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Age determination protocol for hoki (*Macruronus novaezelandiae*)

New Zealand Fisheries Assessment Report 2017/13

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

Horn, P.L.; Sutton, C.P. (2017). Age determination protocol for hoki (*Macruronus novaezelandiae*).

***New Zealand Fisheries Assessment Report 2017/13.* 22 p.**

This report documents the age determination protocol for an important New Zealand middle-depth finfish species: hoki (*Macruronus novaezelandiae*). It describes the most recent scientific methodologies used for otolith preparation and interpretation, ageing procedures, and the estimation of ageing precision, and also describes the changes in these methodologies over time. An otolith reference collection of 480 preparations was compiled and described. Agreed readings and ages determined for the reference set are stored in a reference table in the *age* database. The reference set sample was mostly a random selection from fish stocks and seasons to account for spatio-temporal variations in otolith readability, however the selection process also ensured that a comprehensive range of fish sizes and ages were included.

Digital image examples of otolith reference set preparations are presented and fully illustrate the zone interpretation used in determining fish age for hoki. Associated difficulties and idiosyncrasies related to ageing prepared otoliths are also described.

1. INTRODUCTION

Determining an accurate estimate of age for a fish species is an integral part of fisheries science supporting the management of the fisheries resources in New Zealand. Knowing the age of a fish is critical for estimating growth, mortality rate, population age structure, and age-dependent fishing method selectivity, all important inputs for age-based stock assessments. Information on fish age is also essential for determining biological traits such as age at recruitment and sexual maturity, and longevity.

To maintain accuracy and consistency in ageing fish in New Zealand, the Ministry of Fisheries (now a part of the Ministry for Primary Industries (MPI)) held a fish ageing workshop in Wellington in May 2011, and produced a document "Guideline for the development of fish age determination protocols" based on the workshops results (Ministry of Fisheries Science Group, 2011). From this, it was anticipated that age determination protocols would be developed for every species that was routinely aged for MPI fisheries research projects.

This report describes the age determination protocol for an important New Zealand middle-depth finfish species: hoki (*Macruronus novaezelandiae*) (Figure 1). It is the largest New Zealand finfish fishery, producing landings of 90 000–250 000 t annually since 1987. A substantial Fishstock (HOK 1) for this species falls within Tier 1 of the National Fisheries Plan for Deepwater and Middle-depth Fisheries, with service strategies that promote regular stock assessment, thus utilising routinely collected catch-at-age information. The purpose of this protocol is to provide a practical guide for ageing, and to describe the methodologies and techniques used by NIWA otolith readers to prepare, interpret, and read otoliths, and to convert zone counts into estimates of fish age. This protocol will ensure that the best known methods are documented to ensure that future consistency and accuracy for age estimates of hoki are maintained over time. It will also serve as a valuable training tool for new otolith readers.

No attempt was made to describe protocols related to daily increments in hoki otoliths (usually associated with ageing larval or juvenile fish), investigations into otolith ultrastructure, and chemical composition of hoki otoliths, as these were outside the scope of the current project. Three otolith pairs are present in the otic capsule of bony fishes, i.e., asteriscii, lapilli, and sagittae, but only the sagitta, is usually used in age estimation (Panfili et al. 2002), and is used to age hoki. Therefore, throughout this report, the use of 'otolith' will be synonymous with the sagittal otolith (Figure 1). A glossary describing otolith terminologies and ageing definitions outlined in the "Guideline for the development of fish age determination protocols" is in Appendix 1.

Figure 1: Hoki (*Macruronus novaezelandiae*): whole fish and sagittal otolith. (Fish photo by Peter McMillan, NIWA.)

2. AGE DETERMINATION PROTOCOL FOR HOKI

2.1 Background

Attempts to age hoki (*Macruronus novaezelandiae*) were made using scales (Blagoderov 1978) and otoliths (Kuo & Tanaka 1984a, 1984b; Kenchington & Augustine 1987; Sullivan & Coombs 1989), but none of these studies validated the age estimates after the first 3 years of growth. Different otolith examination methods were used including: untreated whole otoliths that were thinned by grinding on both the proximal and distal surfaces (Kuo & Tanaka 1984a, 1984b), untreated whole otoliths examined in water under reflected light, and untreated transverse sections (about 0.4 mm thick) (Kenchington & Augustine 1987), and otoliths that were broken transversely through or near the centre, the broken surface was ground smooth, then the section was baked in an oven until amber coloured (Sullivan & Coombs 1989). All these preliminary age studies highlighted problems with the interpretation of the early otolith growth zones, i.e., that the zone structure was complex and often the widths between visible zones varied markedly. The first three growth zones are often split or consist of a series of finer zones, and many otoliths exhibit a clear zone inside the first true translucent zone. An inflated estimate of age is derived if all these zones are counted. Consequently, when readers aged a sample from off west coast South Island containing a strong year class of known age, the strong year class could not be identified correctly (Sullivan & Cordue 1994). Because of this problem, Sullivan & Cordue (1994) rejected the otolith readings as an input to the stock assessment.

It was clear, however, that length-frequency distributions of hoki from commercial landings and research trawl surveys often exhibited several modes shorter than 75 cm total length which represented separate year classes of juveniles. By examining the rate of progression of these juvenile length modes between surveys, the age of fish in the length modes was validated and the rate of juvenile growth determined (Horn & Sullivan 1996). An examination of otoliths of fish from these length modes of known age also enabled the pattern of early growth of the otolith to be determined. In addition, relatively strong year classes spawned in 1983 and 1987, and a very weak year class from 1986, were observed to progress through population age distributions sampled annually from 1988 to 1994. These results provided strong validation of the age determination method out to age 8 years. Horn & Sullivan (1996) baked and embedded otoliths before sectioning them transversely, and this has remained the preferred otolith preparation method since then (see Section 2.3).

Further support for the otolith zone interpretation methods described by Kenchington & Augustine (1987) and Horn & Sullivan (1996) was provided from an age validation study using the bomb radiocarbon chronometer method (Kalish et al. 1997). Measurements of $\Delta^{14}C$ from otolith cores plotted against birth date determined from counts of zones in otolith sections were shown to fall on or close to the curve reflecting the increase in ^{14}C in the south-west Pacific Ocean between the late 1950s and early 1970s.

