Ministry for Primary Industries
Manatū Ahu Matua

Literature review of abalone ageing techniques

New Zealand Fisheries Assessment Report 2015/77

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ISSN 1179-5352 (online)
ISBN 978-1-77665-125-2 (online)

December 2015

New Zealand Government

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EXECUTIVE SUMMARY

**Naylor, R. (2015). Literature review of abalone ageing techniques.
New Zealand Fisheries Assessment Report 2015/77. 17 p.**

The estimation of age in abalone using shell growth rings has been achieved using two main techniques. These are the counting of external growth checks on the shell and the counting of internal growth checks within sections of the shell. The internal growth checks may also be viewed by grinding the spire and viewing the checks as a series of concentric rings. External daily growth increments have been validated in *H. madaka*. Age in abalone has also been estimated by a few authors using stable oxygen isotopes. Stable oxygen isotope analyses appear to be the most reliable method of validating the timing of growth check deposition. Commonly used methods of validation such as age estimation from tag-recapture data or the use of length frequency data to determine age are flawed because of the inherent variability of the data.

1 OBJECTIVES

OVERALL OBJECTIVE:

1. To conduct a literature review on abalone ageing techniques using growth rings.

SPECIFIC OBJECTIVES

1. To provide a conceptual description of abalone shell structure and growth.

2. To describe different techniques which have been used to age abalone using shell growth rings or any other morphological characteristics of the shell.

3. To describe the different methods which have been used to validate ageing of abalone using shell growth rings or any other morphological characteristics of the shell.

2 METHODS

The primary sources of information for this review were web-based search engines such as Google Scholar, Scopus, and the ISI Web of Knowledge. Literature that the author was aware of was also consulted. Once relevant articles were found, their bibliographies were examined to identify older articles on the subject, and more recent articles were found by examining papers which had cited that article. The information was then summarised and discussed. The utility of various methods is also discussed. Where appropriate, the theoretical basis for particular methodologies is also presented.

3 SHELL STRUCTURE AND GROWTH

Mollusc shells are constructed primarily from carbonate in the form of calcite, aragonite, or both (Jones et al. 1983). Calcite and aragonite both have the same chemical formula ($CaCo_3$) but the two polymorphs have a different crystal structure (Heinemann et al. 2011). The shell of paua (*Haliotis iris*) is composed of a very fine outer layer of protein (the periostracum) which is about 100–200 nm thick, an outer layer of prismatic calcite and inner layers of nacreous aragonite separated by thin layers of protein (Gray & Smith 2004, Figure 1). In older shells the periostracum is frequently eroded.

The prismatic calcite layer contains rhomboidal calcite crystals (up to 100 nm in diameter) surrounded by a very thin (0.5–3 nm) glycoprotein matrix (Gray & Smith 2004). The inner aragonite layers, usually referred to as nacre, comprise stacks of flat shingle-like crystals or tiles, layered on top of one another, and surrounded by an organic matrix (Gray & Smith 2004, Figure 2). The tiles initially stack up as pyramids, and lateral growth of the tiles continues until the tile layer is continuous (Heinemann et al. 2011, Figure 3). The aragonite layers are periodically separated by a protein layer laid down along the length of the shell. These layers mark interruptions in the deposition of aragonite (Meyers et al. 2008). Sumitomo et al. (2011) examined these layers in *H. gigantea*, and found that they acted mechanically like aragonite, but were much stronger. The abrupt transition between aragonite and this layer also suggests that abalone are able to rapidly alter the biomineralisation process (Sumitomo et al. (2011).

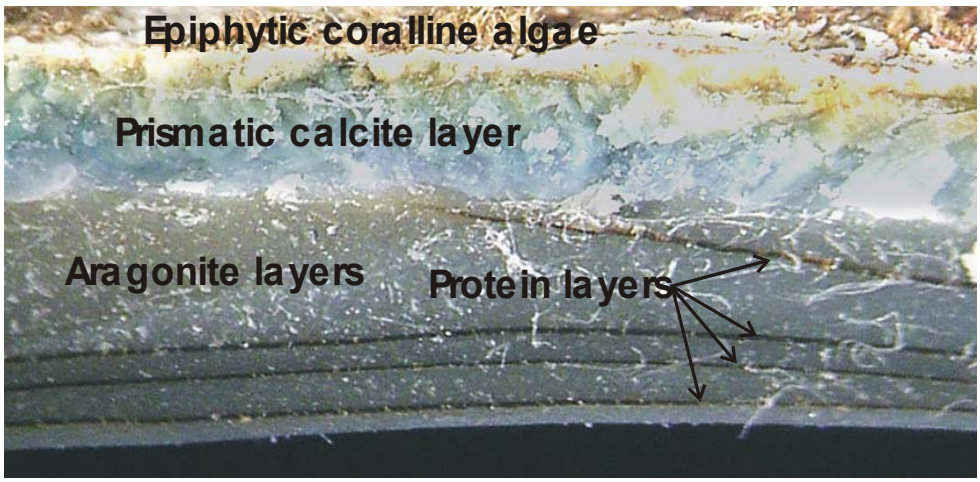

Figure 1: A magnified (about 12 ×) longitudinal cross section of a paua shell. The outer periostracum is not evident and the top of the shell has been colonised by coralline algae.

