INIS-mf--13898

93/40

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1993

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Institute of Geological & Nuclear Science Report 93/40

Institute of Geological & Nuclear Sciences Limited
Lower Hutt, New Zealand
September 1993

RECOMMENDED BIBLIOGRAPHIC REFERENCE

W J Trompetter, G E Coote, 1993. Strontium and fluorine in Tuatua shells. Institute of Geological & Nuclear Sciences Report 93/40. 27 pp.

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ISSN 1171 9184
ISBN 0-478-07075-6

ABSTRACT

This report describes the research to date on the elemental distributions of strontium, calcium, and fluorine in a collection of 24 Tuatua shells (courtesy of National Museum). Variations in elemental concentrations were measured in the shell cross-sections using a scanning proton microprobe (PIXE and PIGME). In this paper we report the findings to date, and present 2-D measurement scans as illustrative grey-scale pictures. Our results support the hypothesis that increased strontium concentrations are deposited in the shells during spawning, and that fluorine concentration is proportional to growth rate.

KEYWORDS:

Microprobe; Tuatua; Shells; Spawning; Growth rate; Strontium; Calcium; Fluorine; Cross-section.

CONTENTS

INTRODUCTION

An enormous amount of research has been undertaken over many years to understand the growth of bivalves and to provide an aging, and a seasonal dating technique. One particular line of research has been to measure elemental composition of the shells using a variety of analytical methods and sampling techniques. Prior to electron probes (EDAX : Energy Dispersive Analysis by X-rays) and proton microprobes (PIXE : Proton Induced X-ray Emission and PIGME : Proton Induced Gamma Ray Emission), measurements were whole shell averages or several discrete samples. At Nuclear Sciences we have a proton microprobe for PIXE and PIGME analysis which allows for much greater resolution of elemental distributions. This is described by Coote, Sparks & Blattner (1982); Coote & Vickridge (1988); and Vickridge (1984). Motivated by the archaeologists' need for a simple seasonal dating technique for shells (Sheppard 1985), microprobe analysis was performed on a collection of Tuatua shells. A collection of 24 shells (courtesy of National Museum) was collected from two Northland beaches over a 14 month period at approximately monthly intervals. Details of this collection are given in Appendix A.

We have previously reported on the experimental methods and some results of the elemental distributions of strontium, calcium, and fluorine of this collection in Trompetter & Coote (1990), Trompetter (1991), and Coote & Trompetter (1992). These elemental concentrations were measured on Tuatua shell cross-sections using a scanning proton microprobe (PIXE) in an effort to identify seasonal patterns.

In this report we report new findings and present measurements of 2-D scans which are much more illustrative than the 1-D scans that we have reported previously. Our results support the hypothesis that an increased strontium concentration is deposited in the shells during spawning, and that fluorine concentration is proportional to growth rate.

METHOD

Variations in elemental concentrations were measured using a proton microprobe (PIXE & PIGME). A Proton Microprobe scans a proton beam from a 3 MV Van de Graaff accelerator across polished cross-sections of Tuatua bivalve shells. As the protons bombard target nuclei, X-rays and gamma rays are emitted. Detectors and analysis equipment are set to count the X-rays or gamma rays of characteristic energies (called energy windows) that correspond to specific elements. Gamma rays emitted by fluorine are detected by a BGO (Bismuth Germanate) crystal and amplified by a photomultiplier, while both calcium and strontium emit X-rays detected by a SiLi (Silicon Lithium) crystal.

The amount of shell that can be scanned is limited by both the maximum range of the beam-scan (4 mm) and the time required to achieve reasonable counting statistics. 1-D scans are rapid and have good counting statistics. However, our system can also perform 2-D scans. These have inferior counting statistics and take longer, but they provide more illustrative information that is useful at an early stage of our investigations. Each 2-D scan was 100 channels across the shell's width (approx 2.5 mm), and 30 channels along the shell (max 4 mm). The dimensions of these scans were chosen to provide optimum conditions to observe the structure within the shell and to obtain a reasonable number of counts per channel within a reasonable amount of time, (1-2 hours).

The proton beam has an energy of 2.5 MeV focused to a 100 x 30 μm beam spot for 30 x 100 point 2-D scans and 10 x 10 μm beamspot for 200 point linear scans. It was found that for beam intensities $>$20 nC/100 μm^2/second that sputtering occurs where the shell surface shatters due to heating (turning it white). At these high beam intensities, sputtering causes elemental migrations which affect profile measurements. At lower intensities and for increased beam scanning speeds, the beam only scorches the shell turning it a dark brown (burning the protein) but leaves the shell material intact for reproducible measurements of elemental profiles. These effects are discussed by J A Cookson (1988).

To display and describe the variations of Ca, Sr and F as clearly as possible, two dimensional scans of the shell were made and plotted in grey-scale pictures.