Fenton et al. (1990) analysed whole hoki otoliths for the naturally occurring radionuclides ^{210}Pb and ^{226}Ra in an attempt to independently verify their age. However, the radiometric technique could not be applied to determine age because the results showed that ^{226}Ra was not incorporated at a constant rate throughout the life of hoki. Uptake of ^{226}Ra was greater in juveniles than in adult fish, possibly resulting from an ontogenetic change in habitat from juvenile to adult fish. A subsequent analysis (Fenton & Short 1995) using otolith cores only found that the radiometric ages of otolith cores were in approximate agreement with those derived by Kenchington & Augustine (1987) using zone counts.

An examination of micro-increment widths in hoki otoliths led Gauldie (1996) to postulate that fish longer than 100 cm were aged about 4–7 years, 2-year-old fish were about 20 cm long, and maximum age was 7 years. These claims were based on the extrapolation of mean micro-increment widths in the juvenile section of the otolith to the total otolith growth axis. This method clearly produces an underestimation of fish age.

Hoki has a moderate life-span, with fish older than 20 years being relatively uncommon in New Zealand. The oldest recorded age determined for a hoki in the New Zealand EEZ is 25 years, for two females (92 cm and 114 cm) captured off west coast South Island in 1988, and a 92 cm male from Chatham Rise in 2001. Most of the commercial and research catch comprises fish aged from about 3 to 12 years.

Hoki are known to spawn over winter, primarily (but not exclusively) in Cook Strait and off the west coast of South Island with peak spawning occurring mainly from June to September (Livingston et al. 2015). Based on biological data collected from the spawning grounds, Horn & Sullivan (1996) chose a birthdate of 1 August as being near the end of the spawning season, and this was adopted as the standard in subsequent hoki ageing studies.

2.2 Methods

Sagittal otoliths are the primary structure used for determining the age of hoki. All methodologies described in the following sections will be associated with age determination using baked and sectioned sagittal otoliths, currently the best practice preparation method. The methodology used for preparing hoki otoliths was developed in the early 1990s, with the aim being to provide robust, permanent, ordered, and compact sets of aged otoliths. The method was subsequently used, occasionally with minor modifications, to prepare otoliths from a variety of deepwater, middle-depth and inshore species. The following sections present additional information pertinent to hoki age determination.

2.3 Otolith preparation and examination

Post extraction, hoki otoliths are cleaned of adhering tissue and blood, and stored in paper envelopes labelled with sample details, including trip code, station number (or landing number for market samples), fish number, date, and fish length and sex. Age determination studies should record sex as a mandatory requirement for each fish selected for analysis because hoki show differential growth between the sexes, where females attain a larger average size than males at a given age (Horn & Sullivan 1996). The envelopes are stored in labelled box files relating to the year of collection and source of the otoliths (i.e., research surveys, commercial catch samples at sea, or on-shore samples of commercial landings), and are archived at NIWA, Wellington.

Whole otoliths selected for preparation are marked with a pencil line dorsoventrally across the nucleus to denote the required cross-section position (Figure 2), and then baked in an oven until amber-coloured (270°C for about 4 min). The baked otoliths are then embedded in blocks of clear epoxy resin (Araldite K142) in flexible silicone moulds. Each block contains 60 otoliths; there are five rows of 12 otoliths, each row being four wide and three high (Figure 3). The anterior and posterior tips of some of the large otoliths are sometimes broken off before embedding to enable all 60 otoliths to fit in each block. In each row, the pencil lines (which are still clearly visible after baking) are aligned with marks on the moulds so that a single saw-cut will bifurcate all 12 otoliths along the marked lines. The cross-sectioning is carried out using a rotary diamond-edged saw (0.3 mm thick) with water lubrication, producing a series of short, resin sub-blocks that fit easily on a microscope stage (Figure 3). Details identifying the block and row number are written on each block section.

For examination and zone counting, the prepared sub-blocks (each with 12 otolith cross-sections) is coated in paraffin oil, illuminated by reflected light with an incident angle of about 45°, and examined under a binocular microscope (×30). A pattern of dark-brown (translucent) and light-brown (opaque) zones is apparent. If the zonation pattern is unclear, improvements can sometimes be achieved by altering the angle of incidence of the illumination, and/or by slightly altering the plane of focus. Subsequently in this document, ‘zone’ refers to the paired structure of one opaque zone inside one translucent zone. The number of translucent zones is counted. Fish length and sex are unknown to the otolith reader.

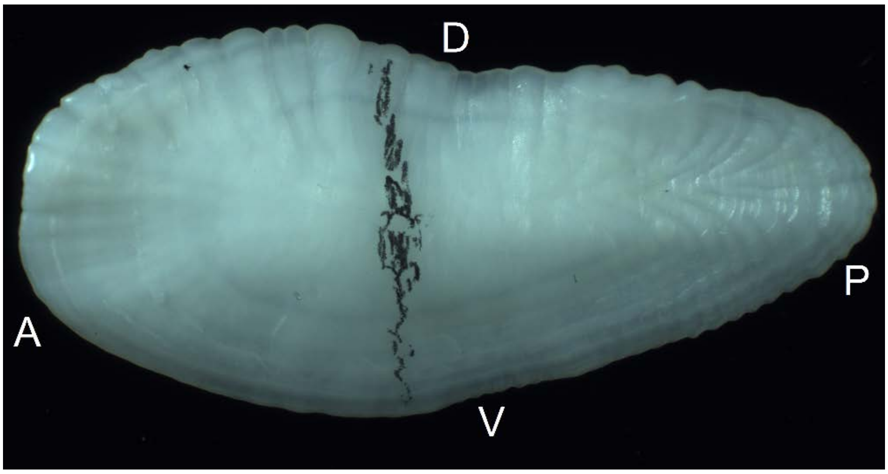

Figure 2: Untreated sagittal otolith (distal surface), with pencil line indicating the position of the cross-section. A, anterior; P, posterior; D, dorsal; V, ventral.

Figure 3: Resin block (block #8 of the hoki reference set), comprising six sub-blocks, holding 60 cross-sectioned baked otoliths in five groups of 12.

