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Potential techniques for ageing paua

New Zealand Fisheries Assessment Report 2015/69

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

Naylor, R.; Neil, H.; Turnbull, J.C.; Max, T.; Fu, D. (2015). Potential techniques for ageing paua.

***New Zealand Fisheries Assessment Report 2015/69.* 21 p.**

Several techniques to estimate age in paua have recently been investigated, including the counting of protein layers in the shell, oxygen isotope ratio techniques and radiocarbon dating. The purpose of this project was to compare the accuracy of the three different techniques listed above and evaluate if the number of protein layers in the shell is an adequately accurate measure of the age of a paua.

The estimated age of nine paua shells was determined using radiocarbon dating. Oxygen isotope ratio estimates of age for these shells either already existed or were obtained. Similarly, counts of the number of protein layers in the shells had already been made or were made. Results from the three age estimation methods are compared here. In three of the nine shells the estimate of age was similar across all three techniques. In the other six shells the number of protein layers in the shell and the estimated isotopic ages were much more similar than the radiocarbon dating results. The estimation of age in paua from radiocarbon dating does not appear to consistently reflect the age estimated by the analysis of oxygen isotope ratios or the number of protein layers in the shell. While refinement of the radiocarbon ageing method may lead to more reliable results, the inconsistency in the results of this method, and the high cost of pursuing this method any further, suggests that radiocarbon ageing is the least favourable of the three methods explored. The similarity in results from the oxygen isotope and protein layer count ageing methods suggests that further work using the oxygen isotope method as a means of ground-truthing the protein layer count method is worth considering.

1. INTRODUCTION

The ability to age paua may allow the development of an age-based stock assessment model, more accurate estimates of growth and the setting of more appropriate regional minimum legal sizes within the paua fishery. However the number of samples that would be required to be analysed, in order to adequately inform an age-based model or determine more appropriate minimum legal sizes, would not be insubstantial and a cost effective technique that performs with a suitable level of accuracy, would need to be used. The potential accuracy of several techniques have recently been investigated, including the counting of protein layers in the shell, oxygen isotope ratio techniques and radiocarbon dating. Both radiocarbon dating and oxygen isotope ratio techniques are more costly than protein layer counts with costs being around $800/per shell for the first two techniques and around $30 per shell to obtain protein layer counts, making this the most economically feasible technique. Preliminary work looking at the accuracy of estimating the age of paua using radiocarbon dating was inconclusive, while previous work using oxygen isotope analysis and counts of protein layers appears more promising. The purpose of this work (Ministry for Primary Industries [MPI] project PAU2014-03) was to compare the accuracy of the three different techniques listed above and evaluate whether the number of protein layers in the shell is a suitable measure of paua age.

The objectives of the project are:

1) Determine the effectiveness of radiocarbon dating, oxygen isotope analysis and growth ring counts for ageing paua to the accuracy of within a year of true age.
2) Compare the effectiveness of the 3 techniques from objective 1 for ageing paua to the accuracy of within a year of true age.

2. METHODS

2.1 Radiocarbon analysis

NIWA provided GNS Science with five paua shells which had already undergone stable oxygen isotope analyses and had been sectioned to count the number of protein layers in the vertical cross section of the spire (Naylor & Neil 2015). That work was done as part of MPI project PAU2011-05. GNS estimated the age of these shells using radiocarbon techniques (Appendix 5). GNS also obtained four paua shells from Museum of New Zealand Te Papa Tongarewa which were collected between 1965 and 1987. The age of these shells was similarly estimated using radiocarbon techniques (Appendix 4), then the shells were forwarded to NIWA for stable oxygen isotope analysis and sectioning to determine the number of protein layers in the shells.

The use of radiocarbon dating for validating ages was developed for fisheries science relatively recently (Kalish 1993), and it has been used most successfully for relatively shallow water species. It is based on levels of radiocarbon in the ocean, which are known to have changed over recent decades. Nuclear testing in the Pacific region introduced a pulse of ^{14}C into the atmosphere between 1950 and 1970, which entered the ocean and quickly became incorporated in the carbonate of hard tissues (coral skeletons, fish otoliths, mollusc shells) in concentrations proportional to those in the water column. The first age-validation studies used otolith core material to position a fish somewhere on the time-line of radiocarbon levels, thus giving it a birth-date. Turnbull (2015) sampled the oldest part of the shell, then subtracted this date from the known date of collection to estimate age at the time of collection. The main results of that report are presented here to allow a comparison of ageing techniques.

2.2 Oxygen isotope analysis

The basis of the oxygen isotope method is that variation in the $^{18}O/^{16}O$ ratio ($\delta^{18}O$) of the carbonate of mollusc shells reflects water temperatures in the sea at the time that the shell was precipitated. In cold

water more of the heavier ^{18}O isotope is precipitated into the shell, while in warmer temperatures relatively more of the lighter ^{16}O isotope is precipitated. This allows the annual temperature cycle preserved along the growth axis of the shell to be used as year tag or marker, which in turn allows growth at length and age to be estimated for individual shells.

Shells for the estimation of oxygen isotope ratios were cleaned of epibiota, and calcite samples were removed from the outer shell with a micro-sampling device at about 2 mm intervals along the growth axis of the shell. Previous studies (Naylor et al. 2007) indicate that this sampling resolution should be sufficient to provide about 10 samples within each estimated season of growth over the length span of interest. Samples were taken along the entire growing axis of the shell if possible. Because the spire is the oldest part of the shell, as well as the highest part, the calcite in this region is often eroded, which prevents sampling. Because paua generally grow between about 20–30 mm in their first year (Poore 1972, Sainsbury 1982, Naylor et al. 2006), if sampling is not possible within 20 to 30 mm of the spire, another isotopic temperature cycle is added to the count of cycles revealed by the isotopes.

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 Vienna Pee Dee Belemnite (VPDB) international standard (Epstein et al. 1953). External reproducibility was ensured via calibration to National Bureau of Standards NBS-19 and internal standards.

Shell length at each sample was measured to the nearest millimetre with vernier callipers and the estimated isotopic temperature for each sample is plotted against shell length. The data vectors and plots were manually inspected, and the length-temperature pairs representing summer high and winter low temperatures were identified. High or low points were chosen only if they were preceded and followed by at least two points supporting the trend of estimated temperature increase or decline. The number of cycles was estimated visually to within half a cycle. Naylor & Neil (2015) estimated cycles by assuming a birthdate at the beginning of August and terminating the cycle at the time of capture to provide a decimal estimate of age.

2.3 Protein layers

After the oxygen isotope samples had been taken from the four historic shells, they were sectioned vertically through the spire with a hand held cutting tool and the number of protein layers was counted under a binocular microscope and then photographed. The number of protein layers in the shells of overseas abalone has been used as a proxy for age. The large body of literature relating to the estimation of age using counts of these layers is variable and conflicting. In New Zealand during the 1980s, counts of these layers were routinely used to age paua (Murray & Ackroyd 1984, Murray 1986, Petherick 1987). There is however, no documented evidence that these layers are laid down on an annual basis. Counting these layers in this project is part of ongoing work to determine whether they may be useful as an ageing tool.