RESULTS AND OBSERVATIONS

Many linear and two dimensional scans using the proton microprobe have been used to investigate how the element concentrations vary throughout a shell in an attempt to understanding of shell growth. Similar patterns were observed in all the shells of the collection.

Common features observed in the strontium and fluorine were:

1. All the shells have an enriched layer of strontium that extends a short distance along the outer surface at the umbo (typically 5-7 mm). This layer corresponds to the first growth of the shell after being spawned. Examples of this are seen in Figures 1a and 5a.

2. Several layers of enriched strontium propagate along the length of the shell in the inner nacreous layer, and curve across the shell width to the outside surface as the layers extend from the umbo towards the tip. The strontium layers correspond to some (but not all) of the dark bands that are observed under the microscope. In Figure 1a the strontium layers can be seen curving around the umbo and along the length. Figures 3a and 4a show a 2-D scan of the strontium layers fanning to the outer surface near the tip. This can also be seen in Figure 5a.

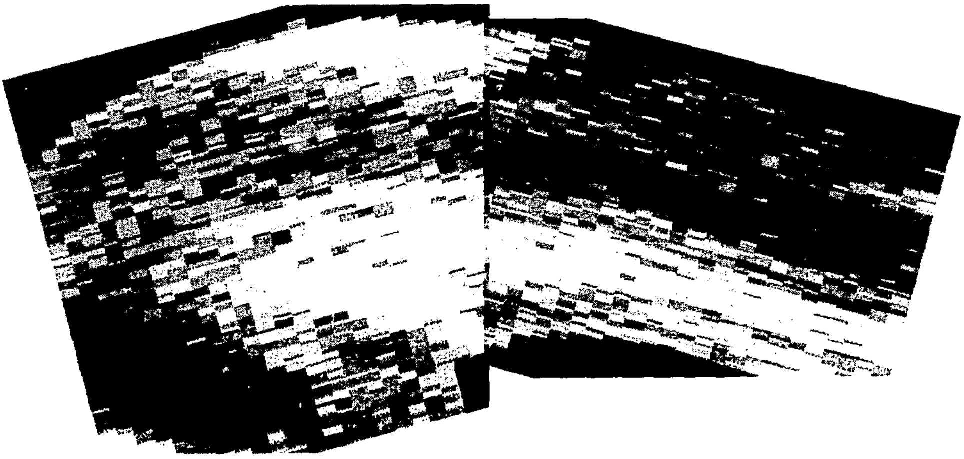
Note: The inner nacreous layer and the outer prismatic layer are disjoint and are not continuous across this boundary. However, it is assumed that the respective layers of high strontium concentrations are related across this boundary.

3. A faint thin layer of Sr and F extends along the outer surface of the shell to the tip. This can be seen in Figures 3a and 4a for the strontium, and Figures 3b and 4b for the fluorine. It is not an edge effect as it does not occur on the inner surface.

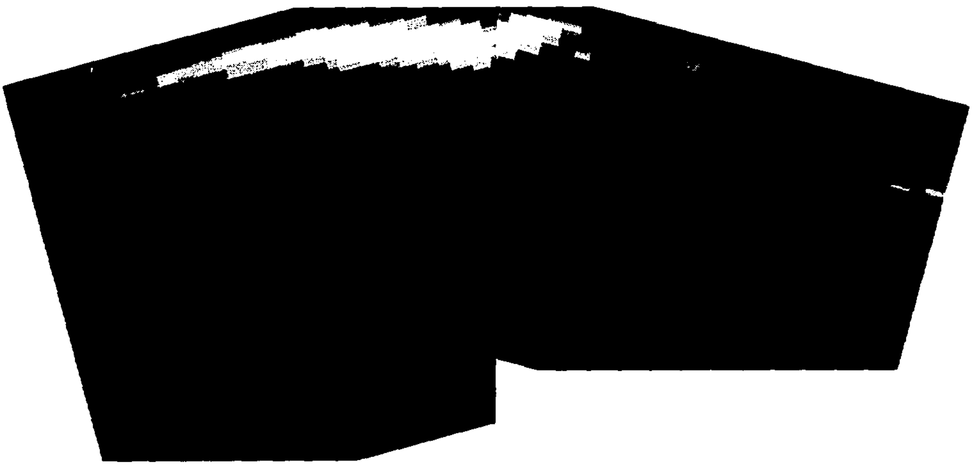
4. The outer prismatic layer contains higher fluorine concentrations than the inner nacreous layer. The prismatic layer is narrow near the umbo, with very high concentrations of fluorine, and then broadens with decreasing fluorine concentration as it extends towards

the tip. The fluorine concentration of shell AA411 can be seen in Figure 5b. This fluorine profile was constructed from six 2-D scans along the length.