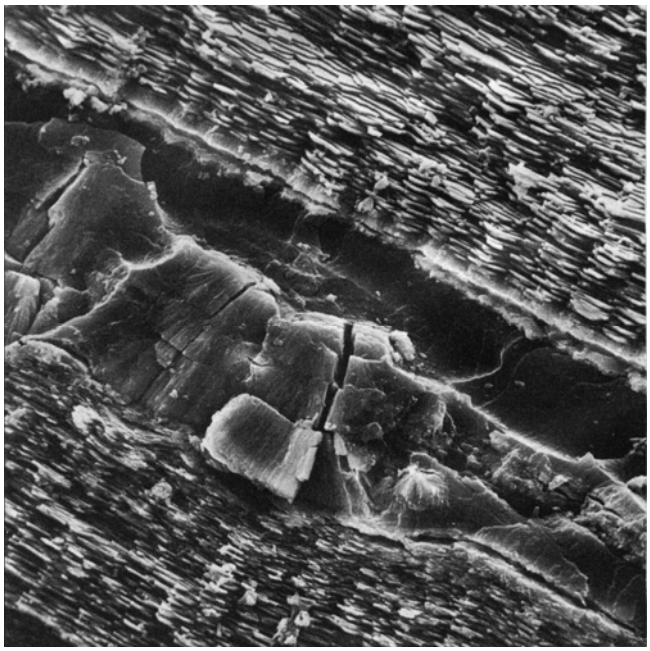

Figure 2: Scanning electron microscope image (magnified about 1000 ×) of a cross section of paua shell showing a protein layer (centre) surrounded by layers of aragonite tiles. Reprinted from *Catch*, with permission from the Ministry of Fisheries.

They suggested that this layer serves a protective role during periods of unfavourable conditions or during conditions of lifecycle transition (Sumitomo et al. (2011). In paua, the thickness of the aragonite tiles is locally very regular over hundreds of tiles, but between the protein layers there

appears to be a cyclical variation in tile thickness between about 0.25 and almost 0.5 μm., where just before, and just after the protein layers, the tile thickness is the lowest (Snow & Pring 2005).

The structure of the calcite and aragonite layers is essentially similar to that in brick and mortar construction where the protein is the mortar. Calcium carbonate alone is not suitable as a structural material because it is inherently brittle (Meyers et al. 2008). The laminated composite form is a highly ordered hierarchical structure, which affords the shell fracture toughness eight times that of monolithic calcium carbonate (Sarikaya et al. 1990).

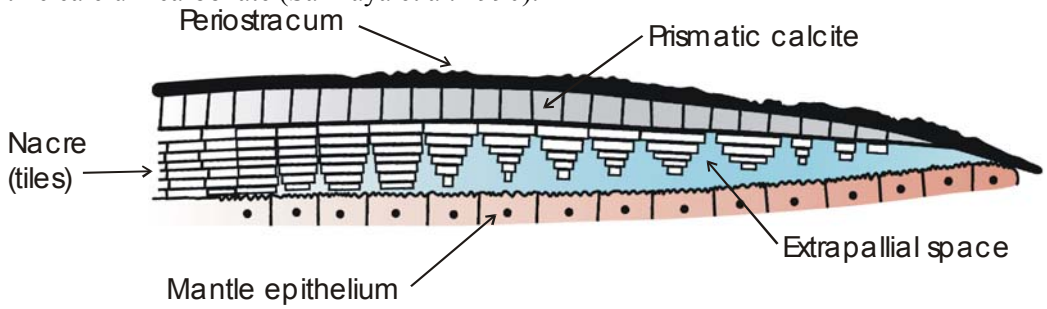

Figure 3: Schematic representation (not to scale) of a vertical cross section of abalone shell. After Heinemann et al. (2011).

New material is added to the outer edges of the shell and to the inside of the shell so that the shell becomes thicker as it becomes larger, and the direction of growth is in the form of a logarithmic spire (Sinclair 1963, i.e. the whorls continually increase in breadth in an unchanging ratio, see Figure 5).

The iridescence (i.e. the way the way the shell changes colour when you look at it from different angles) is caused by the diffraction of light and interference to it, when it passes through the aragonite tiles that comprise the nacre. The thickness of the tiles apparently influences the colour appearance of nacre, eg. thin tiles give the shell a mainly blue appearance and with thicker tiles, only green and red colours are seen (Snow & Pring 2005).

