it appeared that a further protein layer may have been eroded on the spire of the shell causing the loss of a layer. Although slightly chipped to the left of the centre of the spire, further examination revealed that the central region of the spire was not eroded. In shell J, there was some indication that an extra layer towards the outside of the shell may have been missed. Re-examination and a new photo of this shell indicated that the extra layer is very short terminating soon after it began (Appendix 1). Shell H also had a faint line towards the outside of the shell. Re-examination of this shell revealed that that layer also did not continue along the shell. This shell was also re-photographed to make this apparent (Appendix 1).

Table 2: Number of protein layers, marginal increment, the year born inferred from protein layers in the shell, the number of temperature cycles in the shell estimated by stable oxygen isotopes, and shell length (mm). * denotes sexually immature.

Shell	Protein layers	Marginal incr.	Shell cycles	Isotope cycles	Length (mm)
A (George)	6	0.5	6.5	6.7	117
B (George)	5	0.5	5.5	4.7	110
C (George)	7	0	7	7.7	122
D (George)	9	1	10	6.7	125
E (Sth coast)	5	0.9	5.9	6.3	128
F (Sth coast)	7	0.8	7.8	8.3	143
G (Sth coast)	6	0.9	6.9	7.3	152
H (Sth coast)	5	1	6	8.3	146
I (Chalky)	?	?		6.3	123
J (Chalky)	6	1	7	6.3	123
K (Chalky)	7	0	7	7.3	142
L (Chalky)	6	0.2	6.2	7.3	127
M (Akaroa)	4	0	4	4.5	100
N (Akaroa)	6	0.2	6.2	6.5	98
O (Akaroa)	5	0.6	5.6	5.5	101
P (Akaroa)	5	0	5	4.5	98
Q (Motunau)	6	0.5	6.5	4.6	114
R (Motunau)	6	0.5	6.5	4.6	105
S (Motunau)	5	0.3	5.3	5.6	122
T (Motunau)	7	0.4	7.4	5.6	124
U (Okiwi Bay)	4	1	5	3.8	110
V (Okiwi Bay)	7	0.7	7.7	5.8	128
W (Okiwi Bay)	5	1	6	5.8	124
X (Okiwi Bay)	8	0.6	8.6	5.8	113

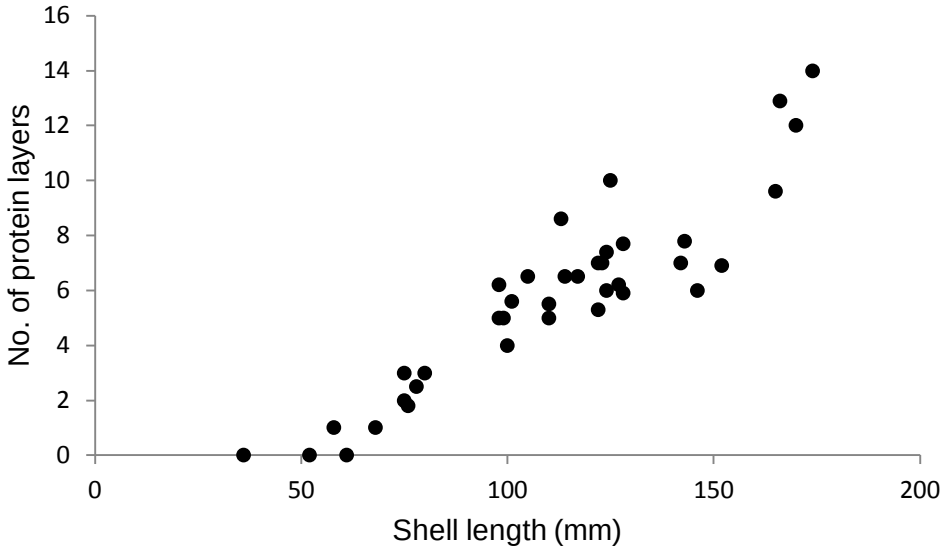


Figure 6: Number of protein layers in vertical cross sections of 38 shells by length.

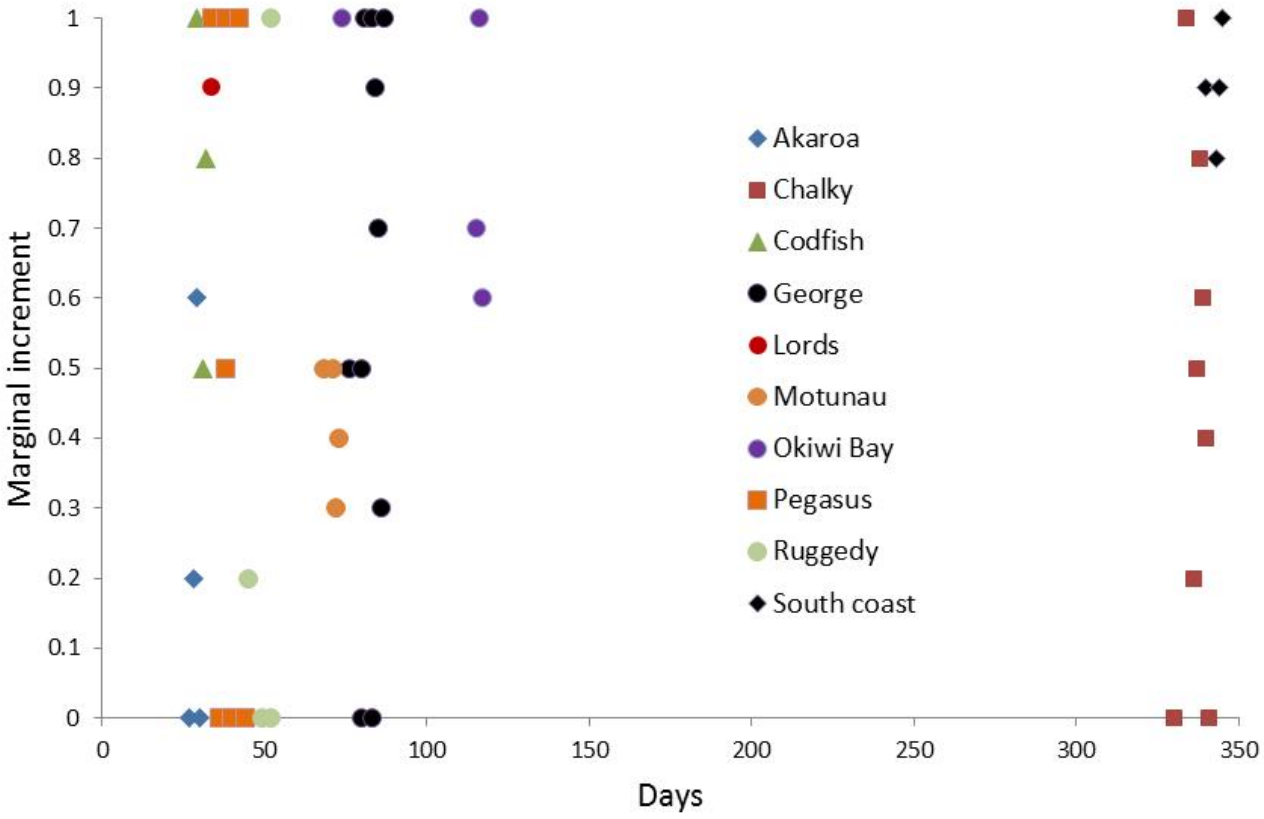


Figure 7: Marginal increments by site and days of the year.

The number of protein layers in vertical cross sections of the shells by length is shown in Figure 6. Protein layers were not obvious in three shells of lengths 36 mm, 52 mm, and 61 mm (Table 2, Appendix 2). These layers appear not to be laid down until the shells are about 60 mm in length. Above about 60 mm in length there appears to be a roughly linear relationship between shell length and the number of

protein layers in the vertical cross section of the shell (Figure 6). Of the nine extra smaller shells from the South coast examined, all were sexually immature (Table 2). Four of these shells had two protein layers and three had one protein layer (Table 2, Appendix 3).

The protein layer is not laid down over the entire surface of the shell at one time. It is laid down in a way that results in a 'blotchy' pattern on the inner surface of the shell. All shells in Table 1, with the exception of shell 6931 (the smallest, SL 36 mm) had recent protein deposition on some part of the shell.

Recently laid down protein layers were readily apparent (e.g., Shell K Appendix 1). Marginal increments by site and days (at time of capture) are shown in Figure 7, where it is apparent that the protein layers are not laid down at the same time of the year, even within sites. Marginal increments in three additional shells from Codfish and one from Lords were also determined and presented to allow a greater comparison between sites.

There appears to be some relationship between the number of protein layers in the shell and the number of temperature cycles in the shell. In 60% of shells the difference between the numbers of cycles is less than 1.

3 CONCLUSIONS

There appears to be a relationship between the number of protein layers in the shell and the age indicated by stable oxygen analyses in most of the shells examined; however, there is no evidence that the protein layers are laid down on an annual basis. A major problem is that the protein layers do not appear to be laid down in shells at the same time of year at the same location. At a couple of sites the deposition of the protein layer appears synchronous, but the overall pattern is less than compelling. If these layers were always laid down at a similar time of year at sites, it may provide some evidence of seasonal or annual deposition. The Shellfish Working Group has suggested that regular monitoring of a local population for a period extending beyond a year may provide evidence about the seasonality of deposition of the protein layer.

Because protein layers appear not to be laid down until a shell length of about 60 mm is attained, at which time the animal might be expected to be two to three years old (Sainsbury 1982, Poore 1972), in shells where the number of protein layers is the same as the estimated age, on at least two occasions, more than one layer must have been laid down during a single year. Murray (1986) notes that these bands are not laid down until paua mature at about four years of age, and Sinclair (1963) notes that *H. iris* do not lay down protein layers until they are 40 mm to 50 mm long. The link between maturity and the deposition of protein layers is unclear, as several immature paua had one or more protein layers in the vertical cross sections of their shells.

An understanding of why these layers are laid down would be useful in assessing the utility of growth checks. Several authors have related their formation to spawning or colder temperatures. Sakai (1960) examined sectioned shells of *H. discus hannai* which had been held in bamboo baskets. He reported that the annual growth checks were associated with spawning. Kojima (1975, cited in Shepherd et al. 1995a) also associated their formation with spawning in *H. discus*. Kim & Chung (1985, cited in Shepherd et al. 1995a) associate the formation of growth checks with winter in *H. diversicolor*, and Poore (1972) found that the growth checks in *H. australis* were formed in late autumn or early winter. Shepherd et al. (1995a) attribute the unusually clear growth checks in *H. mariae* to the coincidence of spawning and winter. Spawning and winter also coincide in New Zealand, which may make it difficult to determine the trigger for laying down the protein layer in *H. iris*. Many of the shells examined in this project, however, had recently laid down protein layers at much warmer times of the year. The problem of the timing of protein layer deposition is further complicated by the fact that this layer is not laid down

over the entire inner surface of the shell at the same time, so that while there may be no recent protein layer under the spire of the shell (the part of the shell sectioned and examined) protein deposition may be occurring on other areas of the inner surface. With the exception of shell 6931 (the smallest, SL 36 mm), all shells in Table 1 had recent protein deposition on some part of the shell.

The large body of literature relating to the estimation of age in abalone using protein layers is variable and conflicting. Internal growth checks in the shells of abalone have been used by a number of authors to estimate age. Shepherd et al. (1995a) estimated growth in *H. mariae* using modal progression analysis as well as tag recapture data and found that there was reasonable agreement between age estimated using these methods and age estimated from counts of growth checks in the shell. Shepherd et al. (1995b) used tag recapture data to estimate age at length in *H. fulgens* and used these ages to confirm the periodicity of shell check formation in abalone between 40 mm and 100 mm. They found that *H. fulgen*s appeared to lay down four growth checks in the first year and three in each subsequent year (Shepherd et al. 1995b). Shepherd & Turrubiates-Morales (1997) found that El Niño events caused the deposition of growth checks in this species. Shepherd & Triantafillos (1997) found that ring deposition in *H. laevigata* was variable between sites. They attributed this to differences in growth rates between sites. They used a combination of length frequency data and growth parameters estimated from tag recapture to determine the timing of growth check deposition in *H. laevigata*, and concluded that while growth checks in the shell did not reliably indicate the age of all individuals, they thought that they should provide 'a probabilistic age for a population sample' (Shepherd & Triantafillos 1997). Shepherd & Huchette (1977) found that in *H. scalaris*, two fine rings were laid down each year, but additional rings were laid down in response to infestation by boring annelids and drilling gastropods. This reduced the utility of the method, because of the high proportion of shells which had to be discarded. A further potential problem with shell growth check methods is that heavily eroded shells or shells heavily infested with boring organisms will frequently have lost part of their growth record. Selecting only very clean shells for analysis may bias age estimates, as these shells are typically faster growing or live in more cryptic habitat where the prevalence of encrusting and boring organisms appears to be lower.