2.4 Otolith interpretation

The zonation pattern is generally clearest on the ventral side of the otolith cross-section (Figure 4), and most counts (and all measurements) are made in this area. The dorsal part of the cross-section is sometimes used, however, if the ventral part is unclear, or as a check on the ventral count if the dorsal part is obviously clear. Although most zone counts (after age 3) are made in the central part of the ventral side (see Figure 4), zonation can sometimes be clearest adjacent to the sulcus on the ventral side, or along the long axis of the section from the primordium to the ventral tip.

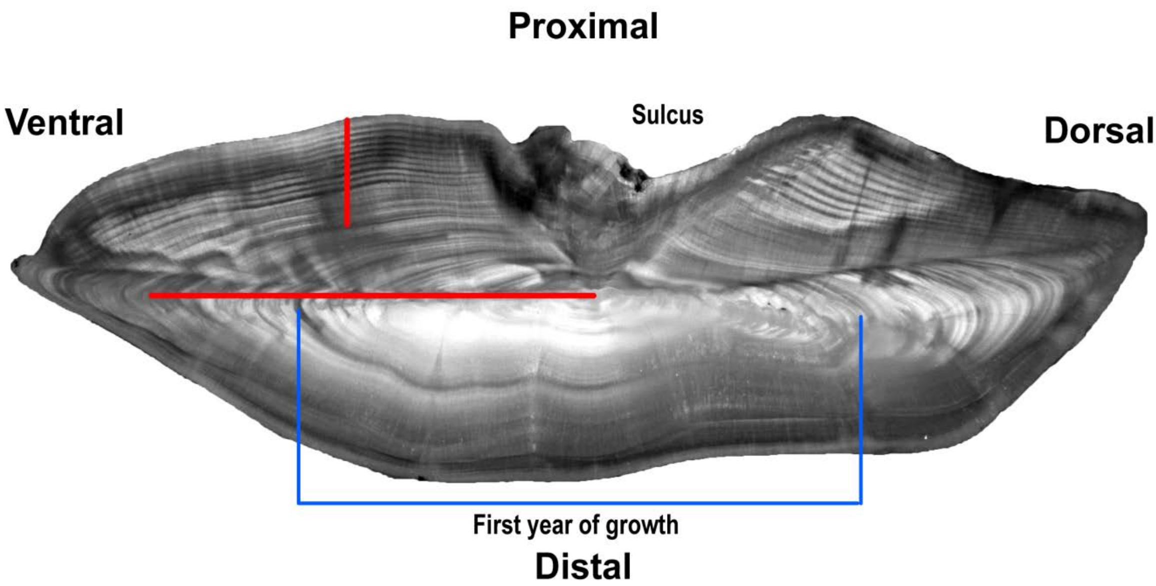

Figure 4: Hoki otolith image (B&W) of a baked transverse cross-section under reflected light, illustrating otolith terminology. Radial measurements are made along the horizontal red line, and subsequent zone counts are generally made in the vicinity of the vertical red line. The blue lines indicate the approximate extent of growth in the first year. Estimated age is 17 years.

Hoki have a post-larval pelagic stage, occurring mainly in offshore waters with post-larval metamorphosis occurring probably during late summer, when the fish are about 20 cm long and 6–9 months old. A relatively clear post-larval zone, probably where juvenile hoki adopt a primarily demersal habit, was observed in about one-third of hoki otoliths (Horn & Sullivan 1996). Kenchington & Augustine (1987) note this characteristic in many, but not all, otoliths. However, multiple post-larval zones of varying strength and clarity are visible inside the first annual zone in most otolith preparations (see Figure 4), and split or sub-annual zones are also often observed out to age 3 years. Sometimes the strength and clarity of sub-annual zones was similar to that of annual zones and this issue led to incorrect age determination in early studies of this species.

Horn & Sullivan (1996) determined mean distances from the primordium to the outer edges of the first three annual dark (translucent) zones on the ventral sides of otoliths sections to help define the likely positions of annual zones when multiple sub-annual zones were also present. To do this they identified otolith sections which appeared to have clear and unambiguous zones at ages 1–3, and the distance from the primordium to each zone was measured. The mean radius of each of the three zones was calculated, and these values were compared to the mean radius of each of the first 3 zones observed on the ventral side of whole otoliths extracted from hoki in distinct length-frequency modes, where each mode almost certainly comprised fish of a single known age (Table 1). The length modes were from samples collected around the South Island in winter, at the time of year when the translucent zone was shown to form in hoki otoliths (Kuo & Tanaka 1984b; Kenchington & Augustine 1987).

Table 1: Measurements from the primordium to the outer margin of the first three translucent zones on the ventral side of otolith sections, and measurements of the distance from the primordium to the ventral edge of whole otoliths collected in winter from fish known to be age 1, 2, or 3 years. CI, 95% confidence intervals.

Distance from primordium to outer margin of translucent zone from otolith sections			
	1st zone	2nd zone	3rd zone
Mean (mm)	2.28	2.89	3.31
N	109	109	109
CI (mm)	±0.24	±0.26	±0.23
Distance from primordium to ventral edge of whole otolith			
	1st zone	2nd zone	3rd zone
Mean (mm)	2.20	2.84	3.40
N	15	30	37
CI (mm)	±0.44	±0.33	±0.32

When a hoki otolith is read, the zone count is recorded along with the distance from the primordium to the first, second, and third annual zones on the ventral side of the otolith section (Figure 5). These radial measurements are used later to determine if the interpretation of the zones in the juvenile region follows an acceptable pattern (using the method of Francis 2001). When reading otoliths with unclear or confusing zonation in the area of juvenile growth, the mean radial measurements from Table 1 can be used to help identify the likely locations of the first three true annual zones. In some occasions, however, when radial measurements are needed to identify the likely locations of annual zones, the pattern of visible zones may be such that there are two ‘logical’ interpretations of the zonation pattern leading to two estimates of age varying by one year (Figure 6). Both interpretations are recorded for use in later analyses of catch-at-age (Francis 2001).