5. The band of fluorine in the outer prismatic layer is occasionally interrupted by growth layers of reduced fluorine. This feature is illustrated in Figures 1b, 2b and 5b. When these scans are compared to the shell, it can be seen that the fluorine corresponds with the bright white layers of the outer prismatic layer.



Figures 1a & 2a. Sr near the umbo.



Figures 1b & 2b. F near the umbo.

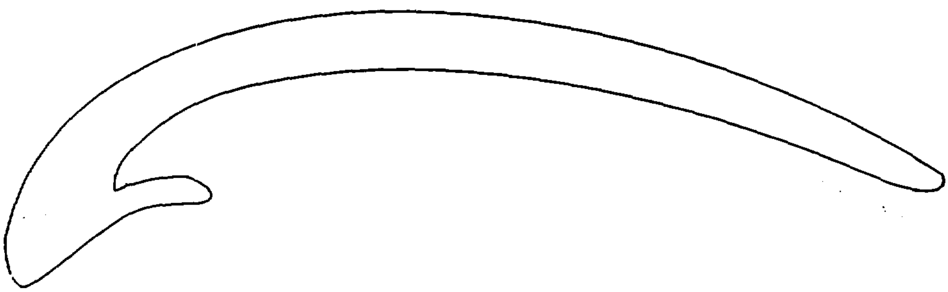


Diagram showing the 2-D scan positions on a shell cross-section.

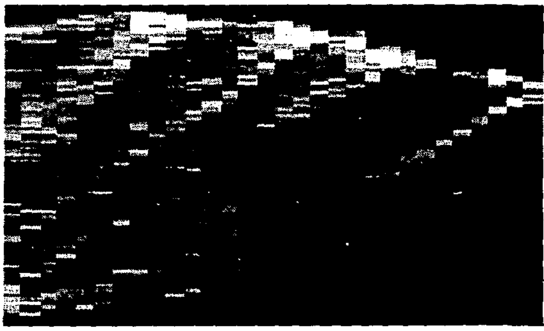


Figure 3a. Tip of AA407

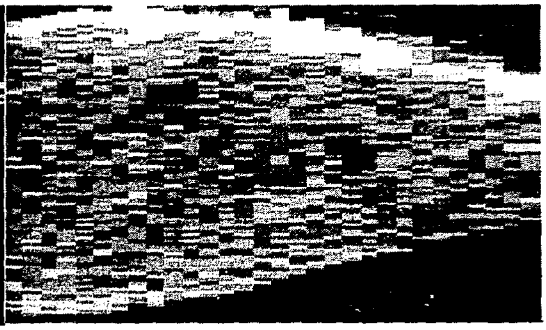


Figure 3b. F in tip of AA407

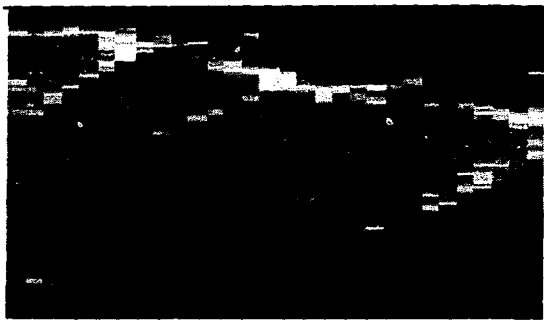


Figure 4a. Sr at tip of AA408.

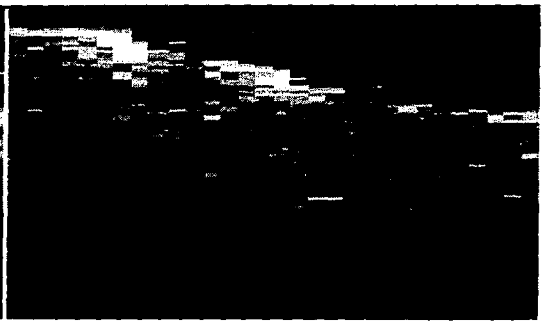


Figure 4b. F at tip of AA408.

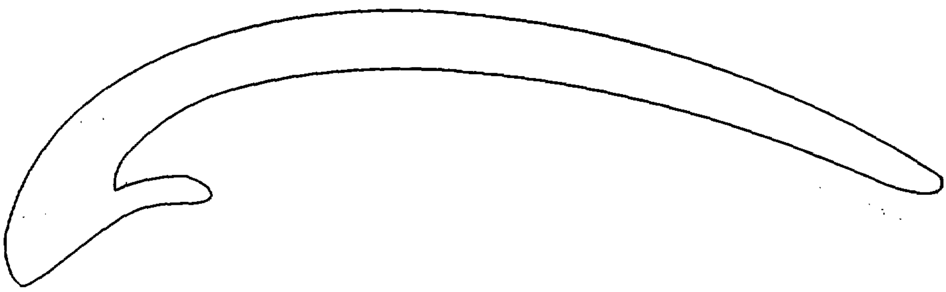


Diagram showing the 2-D scan positions on a shell cross-section.