Prince et al. (1988) found that in *H. rubra* three minor rings were laid down in the first 16 months, one major ring was laid down after 20 months, and major rings were subsequently laid down annually. They constructed an age at length key for *H. rubra* using length frequency data for animals smaller than 80 mm, and tag-recapture data for animals larger than 80 mm. This led them to conclude that the growth checks were laid down in a temporally consistent manner. McShane & Smith (1992), however, found that in other areas the method did not reliably estimate age in *H. rubra*.

Erasmus et al. (1994) used acetate peels and electron microscopy on hatchery raised *H. midae* shells of a known age to determine that three growth checks were formed in the first year of life, and that one was formed in each subsequent year. This is similar to the findings of Prince et al. (1988) for *H. rubra*. Erasmus et al. (1994) found that the deposition of layers was not directly related to environmental variables and suggested that it may be related to an endogenous rhythm associated with growth. From a mechanical perspective, the laminated structure of abalone shell, with alternating layers of calcium carbonate and protein, affords the shell fracture toughness 8 times that of monolithic calcium carbonate (Sarikaya et al. 1990).

In New Zealand counts of growth checks in longitudinal sections of the shells of *H. iris* were routinely used to estimate age in the 1980s (Murray & Ackroyd 1984, Murray 1986, Petherick 1987). According to Murray (1986) 'Tagging has shown the bands to be laid down about once every year.' The tagging studies, however, do not appear to be well documented. Sinclair (1963) also examined growth checks in longitudinal sections of the shell of *H. iris*, and concluded that while they were laid down in response to some 'physiological need' it could not be determined whether the checks were annual or biannual. Schiel & Breen (1991) compared growth predicted by internal ring counts and growth predicted by tag-recapture data from three regions around Stewart Island and from three regions of the Marlborough Sounds. They concluded that in these areas, counts of growth checks over-estimated age (i.e., more than one growth check was laid down each year). It is useful to note that Murray (1986) counted rings in the

thick region at the base of the posterior of the shell. The number of protein layers in this region may sometimes be much greater than the number of protein layers in the spire region (Naylor 2011b).

Although this study indicates that there is some relationship between the number of protein layers in the shell and the age of the shell, further understanding of the timing of the deposition of the these layers is required to assess the likely utility of these layers as a proxy for age. If paua populations could be reliably aged by counting internal growth layers within the shell, the potential exists to adopt an age based stock assessment model with a consequent improvement in the precision of assessment projections.

4 ACKNOWLEDGEMENTS

This project was funded by the Ministry for Primary Industries project PAU 2011/01. We thank Pete Notman and Dave Rae for the collection of shell samples, Caoimhghin Ó Maolagáin for sectioning shells, Michael Uddstrom for providing SST data, and Chris Francis for advice on ageing protocols. Thanks also to Peter Horn for reviewing the draft report.

5 REFERENCES

Epstein, S.; Buchsbaum, R.; Lowenstam, H.A.; Urey, H.C. (1951). Carbonate–water isotopic temperature scale. *Bulletin of the Geological Society of America 62*: 417–426.

Epstein, S.; Buchsbaum, R.; Lowenstam, H.A.; Urey, H.C. (1953). Revised carbonate–water isotopic temperature scale. *Bulletin of the Geological Society of America 64*: 1315–1326.

Erasmus, J.; Cook, P.A.; Sweijd, N. (1994). Internal shell structure and growth lines in the shell of the abalone, *Haliotis midae*. *Journal of Shellfish Research 13*: 493–501.

Kim, J.W.; Chung, S.C. (1985). On the growth of abalones, *Sulculus diversicolor diversicolor* (Reeve), and *S. diversicolor aquatills* (Reeve) in Cheju Island. *Bulletin of Marine Research of Research Institute of Cheju National University 9*: 39–50 (in Chinese).

Kojima, H. (1975). A study on the growth of the shell of *Haliotis discus discus* (Gastropoda: Ha1iotidae)-I, Age and growth. *Aquaculture 23*: 1–6 (in Japanese).

McShane, P.E.; Naylor, J.R. (1996). Variation in spawning and recruitment of *Haliotis iris* (Mollusca:Gastropoda). *New Zealand Journal of Marine and Freshwater Research 30*: 327–334.

McShane, P.E.; Smith, M.G. (1992). Shell growth checks are unreliable indicators of age of the abalone *Haliotis rubra* (Mollusca: Gastropoda). *Australian Journal of Marine and Freshwater Research 43*: 1215–1219.

Muñoz-Lopez, T. (1976). Resultados preliminares de un metodo para determinar edad en abulones (*Haliotis* spp.) de Baja California. *Memorias del Simposio sobre Recursos Pesqueros Masivos de Mexico Ensenada, B.C.*, del 28 a1 30 de Septiembre, 281–301 (in Spanish).

Murray, T. (1986). Paua shell and its growth. Shellfisheries Newsletter 30: 10, in *Catch 13* (3).

Murray, T.; Ackroyd, J. (1984). The New Zealand paua fishery. An update and review of biological considerations to be reconciled with management goals. Internal Report No. 5. Fisheries Research Division. 25 p.

Naylor, J.R. (2011a). Literature review of abalone ageing techniques. Final Research Report to the Ministry of Fisheries for project SEA2010-16. (Unpublished report held by Ministry for Primary Industries, Wellington.) 15 p.

Naylor, J.R. (2011b). Paua ageing methodology. Presentation to the Shellfish Working Group on the 18th October 2011.

Naylor, J.R.; Andrew, N.L.; Kim, S.W. (2006). Demographic variation in the New Zealand abalone *Haliotis iris*. *Marine and Freshwater Research 57*: 215–224.

Naylor, J.R.; Breen, P.A. (2008). Fine-scale growth in paua populations. Final Research Report for Ministry of Fisheries Project PAU 200604. March 2008. (Unpublished report held by the Ministry for Primary Industries, Wellington.) 33 p.

Naylor, J.R.; Manighetti, B.M.; Neil, H.L.; Kim, S.W. (2007). Validated estimation of growth and age in the New Zealand abalone *Haliotis iris* using stable oxygen isotopes. *Marine and Freshwater Research 58*: 354–362.

Petherick, C. (1987). Preliminary paua survey of the Wairarapa coast. Ministry of Fisheries Internal Report. Central Fishery Management Area. 87/2.

Poore, G.C.B. (1972). Ecology of New Zealand abalones, *Haliotis* species (Mollusca: Gastropoda): 3. Growth. *New Zealand Journal of Marine and Freshwater Research 6*: 534–59.

Prince, J.D.; Sellers, T.L.; Ford, W.B.; Talbot, S.R. (1988). A method for ageing the abalone, *Haliotis rubra* (Mollusca: Gastropoda). *Australian Journal of Marine and Freshwater Research 39*: 167–175.

Ridgway, N.M.; Heath, R.A.; Marott, C. (1979). Southwest Pacific Ocean: surface salinity. Miscellaneous series/New Zealand Oceanographic Institute 26 (Map).

Sainsbury, K.J. (1982) Population dynamics and fishery management of the paua, *Haliotis iris.* 1. Population structure, growth, reproduction and mortality. *New Zealand Journal of Marine and Freshwater Research 16*: 147–161.

Sakai, S. (1960). The formation of the annual ring in the shell of the abalone *Haliotis discus* var *hannai* Ino. *Tohoku Journal of Agricultural Research 11*: 239–244.

Sarikaya M.; Gunnison, K.E.; Yasrebi M.; Aksay, J.A. (1990). Mechanical property-microstructural relationships in abalone shell. *Materials Research Society Proceedings 174*: 109–116.

Savin, S.M.; Abel, L.; Barrera, E.; Hodell, D.; Kennett, J.P.; Murphy, M.; Keller, G.; Killingley, J.; Vincent, E. (1985). The evolution of Miocene surface and near-surface marine temperatures: oxygen isotopic evidence. The Miocene Ocean: Paleoceanography and biogeography. *Geological Society of America Memoir 163*: 42–82.

Schiel, D.R.; Breen, P.A. (1991). Population structure, ageing, and fishing mortality of the New Zealand abalone *Haliotis iris*. *Fishery Bulletin 89*: 681–691.

Shepherd, S.A.; Al-Wahaibi, D.; Al-Azri, A.R. (1995a). Shell growth checks and growth of Omani abalone *Haliotis mariae. Maine and Freshwater Research 46*: 575–582.

Shepherd, S.A.; Avalos-Borja, M.; Quintanilla, M. (1995b).Toward a chronology of *Haliotis fulgens,* with a review of abalone shell microstructure. *Marine and Freshwater Research 46*: 607–15.

Shepherd S.A., Huchette, S. (1997). Studies on southern Australian abalone (genus *Haliotis*) XVIII. Ring formation in *H. scalaris*. *Molluscan Research 18*: 247–252.

Shepherd S.A, Triantofillos, L. (1997). Studies on southern Australian abalone (genus *Haliotis*) XVII. Ring formation in *H. laevigata*. *Molluscan Research 18*: 233–245.

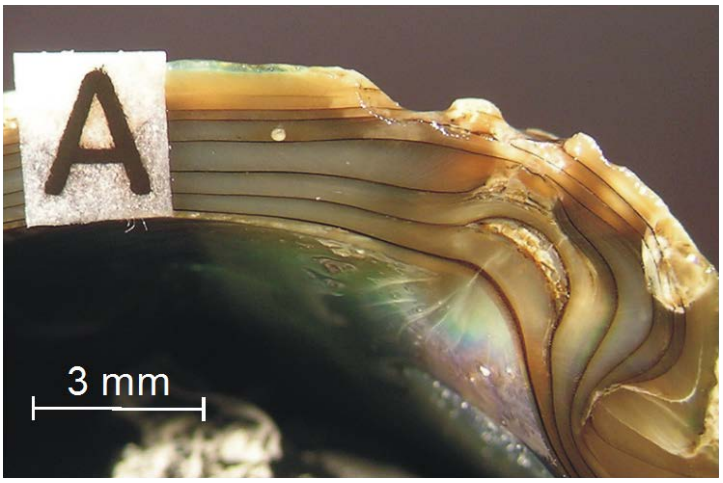
Shepherd S.A., Turrubiates-Morales, J.R. (1997). A practical chronology for the abalone *Haliotis fulgens*. *Molluscan Research 18*: 219–226.

Sinclair, M. (1963). Studies on paua, *Haliotis iris* Martyn in the Wellington district, 1945-46. *Zoology Publications from Victoria University of Wellington* 35 p.

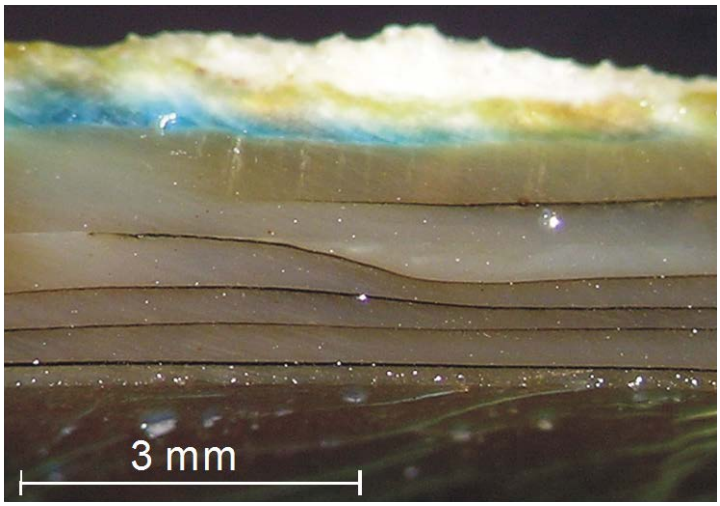
Uddstrom, M.J.; Oien, N.A. (1999). On the use of high-resolution satellite data to describe the spatial and temporal variability of sea surface temperatures in the New Zealand region. *Journal of Geophysical Research* 104: 729–751.

Urey, H.C.; Lowenstam, H.A.; Epstein, S.; McKinney, C.R. (1951). Measurement of paleotemperatures and temperatures of the Upper Cretaceous of England, Denmark, and the southeastern United States. *Bulletin of the Geological Society of America 62*: 399–416.

