Ministry for Primary Industries Manatū Ahu Matua



Fine-spatial scale assessment of growth rings in paua (*Haliotis iris*) New Zealand Fisheries Assessment Report 2016/39

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ISSN 1179-5352 (online) ISBN 978-1-77665-313-3 (online)

July 2016



New Zealand Government

Growing and Protecting New Zealand

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EXECUTIVE SUMMARY

Naylor, R.; Notman, P. (2016). Fine-scale assessment of growth rings in paua (Haliotis iris).

New Zealand Fisheries Assessment Report 2016/39. 11 p.

Internal growth checks in the shells of abalone have been used by a number of authors to estimate age; however, evidence regarding their utility is variable and conflicting. Recent work showed that for *Haliotis iris* there was a reasonable relationship between the number of protein layers in the shell and the number of seasonal temperature cycles experienced by the shell estimated using stable oxygen isotopes. It was also apparent, however, that new protein layers under the spire of the shell were not all laid down at the same time of the year. This apparent lack of seasonality was investigated in the current work.

Protein layer deposition at the shell spire is clearly not driven by either temperature or spawning activity. The deposition of protein layers on the inner surface of shells appears to be a continuous process. A dramatic fall in the gonad index between August and October 2014 is highly likely to indicate a spawning event in the area over that period. A sharp decline in water temperature at this time may have provided a cue for spawning.

1. INTRODUCTION

Internal growth checks in the shells of abalone (*Haliotis* sp.) have been used by a number of authors to estimate age (e.g. Shepherd et al. 1995a, 1995b); however, the large body of literature relating to the estimation of age in abalone using protein layers is variable and conflicting.

Recent work in Project PAU2011-05 investigated the utility of growth checks in the shell of paua (*H. iris*) as a fast and simple technique to estimate age. The results showed that there was often a reasonable relationship between the number of growth checks in longitudinal sections of paua shell and the number of seasonal temperature cycles experienced by the shell estimating using stable oxygen isotope analysis (Naylor & Neil 2015).

The Shellfish Working Group, however, identified results that indicated that the growth checks may not be laid down on a seasonal basis (Naylor & Neil 2015). These were marginal increment data, which indicated that even at the same site, new checks under the spire of the shell, did not appear to be laid down at the same time of the year. To investigate this apparent lack of seasonality, the Working Group suggested that one coastal site near Wellington be sampled at monthly intervals over a 16 to 18 month period to monitor the seasonal timing of growth check deposition.

Several authors have related the formation of growth rings in abalone shell 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) attributed the unusually clear growth checks in *H. mariae* to the coincidence of spawning and winter.

The objectives of this project (PAU2013-02) were to look at the timing of protein layer deposition in the shells of paua and to look at the relationship between spawning and the formation of protein layers.

Stated objectives were:

- 1) To clarify growth ring and marginal increment formation in paua shells
- 2) To assess paua growth across various developmental phases of maturity.

2. METHODS

2.1 Timing of protein layer deposition

Paua samples were taken from Reef Bay on Wellington's south coast (Figure 1) at monthly intervals over a 17 month period beginning at the end of March 2014. On each sampling occasion, twenty paua over the available size range between about 60 mm and 130 mm were collected from the same site.



Figure 1: Location of sampling site Reef Bay on Wellington's south coast.

Shells were measured, externally tagged and the details of capture location and date were recorded. Shells were then sectioned vertically though the spire (Figure 2) with a cut-off wheel attached to a Dremel power tool, and the cut section surfaces at the spire were coated in oil and examined under a binocular microscope.



Figure 2: Vertical cross section of the shell showing the location of the spire and the examined section.

The number of protein layers at the spire was counted and recorded, and each section was photographed. As well as counting the number of protein layers in the shell, the thickness of calcium carbonate (aragonite) deposited since the last protein layer was also measured using the open source image processing program ImageJ. The thickness of the previous complete calcium carbonate layer, and the average thickness of all previous layers, were also measured, and the marginal increment was calculated both as a proportion of the previous layer and as a proportion of the average thickness of all layers. Because the aragonite layers adjacent to the spire of the shell were often convergent or irregular, measurements were made at the point to the left of the spire where the protein layers first appeared to become approximately parallel.