The precipitation of shell material occurs within the extrapallial space between the shell (or periostracum) and the mantle (Lin & Meyers 2005, Figure 3). The mantle is a soft, thin sheet of tissue next to the shell that surrounds the adductor muscle at its attachment point and covers the body organs. The periostracum seals the extrapallial space and also provides support for the first layers of calcite deposited (Marin & Luquet 2004).

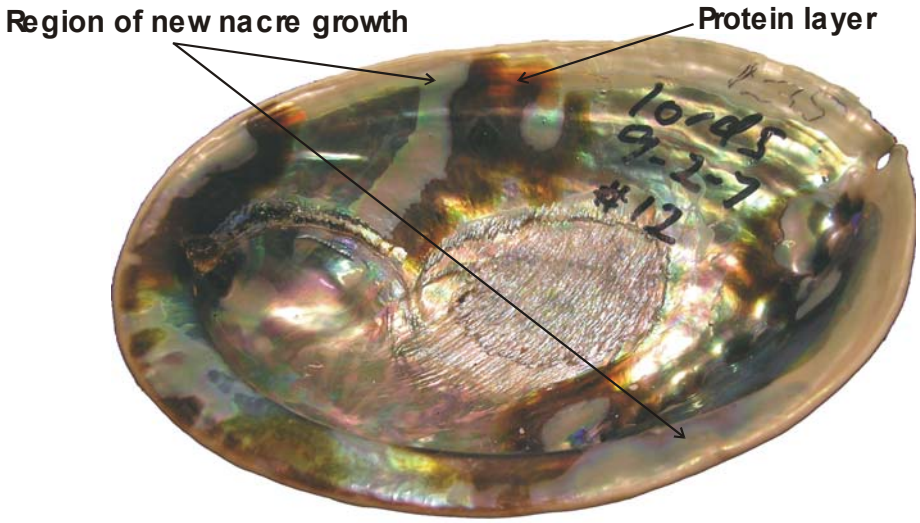

Figure 4: Photograph of the inside of a paua shell (135 mm in length) showing the area of nacre deposition and the protein layer. The region of nacre deposition is characterised as being dull.

Calcium and bicarbonate ions are taken up by the gills, gut, mantle epithelium, or body surface, from the surrounding water and transported to the epithelial cells where they are stored until required (Marin & Luquet 2004). They are then actively pumped from the mantle epithelium into the sealed extrapallial space to form a supersaturated solution which allows the crystallisation of calcium carbonate (Marin & Luquet 2004). A calcifying matrix is also secreted into the extrapallial space and this interacts with the mineral ions such that they self assemble in a precise manner to form crystals with well defined morphologies (Marin & Luquet 2004). The calcifying matrix is also thought to be the main nucleating agent (i.e. involved in the formation of a focal centre around which the crystal lamellae can orientate themselves). The matrix allows crystal formation only when appropriate; it selects the appropriate polymorph of calcium carbonate and regulates the shape, size, and orientation of crystals (Marin & Luquet 2004). The matrix is a complex combination of proteins, glycoproteins, proteoglyycans, polysaccharides and chiton (Marin & Luquet 2004). The region of nacre deposition can be seen with the naked eye as a dulled area (Heinemann et al. 2011). This is usually most noticeable as a dull rim around the outer edge of the inside of the shell, but it is also sometimes patchily distributed further into the shell (Figure 4).

The mediation of crystallisation in gastropod shells is extremely complex and not yet fully understood. It is, however, the subject of an extensive and rapidly advancing body of work in the field in biomimetics, which seeks to understand the production of materials in nature, and from this understanding, develop advanced synthetic materials (Lin & Meyers 2005). It appears likely that the biomediation of shell deposition involves a complex suite of biomineralisation genes which cause the production and secretion of a complex suite of proteins into the extrapallial space at the appropriate time. Jackson et al. (2007) found that hundreds of proteins were secreted from the mantle of *H. asinina.* They also isolated nine genes at the edge of the mantle, some of which were only present at certain times of shell development (Jackson et al. 2007). The gene regulatory networks that control the deposition of shell material and the chemical pathways used to achieve this are not yet fully understood.

4 AGEING TECHNIQUES

The estimation of age in abalone using shell growth rings has been achieved using two main techniques. These are the counting of external growth checks on the shell and the counting of internal growth checks within sections of the shell. The internal growth checks may also be viewed by grinding the spire and viewing the checks as a series of concentric rings (Muñoz-lopez 1976, cited in Prince et al. 1988). External daily growth increments have been validated in *H. madaka* (Tsuiki et al. 2004). Age in abalone has also been estimated by a few authors using stable oxygen isotopes.

External growth checks on the shell

External growth checks on the shell have been used to estimate the age of abalone, and in some species, it is thought that the checks are laid down on an annual basis. Poore (1972) found external growth checks on the shells of *H. australis* and *H. virginea* at Kaikoura, but did not find them on the shells of *H. iris*. By tagging, he was able to determine that the growth checks were annual in *H. australis* and assumed that the same was likely to apply to *H. virginea*.