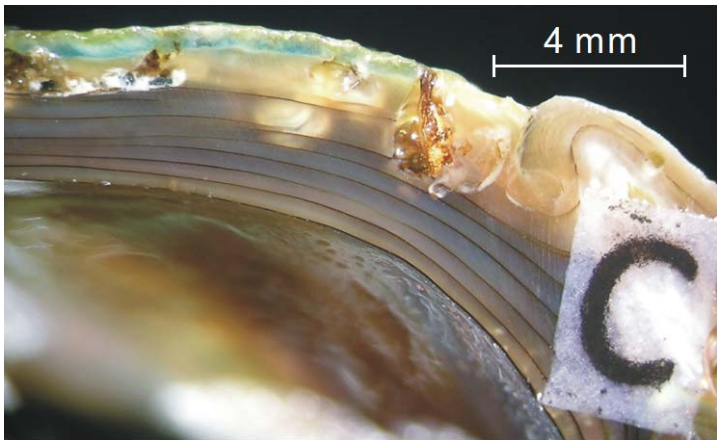
APPENDIX 1. VERTICAL CROSS SECTIONS THOUGH THE SPIRES OF SHELLS.



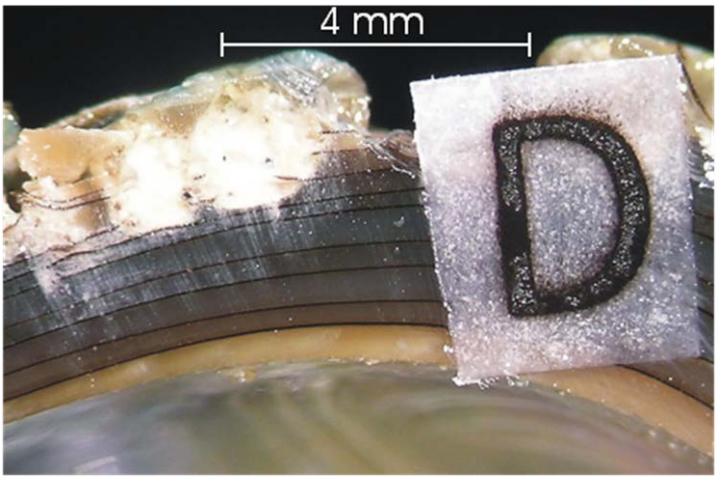
A. Magnified (approximately 7 ×) vertical section adjacent to the spire of shell A (117 mm) showing 6 growth checks.



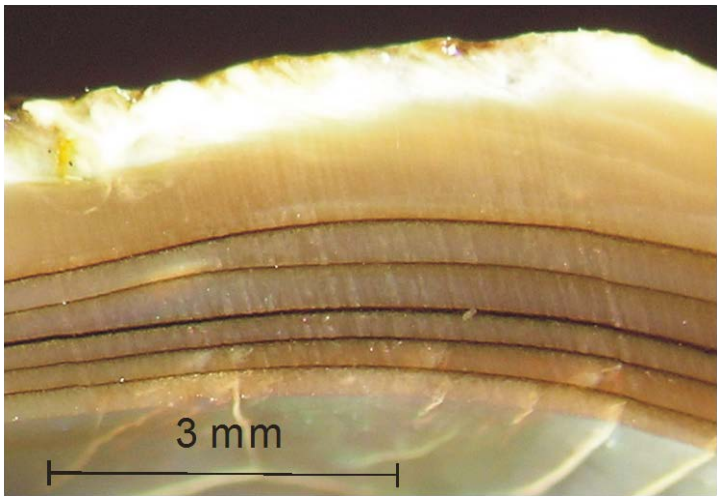
B. Magnified (approximately 15 ×) vertical section adjacent to the spire of shell B (110 mm) showing 5 growth checks.



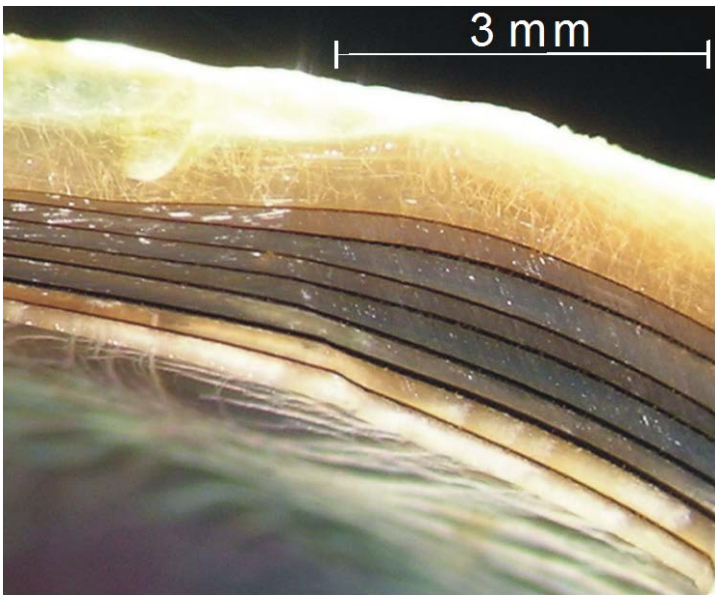
C. Magnified (approximately 6 ×) vertical section adjacent to the spire of shell C (122 mm) showing 7 growth checks.



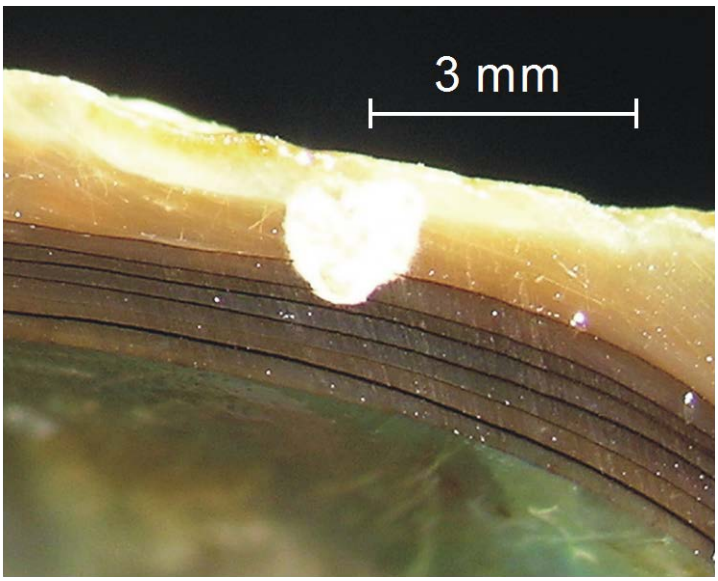
D. Magnified (approximately 10 ×) vertical section adjacent to the spire of shell D (125 mm) showing 9 growth checks.



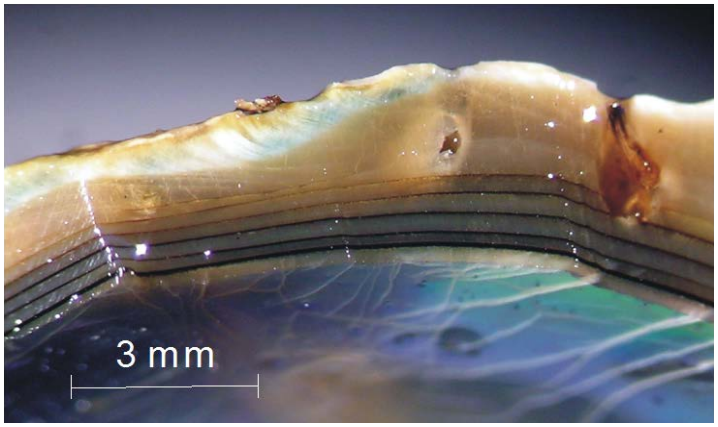
E. Magnified (approximately 15 ×) vertical section adjacent to the spire of shell E (128 mm) showing 5 growth checks.



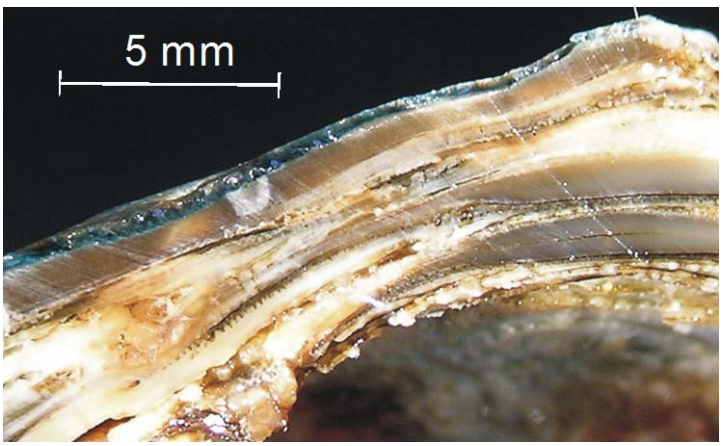
F. Magnified (approximately 16 ×) vertical section adjacent to the spire of shell F (143 mm) showing 7 growth checks.



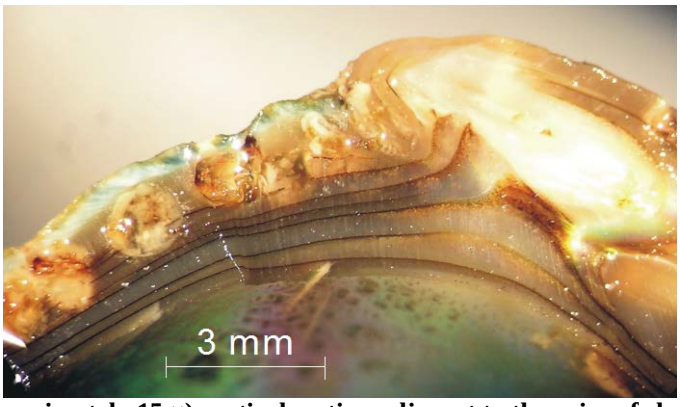
G. Magnified (approximately 12 ×) vertical section adjacent to the spire of shell G (152 mm) showing 6 growth checks.



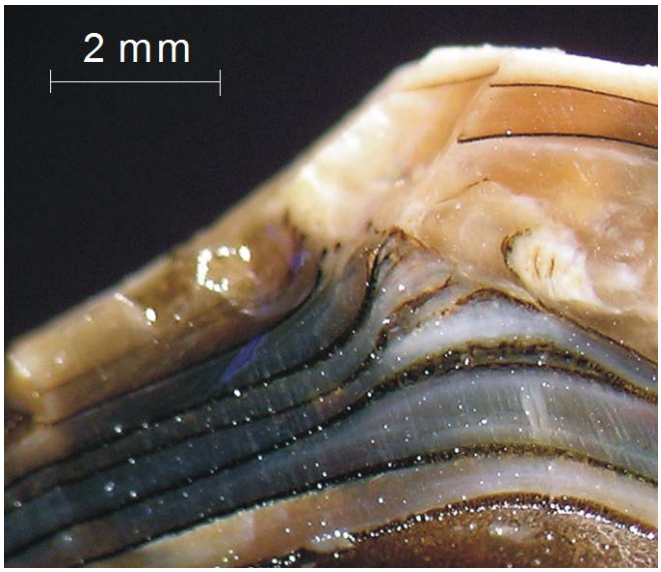
H. Magnified (approximately 20 ×) vertical section adjacent to the spire of shell H (146 mm) showing 5 growth checks.



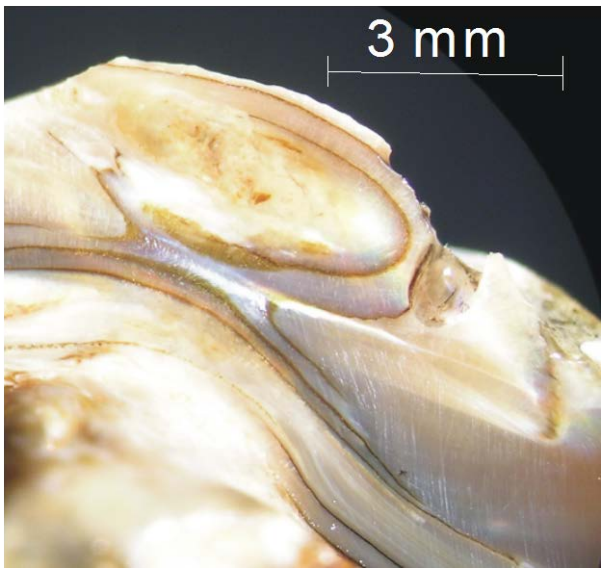
I. Magnified (approximately 6 ×) vertical section adjacent to the spire of shell I (123 mm). Growth checks not discernible.