The number of protein layers in the shells was plotted against shell length and the mean marginal increments from each month were also plotted. The shell layers referred to in this document are indicated in Figure 3. Marginal increment values range from zero to one or greater, where zero is the stage when a protein layer has recently been laid down on the inside of the spire of the shell, with no subsequent aragonite deposition. A marginal increment of one or greater indicates that a protein layer is likely to be laid down soon based on the

thickness of the previous layer or layers. Because the values of zero and one are effectively the same in terms of the actual or expected timing of protein layer deposition respectively, mean values over the range from zero to one are not informative with respect to the timing of layer deposition. For example, 10 values of zero and 10 values of 1 would produce a mean marginal increment of 0.5, which does not suggest that protein layers have just been laid down or are expected to be laid down. To adjust marginal increment values to reflect their proximity to protein layer deposition, values greater than 0.5 were replaced with (1- the value). In this way, marginal increment values range from 0 to 0.5, where 0 is the value closest to protein layer deposition and 0.5 is the value most distant from it. Values larger than 1 were considered to be equal to 1.



Figure 3: Vertical cross section of the shell showing outer calcite layer, the three aragonite layers, and the two protein layers.

2.2 Timing of spawning

The temporal relationship between spawning and the deposition of protein layers was assessed by estimating the timing of spawning and comparing this with the timing of protein layer deposition.

Gonads were removed and preserved in 10% formalin to harden the tissue. Gonads were then sectioned transversely at a point one-third the distance up from the tip of the conical appendix (McShane & Naylor 1996, Counihan et al. 2001). The surface areas of gonad and digestive gland were calculated using the open source image processing program ImageJ. The gonad index was calculated (after Newman 1967) as:

(surface area of gonad in the section / total surface area of the section) \times 100.

Spawning is indicated by a large drop in the gonad index followed by a subsequent recovery (McShane & Naylor 1996).

Because temperature has also been implicated in the formation of growth checks in abalone a HOBO pendant subtidal temperature logger was also deployed at the site at a depth of about 6 m on 16 May 2014 to see whether there is any relationship between water temperature and spawning or growth check formation. Water temperature was recorded every two hours.

3. RESULTS

3.1 Timing of protein layer deposition

Shells were collected on 17 occasions at about monthly intervals between 31 March 2014 and 13 August 2015. All shells were sectioned; however, 21 of the 340 shells (6.2%) had fungal infections which meant that the number of protein layers could not be ascertained. An example of an infected and unreadable shell is shown in Figure 4.

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Figure 4. Vertical cross section through the spire of Shell N188 showing fungal infection.

Images for almost all of the remaining shells were sharp, readable and the number of protein layers adjacent to the spire of the shell was unambiguous. There are too many images (340) for them all to be presented in this document, but three examples showing 4, 4, and 5 protein layers respectively are shown in Figure 5. All images and shells have been archived by the Ministry for Primary Industries' database manager.

The marginal increments for shells were calculated both as growth since the last protein layer as a proportion of the previous layer and as growth since the last protein layer as a proportion of the average thickness of all layers. Usually there was little difference between the estimates, but unusually thin or thick layers of aragonite sometimes resulted in relatively large differences in the two estimates (Figure 6). Because there was usually little difference in marginal increment values calculated in either manner, increment plots over time were estimated relative to the thickness of the last aragonite layer.

The relative timing of protein layer deposition on the shell beneath the spire is shown in Figure 7. A value of zero or close to zero indicates that a protein layer has just been laid down beneath the spire (e.g. Figure 5, bottom image) or it is expected to be laid down, based on the thickness of the previous aragonite layer.

Mean marginal increments were lowest during July and June of 2014 and 2015 respectively, but the differences are not significant and the variance associated with the estimates is high (Figure 7). General observations of inner shell surfaces showed protein visible on all shells at all times of the year. Consequently, it is apparent that the deposition of protein is a continuous process rather than a seasonal or spawning related phenomenon.

There were no obvious patterns in the timing of deposition of the protein layers beneath the spire over time (Figure 7). All shells, at all times of the year had recently laid down protein on some part of the inside of the shell so it appears that the deposition of this layer may be a continuous process rather than a seasonal or spawning related phenomenon.

The relationship between shell length and the number of protein layers in the vertical cross section of the shell is shown in Figure 8. There is a moderate correlation between length and the number of protein layers (corr=0.51, R squared = 0.26), but the variance is reasonably large.



Figure 5: Vertical cross sections through the spire of Shells A13, N583, and 8 (from top to bottom) showing 4, 4, and 5 protein layers respectively. In the bottom shell a new protein layer has just been laid down.