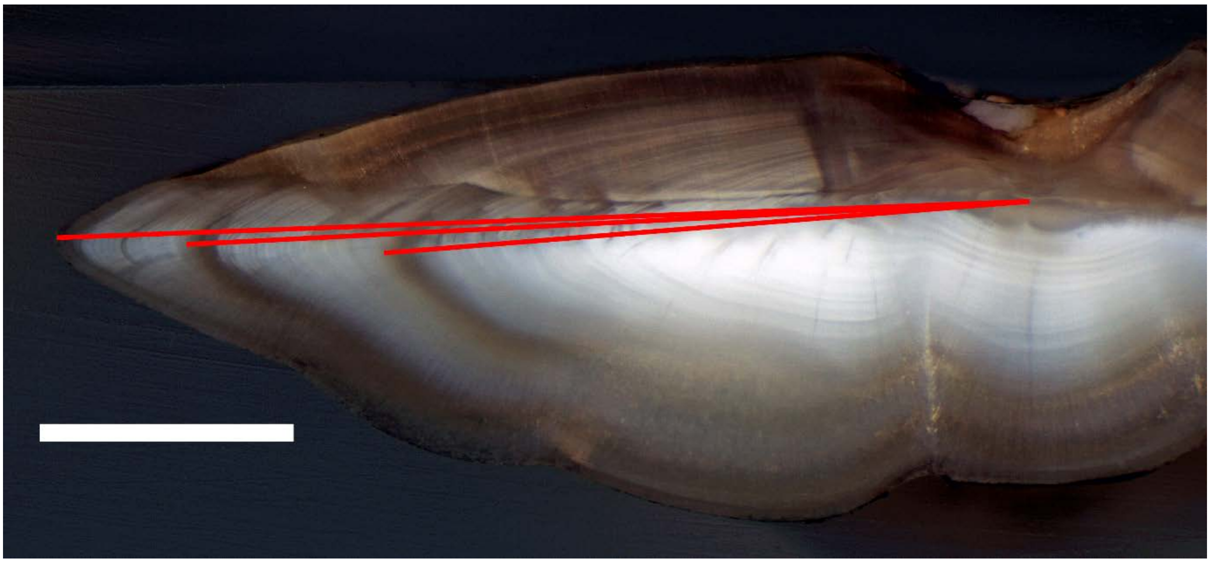

Figure 5: Otolith section image for a 60 cm male hoki with three distinct dark zones, and red lines indicating the measured radial distances to the outer extents of these zones (reference set otolith #094). This fish was sampled during winter and exhibits a dark margin. Other sub-annual zones are apparent in the first three years of growth. White bar = 1 mm.

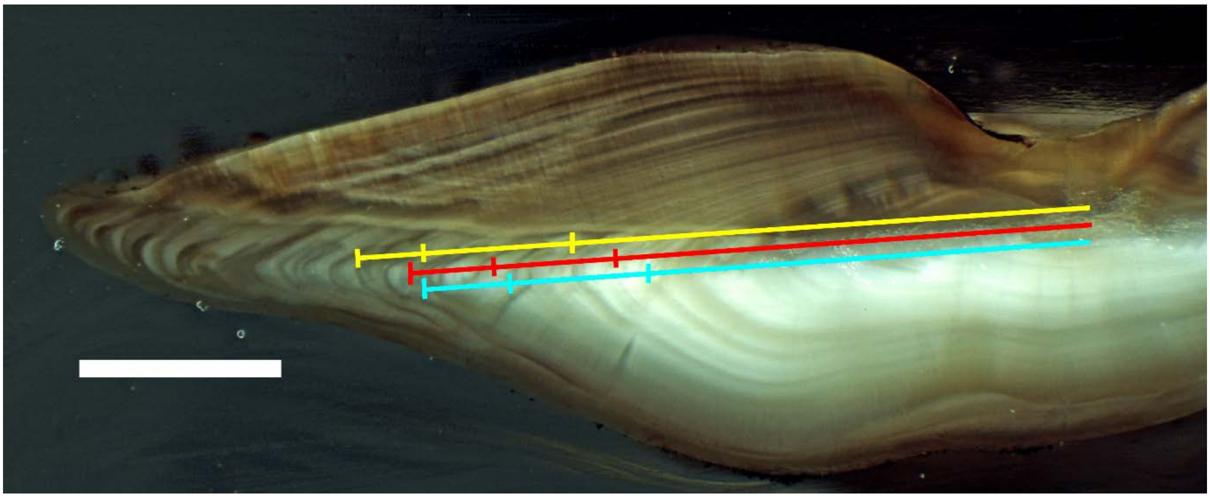

Figure 6: Otolith section image for a 91 cm male hoki estimated to be either 10 or 11 years old owing to uncertainly within the region of juvenile growth (reference set otolith #209). The tick marks on the red line show the calculated mean radial distances to the first three annual zones (from Table 1). The blue and yellow lines, with tick marks, show the two most likely interpretations of the zones visible in the section. The blue interpretation implies an age of 11, and the yellow interpretation an age of 10. White bar = 1 mm.

The main assumptions made and processes followed when interpreting zones (and counting translucent zones) in transversely sectioned hoki otoliths are:

1. The translucent zone (dark in baked cross-section preparations) first becomes visible in early winter.
2. The theoretical 'birthday' for all hoki is 1 August.
3. For otoliths from fish sampled during winter (i.e., the spawning season fisheries), the margin is generally translucent. This translucent zone is counted, as fish from the spawning fisheries are aged as though they have all just had their birthday (even though they may have been captured just before 1 August).
4. When the locations of any of the first three annual (translucent) zones is not clear, measure out from the primordium 2.2 mm, 2.9 mm, and 3.4 mm and search around these distances to identify zones that are likely to represent the winter growth in each of these three years. Most false checks occur in this area of the otolith.

Zone width tends to decline steadily up to about age 6, and then becomes relatively uniform and often quite clear. Sometimes, however, more than one reading from more than one region is required to attain a final zone count. Zone deposition on different parts of the otolith section may not always appear to be equal, and if discrepancies occur between counts, the default is to use the higher estimate.

The conversion of a zone count to an age estimate involves considering the relationship between the date of the increment formation, the date of capture, and the nominal birthdate (Panfili et al. 2002). This is relatively straightforward for hoki because the counted translucent zone begins to appear in early winter just before the chosen birthdate (1 August). So the zone count corresponds with the age of the fish in whole years. Three categories of hoki age samples are produced routinely. Winter spawning fishery samples (generally from mid-June to mid-September) are characterised by otoliths with translucent (dark) margins. Non-spawning fishery samples extend over the remaining nine months of the year, and are characterised by otoliths with opaque (light) margins of varying widths. Trawl survey samples are generally collected during the summer (characterised by medium width opaque margins), but occasionally during the winter spawning season (translucent margins).