Examining *H. lamellose*, Bolognari (1953) thought that the second external growth check was formed after the first year of life, and that subsequent checks were laid down on an annual basis. There appears to be no evidence to support the annual basis of growth check formation. He noted that some lines were clearer than others and that in larger abalone the checks were not evident because of epiphytes on the shell. Bolognari (1953) also thought that the growth checks were formed during spawning.

Forster (1967) frequently found external growth checks on the shells of *H. tuberculata* but they were not always evident. He found reasonable agreement between growth estimated by tagging and the growth between one growth check and the next; however some slowly growing tagged abalone were not included in the analyses to avoid the possibility of confusing annual growth checks with checks caused by the disturbance associated with tagging. This highlights the major problem associated with this type of ageing, namely that while growth checks may be formed annually, they may also be formed at other times of the year in response to other physical factors.

External growth checks on the shells of *H. iris* are sometimes conspicuous on clean, fast growing shells (Figure 5). They may also be formed annually. The shell in Figure 5 is from the south coast of PAU 5A. External growth checks are apparent as darker bands in the shell. This shell had previously been examined to determine the water temperatures at various shell lengths using stable oxygen isotope methods (Naylor & Breen 2008). External growth checks where the shell length at the check coincides with the shell length when the estimated isotopic temperature was lowest during a seasonal cycle, are indicated with arrows in the Figure 5. This shell appears to be very fast growing and is devoid of encrusting organisms. While not uncommon, specimens like this are not typical of the fished population of paua in most areas.

Shepherd et al. (1995a) found primary and secondary growth checks on the shells of *H. mariae*. By estimating growth using modal progression analysis of length frequency data and with tag recapture data, they concluded that the primary checks were laid down in winter, and that the secondary rings were laid down in early summer at about the time of the monsoon. They distinguished primary and secondary bands by viewing the exterior of the shell with strong transmitted light, where primary checks were conspicuous as translucent bands. Shepherd et al. (2000) found that the external growth checks in *H. Kamtschatkana* were annual comparing growth estimated from tag recapture data and from modal progression analysis, with growth between the shell checks.

In clean *H. iris* shells (i.e. not covered with epiphytes), similar translucent bands are visible using transmitted light (Figure 6). In Figure 6 all external bands appear as translucent bands; however, one translucent band is visible which stable oxygen analysis indicated was not an annually formed band.

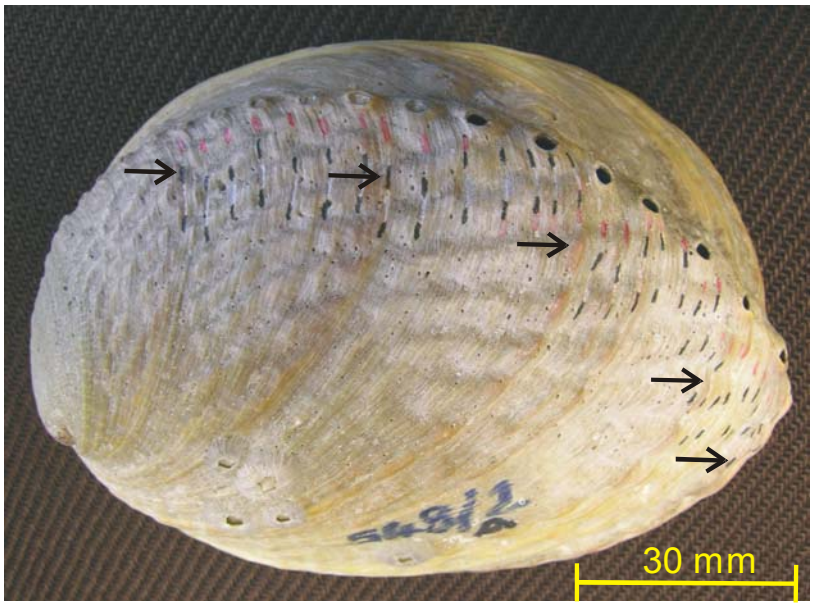

Figure 5: Shell of paua S48/2A from PAU 5A. Arrows indicate growth checks at lengths coinciding with minima in the temperature cycle estimated using stable oxygen isotopes.

It would appear (albeit only in one shell) that while viewing the shell with strong transmitted light is another method of identifying growth checks on the shell, for *H. iris*, the method does not distinguish between annual growth checks and those formed at other times of the year. Examination of a larger sample using the method may be useful, as this type of examination is not very time consuming.

External shell checks may have some utility in estimating age in juvenile abalone, but because these checks may be laid down more than once a year in some cases, and because, especially with *H. iris*, older shells are usually heavily encrusted with coralline algae and other organisms, the utility of the method as an ageing tool appears to be very limited.