Figure 6: Marginal increments calculated relative to the thickness of the last aragonite layer compared with marginal increments calculated relative to the mean thickness of all aragonite layers for paua sampled at Reef Bay on Wellington's south coast. Dotted line indicates a one to one relationship.



Month & Year





eriod between

March 2014 and August 2015. From the beginning of sampling the index remained relatively constant at between about 80 and 90 until the end of July 2014 (Figure 9). By late August and until late October the index fell dramatically to between about 30 and 40 until near the end of October. By the end of November, the index had risen again and fluctuated between about 70 and 85 until the end of sampling in August 2015 (Figure 9).

The dramatic fall in the index between August and October 2014 is highly likely to signal a spawning event in the area over that period. A slight decline in the index between March and the end of May 2015 (Figure 9) may signal a partial spawning event at that time, but this evidence is not compelling.

The HOBO pendant temperature logger deployed at the site on 16 May 2014 was retrieved and replaced on 28 January 2015, but had stopped recording on 27 September 2014, probably because of a drained battery. It was retrieved again on the 13 August 2015. In 2014 water temperature at a depth of about 6 m ranged between about 10.5 °C and 15 °C over the period of recording, and in 2015 water temperature ranged between 10.4 °C and 18 °C over the period of recording (Figure 10). Bottom water temperature, especially in 2015, oscillated on an approximately monthly basis, but generally followed an expected trend of warmer in summer and colder in winter months (Figure 10).

In 2014, the sharp water temperature decline during July (Figure 10) corresponds with a sharp decrease in the gonad index (Figure 9).



Figure 9: Gonad index and 95% confidence intervals for paua sampled at Reef Bay on Wellington's south coast.



Figure 10: Bottom water temperature (°C) at the Reef Bay sampling site (6 m depth) for months monitored in 2014 (top) and 2015 (bottom).

4. DISCUSSION

There were no apparent seasonal trends in the formation of protein layers beneath the spire of shells, and all shells examined had recent protein deposits visible on some part of the inner shell, suggesting that the deposition of these layers is a continuous process and occurs on different inner surfaces of the shell at different times.

The large body of literature relating to the estimation of age using growth checks is variable and conflicting. Several authors suggest that the number of protein layers in the shell may usefully indicate age in abalone. 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. For the same species in different areas, McShane & Smith (1992) found that the method did not reliably estimate age. Erasmus et al. (1994) examined hatchery raised *H. midae* shells of a known age and found that three growth checks were formed in the first year of life, and that one was formed in each subsequent year.

The cues for the deposition of protein layers are most often reported as spawning, the onset of winter, or both. Sakai (1960) reports that the annual growth checks are associated with spawning in *H. discus hannai*, and Kojima (1975, cited in Shepherd et al. 1995a) associates their formation with spawning in *H. discus*. Poore (1972) found that the growth checks in *H. australis* were formed in late autumn or early winter. Kim & Chung (1985, cited in Shepherd et al. 1995a) also associate the formation of growth checks with winter in *H. discus*.

In this study, there are no apparent trends in the timing of protein layer deposition beneath the spire of the shell and therefore no apparent relationship between the deposition of this layer and either temperature or spawning. In 2014, a sharp water temperature decline during July did correspond with a sharp decrease in the gonad index, but there is no evidence to suggest a causal relationship. There is anecdotal evidence; however, that spawning in paua is triggered by large storms and an associated drop in water temperature (Graeme Moss, NIWA, pers. comm.).

There appears to be a reasonable relationship between paua shell length and the number of protein layers in the shell although there is a lot of variability associated with this. This large variation is not surprising given the large variation in growth rates which is known to occur in paua, even within the same area (e.g. Naylor et al. 2006).

The deposition of protein beneath the spire of the shell is clearly not driven by either a fall in temperature or spawning. The process of protein layer deposition appears to be continuous, i.e. part of a new protein layer is being laid down on some part of the inner surface of the shell almost all of the time. A more important question is probably the proportion of times that the number of protein layers coincides with the known age of the shell. This has been examined in other projects (e.g. PAU2011-05, Naylor & Neil 2015)

5. ACKNOWLEDGMENTS

This project was funded by the Ministry for Primary Industries project PAU2013-02. We thank Jeff Forman, Owen Anderson, Roberta D'Archino, and Steve Mercer for help with the sample collections, Neill Barr for configuring and downloading the temperature data, and Peter Horn for reviewing the draft report.

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