Otolith readers know the source of the sample they are reading (i.e., either spawning season, non-spawning season, summer survey, or winter survey), and they know that otoliths are prepared roughly in temporal order (i.e., early season first, and late season last). The timing of the deposition of the newly formed zones may vary slightly between individual fish, stocks and years, but by knowing the sample source, readers are able to anticipate the expected otolith margin in comparison to what they actually see. The correct interpretation of the otolith margin is only an issue for otoliths collected very late in the non-spawning season when a narrow translucent margin may be apparent (but is not counted by the reader), or otoliths collected very early in the spawning season when a wide opaque margin is apparent (i.e., no translucent material is yet apparent, so the reader must add 1 to the zone count).

No readability scale is currently used when ageing hoki otoliths, although the readability of the juvenile zones was recorded in the past. However, it was found to be non-useful data, so its collection ceased in 2008. Consequently, a readable otolith preparation has either a single age interpretation (with associated radial measurements), or an interpretation where there are two possible ages with a 1-year difference.

2.5 Characteristics of sections

A good hoki otolith section is one where the first three annual zones are clear and unambiguous (even though sub-annual zones might still be apparent) and where subsequent annual zones are clear and distinct and probably exhibit a declining width as the margin is approached (Figure 7). Figures 8–16 illustrate other examples of both clear and complex zone counts in a range of hoki otolith sections.

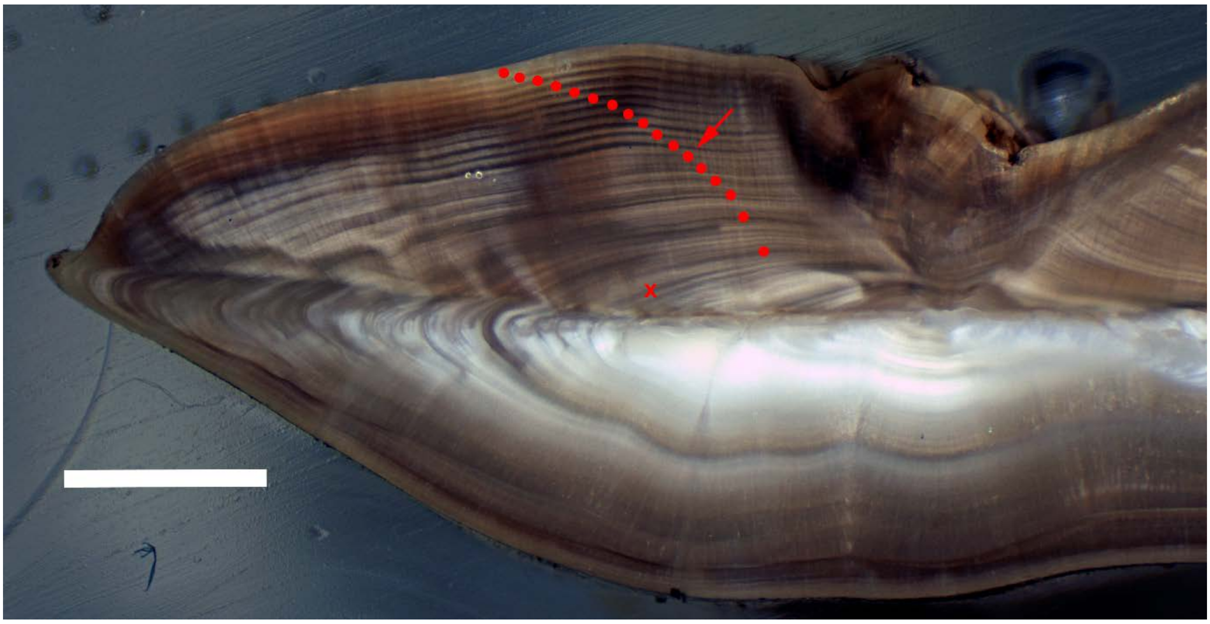

Figure 7: Otolith section image for a 103 cm female hoki that can be clearly aged as 17 years (reference set otolith #001). The counted dark zones are indicated by red dots. The dot indicated by the red arrow shows the least clear zone in the section (the 7th), but this zone is apparent across the entire ventral section, so is clearly a true zone. The red × indicates the likely location of the settlement zone inside the 1st annual zone. Other sub-annual zones are apparent in the first three years of growth. White bar = 1 mm.

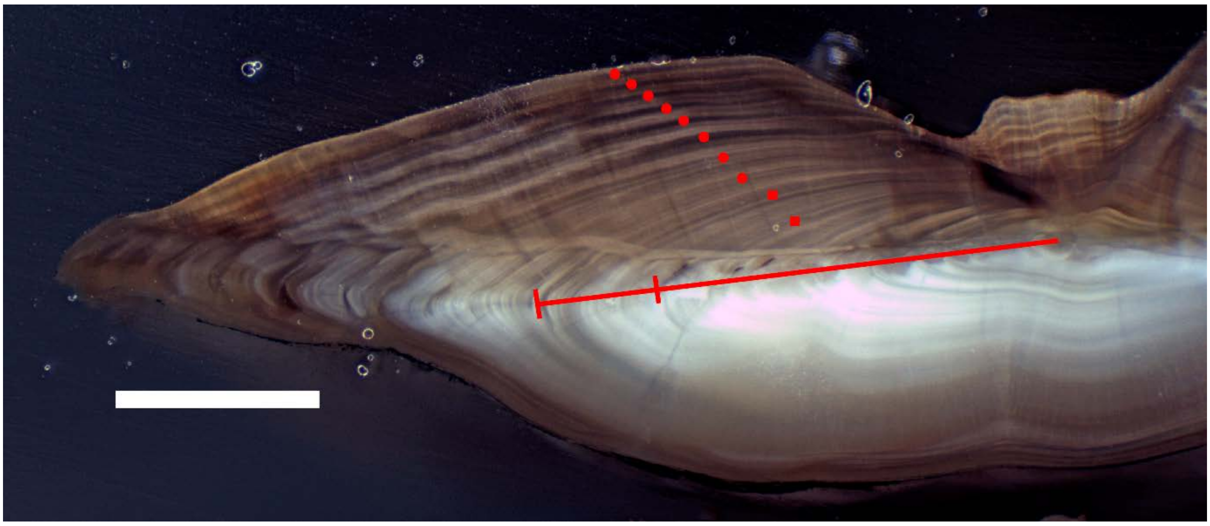

Figure 8: Otolith section image for an 82 cm male hoki aged as 10 years (reference set otolith #013). The counted dark zones are indicated by red dots. The 1st annual zone is faint at the long axis (i.e., the first tick mark on the red line), although it is more apparent nearer the sulcus. The 2nd annual zone is clear throughout the section (denoted by the terminal tick mark on the red line). The 3rd annual zone is also faint and indistinct at the long axis. White bar = 1 mm.