2.4 Effectiveness of techniques

A generalised linear model was used to regress the number of protein layers and the age estimated by radiocarbon dating against the isotopic estimate of age. We assumed that the age estimated as the number of seasonal temperature cycles recorded in the carbonate of the shell was the true age of the shell.

3. RESULTS

3.1 Age estimates

The stable oxygen isotope ratio profiles for the four historic shells are shown in Figure 1. In all four shells, sampling could not be done along the first 20 mm of the shell because the calcite layer had been eroded. In the estimation of age from isotope ratios the first cycles in all four shells were therefore completed to adjust estimates for the unsampled portion. The age estimate for shell A is uncertain due to some sharp fluctuations in the isotopic ratio, but the cycles probably represent 5 seasonal cycles. The isotopic ratio cycles for shells B, C, and D are relatively clear (Figure 1) and appear to represent 3, 4.5, and 3 seasonal cycles respectively (Figure 1, Table 1). Estimated ages from oxygen isotope profiles for shells E, G, Q, R, and U are from Naylor & Neil (2015). Images of vertical cross sections from shells A, B, C, and D are shown in Appendix 1. The number of protein layers in the remaining shells are from Naylor & Neil (2015). Radiocarbon age estimates (Turnbull 2015), oxygen isotope age estimates and the number of protein layers are shown in Table 1.

Table 1. Shell location, date of capture, age estimates based on radiocarbon dating and oxygen isotope analysis, number of protein layers in the shell and shell length. Radiocarbon ages from Turnbull (2015).

ID	Location	Date of capture	^{14}C-based age	Protein layers	^{18}O-based age	Length (mm)
A	Te Kaha, Bay of Plenty	1/1/65	5?	5	5	109
B	Island Bay, Wellington	5/10/75	7	2	3	93.5
C	Ahipara Bay, Northland	16/1/87	0–13	5	4.5	106.5
D	Flat Point Wairarapa	6/10/79	8	2	3	89
E	South Coast, Fiordland	6/12/05	5	5	5.9	128
G	South Coast, Fiordland	6/12/05	7	7	6.9	152
Q	Motunau, Canterbury	15/3/12	2	5	6.5	114
R	Motunau, Canterbury	15/3/12	1	4	6.5	105
U	Okiwi Bay, Kaikoura	26/4/12	1	4	5.0	110

Full details of the measurements from the stable isotope analyses are given in Appendix 3.

For three shells, A, E, and G, estimates of age from radiocarbon dating and from oxygen isotope analyses are similar, as are the number of protein layers in the shells (Table 1). For shells B and D, the ages estimated by radiocarbon dating are 3.5 and 5 years older than the estimates from oxygen isotope analyses (Table 1). These shells are 93.5 mm and 89 mm respectively and are from areas of fast growth. The number of protein layers in these shells is one less than the estimated isotopic age (Table 1).The estimated age for shell C from radiocarbon dating is not conclusive (between 0 and 13 years) and the number of protein layers is similar to the estimated isotopic age (Table 1). The estimated age from radiocarbon dating for shells Q, R, and U, are 4.5, 5.5, and 4 years lower respectively than isotopic estimates (Table 1). The growth rates suggested by the radiocarbon age estimates for these shells exceed published estimates of growth. The number of protein layers in these shells is also lower (by between 1 and 2.5 years) than the isotopic estimates (Table 1).

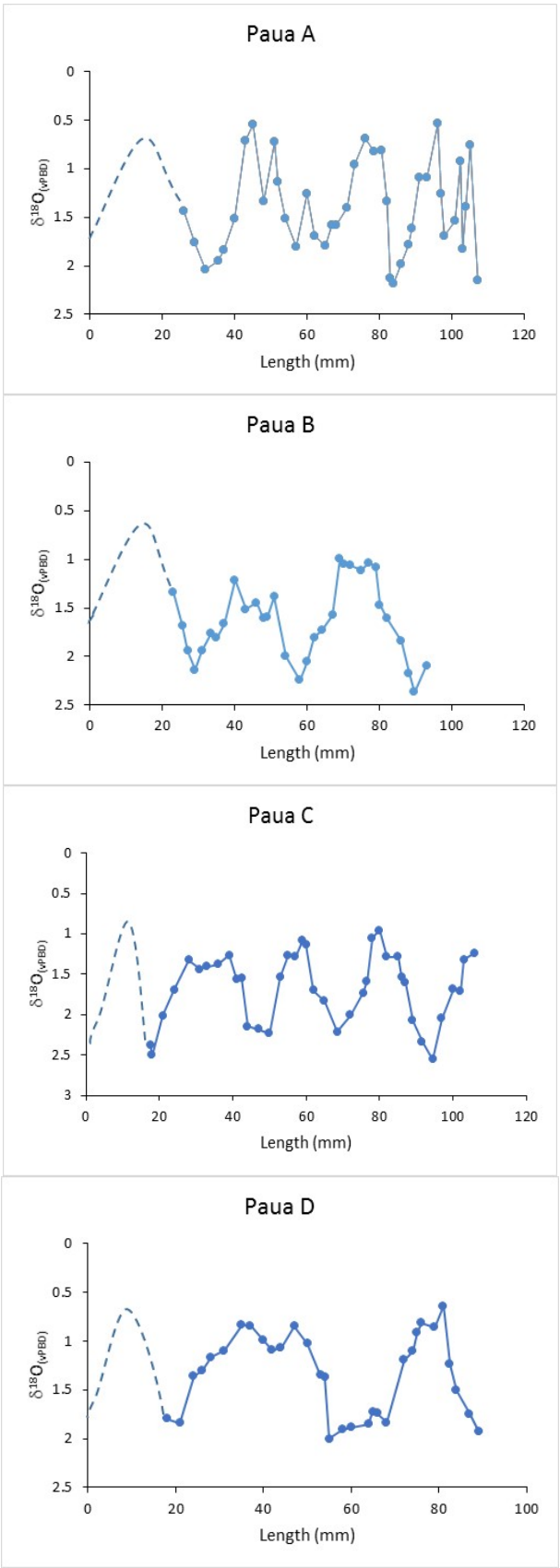

Figure 1: Oxygen isotope profiles of historic shells. Dotted line represents cycle added to compensate for unsampled portion of the shell.

3.2 Relationships between age estimates

The regressions of radiocarbon age estimates and the number of protein layers against estimated isotopic age are shown in Figures 2 and 3 respectively.