Figure 5a: Strontium in shell AA411

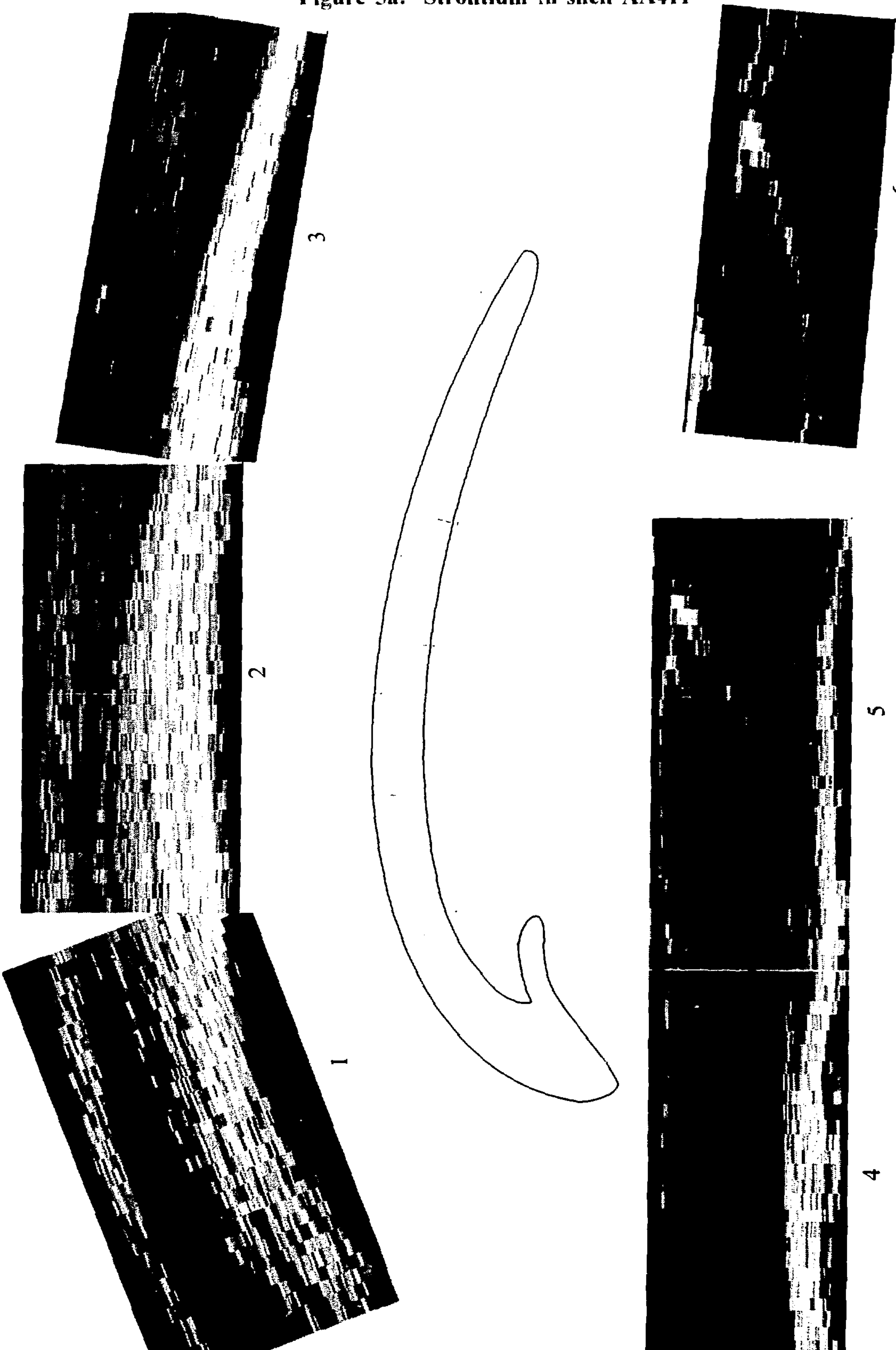


Figure 5b: Fluorine in shell AA411

STRONTIUM ANALYSIS

From the features of the strontium rich layers (SRLs) listed above, a number of observations can be made about the growth of shells.