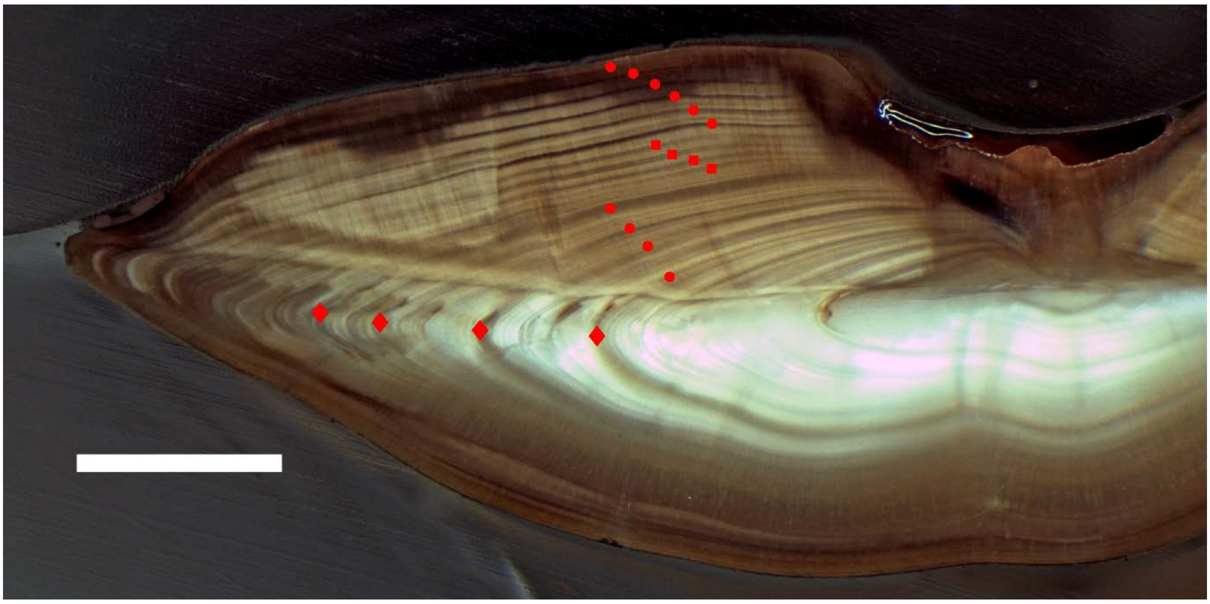

Figure 9: Otolith section image for a 105 cm female hoki aged as 14 years (reference set otolith #022). The counted dark zones are indicated by red dots (and the first four juvenile zones also indicated by red diamonds on the long axis). This image demonstrates some difficult interpretation in the midsection of the otolith. The first two annual zones are clear on the long axis, and the 3rd and 4th zones are clear nearer the sulcus. The 5th zone is relatively broad, but indistinct, and is followed by four zones that are distinct, but narrow relative to the final five zones. Assuming zone width is related to actual fish growth, it appears that this hoki experienced a period of unusually slow growth between its 5th and 9th birthdays. White bar = 1 mm.

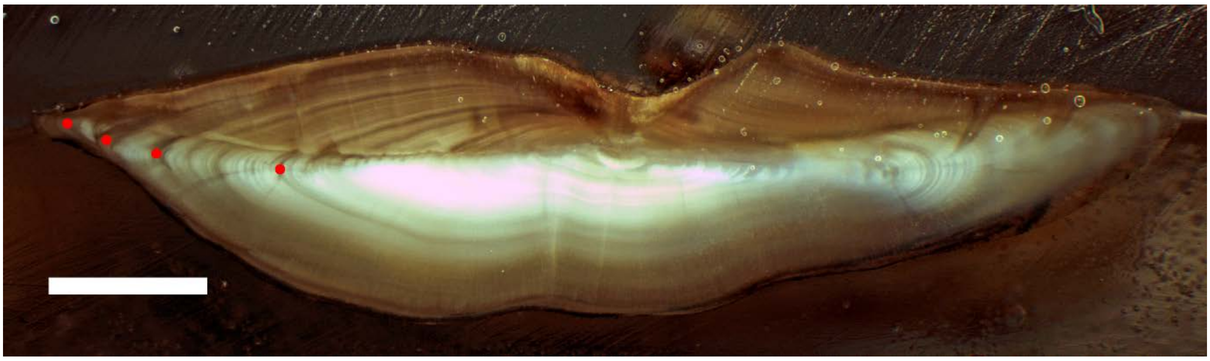

Figure 10: Otolith section image for a 68 cm male hoki aged as 4 years (reference set otolith #028). The counted dark zones are indicated by red dots. The 1st annual zone is very clear across the section. Between the 1st and 2nd annual zones is a clear sub-annual zone. The presence of the sub-annual zone was determined by radial measurements, coupled with the observation that this sub-zone merged with the 2nd annual zone in the proximal area of the section. White bar = 1 mm.