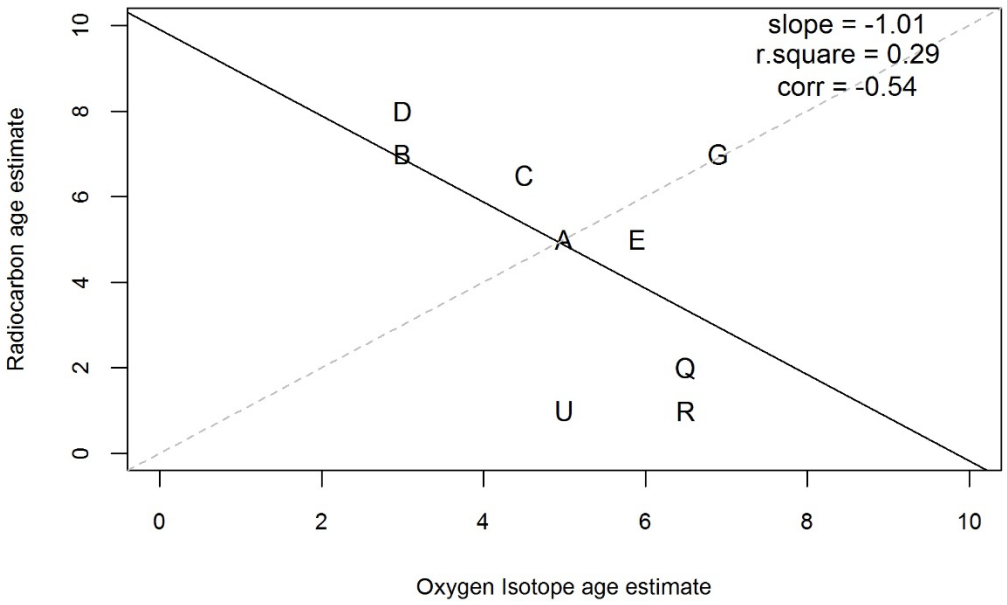

Figure 2: Regression of radiocarbon and oxygen isotope age estimates. Dotted line indicates a 1 to 1 relationship.

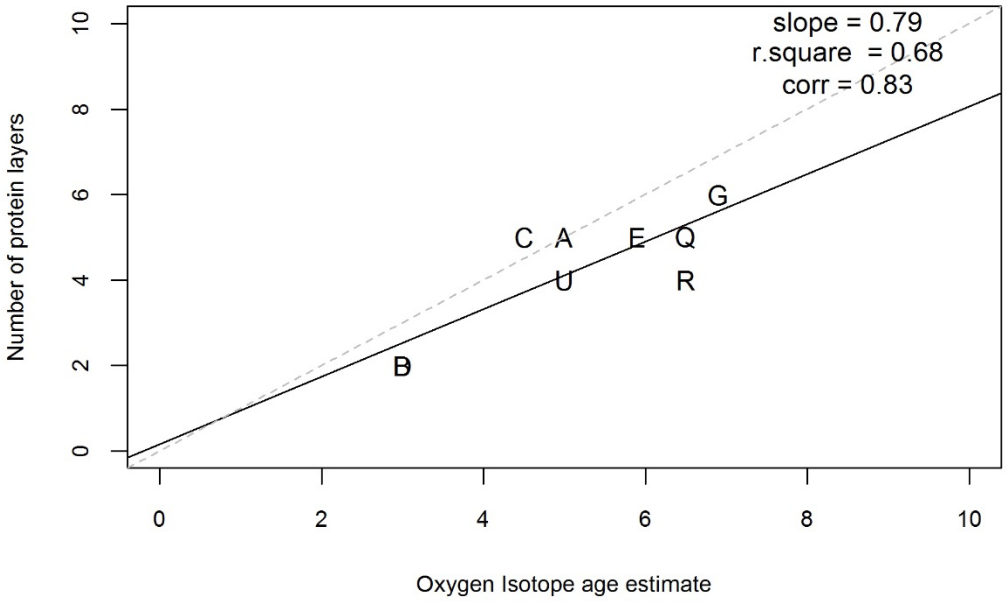

Figure 3: Regression of the number of protein layers in the shell and oxygen isotope age estimates. Dotted line indicates a 1 to 1 relationship.

Radiocarbon age estimates were inversely correlated with oxygen isotope age estimates and the associated variance was large (Figure 2). There was a good correlation between the number of protein

layers in the shell and the age estimated by oxygen isotope methods, but the relationship was not one to one (Figure 3).

4. DISCUSSION

The ability to age paua may allow the development of age-based stock assessments which may be more precise than assessments based on the current length-based model. The oxygen isotope method for ageing paua has been validated by examining shells which had been tagged in previous work, where it was found that known length increments determined by tagging corresponded with the increments determined by stable oxygen isotope analysis (Naylor et al. 2007).

Although there appears to be a good correlation between the number of protein layers in the shell and the age estimated by oxygen isotope ratios, the relationship is not one to one. It appears that the protein layers in the shell are not always laid down at the same time each year, even at the same site, so it has been suggested that the deposition of these layers is not seasonal (Naylor & Neil 2015).The seasonality of protein layer deposition is currently being investigated and needs to be resolved before the utility of this ageing method can be properly assessed.

While radiocarbon age estimates were similar to oxygen isotope estimates in three of the nine shells, radiocarbon dating does not appear to consistently reflect the age estimated by the analysis of oxygen isotope ratios in the shell. Some estimates made using the radiocarbon method were either markedly higher or lower than the oxygen isotope estimates. Where the radiocarbon estimates of age are lower than oxygen isotope estimates, the implied growth rates are much faster than any reported in the literature. In two cases where the radiocarbon estimates of age are much higher than oxygen isotope estimates, the implied growth rates also appear implausible as the paua are from areas of known fast growth and are less than 100 mm long.

It is possible that more reliable results could be achieved with an improved ^{14}C surface water history for the waters around New Zealand and with improved shell sampling techniques (Turnbull 2015). However, because both radiocarbon dating and oxygen isotope ageing methods are much more expensive than counting protein layers in the shell (approximately $800/shell for the first two methods compared to approximately $30/shell for the latter) the utility of both of these techniques is more likely to be in the validation of less expensive ageing methods, including the count of protein layers investigated in this project.

5. ACKNOWLEDGMENTS

We are grateful to Jocelyn Turnbull from GNS Science for producing the radiocarbon age estimates and to Peter Horn for reviewing this report. The work was funded by the Ministry for Primary Industries project PAU2014-03.