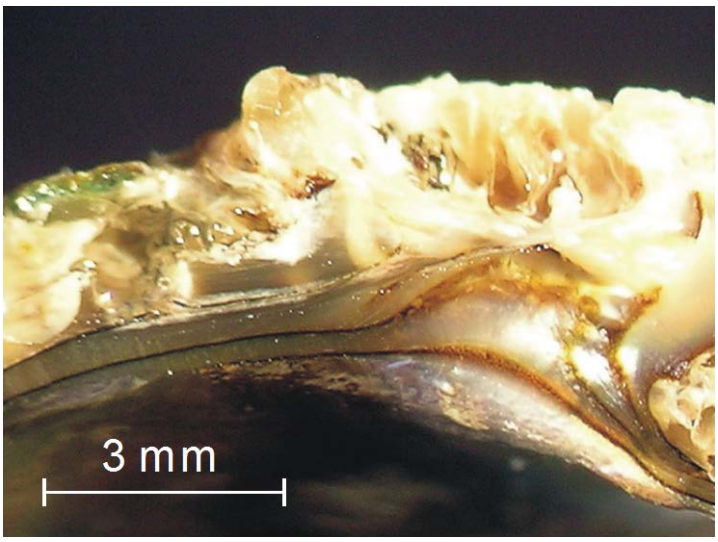
J. Magnified (approximately 15 ×) vertical section adjacent to the spire of shell J (123 mm) showing 6 growth checks.



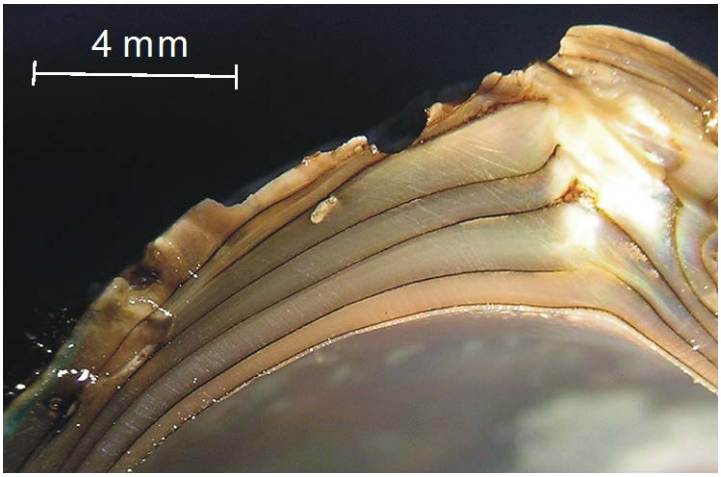
K. Magnified (approximately 12 ×) vertical section adjacent to the spire of shell K (142 mm) showing 7 growth checks.



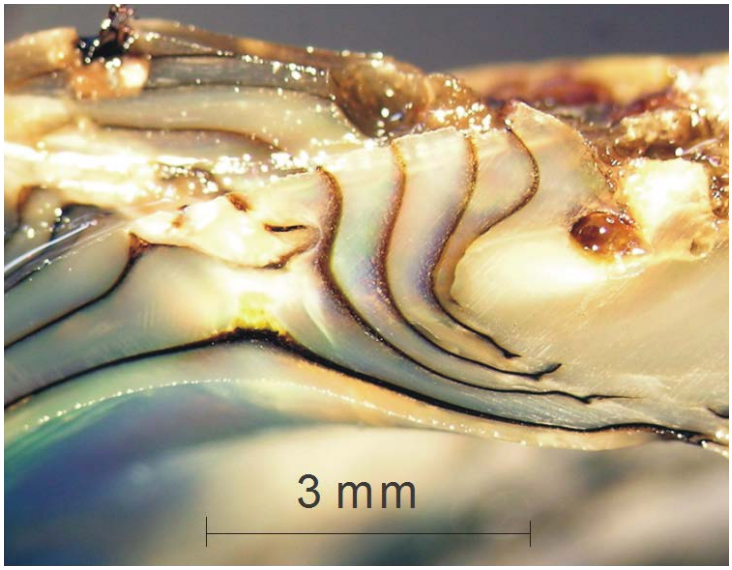
L. Magnified (approximately 7 ×) vertical section adjacent to the spire of shell L (127 mm) showing 6 growth checks.



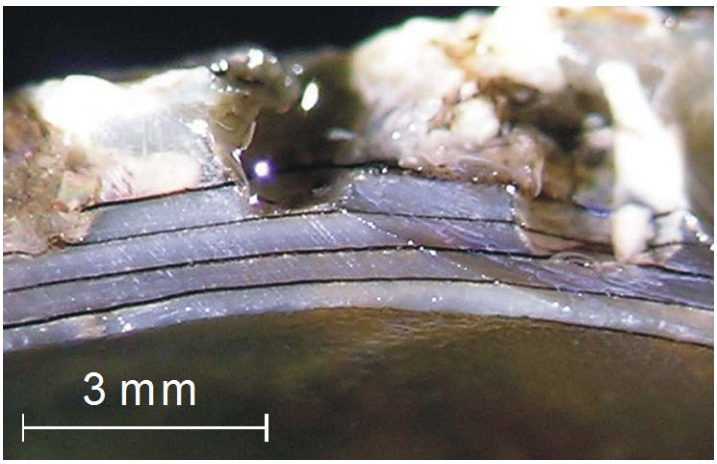
M. Magnified (approximately 10 ×) vertical section adjacent to the spire of shell M (100 mm) showing 4 growth checks.



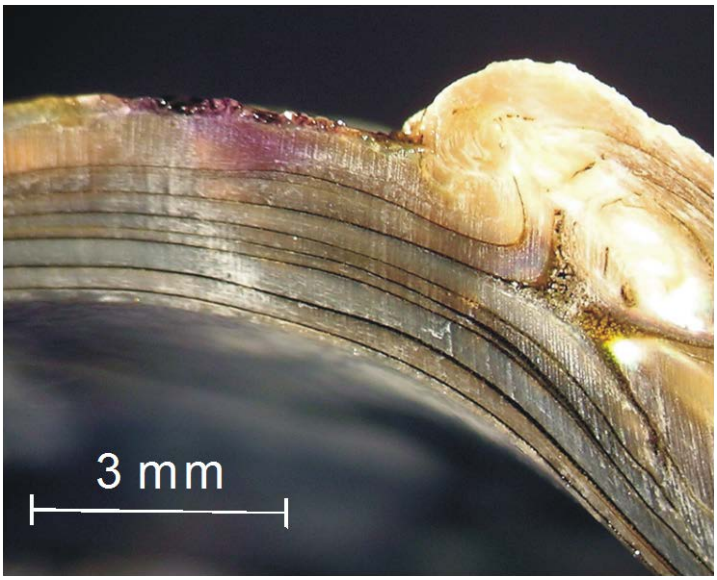
N. Magnified (approximately 7 ×) vertical section adjacent to the spire of shell N (98 mm) showing 6 growth checks.



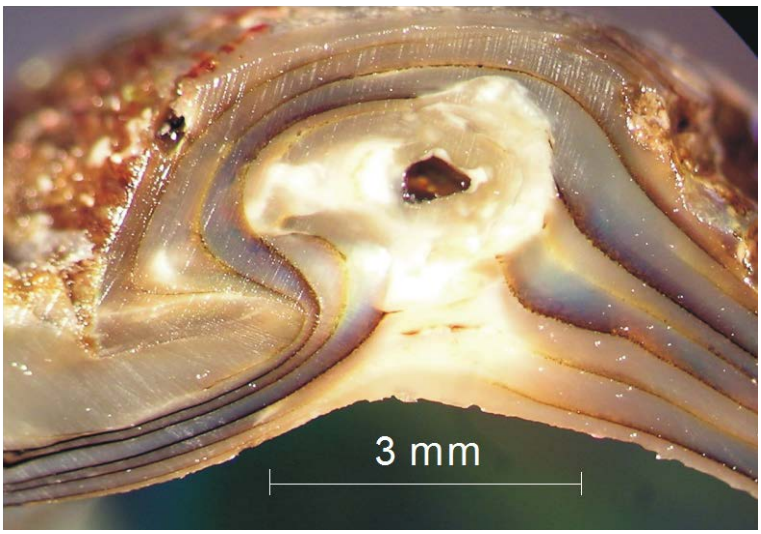
O. Magnified (approximately 9 ×) vertical section adjacent to the spire of shell O (101 mm) showing 5 growth checks.



P. Magnified (approximately 11 ×) vertical section adjacent to the spire of shell P (98 mm) showing 5 growth checks.

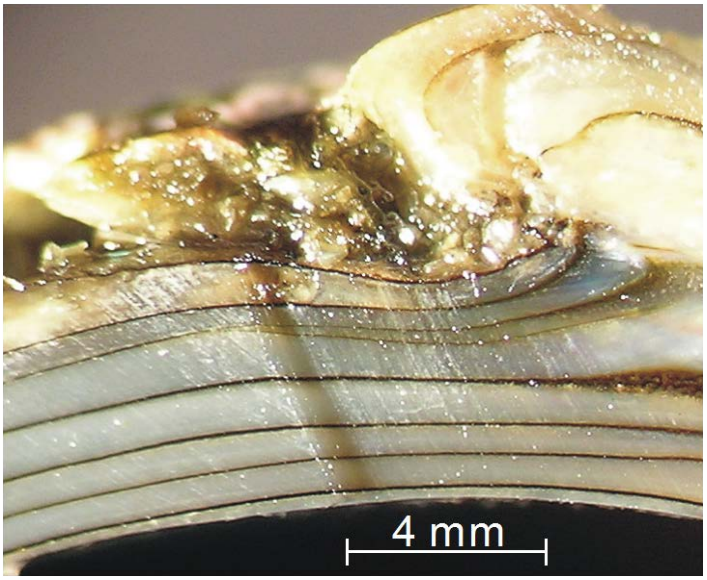


Q. Magnified (approximately 11 ×) vertical section adjacent to the spire of shell Q (114 mm) showing 6 growth checks.

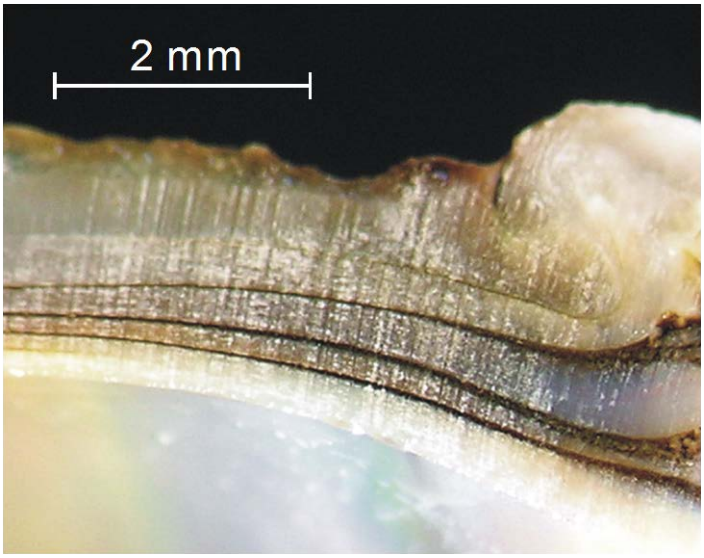


R. Magnified (approximately 24 ×) vertical section adjacent to the spire of shell R (105 mm) showing 6 growth checks.

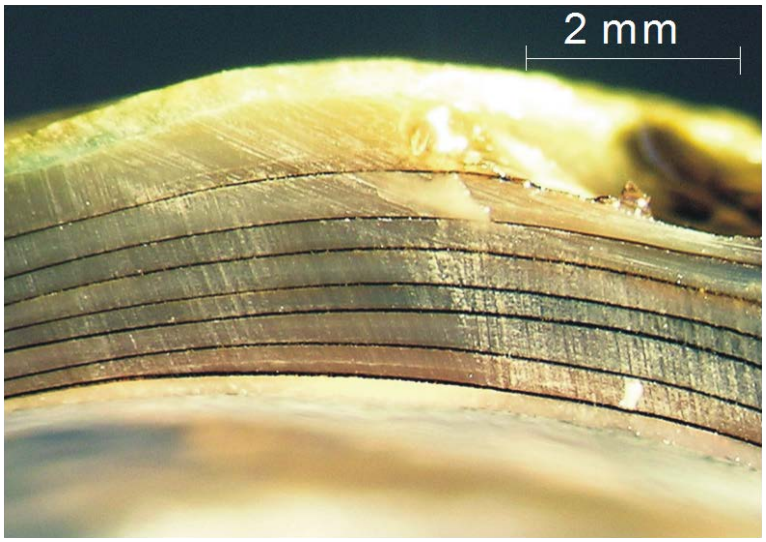
S. Magnified (approximately 18 ×) vertical section adjacent to the spire of shell S (122 mm) showing 5 growth checks.