External daily growth checks on the shell

External daily growth increments have been validated in *H. madaka* (Tsuiki et al. 2004). Many molluscs form microscopic structures on their shells on a daily basis (Rhoads & Pannella 1970, Wilbur 1972). Scallops lay down concentric fine ridges on their shells which are variously referred to as lamellae, striae or microstriae (Owen et al. 2002, Smith et al. 2007), and it has been suggested that in some species, these are laid down daily (Clark 1968, 1975; Parsons et al. 1993).

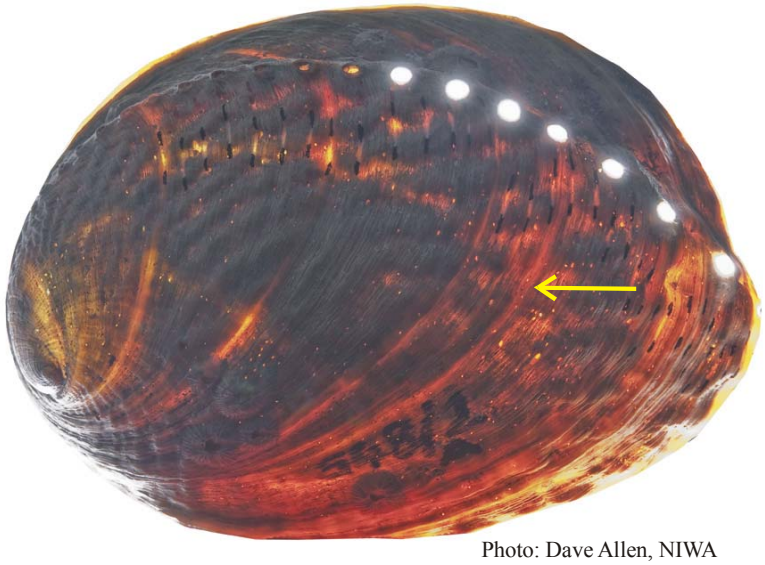

Photo: Dave Allen, NIWA

Figure 6: Shell of paua S48/2A viewed with strong transmitted light. Arrow indicates a translucent band not indicated as an annually formed band using stable oxygen isotopes.

In clean shells of *H .iris* these lamellae are readily visible under a binocular microscope (Figure 7). Validation of the temporal nature of these lamellae in *H. iris* could be made by tagging as long as the tagging process did not cause temporary growth cessation. Counting the number of lamellae over cycles of oxygen isotopic values in the shell might also indicate whether lamellae are formed on a daily basis in *H. iris*. Casual examination of the width of lamellae in Figure 7 (i.e. about 10 per mm.) does not exclude the possibility that they are laid down on a daily basis.

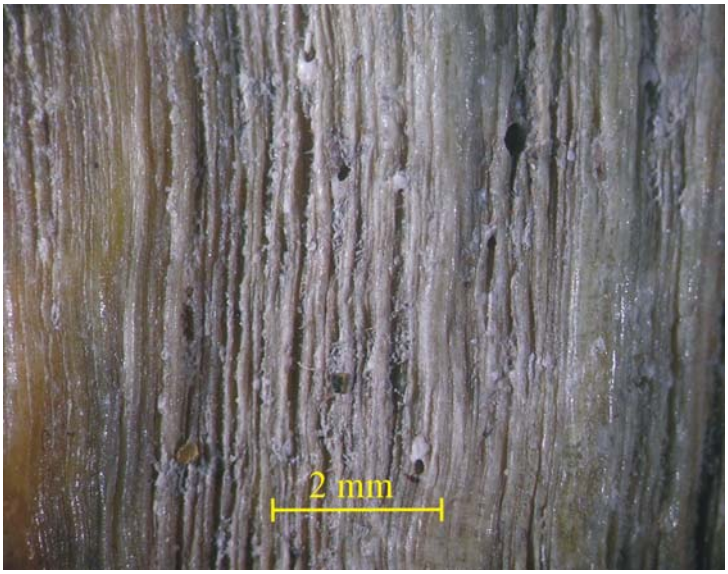

Figure 7: Magnified (about 12 ×) section of the exterior of paua S48/2A showing rows of lamellae.

Internal growth checks in the shell

Internal growth checks in the shells of abalone have been used by a number of authors to estimate age. Sakai (1960) examined sectioned shells of *H. discus hannai* which had been held in bamboo baskets. He was able to determine that the growth checks formed annually at about the time of spawning. He was able to distinguish annual checks from disturbance checks on the basis that the disturbance checks faded as they approached the nacreous layer, while the annual rings appeared as opaque fault lines in that layer.

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, 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).

Muñoz-lopez (1976, cited in Prince et al. 1988) developed a different approach to examining growth checks in the shells of abalone. The spire of the shell was ground with abrasive paper until a small hole was visible in the spire. This resulted in the exposure of concentric rings of nacre surrounding the hole. The area was then polished with finer paper to accentuate the rings (Figure 8). This method has subsequently been adopted by Prince et al. (1988), McShane & Smith (1992), Shepherd et al. (1995 a,b), Shepherd & Avalos-Borja (1997), Shepherd & Triantofillos (1997), Shepherd & Turrubiates-Morales (1997), Shepherd & Huchette (1997), Shepherd et al. (2000), and Naylor (2010).