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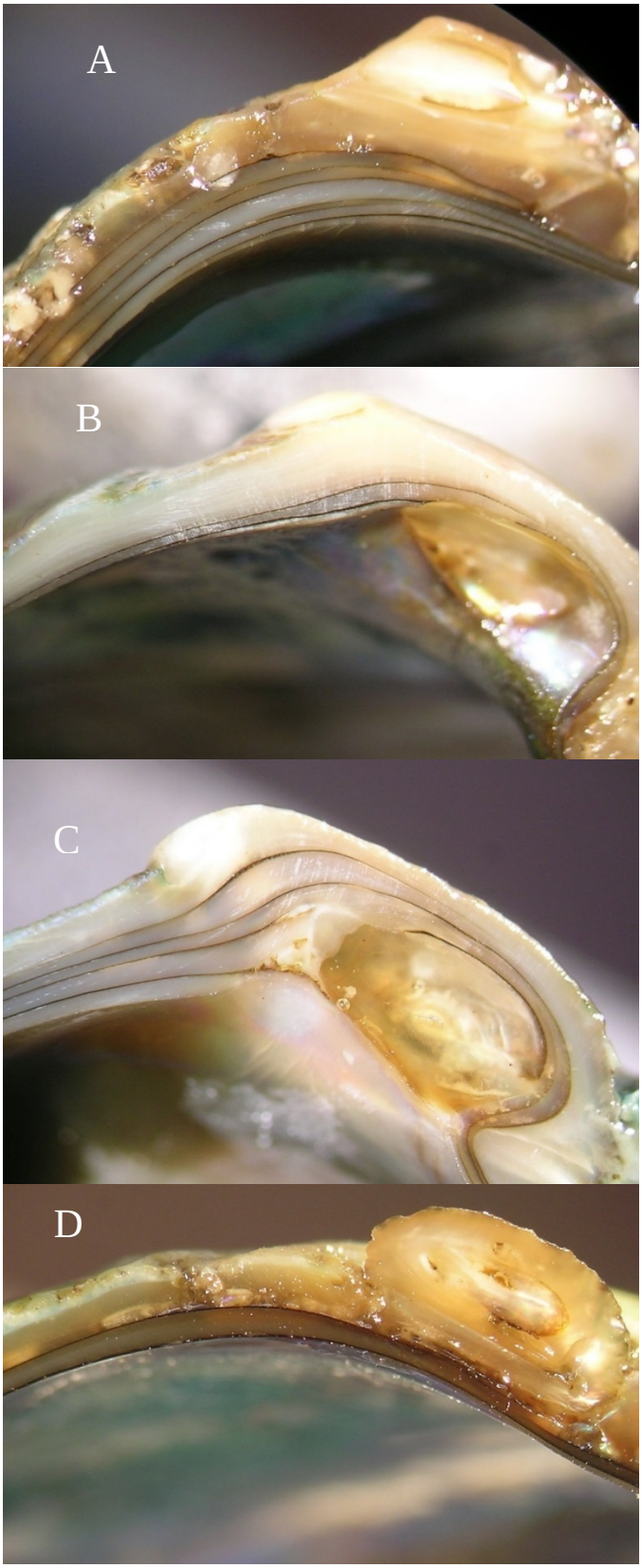
Appendix 1: Vertical cross sections of historic shells A, B, C, and D.



Appendix 2: Precision Of Mass Spectrometer Measurements

Precision of Measurements

All values reported relative to vPDB, where $\delta^{18}O$ has a value of -2.20‰ for NBS19 calcite.

Internal precision of measurements is 0.01-0.03 for $\delta^{18}O$. External precision is 0.08‰ for $\delta^{18}O$, relative to vPDB.

Appendix 3: Oxygen Isotope Results Tables

Table A2: *Paua A-D* Data Tables

Paua A

Sample no.	Length (mm)	$\delta^{18}O_{(vPBD)}$
1	107	2.151
2	105	0.757
3	104	1.391
4	103	1.829
5	102.5	0.925
6	101	1.540
7	98	1.690
8	97	1.256
9	96	0.530
10	93	1.091
11	91	1.095
12	89	1.615
13	88	1.785
14	86	1.983
15	84	2.180
16	83	2.126
17	82	1.336
18	80.5	0.815
19	78.5	0.818
20	76	0.691
21	73	0.962
22	71	1.401
23	68	1.585
24	67	1.580
25	65	1.797
26	62	1.688
27	60	1.252
28	57	1.801
29	54	1.515
30	52	1.140
31	51	0.726
32	48	1.337
33	45	0.548
34	43	0.715
35	40	1.519
36	37	1.839
37	35.5	1.949
38	32	2.039
39	29	1.759
40	26	1.432

Paua B

Sample no.	Length (mm)	$\delta^{18}O_{(vPBD)}$
1	93	2.094
2	89.5	2.363
3	88	2.173
4	86	1.840
5	82	1.603
6	80	1.470
7	79	1.078
8	77	1.035
9	75	1.113
10	72	1.060
11	70	1.051
12	69	0.992
13	67	1.574
14	64	1.731
15	62	1.802
16	60	2.046
17	58	2.240
18	54	1.999
19	51	1.384
20	49	1.590
21	48	1.607
22	46	1.446
23	43	1.511
24	40	1.213
25	37	1.664
26	35	1.809
27	33.5	1.761
28	31	1.944
29	29	2.136
30	27	1.940
31	25.5	1.678
32	23	1.343

Table A2 continued.

Paua C			Paua D		
Sample no.	Length (mm)	$\delta^{18}O_{(vPBD)}$	Sample no.	Length (mm)	$\delta^{18}O_{(vPBD)}$
1	106	1.241	1	89	1.923
2	103	1.316	2	87	1.745
3	102	1.714	3	84	1.506
4	100	1.685	4	82.5	1.239
5	97	2.045	5	81	0.638
6	94.5	2.557	6	79	0.856
7	91.5	2.331	7	76	0.813
8	89	2.076	8	75	0.906
9	87	1.607	9	74	1.103
10	86	1.530	10	72	1.191
11	85	1.282	11	68	1.835
12	82	1.283	12	66	1.738
13	80	0.955	13	65	1.722
14	78	1.049	14	64	1.851
15	76.5	1.586	15	60	1.883
16	75.5	1.734	16	58	1.908
17	72	2.010	17	55	1.999
18	68.5	2.211	18	54	1.368
19	65	1.830	19	53	1.346
20	62	1.698	20	50	1.027
21	60	1.136	21	47	0.848
22	59	1.077	22	44	1.071
23	57	1.284	23	42	1.091
24	55	1.262	24	40	0.993
25	53	1.536	25	37	0.841
26	50	2.235	26	35	0.833
27	47	2.183	27	31	1.099
28	44	2.148	28	28	1.164
29	42.5	1.554	29	26	1.300
30	41	1.559	30	24	1.358
31	39	1.263	31	21	1.838
32	36	1.381	32	18	1.794
33	33	1.408			
34	31	1.436			
35	28	1.325			
36	24	1.691			
37	21	2.014			
38	18	2.498			
39	17.5	2.383			

Appendix 4: Results Of Radiocarbon Analysis Of Paua *(Haliotis Iris)* Shell

Phase 1

Methods

Five paua shells were supplied. These were all spawned at the Mahanga Bay aquarium, which receives water directly from the harbour intake, and the water is circulated rapidly, so that we expect the aquarium water to reflect the ^{14}C content of the harbour seawater.

The paua were all collected on the same date, 5 March 2013. The oldest animal was spawned in 2000, the youngest in 2009. For each animal, two subsamples were taken, from what was expected to be the youngest and oldest parts of the shell. Figure 1 shows the samples taken for one of the shells; photos of the other shells were also kept should they need to be referred to. Surface material was removed and a shell section ~1 cm square was selected for measurement. Each sample was etched to remove surface contamination and then hydrolyzed to produce CO_2 gas, which was reduced to graphite and measured by high precision accelerator mass spectrometry. Results are reported in $\Delta^{14}C$, which is the ^{14}C content relative to a standard, and corrected for radioactive decay since the time the shell grew. We used a decay correction based on the expected age of the shell section, so the decay correction is larger for the old parts of the shells than for the young parts.