High strontium concentrations were observed in the first growth of shells and in several layers in the shell's later life. In most shells the strontium rich growth layers were seen to extend nearly to the tip before fanning to the outer edge. Only one or two strontium rich layers curved to the outer shell edge in the midsection of these shells. This can be attributed to an exponential shell growth rate where they grow very rapidly in the early part of their life, and then grow very slowly in their later life, behaviour similar to human growth rates. Perhaps 80% of the shell growth occurs in approximately two years whilst the last 20% of the shells growth may represent 2-6 years growth (von Beralaffy's growth rate equation for shells is described in Rhoads and Lutz (1980)). The strontium layers do not always occur at exactly regular spacings or regular intervals taking an exponential growth rate in to account. To investigate the timing and correlation of the strontium rich layers between the shells collected at successive times, the distance between the "most recent" or "the last" strontium layer and the tip (measured along the outer edge) was plotted in Figure S1 against collection date. It is observed that the positions of the peaks are in two groups that are vertically displaced by 0.9 mm. Each group can be described by two lines, one of which has a near constant value during the winter half of the year (February-August) and the other has an increasing slope during the summer half of the year (August-February). The group at the bottom corresponds to an event in January-March. An important feature of this plot is to note that the shells that are in the top group (389, 411, 399, and 392) did not deposit a strontium rich layer (SRL) at the same time as the shells in the bottom group. Also the "latest" marking of shells AA391 and AA402 appears to be from earlier and later marking events respectively. This means that the mechanism that is responsible for the SRLs does not affect every shell every time.

The most likely cause of the SRLs is spawning. The first growth of the shell occurs when the shell is spawned as an embryo and it deposits a SRL. Then later in life once it is sexually mature it will itself spawn other embryos and deposit further SRL's. The relation between spawning and strontium concentration is supported by Bidwell *et al.* (1990) and Gallager *et al.* (1989) who report that strontium is required for the normal embryonic development of marine mollusca including bivalves.

Spawning is believed to occur in "seasons", although the timing of spawning and the critical factors which trigger the event are not accu ately known. Currently they are inferred by monitoring the condition factor of shells and observation of larvae in plankton (Booth (1983), Redfearn (1987)).

It is interesting to observe in Figure 5a how the SRLs propagate along the shell. They are quite widely spaced in the first half of the shell converging to a narrow band in the second half before fanning to the outer edge of the shell. These bands occur in the later stages of shell growth. This suggests that shell growth is predominantly along the shell length in the early stages of shell life. As the shell gets older it deposits material on the inner shell surface, growing across the width of the shell as well as along the length of the shell.

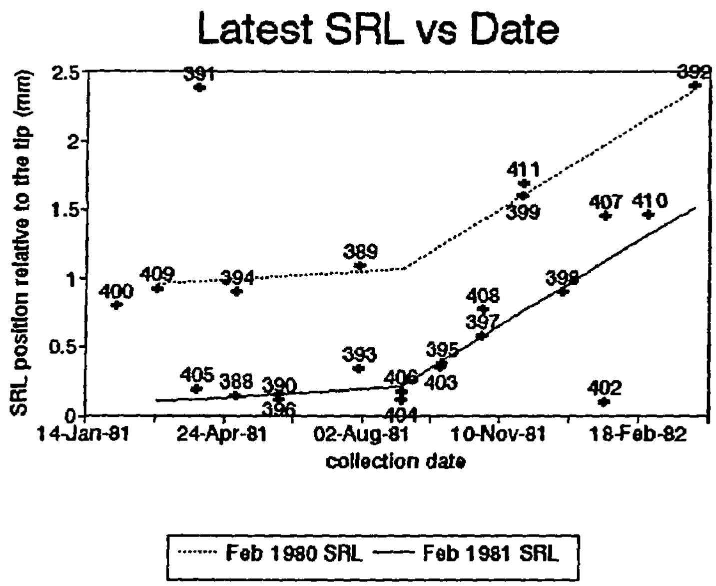


Figure S1. The distance of the most recent strontium rich layer (SRL) to the tip (measured along the outer edge) is plotted against collection date for shells collected at successive times.

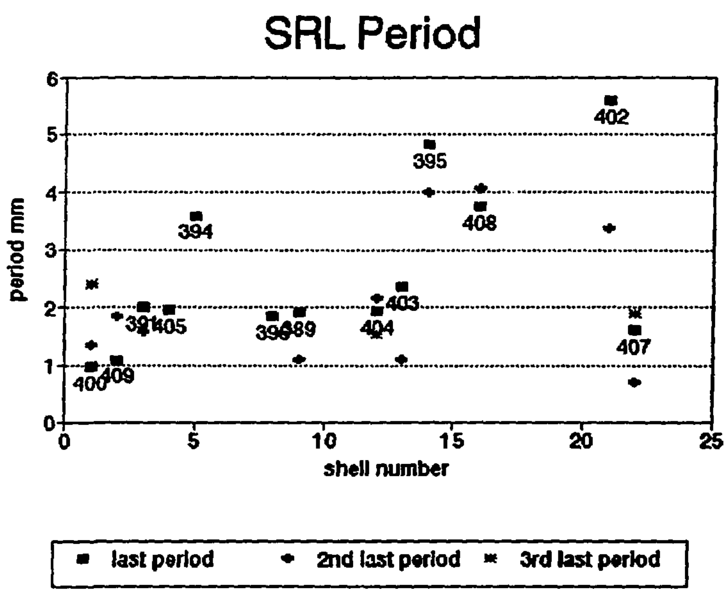


Figure S2. The spacings between the SRL's have been measured and plotted for each shell.