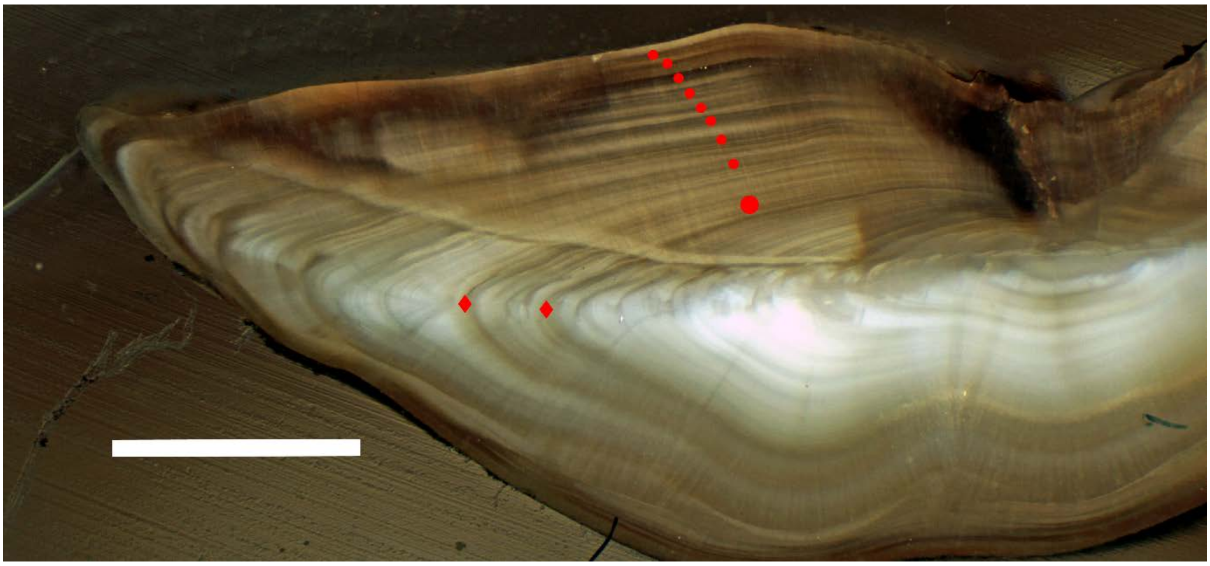

Figure 11: Otolith section image for a 97 cm female hoki aged as 9 years (reference set otolith #059). The counted dark zones are indicated by red dots. The 1st annual zone (indicated by the largest red dot) is clearly split, with its two dark components (indicated on the long axis by red diamonds) being apparent across much of the ventral section. Additional sub-annual zonation is apparent in the first year of growth. White bar = 1 mm.

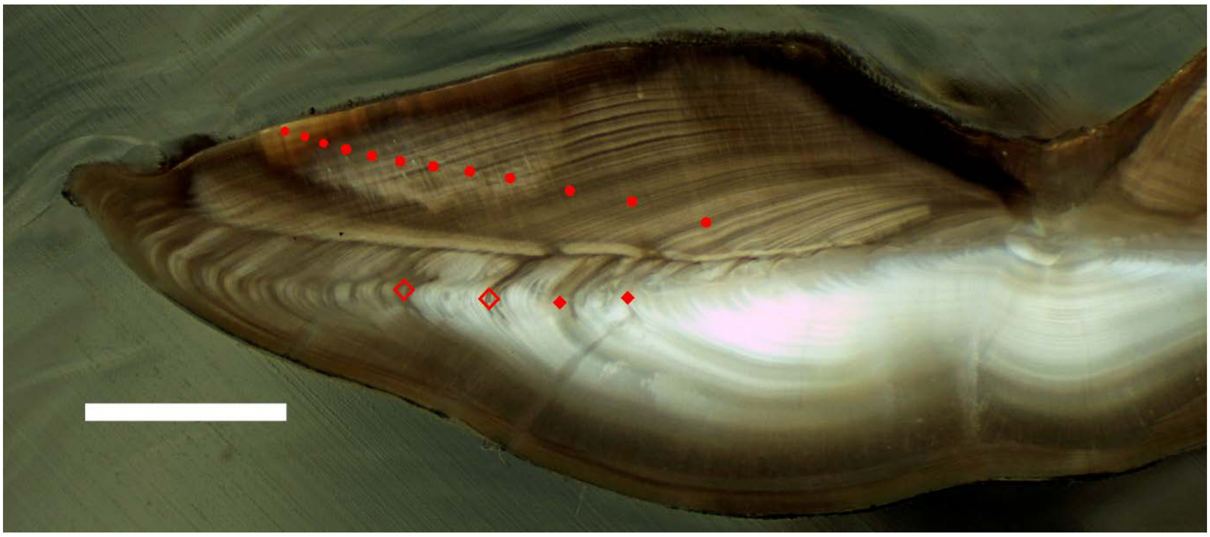

Figure 12: Otolith section image for a 95 cm male hoki aged as 12 years (reference set otolith #065). The counted dark zones are indicated by red dots. The 1st and 2nd annual zones are both split on the long axis; the components of the 1st zone are shown by small red diamonds, with open diamonds for the 2nd zone. The 4th to 6th zones exhibit a double banding structure along the long axis and, particularly, between the row of red dots and the sulcus. White bar = 1 mm.