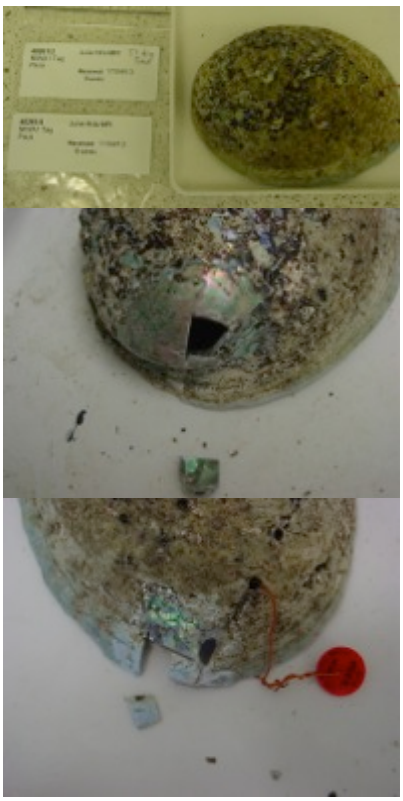


Figure 1. Sampling of NIWA1 Tag6350 (spawned 2003). Top: original shell. Middle: sampling of oldest part of shell. Bottom: sampling of youngest part of shell.

Results

Figure 2 shows the measured values. All of the young (nominally 2013) subsamples have consistent $\Delta^{14}C$ of 41.6 ± 1.2‰. Reported uncertainties are 1.6‰, there is no significant difference amongst these results. The two youngest shells do have slightly (but not significantly) lower $\Delta^{14}C$ values. Water collected from the aquarium inlet on 2 May, 2013, has a $\Delta^{14}C$ significantly lower than these shells, at 38.5±1.5‰.

A small difference between the water and shell $\Delta^{14}C$ is not unexpected, since the water is an instantaneous sample that reflects the $\Delta^{14}C$ on the date of collection, whereas the shell sample is an average over at least one season with potentially varying $\Delta^{14}C$ during that time. For example, Druffel & Griffin (2008) showed that day-to-day variability of 10–15‰ can be superimposed on the long-term and seasonal trends in surface ocean $\Delta^{14}C$.

The oldest parts of the shells show a trend to larger $\Delta^{14}C$ values, consistent with a downward trend in surface ocean $\Delta^{14}C$, but the trend is much smaller than expected, and is only apparent in the two oldest shells (spawned in 2000 and 2003, with $\Delta^{14}C$ of 48.0±1.6 and 49.2±1.6‰, respectively). These two samples are not statistically distinguishable from one another. The remaining three older samples have $\Delta^{14}C$ similar to, or lower than, the $\Delta^{14}C$ of the young part of the samples.

Discussion

A downward trend in $\Delta^{14}C$ in surface ocean water, and therefore in $\Delta^{14}C$ in the shell material was expected, with a magnitude of about 3‰/yr. This would predict a difference of ~40‰ between the older and younger parts of the shell spawned in 2000. In fact the trend is much smaller, at about 0.5‰/yr. We consider three possible explanations for this unexpected result.

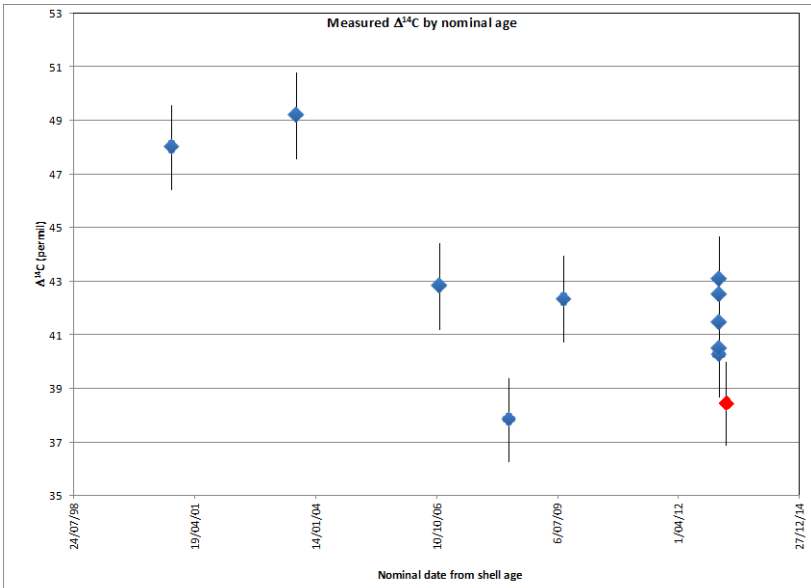


Figure 2. Measured $\Delta^{14}C$ for shell samples, shown by nominal age of the shell material measured. For the youngest part of each shell, age is assumed to be the date of collection (March 2013), and for the older parts of the shells, age is assumed to be the spawning date. Red point is the seawater sample collected at Mahanga Bay in May 2013.

Explanation 1.

All five animals were spawned inside the Mahanga Bay aquarium and were raised there for ~18 months before being released into the harbour. The water being pumped into the aquarium (and hence the water that the paua take up and that reflects their $\Delta^{14}C$) is expected to have the same $\Delta^{14}C$ as the harbour water, so that the paua carbon content would reflect that of the seawater at the time. If the aquarium water $\Delta^{14}C$ were altered relative to the seawater $\Delta^{14}C$, then this would give spurious results. If the food (seaweed?) that the paua eat did not grow in local seawater at about the same time, then this would also result in a spurious $\Delta^{14}C$ content in the paua. However, we might expect that either of these possibilities would result in $\Delta^{14}C$ close to the atmosphere of the time (~90‰ in 2000), rather than the observed values that are lower than the atmosphere of the time (Figure 3).

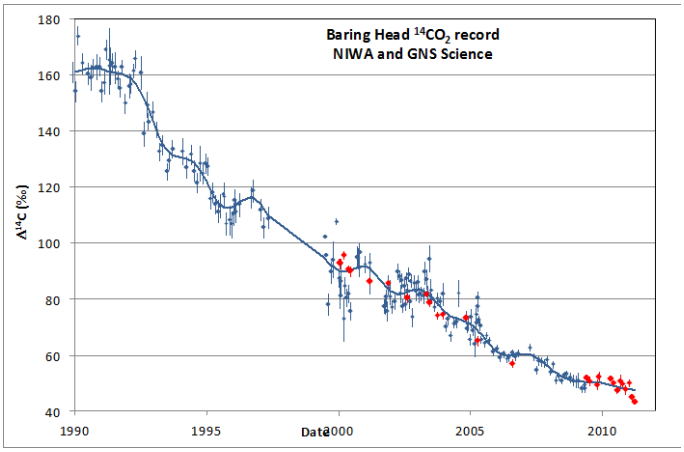


Figure 3. Baring Head (Wellington) atmospheric $\Delta^{14}CO_2$ record (Currie et al., 2009; unpublished GNS/NIWA data).

Explanation 2.