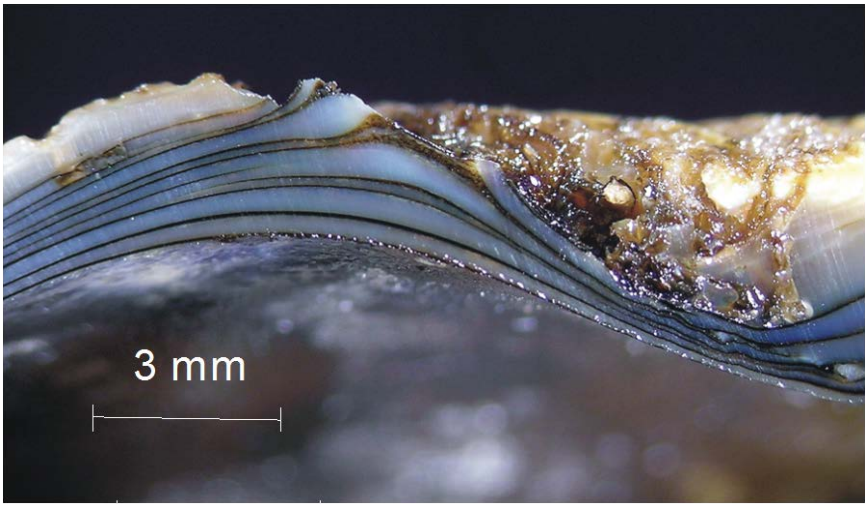
T. Magnified (approximately 7 ×) vertical section adjacent to the spire of shell T (124 mm) showing 7 growth checks.



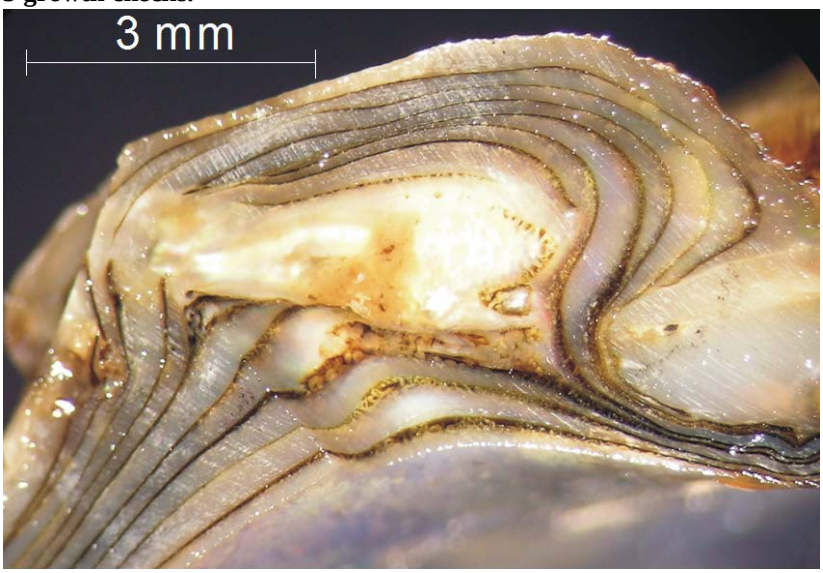
U. Magnified (approximately 17 ×) vertical section adjacent to the spire of shell U (110 mm) showing 4 growth checks.



V. Magnified (approximately 13 ×) vertical section adjacent to the spire of shell V (128 mm) showing 7 growth checks.

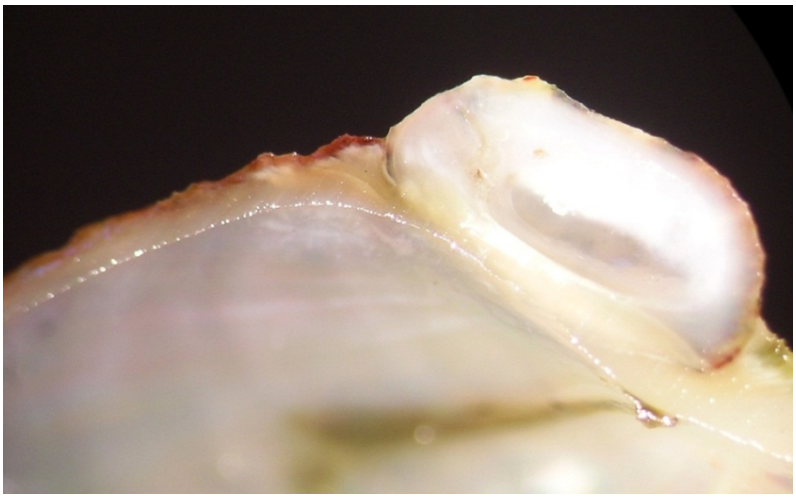


W. **Magnified (approximately 11 ×) vertical section adjacent to the spire of shell W (124 mm) showing 5 growth checks.**

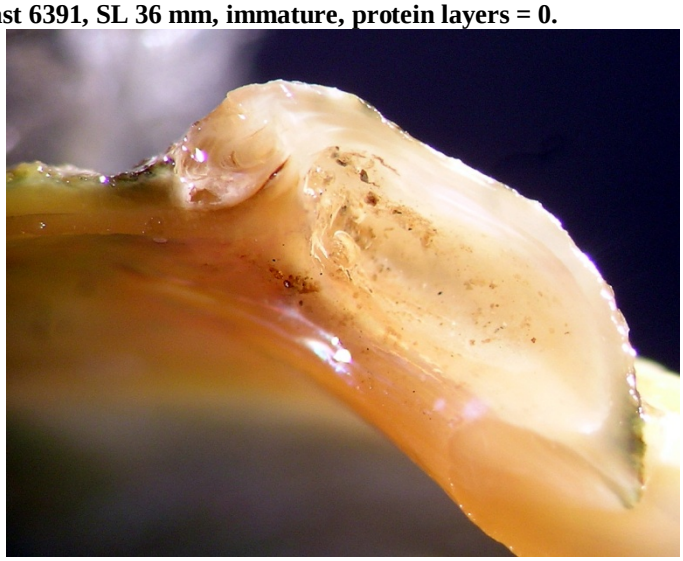


X. Magnified (approximately 12 ×) vertical section adjacent to the spire of shell X (113 mm) showing 8 growth checks.

APPENDIX 2. VERTICAL CROSS SECTIONS THROUGH THE SPIRES OF ADDITIONAL SHELLS EXAMINED.

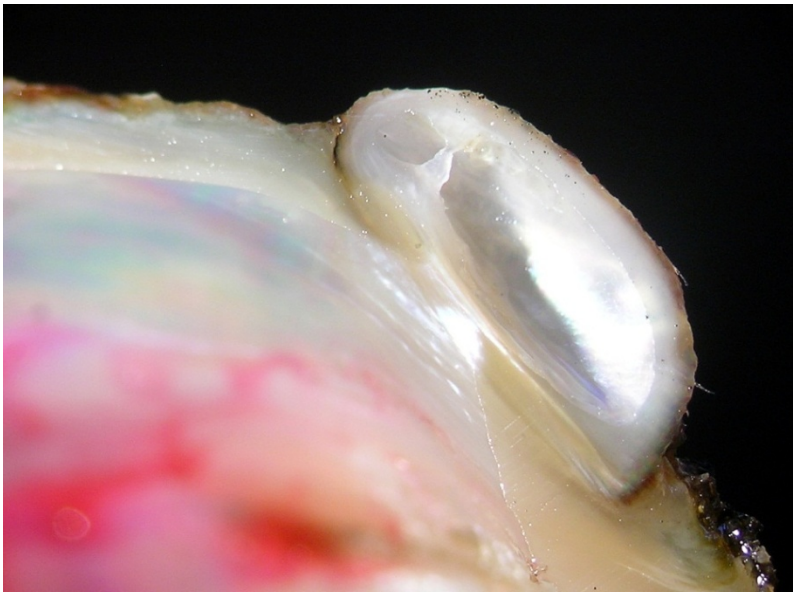


Sth coast 6391, SL 36 mm, immature, protein layers = 0.



Wellington 52, SL 52 mm, protein layers = 0.

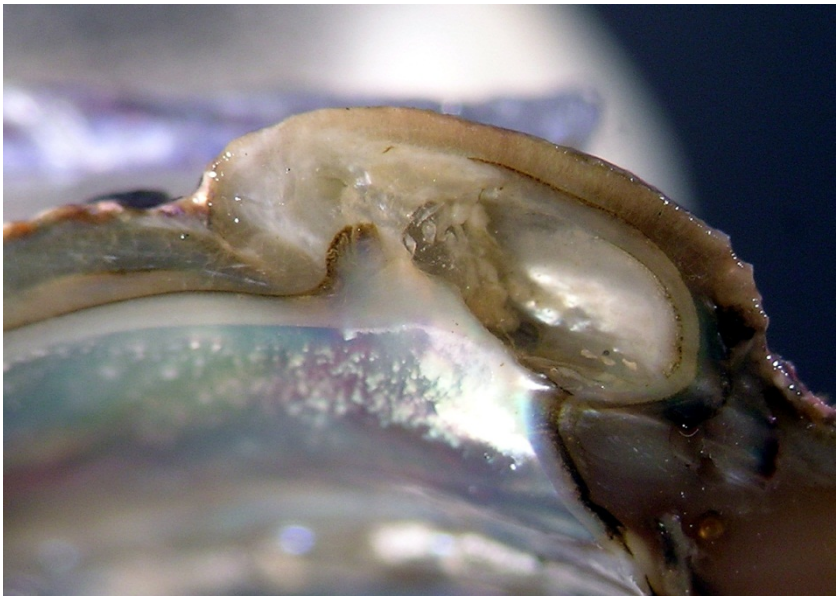
Sth coast 6310, SL 58 mm, immature, protein layers = 1.



Sth coast 6385, SL 61 mm, immature, protein layers = 0.

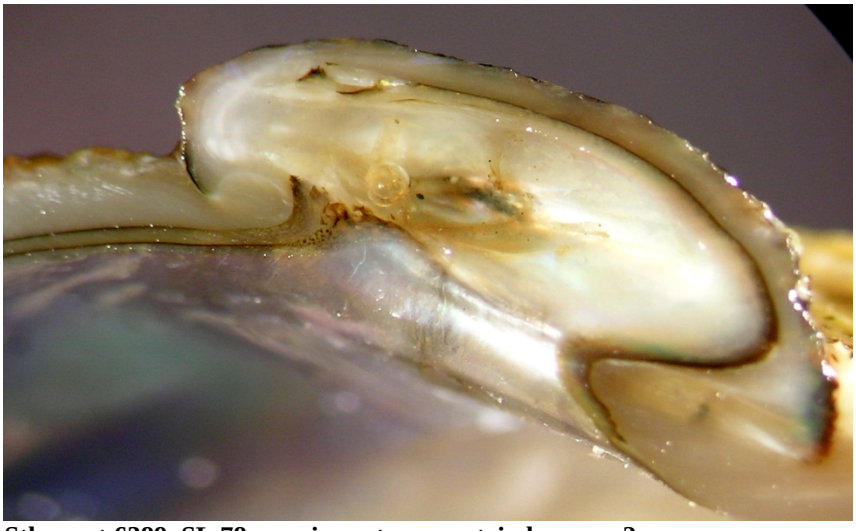
Sth coast 6397, SL 68 mm, immature, protein layers = 2.

Sth coast 6316, SL 75 mm, immature, protein layers = 2.



Sth coast 6370, SL 75 mm, immature, protein layers = 2.

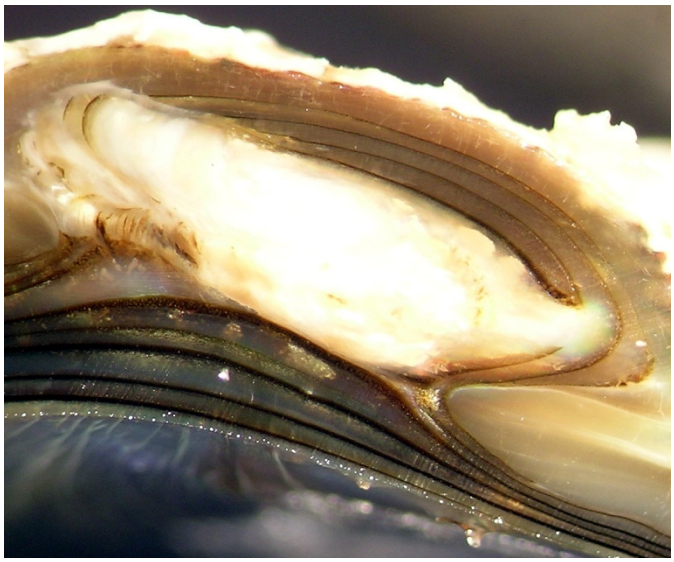
Sth coast 6379, SL 76 mm, immature, protein layers = 1.