Shepherd et al. (1995b) used the method of Muñoz-lopez (1976, cited in Prince et al. 1988) and also examined vertical sections in the same shells of *H. fulgens*. While they found that early fine layers were clearer in vertical sections, they advocated the use of horizontal sections (e.g. Figure 7) as they were much easier to prepare, and in larger shells there was no significant bias associated with the method. They note that in larger shells early growth was frequently eroded. *H. fulgens* 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) also 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.

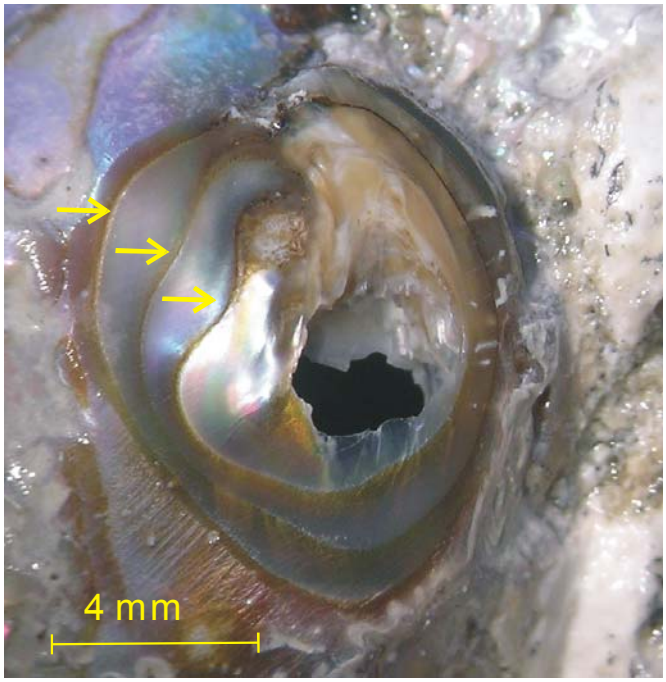

Figure 8: Magnified (about 12 ×) polished spire of shell D816. Arrows indicate three growth checks.

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. In Figure 7 they are being viewed from above, and in Figure 1 they are being viewed from the side, and according to Shepherd et al. (2000) readings from each method are comparable. The horizontal sections (i.e. those looked at from above) have practical advantages because they are easier to prepare and read than vertical sections (i.e. those looked at from the side (Shepherd et al. 2000).

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.

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.

Murray (1986) reports that the growth checks in *H. iris* are not laid down until maturity at about 4 years of age. Sinclair (1963) also notes that *H. iris* shells between 40 mm and 50 mm had no growth checks. 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, and Poore (1973) found that *H. australis* spawned twice, once in spring and again in late summer to early autumn. Kim & Chung (1985, cited in Shepherd et al. 1995a) also associate the formation of growth checks with winter in *H. diversicolor*. It seems likely that spawning or winter may cause the formation of growth checks in abalone. Shepherd et al. (1995a) attribute the unusually clear growth checks in *H. mariae* to the coincidence of spawning and winter. *H. iris* usually spawn in late summer or early autumn but may spawn again around September, and as late as October (Poore

1973, Sainsbury 1982, Wilson & Schiel 1995, Hooker & Creese 1995, McShane & Naylor 1996). The cause of growth check formation in *H. iris* is not yet known.

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*. McShane & Smith (1992); however, found that in other areas the method did not reliably estimate age in *H. rubra*.

Proudfoot et al. (2008) examined sectioned *H. midae* shells under a fluorescent microscope. Under UV light the alternate protein and aragonite layers fluoresced. The advantage of the method is that the bands can easily be identified and counted in rough cut sections of shell, eliminating the need for polishing (Proudfoot et al. 2008). For *H. iris* alternate bands in rough cut shell sections are readily apparent (see Figure 1), so this technique is unlikely to improve the readability of internal growth checks in paua.

A potential problem with internal and external 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.

The large body of literature relating to the estimation of age using growth checks is variable and conflicting. It is clear; however, that if growth checks are to be used for this purpose, validation of the method is required for different species and stocks (Beamish & McFarland 1983).

5 VALIDATION METHODS

There are three main methods that have been used to validate ageing of abalone shell using growth rings. The methods generally compare estimates of age made by growth checks with estimates of age inferred by tag-recapture data, length frequency analysis, or stable isotope analysis.