The subsamples of the paua shells that were taken do not reflect the oldest parts of the shells, and we are instead sampling a young part of the shell, or a mixture of the first few years of growth. The young parts of the shells give consistent results from all five shells, and these values are consistent with the extrapolated atmospheric $\Delta^{14}CO_2$ value at Baring Head in 2013 (latest Baring Head measurements are late 2011 at 43‰, and the trend predicts a decrease of 3-5‰/yr).

Explanation 3.

The paua measurements accurately reflect the surface ocean $\Delta^{14}C$ in Wellington Harbour, and that surface ocean $\Delta^{14}C$ is not changing at the expected rate. If this is the case, it is unknown whether

this would be localized to Wellington Harbour, or whether it applies more generally to New Zealand coastal and open ocean waters.

Model and observations predict a general downward trend in surface ocean $\Delta^{14}C$, due to the assimilation of "bomb ^{14}C" from atmospheric nuclear weapons testing in the 1960s. Naegler & Levin (2009) modeled the global ^{14}C distribution, and predict a ~5‰/yr decrease in surface ocean $\Delta^{14}C$ in the 2000s. Druffel et al. (2010) showed a downward trend of 3‰/yr in Northeast Pacific Ocean DIC from 1994-2004. Similarly, Santos et al (2011) examined $\Delta^{14}C$ at a coastal site in California from 2004-2010, and showed a downward $\Delta^{14}C$ trend of about 3‰/yr. In both studies, there was variability through the year, attributed to small scale eddies, upwelling of older ocean water, and runoff events from a local river (Hinger et al., 2010).

There are no available time series of $\Delta^{14}C$ in New Zealand coastal waters, or indeed in any Southern Ocean areas that reach to the 2000s. However, Graven et al. (2012) compiled global ocean $\Delta^{14}C$ data through to the 2000s, and used two models to understand the observed changes through time. They showed that at 40°S (Wellington), surface ocean $\Delta^{14}C$ should be similar to that of the overlying atmosphere (Figure 3), which is what we observe in the 2013 paua measurements.

Graven's study demonstrates that surface ocean $\Delta^{14}C$ was evolving during the 1990s and 2000s, initially being lower than that of the atmosphere, but at some point, switching so that the surface ocean $\Delta^{14}C$ is higher than that of atmosphere. During this transition period, surface ocean $\Delta^{14}C$ will stay roughly constant. One of the models used in the Graven study predicts that this switch occurred in the mid-2000s at 40°S, whereas the other model predicts that the switch has not yet occurred. Insufficient observations are available to test the accuracy of these predictions. Therefore, it is certainly possible, if not probable that this switch in surface ocean $\Delta^{14}C$ trend occurred during the 2000s at Wellington, resulting in roughly constant $\Delta^{14}C$ through the period of the paua analysis.

A further possible explanation is that different ocean currents have differing $\Delta^{14}C$ content. Southern Ocean surface waters have much lower $\Delta^{14}C$ than the overlying atmosphere, even in the 2000s, due primarily to upwelling of deep (^{14}C-depleted) waters (Graven et al., 2012). Interannual changes in the ocean currents at the Mahanga Bay site could result in changes in the $\Delta^{14}C$ content that overwhelm the expected temporal trend.

Conclusions

The young parts of the paua shells (from 2013) gave $\Delta^{14}C$ measurements with seawater collected at the same time. These results are also consistent with predictions that surface ocean $\Delta^{14}C$ would be similar to that of the overlying atmosphere.

The oldest parts of the paua shells, which are known from other methods to be up to 13 years old, show slightly higher $\Delta^{14}C$ values than the younger parts of the shells, but they indicate a downward trend of only about 0.5‰/yr, rather than the 3-5‰/yr documented in observations from other parts of the world and from modeled predictions.

The most likely explanation is that the sampling of the "old" parts of the shells was problematic, and resulted in sampling a mix of shell growth layers rather than the oldest part of the shell.

Possible further work with MPI

1. Test the methodological explanations one and two. Make measurements of historic (2000-2010) Mahanga Bay/Wellington Harbour $\Delta^{14}C$ from other materials such as short-lived shells of known age. Observe whether the $\Delta^{14}C$ matches that measured in the paua samples, or if they show higher historic $\Delta^{14}C$ as per our initial expectations.
2. Test whether the observed $\Delta^{14}C$ trend from Mahanga Bay is consistent around New Zealand, or is localized to Mahanga Bay/Wellington Harbour. This would determine whether the paua ageing method could be applied at other locations.

Phase II

This report documents phase II of the radiocarbon analysis of paua. Phase I was performed in 2013 and Phase II in 2014.

The principle

The time history of the radiocarbon (^{14}C) content of dissolved inorganic carbon and hence paua shell is variable enough through time that the ^{14}C content of the oldest part of paua shells can be used to determine the shell age. There is a very small pool of literature suggesting that this is possible, but no reliable established method. The method depends on (i) paua laying down their carbonate shell sequentially, (ii) being able to isolate and measure the oldest part of the shell, and (iii) a good knowledge of the time history of ^{14}C in the coastal waters where the paua grow so that the age can be determined.

Phase I results

In Phase I, we tested paua that were spawned in the Ministry for Primary Industries (MPI) Mahanga Bay aquarium and then released to the adjacent Wellington Harbour. The spawning and collection dates were both known. At this stage we did not have any reliable information about the time history of ^{14}C in New Zealand coastal waters, basing our knowledge on the modeled global average surface water ^{14}C history. For the paua collected in 2013, the older parts of each shell had higher ^{14}C content than the youngest parts of the shell, consistent with the expected downward trend in surface ocean ^{14}C during the 2000s. Yet although the observed pattern was correct, the magnitude of the difference was smaller than expected. We had several hypotheses that might explain this, with the most likely being that we had incorrectly sampled the shell material, so that we had not actually measured the ^{14}C in the oldest part of the shell.

Phase II experiment

Four shells were obtained from the Museum of New Zealand Te Papa Tongarewa (Te Papa) collection, courtesy of Te Papa conservator Bruce Marshall, collected at known dates from 1965 to 1987 from four different sites around New Zealand (Table 1). Five shells obtained from Reyn Naylor (NIWA) were collected in 2005 and 2012. These five shells had known collection dates and locations, and had been analysed for age by both protein layer counting and oxygen-18 (^{18}O) seasonal cycle analysis (Table 1).

All shells appeared to be intact without erosion of the spire (the oldest part of the shell). First the outer surface was cleaned to remove debris. Two subsamples were then taken from each shell. One sample was taken from the outer edge, representing the youngest part of the shell, which should have a ^{14}C content reflecting the surface water at the time of shell collection. A second sample was taken by grinding carbonate material from the outer surface at the spire, which should represent the oldest part of the shell (Figure 4). A major challenge was collecting sufficient material from the spire and in some cases we suspect that the subsample may include a mixture of several years' growth.

Samples were then hydrolysed to CO_2 gas, reduced to graphite, and the carbon isotopes (^{12}C, ^{13}C and ^{14}C) measured by high precision accelerator mass spectrometry. Results are reported as fraction modern ($F^{14}C$), a measure of the ^{14}C content of the sampled material relative to the absolute radiocarbon "modern" standard value (Reimer et al., 2004; Stuiver &Polach, 1977).