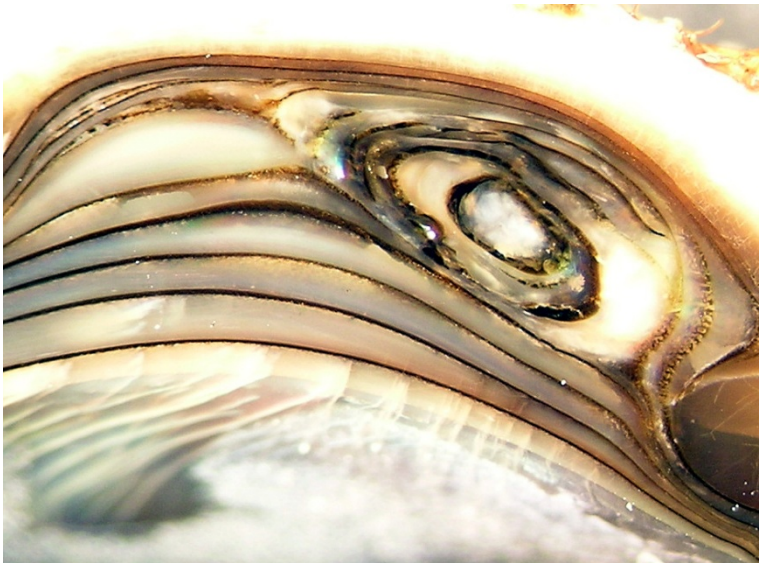
Sth coast 6389, SL 78 mm, immature, protein layers = 2.

Sth coast 6352, SL 80 mm, immature, protein layers = 2.

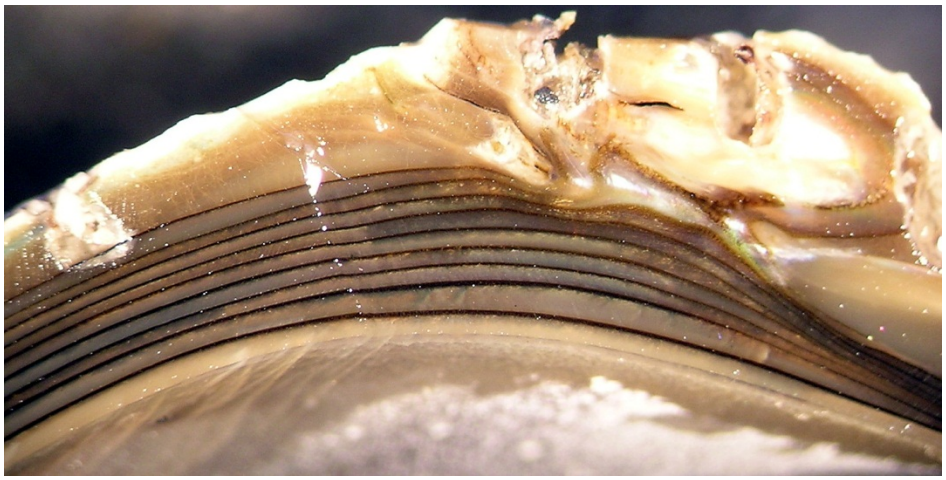
Sth coast 6382, SL 99 mm, immature, protein layers = 4.



Sth coast 128, SL 165 mm, protein layers = 9.



Dusky 19, SL 166 mm, protein layers = 12.

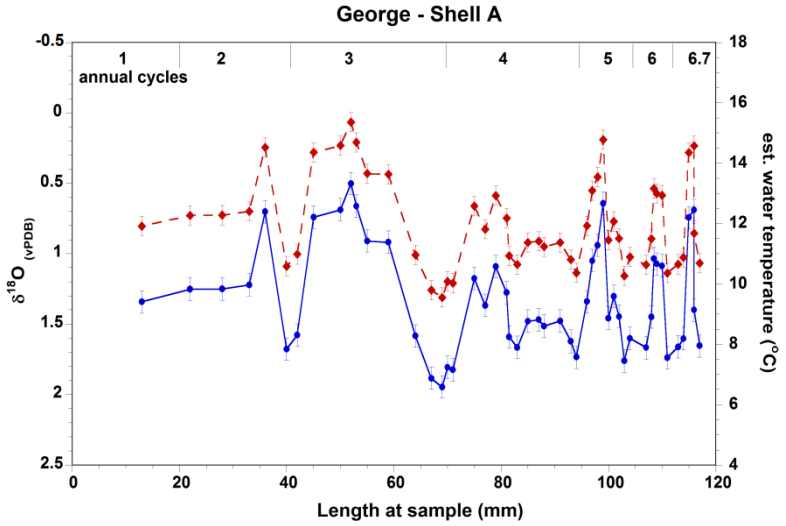


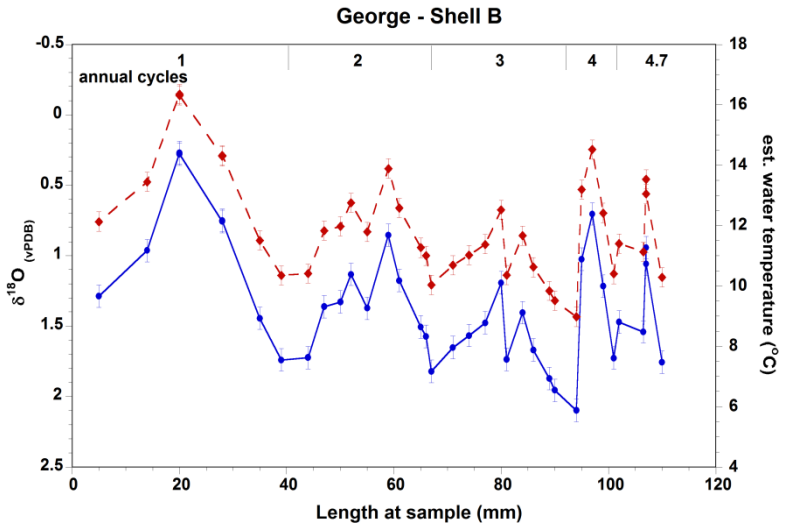
Sth coast 139, SL 170 mm, protein layers = 9 (Shell 3 mm thick).

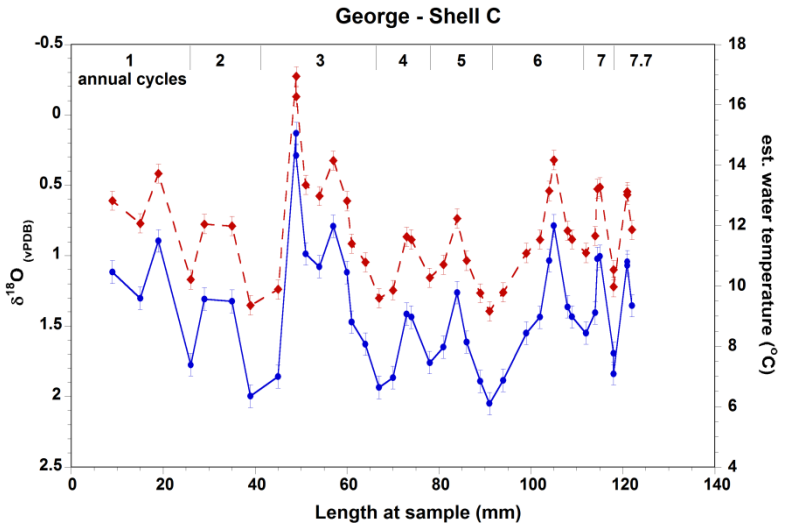
Pegasus 67, SL 174 mm, protein layers = 13 (Shell 6 mm thick).

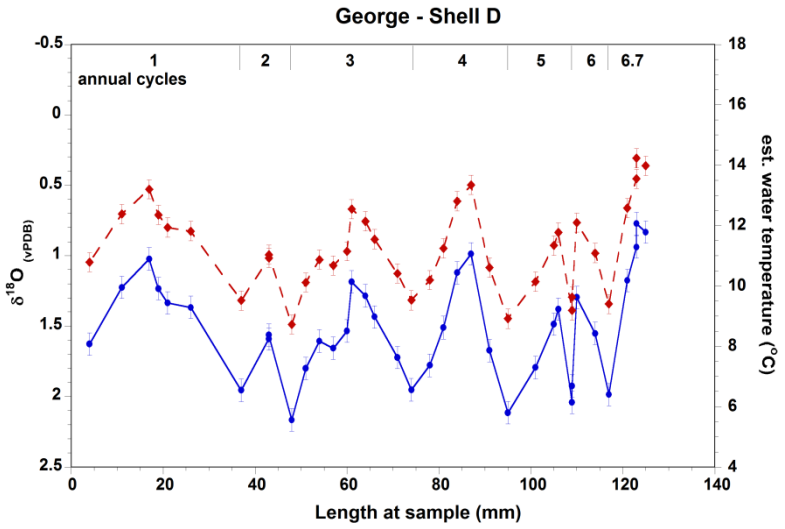
APPENDIX 3: OXYGEN ISOTOPE PROFILES AND ESTIMATED WATER TEMPERATURE PROFILES FOR SHELLS SAMPLED.

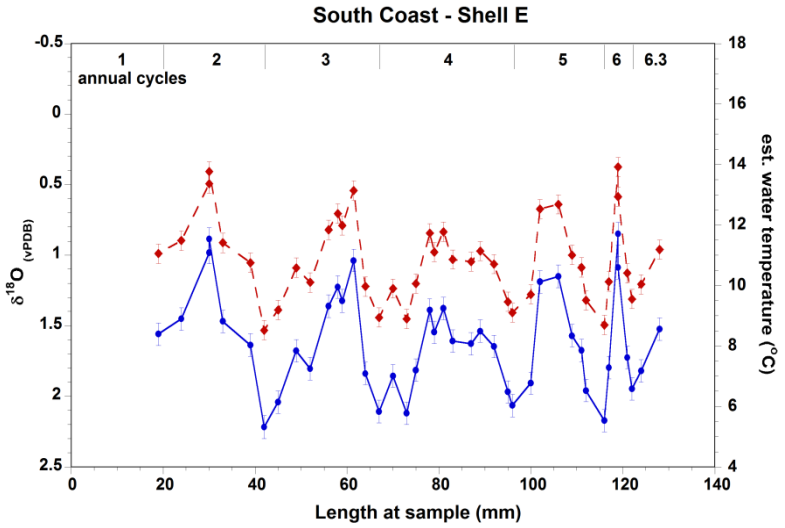
Blue (lower) line = $\delta^{18}O$, red (upper) line = ^{o}C.