As the shells are collected at successive time intervals, more and more strontium depleted material is deposited on the shell's inner surfaces, increasing the distance to the last SRL. From the slopes of the lines in Figure S1 we can estimate the deposition rate or growth rate of the shells for the winter and summer periods.

In Figure S2 the spacings between SRLs that have been measured are plotted for each shell. Although the SRLs are not regularly spaced, they appear to be integral amounts of approximately 1 mm, 2 mm, 3.75 mm. The reason for the regular spacing of SRLs within particular shells is not clear. There are probably a number of factors which contribute to how regularly a particular shell spawns. Also growth rate may not decrease to zero as the von Beralaffy growth equation suggests but it may reduce to a constant value such as 1 mm per year.

FLUORINE ANALYSIS

The presence of fluorine in these shells was an unexpected bonus, discovered only recently and first reported in Coote and Trompetter (1992). The presence of fluorine is unexpected as it is not taken up by calcium carbonate because there is no space large enough for it within the crystal structure. However, supporting evidence was found in precipitation experiments by Kitano and Okumara (1973) and studies by Ichikuni (1979) who found that fluorine was coprecipitated as CaF_2 with calcium carbonate especially aragonite.

A number of observations of the fluorine concentration profiles the shells measured are listed below:

- The band of fluorine rich material in the outer prismatic layer was highest near the umbo and reduced approximately linearly as the band reached the tip. A grey-scale picture of the fluorine can be seen in Figure 5b and a graph of the fluorine profile can be seen in Figure F3. The fluorine concentration is unlikely to be due to absorption through the exterior surface as the band of fluorine should be broadest at the oldest part of the shell. However, the reverse is observed and it is more likely that the fluorine is incorporated into the shell structure during deposition. The rate of growth is also maximum near the umbo in the early part of the shell's life and thereafter falls off linearly (for an exponential growth rate) as the shell ages. In the next section the theoretical growth rate is compared with the measured fluorine concentration profile.

- Banding of the fluorine in the outer prismatic layer could be due to slow growth in winter periods. Based on the number of strontium rich layers observed at the tip region there would be a corresponding number of winter growths and hence bands in the fluorine. However, there are no significant breaks in the fluorine concentration near the tip region to support this. One possible explanation is that perhaps the growth rate has fallen below a certain threshold.

Growth rate and fluorine concentration comparison

In the literature eg Rhoads and Lutz (1980), the growth rate of shells is described by von Beralaffy's exponential growth equation -

$$l = L_{max}(1\text{-}\exp(-kt)) \qquad (1)$$

where l is length, L_{max} is the maximum length that shells growth in a given population, k is a growth constant, and t is time.

To get a relation for the rate of growth we differentiate the growth equation -

$$dl/dt = k.L_{max}.\exp(-kt)$$
$$= k(L_{max} - l) \qquad (2)$$

From equation (2) it can be seen that growth rate decreases as the length increases. Equations (1) and (2) are plotted in figures F1 and F2 respectively. The values for the constants used are; L_{max} = 40 mm and k = 1.5 years which are from earlier measurements made on the collection of Tuatua shells and reported in Trompetter (1991).

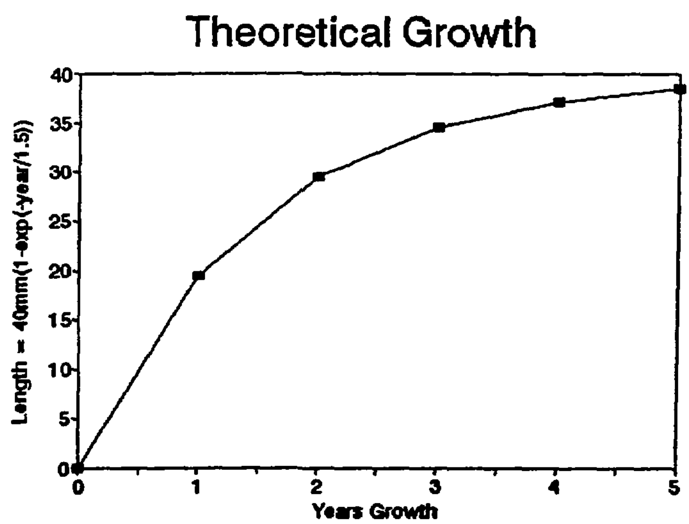


Figure F1. Plot of the theoretical growth of a shell.