Phase II results

An essential starting point for ageing paua using radiocarbon is knowledge of the time history of ^{14}C in the waters in which the paua shell grows. Global modelling studies have produced estimates of global average surface water ^{14}C through time (Naegler & Levin, 2009). There have been a few large-scale surface ocean ^{14}C measurement programs in over the years, but only a handful of measurements specific to New Zealand coastal waters (Hogg et al., 1998). While we might expect New Zealand coastal waters to roughly follow the global modelled surface water ^{14}C curve, the detail is unknown. We used the young part of each paua shell and its known age (collection date) to provide an initial basis for a New Zealand coastal water ^{14}C history (Figure 5, red points). We also include a set of four measurements from shells collected in Tairoa Harbour in 1993 – 1995 and published by Hogg et al.

(1998). We find a surprisingly good agreement between the paua measurements and the modelled prediction (data not shown). This gives us confidence that the proposed method is reasonable and sensible. We fit a curve to our young paua data, to provide an estimated New Zealand coastal water ^{14}C history from the 1960s until the present (Figure 5, red line).

Next, we use the measurement of oldest part of each shell to estimate the growth period of each paua. An initial inspection shows that the results are sensible (Figure 5, light blue points). In the early part of the record, when the surface water $F^{14}C$ is increasing through time, the oldest part of the shell always has lower $F^{14}C$ than the youngest part of the same shell. In the later part of the record, where surface water $F^{14}C$ is declining, the oldest part of the shell has higher $F^{14}C$ than the young part of the shell. During the transition period in the 1970s-1980s, where surface water $F^{14}C$ is fairly constant, the old and young parts of the shell have similar $F^{14}C$.

We then infer the year of growth of the oldest part of each shell by matching its $F^{14}C$ value to the red ^{14}C history curve and determining the year at which the shell material grew; these are shown as the dark blue points. The age is determined from the collection date minus the inferred growth year of the oldest part of the shell (Table 1). For the shell collected in 1965, we cannot determine the age with any confidence, since we do not have any previous surface water ^{14}C measurements. For the shell collected in 1987, the shell could be up to 13 years old, but it is difficult to determine through this period of flat $F^{14}C$ in the surface ocean history and is an inherent limitation of the method.

The five shells labelled E, G, Q, R and U have estimated ages from both ^{18}O analysis and protein layer counting analysis, shown in Table 1. Samples E and G, both collected from the Wellington South Coast in 2005, both agree very well across all methods. We obtain much younger ages for shells Q, R and U, all collected in 2012. The Te Papa shell ages are not known, but our estimated ages of 7 and 8 years appear to be reasonable.

The very young estimated ages for shells Q, R and U could be due to two possibilities.

1. Despite efforts to sample the oldest part of the shell, we may have inadvertently sampled and analysed a mixture of growth years from the shell, resulting in an underestimate of the age. This possibility could be tested by doing the same analysis on new shells, and using much smaller amounts of sample material. There is a trade-off in using less sample material, as accuracy is lost, but we are confident that using about half as much material would still be successful. Alternatively, a new technique for laser ablation ^{14}C analysis of carbonates such as shell is being pioneered in an overseas laboratory. This method requires much less sample material and could be used to scan across the entire shell surface, providing a scan of ages rather than discrete points. The measurement precision is necessarily lower.
2. We only have a few points from which to interpolate the surface water time history, and it is possible that more data on the time history would resolve this issue. Certainly, if the ^{14}C method for ageing paua is to be pursued, a more detailed New Zealand surface water ^{14}C history will be needed. In this dataset, we implicitly assume that surface waters at all New Zealand coastal sites are consistent, whereas it is quite reasonable to expect variations in the ^{14}C content at different sites. This should be addressed in any future study.

Conclusion

Ageing of paua using ^{14}C is feasible. Initial results show good agreement between the ^{14}C method, ^{18}O and protein layer counting methods for some samples. In some samples, we obtain ages that are significantly too young, suggesting that further refinement of our sampling technique for the oldest part of each shell is required. In these results, the ^{14}C shell ages should be considered minimum ages.

The surface water ^{14}C history is of broader scientific significance, as it is an indicator of the rate of ocean carbon uptake. Ocean carbon uptake is the major sink for human-produced carbon dioxide, the driver of the current observed global warming. The rate of ocean carbon uptake is a critical component of future predictions of global warming. Thus this research may have some interest to other New Zealand government agencies.

As all three ageing methods have some limitations and complications, any single method may be insufficient, and a combination of all three methods may be necessary to ensure accurate shell ages and growth periods are obtained.

Possible future work

This pilot study demonstrates the feasability of the ^{14}C method for paua ageing, which could also be applied to other shellfish species. Further work is needed to refine the method and obtain robust shell ages.

Further measurements of the New Zealand surface water ^{14}C history is essential to improved ages. Ideally, this would include obtaining and measuring known age shell (preferably but not necessarily paua) samples from all sites of interest around New Zealand. Samples collected across the years of primary interest from the late 1990s until present would be needed (5 to 10 samples from each site).

Paua shells with known collection dates from the sites of interest can then be used to determine their ages using ^{14}C. We would continue to refine our sampling method to ensure that the oldest part of the sample is measured.

Costs of future work will be dependent on the scope of the project. It may be useful to approach other government agencies to partially support this work. Costs for a larger scope study could be reduced by making this a graduate student thesis project, recognising that this would be likely to extend timeframes required.

ID	Location	Date of collection	$F^{14}C$ (young)	$F^{14}C$ (oldest)	^{14}C-based age	Protein layer age	^{18}O-based age
M.032593	Te Kaha, Bay of Plenty	01/01/65	1.0449 ± 0.0013	0.9958 ± 0.0013	5?		
M.059119	Island Bay, Wellington	05/10/75	1.0908 ± 0.0014	1.0718 ± 0.0013	7		
M.275244	Flat Point, Wairarapa	06/10/79	1.0918 ± 0.0013	1.0845 ± 0.0013	8		
M.087460	Ahipara Bay, Northland	16/01/87	1.0880 ± 0.0013	1.0876 ± 0.0013	0-13		
E	South Coast, Wellington	06/12/05	1.0652 ± 0.0013	1.0733 ± 0.0013	5	5	5.9
G	South Coast, Wellington	06/12/05	1.0661 ± 0.0013	1.0758 ± 0.0013	7	7	6.9
Q	Motunau, Canterbury	15/03/12	1.0421 ± 0.0013	1.0514 ± 0.0013	2	5	6.5
R	Motunau, Canterbury	15/03/12	1.0466 ± 0.0013	1.0491 ± 0.0013	1	4	6.5
U	Okiwi Bay, Marlborough	26/04/12	1.0433 ± 0.0013	1.0481 ± 0.0013	1	4	5.0

Table 1: ^{14}C results and comparison with other methods for the nine paua shells used in this study.