Modal analysis of length-frequency

Modal analysis of length-frequency data has been used by numerous authors to estimate growth and age. Poore (1972), and Sainsbury (1982) analysed length distributions to estimate growth in *H. iris* and Newman (1968) estimated growth in juvenile *H. midae* using length frequency distributions. By examining gonad indices, he also linked cohorts to approximate spawning dates (Newman 1968). Estimates of age have been made for isolated cohorts (eg. Day & Leorke 1986 for *H. rubra*) but only for juvenile abalone. Shepherd (1988) was able to separate length frequency modes in juvenile *H. laevigata*, but where the size classes were larger than 110 mm, the modes contained several age classes.

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 ages estimated using these methods and ages estimated from counts of growth checks in the shell. Shepherd & Triantofillos (1997) also 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*.

The main problem with this approach arises from growth variability in abalone. Variation in the growth of abalone worldwide is well documented (e.g. Shepherd 1988). Variation in the growth of paua within the same population is also well documented (Sainsbury 1982, Poore 1972, McShane &

Naylor 1995a, Naylor et al. 2006). This variation means that cohorts merge as they become older. Even the most casual examination of plotted tag-return data for paua (see for example the plots in Naylor & Andrew 2002) makes it abundantly clear that this method is very unlikely to provide reliable estimates of age. To complicate matters further, growth variation may also occur between the sexes (Hearn 1986, cited in Day & Fleming 1992). Variable recruitment between years will also make the separation of cohorts more difficult, although a large pulse of recruitment may produce a strong cohort which can be more easily followed. In *H. iris*, this variation is well documented, both between locations and between years (Day & Loerke 1986, Shepherd 1988, Sainsbury 1982, Poore 1972, Hooker & Creese 1995, McShane & Naylor 1996). Another factor precluding this method as a useful validation tool is that mean values are produced (Day & Fleming 1992).

Prince et al. (1988) 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.

A review of the methods used to separate modes is not a constructive avenue within the context of this review. The method will not reliably validate estimates of age made by the counting of growth checks in or on the shells of *H. iris*.

Tag recapture data

Poore (1972) used tag recapture to determine that external shell checks on the shells of *H. australis* were annual. Forster (1967) did the same for *H. tuberculata*. External growth checks are not often evident on the shells of *H. iris*; as older paua are usually encrusted with coralline algae.

For the validation of growth checks, tag recapture data is more often used to predict age at length. Shepherd et al. (1995a) found primary and secondary growth checks on the shells of *H. mariae*. By estimating growth using modal progression analysis and using tag recapture data they thought that the primary checks were laid down in winter, and that the secondary rings were laid down in early summer at about the time of the monsoon. They distinguished primary and secondary bands by viewing the exterior of the shell with strong transmitted light, where primary checks were conspicuous as translucent bands. Shepherd et al. (2000) found that the external growth checks in *H. kamtschatkana* were annual by comparing growth estimated from tag recapture data and from modal progression analysis, with growth between the shell checks.

Shepherd et al. (1995b) used tag recapture data 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.

Incremental growth data from tag-recapture programmes can not reliably provide age estimates to validate ageing using growth checks. This is primarily because early growth (i.e. in paua less than about 70 mm) cannot be determined by tag recapture techniques. Apart from the difficulty in locating sufficient numbers of small animals to tag, because they are cryptic at this stage, it is very difficult to re-locate them, especially if they are residing under large boulders. Even if growth could be estimated for juvenile paua by tag-recapture, because of the well documented variable growth rates of paua within populations (e.g., McShane & Naylor 1995a, Sainsbury 1982) paua of a certain length at release will grow different amounts over the following and subsequent years. Because of this, the range of ages a paua could be at a particular length increases with length. This means that a paua 125 mm in length is likely to be between, for example, 4 years old and 10 years old. This is not informative in determining whether the number of growth checks present in the shell are annual or otherwise. This type of analysis may, however, be useful in discrediting an ageing method if the method indicated an age well outside that indicated by tagging data.

Stable isotope analysis

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.

Samples of calcite are taken with a micro-sampling device at about 2 mm intervals along the growing axis of the shell (Figure 4). These are then analysed for oxygen isotopes using a mass spectrometer, and the isotopic temperature of paua shell calcite can then be estimated by solving the carbonate paleotemperature equation of Epstein et al. (1953). The water temperatures at the time of shell precipitation are then graphed along an axis corresponding to shell length. This plot reveals a series of annual cycles reflecting seasonal warm-cool oscillations, and differences in shell length associated with high or low temperatures. Growth increments can then be calculated as the differences in length between successive low temperature or high temperature data pairs (Naylor & Breen 2008). As long as the entire shell is sampled, the number of warm-cool oscillations will also indicate the age of the shell at the time of capture.

Despite the fact that 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*, the method has not often been used to determine growth or age in abalone. Since the 1950s, it has been widely used in palaeoenvironmental studies on foraminifera (e.g. Emiliani 1955; Kroon & Ganssen, 1989) and is increasingly applied to ecological investigations of living plankton (Peeters et al. 2002). The method has also been used to explore life history patterns in benthic algae, corals, bryozoans, polychaetes, arthropods, bivalves, gastropods, cephalopods and vertebrates Wefer & Berger (1991).