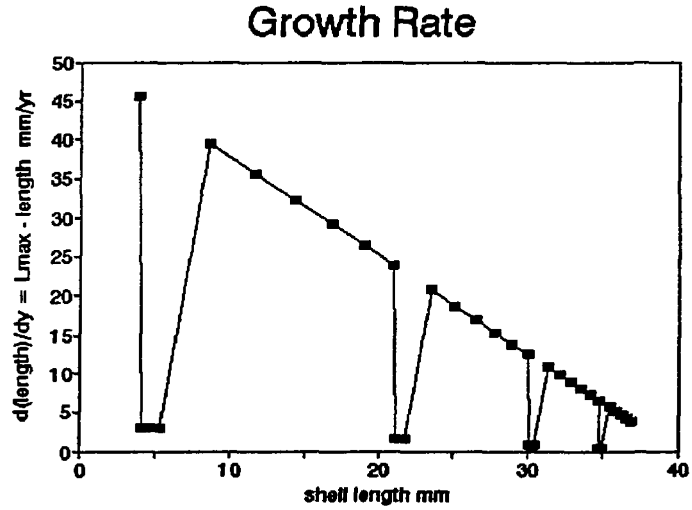


Figure F2. Plot of the theoretical growth rate.

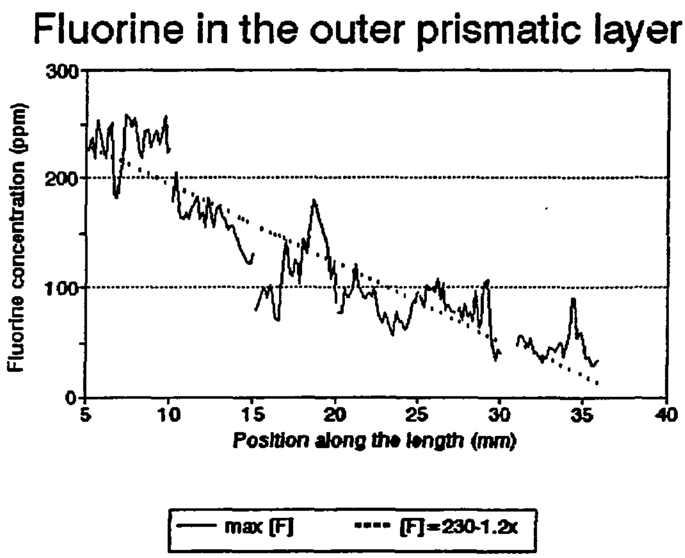


Figure F3. Measured profile of the fluorine concentration [F] in the outer prismatic layer of shell AA411 with the line of best fit.

The profile of fluorine concentration along the outer prismatic layer of the shell cross-sections is shown in Figure F3. The fluorine concentration decreases linearly with length, and is therefore proportional to growth rate. The line of best fit can be used to formulate an expression for the growth rate of a shell namely:

[Fluorine concentration : ppm] = 230 - 1.2 x [shell length : mm]

SUMMARY

Strontium : The strontium concentration appears to be an indicator of spawning. This has yet to be confirmed by measurements in recently spawned shells. However, from our research and the research on strontium in embryo shells, it seems a reasonable assumption.

From the spacings of the strontium rich layers (SRLs) a number of features of spawning and growth rate can be made:

1. In the shells measured, most of the SRLs were found in the very last part of the shells growth. This implies that spawning occurs in the later stages of the shells life once the shell reaches a sexually mature age.

2. Monitoring the distance to the "latest" marking for shells collected at monthly intervals revealed that shells did not deposit a SRL every year.

3. The spacings between the SRLs in some shells are regularly spaced. Hence this implies that once the shell has reached a certain age, its growth rate may approach a constant rather than continually decreasing.

4. Some SRLs were double bands. The possible implications of this are either that spawning occurred twice in that season or that the physiological conditions of spawning (that are responsible for the deposition of the SRLs) occurred twice before the shell actually spawned.

5. When shells are sexually mature and have started depositing SRLs it has been observed that they do not necessarily deposit a SRL each year.

Fluorine : The fluorine concentration appears to be proportional to growth rate. The reason for the banding in the fluorine is unknown.

FUTURE POSSIBILITIES

Measurements of the strontium profiles of shells can provide marine researchers with the spawning history of individual shells with information such as the frequency and timing of reproduction.

Applications for growth rate analysis using these SRLs also exist once the spawning patterns of the shellfish are understood. Once spawning and growth rate is understood then it may be possible to apply the measurement of strontium rich layers (SRLs) to seasonal dating.

Because fluorine concentrations are proportional to growth rate we are provided with an estimate of growth rate. This may have applications in the management of the shellfish resource.

ACKNOWLEDGEMENTS

We gratefully acknowledge the following people:

Dr Ian Vickridge of IGNS for writing software and assistance with producing the 2-D greyscale pictures used in this report.