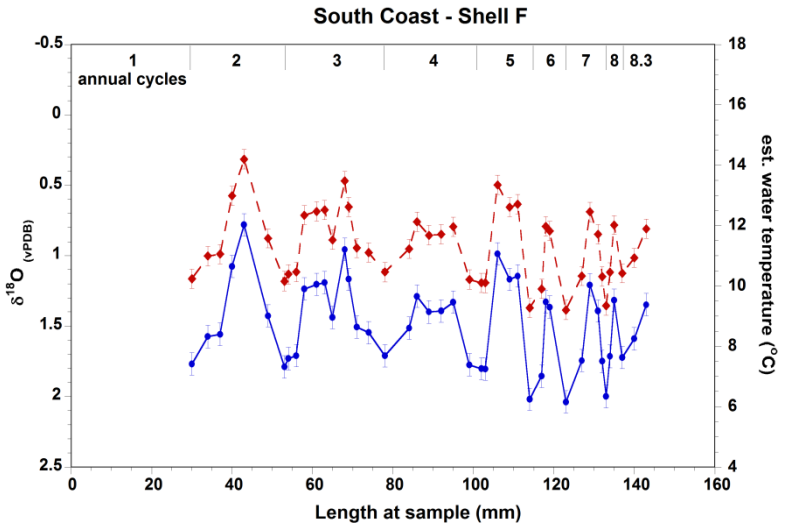


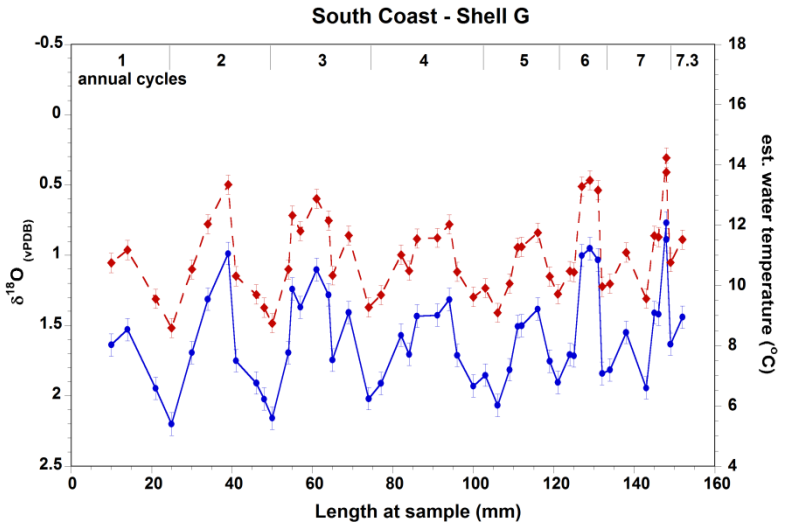


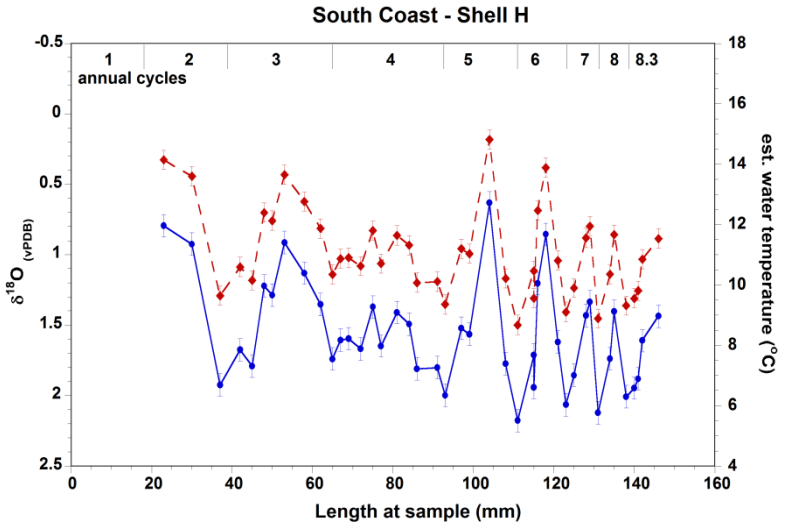

George - Shell C
annual cycles
1
2
3
4
5
6
7
7.7
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

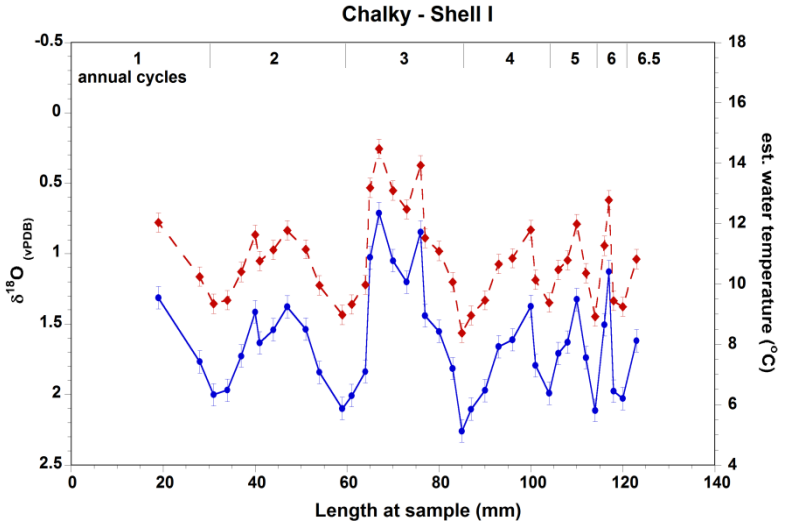

George - Shell D
annual cycles
1
2
3
4
5
6
6.7
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

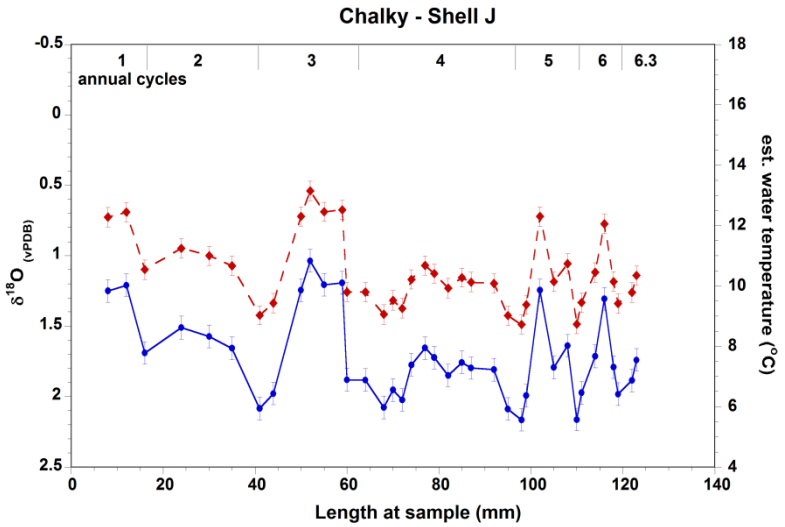

South Coast - Shell E
annual cycles
1
2
3
4
5
6
6.3
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

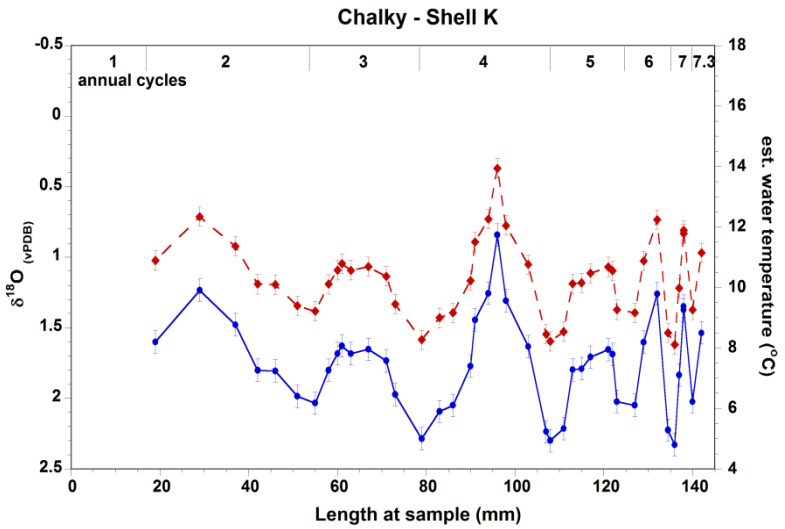

South Coast - Shell F
annual cycles
1
2
3
4
5
6
7
8
8.3
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

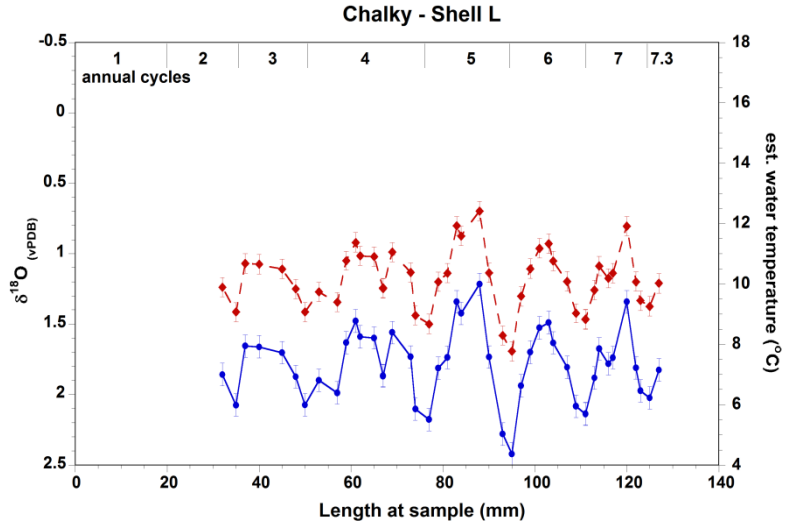

South Coast - Shell G
annual cycles
1
2
3
4
5
6
7
7.3
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

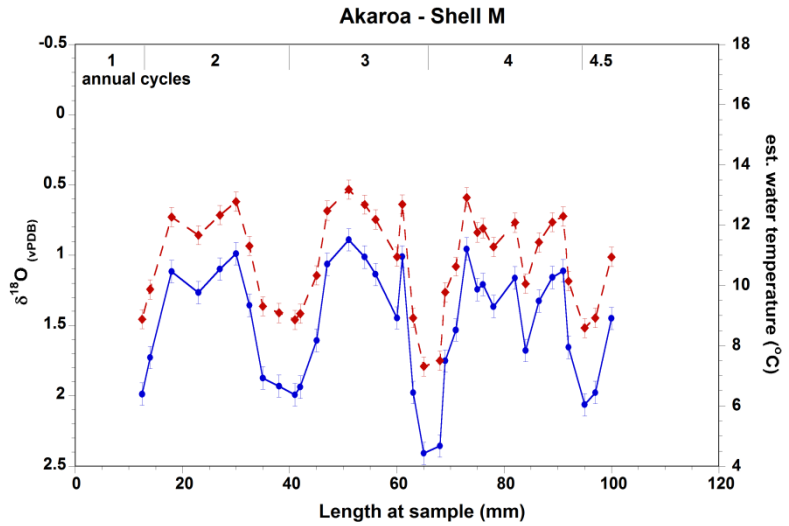

South Coast - Shell H
annual cycles
1
2
3
4
5
6
7
8
8.3
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

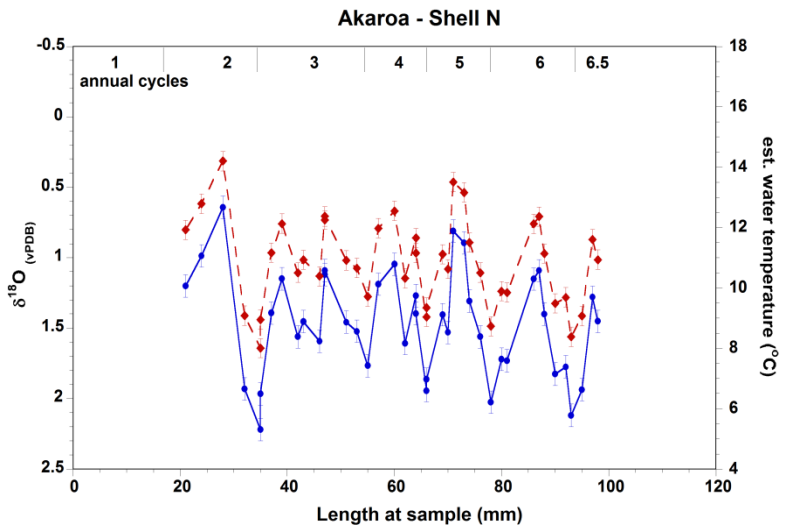

Chalky - Shell I
annual cycles
1
2
3
4
5
6
6.5
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

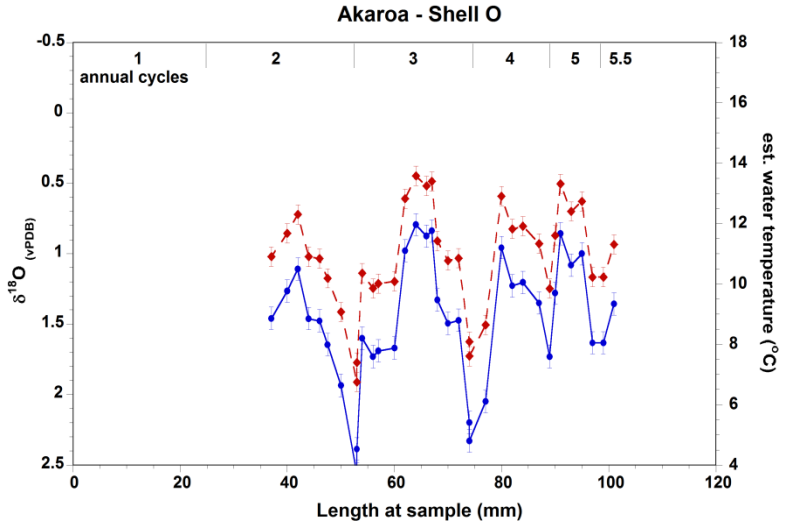

Chalky - Shell J
annual cycles
1
2
3
4
5
6
6.3
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