Relatively few studies have been attempted on abalone. Lee & Chen (2002) estimated age in three *H. diversicolor* shells using stable oxygen isotopes. They also used stable carbon isotope analysis to distinguish between wild and released abalone (Lee & Chen 2002). Gurney et al. (2006) estimated the age and growth of two *H. rubra* shells using the method, and Naylor et al. (2007) used the method to estimate growth and age in five *H.iris* shells. These shells had been tagged in previous work (McShane & Naylor 1995b) and known length increments determined by tagging corresponded with increments determined by stable oxygen isotope analysis (Naylor et al. 2007). Naylor & Breen (2008) estimated growth in 30 shells from 10 sites in PAU 5A (Fiordland) using the same method and concluded that the method appeared to work well. The growth increments estimated from stable isotope analyses were comparable to tag-recapture data from Chalky Inlet and the growth curves fitted to both data sets were similar. Roussell et al. (2011) examined three *H. diversicolor* shells using stable oxygen isotopes and found that 57% of the external growth checks on the shell corresponded to the lowest winter temperatures indicated by isotopic analyses. They also found that in all three shells, no growth checks were associated with the first lowest winter temperatures indicated by isotopic analysis. Roussell et al. (2011) suggest that the approach developed by Shepherd & Avalos-Borja (1997) may be more cost effective than isotopic analyses. They used scanning electron microscopy and determined that the thickness of the aragonite tiles in *H. corrugate* followed a seasonal pattern (Shepherd & Avalos-Borja 1997). Snow & Pring (2005) report a similar pattern in *H. iris*. While the thickness of the tiles could be used to infer seasonal cycles, each cycle of tile thickness variation is within the protein layers, i.e. the aragonite tiles are most thin just before and just after the protein layer is deposited. As the protein layers (i.e. growth checks) are clearly visible in *H. iris*, examination of tile thickness is unlikely to provide further information on growth or age.

Bomb radiocarbon analysis

Leaf et al. (2008) used bomb radiocarbon analysis to support an age at length relationship in the red abalone *H. rufescens*. The relationship was estimated from tag recapture data and age was estimated using the Von Bertalanffy growth function. The basis of this method is that naturally occurring levels of ^{14}C were greatly enhanced because of atmospheric testing of thermonuclear devices during the 1950s and 1960s. During ocean-atmosphere gas exchange processes ^{14}C levels also became elevated in ocean surface waters, and elevated ^{14}C levels were subsequently incorporated into the shells of many marine organisms (Leaf et al. 2008).

Leaf et al. (2008) examined four samples from one shell which was thought to have been collected in 1968 and compared ^{14}C levels from the samples to two reference series from fish otoliths to estimate age at sample formation. The method, albeit with a very small sample size, appeared to work. This approach will not be useful in validating or determining age in paua. This is because the increase in oceanic ^{14}C levels occurred between 1958 and 1968 (pers. comm. Helen Neil, NIWA) after which there is a very gradual decrease in ^{14}C levels over time. Unless the birth year of the paua is earlier than about 1955, shell samples cannot be associated with the bomb growth curve and the current slow decrease in ^{14}C levels does not allow the resolution for age validation.

Ratios of magnesium/calcium in mollusc shells also reflect water temperature (Vasil'ev 2005); however, the methodology is not as well developed as stable isotope analysis (pers. comm. Russell Frew, University of Otago). Trace element analyses have so far been used primarily on ostracods, foraminifera, and otoliths.

Hatchery reared abalone

Tsuiki et al. (2004) found and validated the formation of daily growth rings in *H. madaka*. They used aquarium held and marked abalone and abalone marked and released into the wild to confirm that the lamellae on the external surface of the shell were formed on a daily basis. Abalone held in aquaria were marked by immersion in alizarin complexone at fourteen day intervals. When viewed under fluorescent microscopy, the mean number of increments on the shell surface was 13.7 (n=21, S.D. = 1.7, Tsuiki et al. 2004). The tagged and released abalone were at liberty for 372 days. To estimate length at release, 372 increments were counted inwards from the edge of the shell, and the shells were measured at that point. There was a good correspondence between estimated and actual lengths at release (Tsuiki et al. 2004).

The use of hatchery rearing to produce abalone of a known age for the validation of growth checks may be confounded if the stimuli which cause the deposition of checks are not present in the hatchery environment.

Conclusion

Stable oxygen isotope analyses appear to be the most reliable method of validating the timing of growth check deposition. Commonly used methods of validation such as age estimation from tag-recapture data or the use of length frequency data to determine age are flawed because of the inherent variability of the data.

6 ACKNOWLEDGEMENTS

This work was funded by Ministry of Fisheries project SEA201016. Thanks to Owen Anderson for reviewing the manuscript.

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