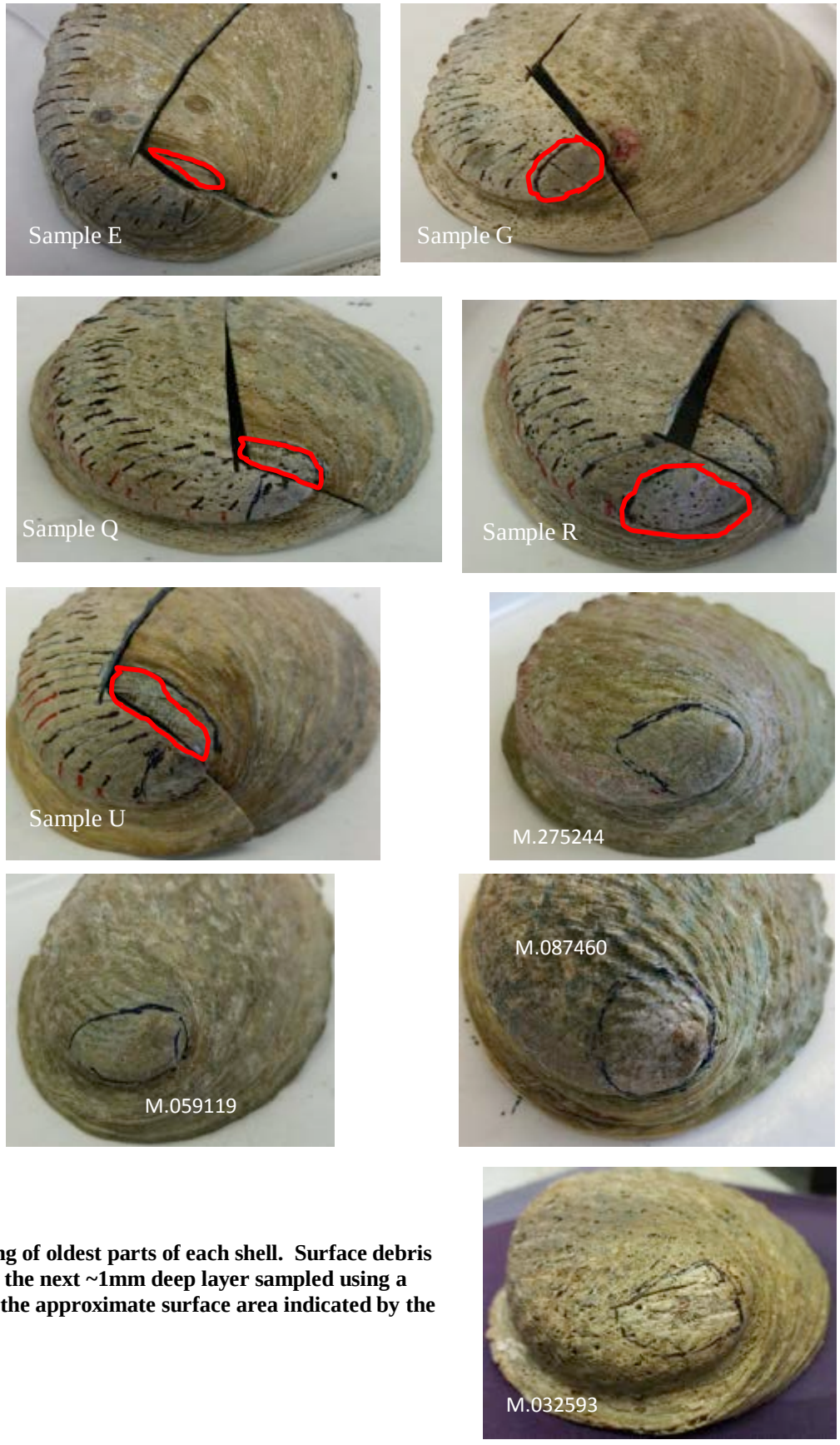


Figure 4: Sampling of oldest parts of each shell. Surface debris was removed and the next ~1mm deep layer sampled using a dremel tool, over the approximate surface area indicated by the red or black line.

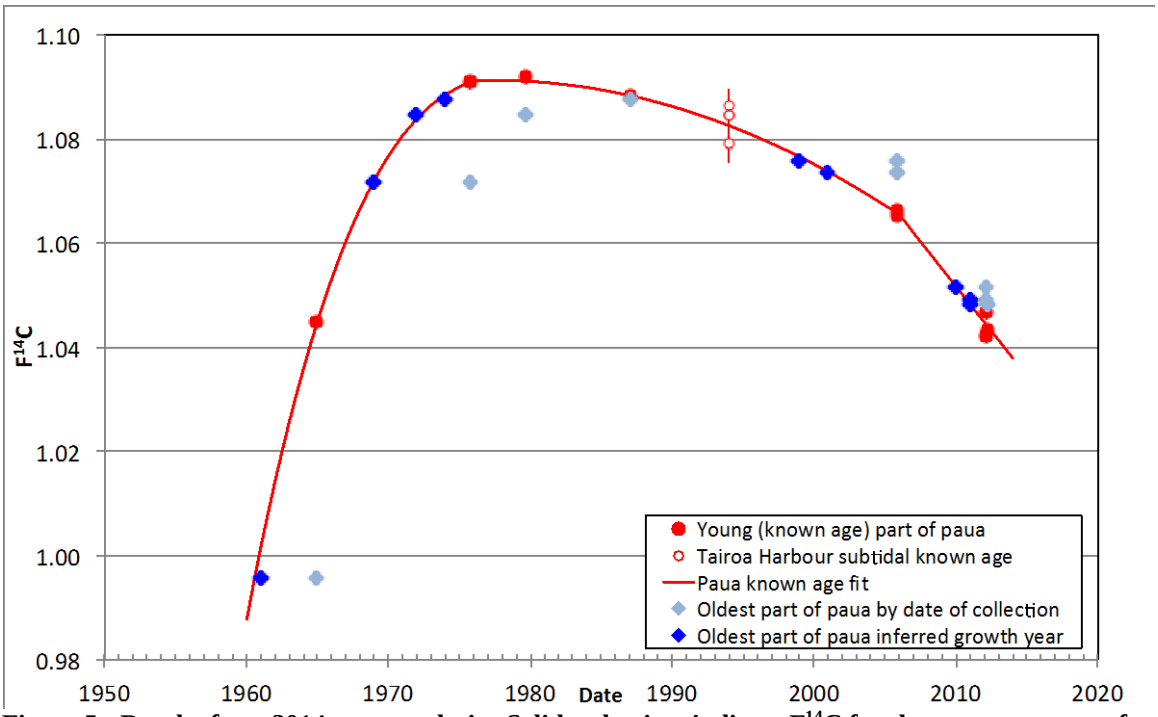


Figure 5: Results from 2014 paua analysis. Solid red points indicate $F^{14}C$ for the youngest parts of each shell, for which the age is assumed to be the same as the date of collection. Three open red symbols are for shell material collected from Tairoa Harbour in 1994 (Hogg et al., 1998). A curve was fitted to the known age paua. The curve fits the Tairoa Harbour data well, although they were not included in the fit. The light blue points indicate the measured $F^{14}C$ of the oldest part of each paua, plotted using the collection date.

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