Mr Chris Purcell of IGNS for maintaining the 3 MV accelerator and assistance with the accelerator operation.

Dr Foss Leach of the National Museum for providing the collection of shells used in this study.

APPENDIX A

Table of shell data (dimensions are measured from the shells tip in mm):

No	National Museum N	Collection date	Location	Length of X-section	1st Peak	2nd Peak	3rd Peak	4th Peak
1	AA400	05-Feb-81	Gap	31.0	0.80	1.75	3.10	5.50
2	AA409	06-Mar-81	Gap	32.0	0.92	2.00	3.85	
3	AA391	05-Apr-81	Gap	35.5	2.38	4.38	5.95	
4	AA405	05-Apr-81	Waik	36.4	0.19	2.15		
5	AA395	03-May-81	Gap	39.5	0.90	4.50		
6	AA388	03-May-81	Waik	39.0	0.14			
7	AA390	03-Jun-81	Gap	38.0	0.11			
8	AA396	03-Jun-81	Waik	36.0	0.15	2.00		
9	AA389	31-Jul-81	Gap	35.0	1.09	3.00	4.09	
10	AA393	31-Jul-81	Waik	40.0	0.33			
11	AA406	30-Aug-81	Gap	36.0	0.18			
12	AA404	30-Aug-81	Waik	36.0	0.15	2.30	3.85	4.75
13	AA403	28-Sep-81	Gap	37.0	0.35	2.70	3.80	
14	AA395	29-Sep-81	Waik	39.5	0.37	5.20	9.20	
15	AA397	28-Oct-81	Gap	34.0	0.57			
16	AA408	29-Oct-81	Waik	37.0	0.77	4.54	8.62	
17	AA399	28-Nov-81	Gap	38.0	1.60			
18	AA411	29-Nov-81	Waik	39.0	1.69			
19	AA401	25-Dec-81	Gap	35.3	--			
20	AA398	25-Dec-81	Gap	38.0	0.90			
21	AA402	25-Jan-82	Gap	37.5	0.10	5.69	9.08	
22	AA407	25-Jan-82	Waik	38.0	1.45	3.05	3.75	5.65
23	AA410	25-Feb-82	Waik	34.0	1.46			
24	AA392	29-Mar-82	Waik	36.0	2.40			

REFERENCES

G E Coote, R J Sparks and P Blattner, 1982. Nuclear microprobe measurement of fluorine concentration profiles, with application in archaeology and geology. *Nucl Instr Meth 197*: 213-221.

G E Coote and I C Vickridge, 1988. Application of a nuclear microprobe to the study of calcified tissues. *Nucl Instr Meth B30*: 393-397.

I C Vickridge, 1985. The INS nuclear microprobe; description and applications. *NZ J Technology 1*: 61-66.

R A Sheppard, 1985. Using shells to determine season of occupation of prehistoric sites. *NZ J Archaeology 7*: 77.

W J Trompetter and G E Coote, 1993. Aging and seasonal dating of tuatua shells using strontium markings. Proc 4th Australian Archeometry Conference, 1991 (in press).

W J Trompetter, 1991. Seasonal patterns in Tuatua shellfish; a search based on microprobe and electron microscope analysis. MSc thesis Victoria University.

G E Coote and W J Trompetter, 1993. Strontium and fluorine markings in shells of a mollusc (paphies subtriangulata) with potential importance in biology and archaeology. *Nucl Instr Meth B77*: 501-504.

J A Cookson, 1988. Specimen damage by nuclear microbeams and its avoidance. *Nucl Instr Meth B30*: 324-330.

D C Rhoads and R A Lutz, 1980. Skeletal growth of aquatic organisms. Plenum Press, New York.

J P Bidwell *et al.*, 1990. The effect of strontium on embryonic calcification of aplysia californica. *Biol Bull 178*: 231-238.

S M Gallager *et al.*, 1989. Strontium is required in artificial seawater for embryonic shell formation in two species of bivalve mollusca. Origin, History and Modern Aspects of Biomineralisation in Plants and Animals. R Crick, ed., Plenum press, New York: 349-366.

J D Booth, 1983. Studies on twelve common bivalve larvae, with notes on bivalve spawning seasons in New Zealand. *NZ J Marine Freshwater Res 17*: 231-265.

P Redfearn, 1987. Larval shell development of the northern tuatua, *Paphies subtriangulata (Bilvalia Mesodesmatidae)*. *NZ J Marine Freshwater Res 21*: 65-70.

Y Kitano and M Okumura, 1973. Coprecipitation of fluoride with calcium carbonate. *Geochemical Jl 7*: 37-49.

M Ichikuni, 1979. Uptake of fluoride by aragonite. *Chem Geol 27*: 207-214.