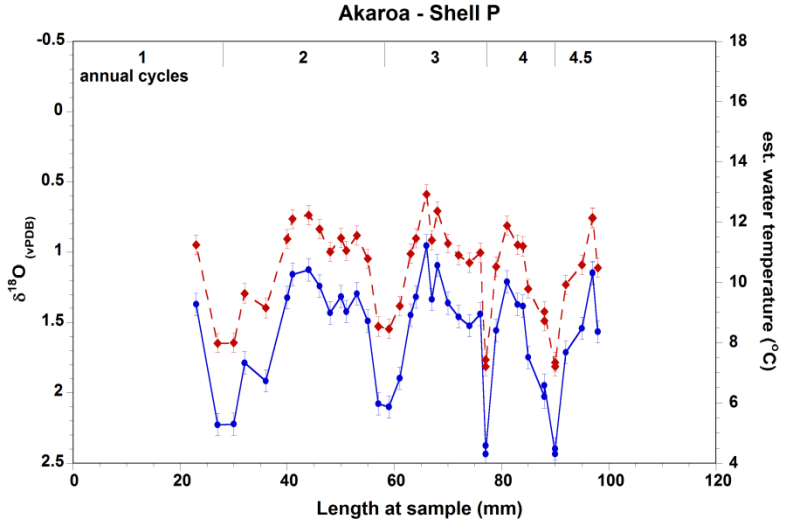

Chalky - Shell K
annual cycles
1
2
3
4
5
6
7
7.3
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

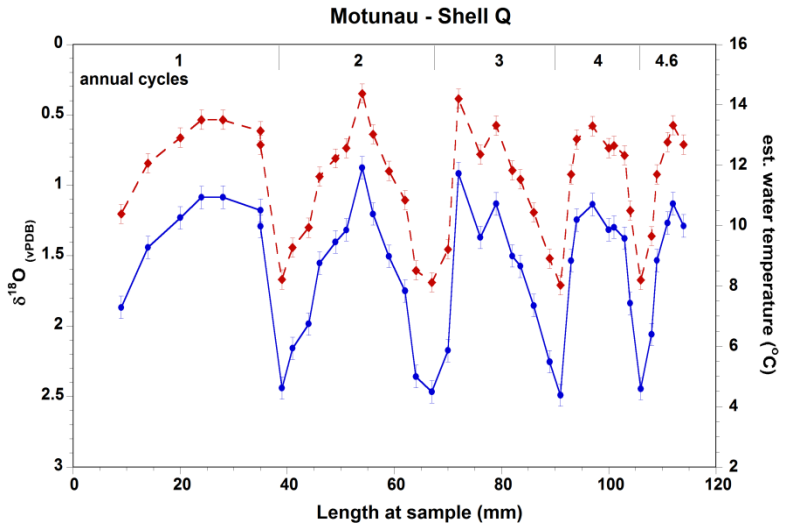

Chalky - Shell L
annual cycles
1
2
3
4
5
6
7
7.3
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

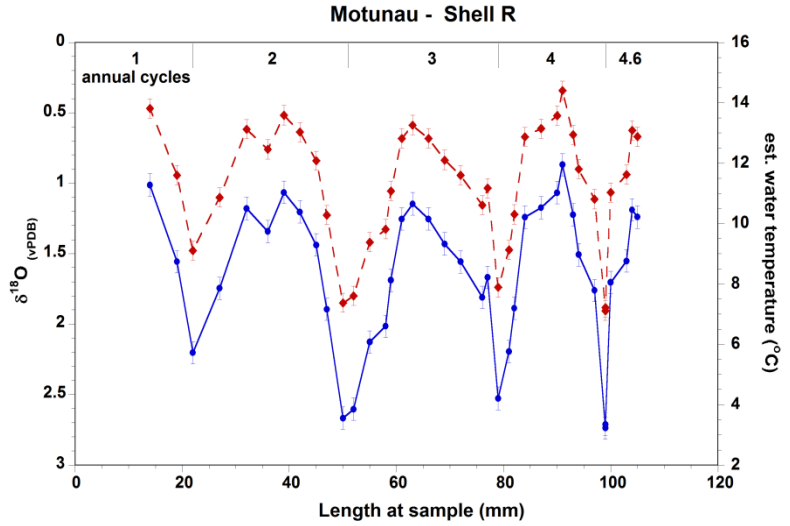

Akaroa - Shell M
annual cycles
1
2
3
4
4.5
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

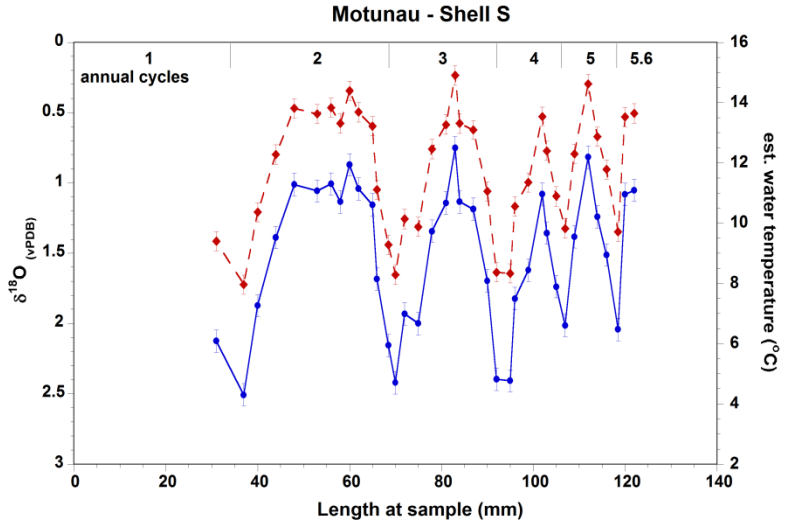

Akaroa - Shell N
annual cycles
1
2
3
4
5
6
6.5
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

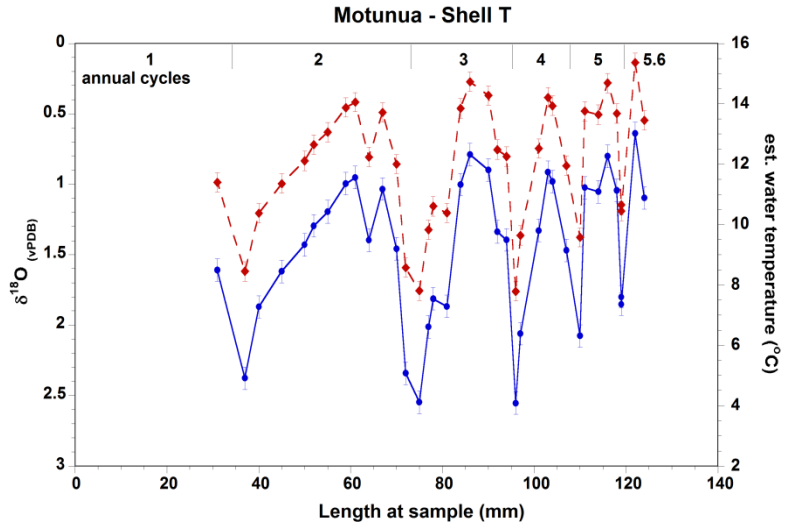

Akaroa - Shell O
annual cycles
1
2
3
4
5
5.5
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

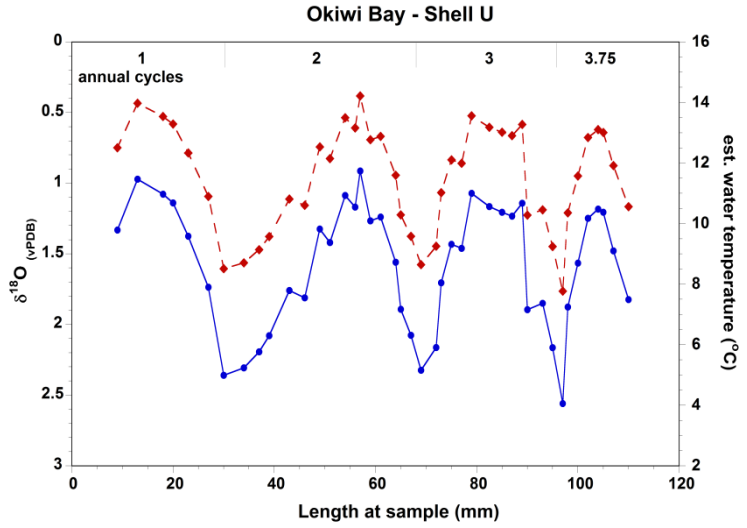

Akaroa - Shell P
annual cycles
1
2
3
4
4.5
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

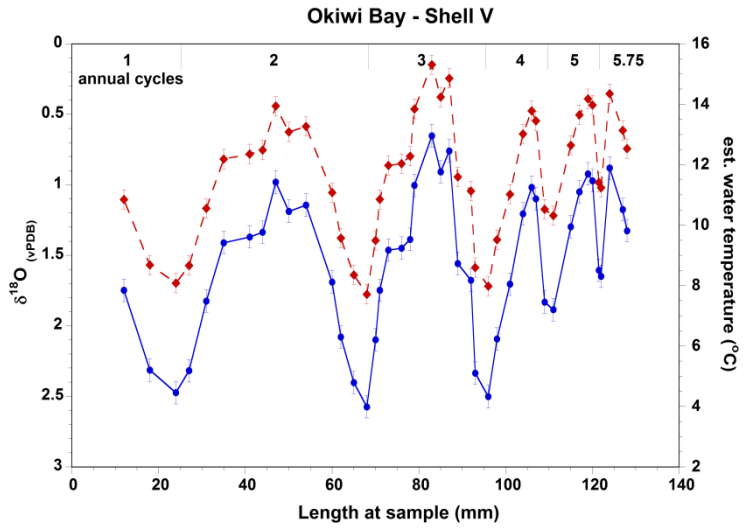

Motunau - Shell Q
annual cycles
1
2
3
4
4.6
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

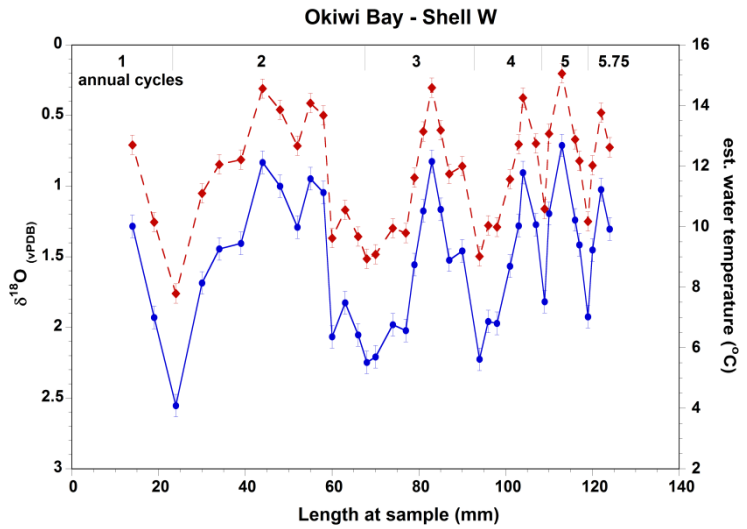

Motunau - Shell R
1
annual cycles
2
3
4
4.6
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)

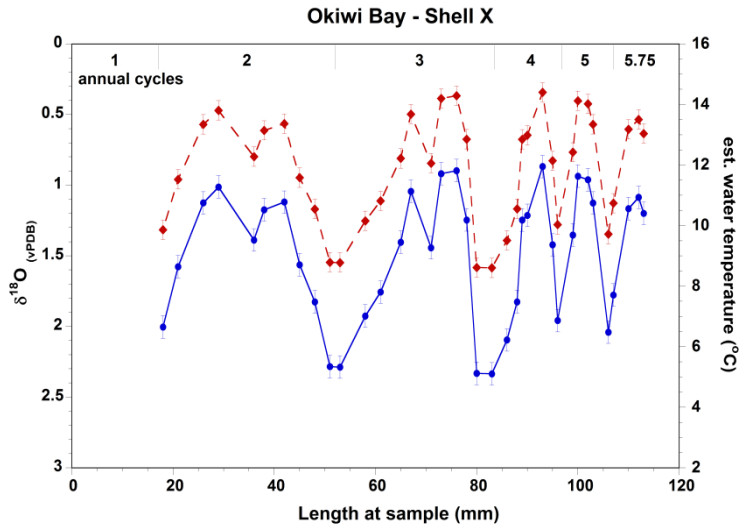

Motunau - Shell S
1
annual cycles
2
3
4
5
5.6
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)


Motunua - Shell T
1
annual cycles
2
3
4
5
5.6
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)


Okiwi Bay - Shell U
annual cycles
1
2
3
3.75
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)


Okiwi Bay - Shell V
annual cycles
1
2
3
4
5
5.75
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)


Okiwi Bay - Shell W
annual cycles
1
2
3
4
5
5.75
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)


Okiwi Bay - Shell X
1
annual cycles
2
3
4
5
5.75
δ18O (vPDB)
est. water temperature (°C)
Length at sample (mm)
0
0.5
1
1.5
2
2.5
3
16
14
12
10
8
6
4
2
0
20
40
60
80
100
120