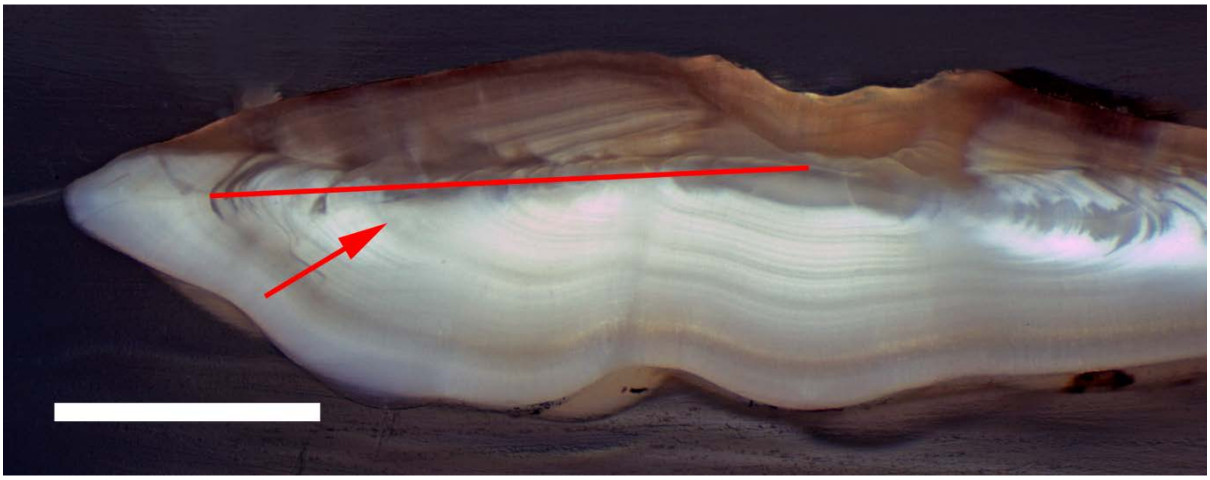

Figure 13: Otolith section image for a 38 cm male hoki aged as 1 year (reference set otolith #086). The red line indicates the radius to the 1st annual zone. The red arrow indicates the relatively clear settlement zone. The fish was sampled in January, and there is a broad opaque (light) margin outside the 1st annual zone. White bar = 1 mm.

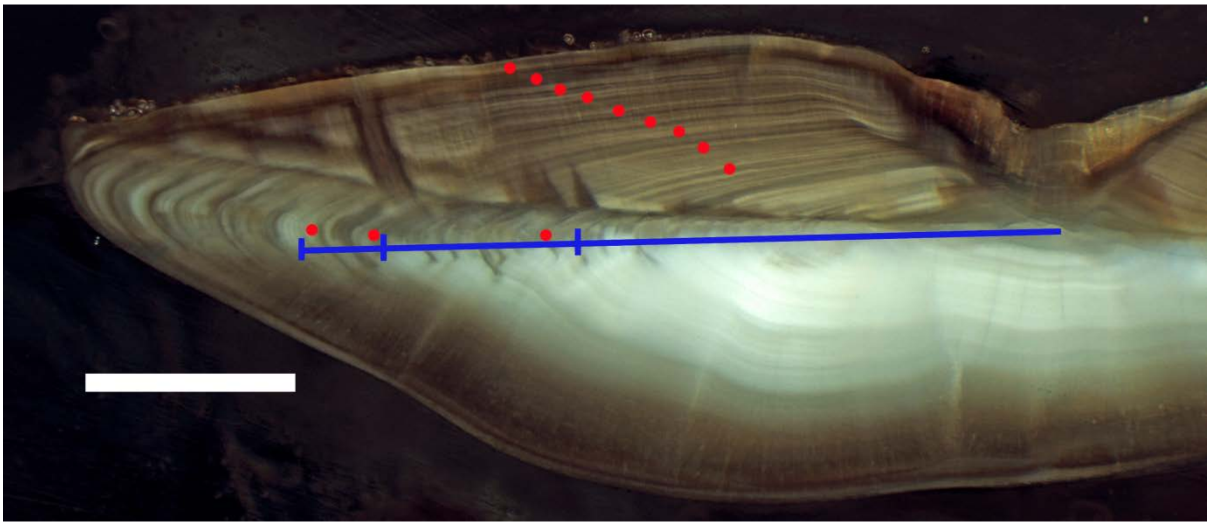

Figure 14: Otolith section image for a 97 cm female hoki aged as 9 years (reference set otolith #211). The counted dark zones are indicated by red dots. The 1st and 2nd annual zones are relatively indistinct on the long axis, and the zonation pattern is also not clear near the sulcus. The locations of the 1st, 2nd and 3rd zones along the long axis (indicated by red dots) were determined using mean radial measurements (indicated by the blue line with tick marks). White bar = 1 mm.

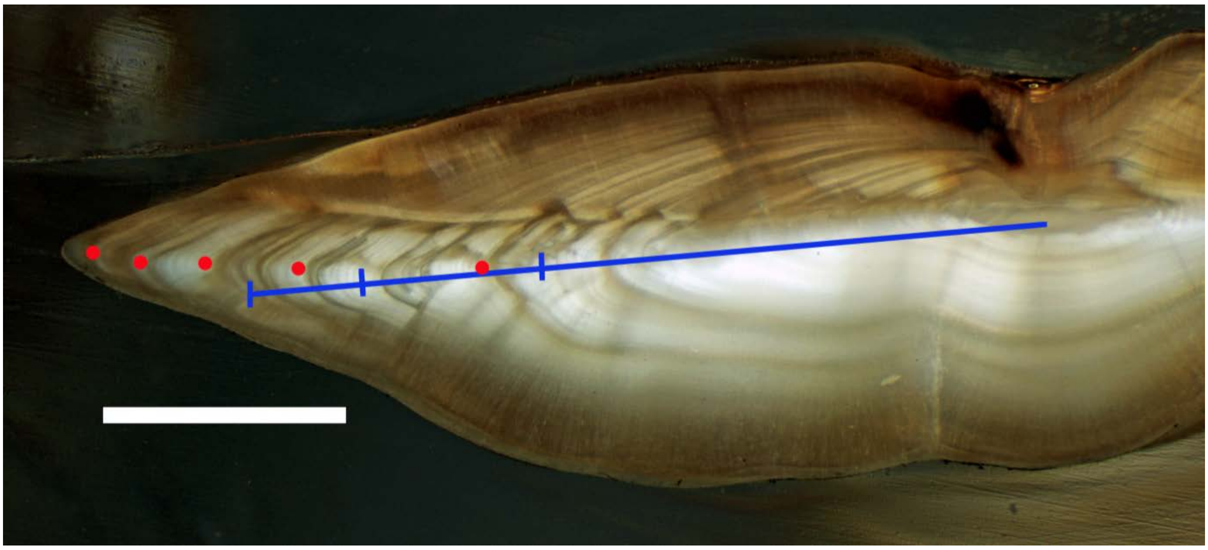

Figure 15: Otolith section image for a 75 cm female hoki aged as 5 years (reference set otolith #035). The outer edges of the counted dark zones are indicated by red dots. The 1st, 2nd and 3rd annual zones were determined using mean radial measurements (indicated by the blue line with tick marks). There are strong and distinct sub-annual zones inside both the 1st and 2nd annual zones. The age of this fish was determined by counting zones along the long axis from the primordium to the ventral tip because the zonation pattern is not clear towards the proximal surface of the otolith. White bar = 1 mm.

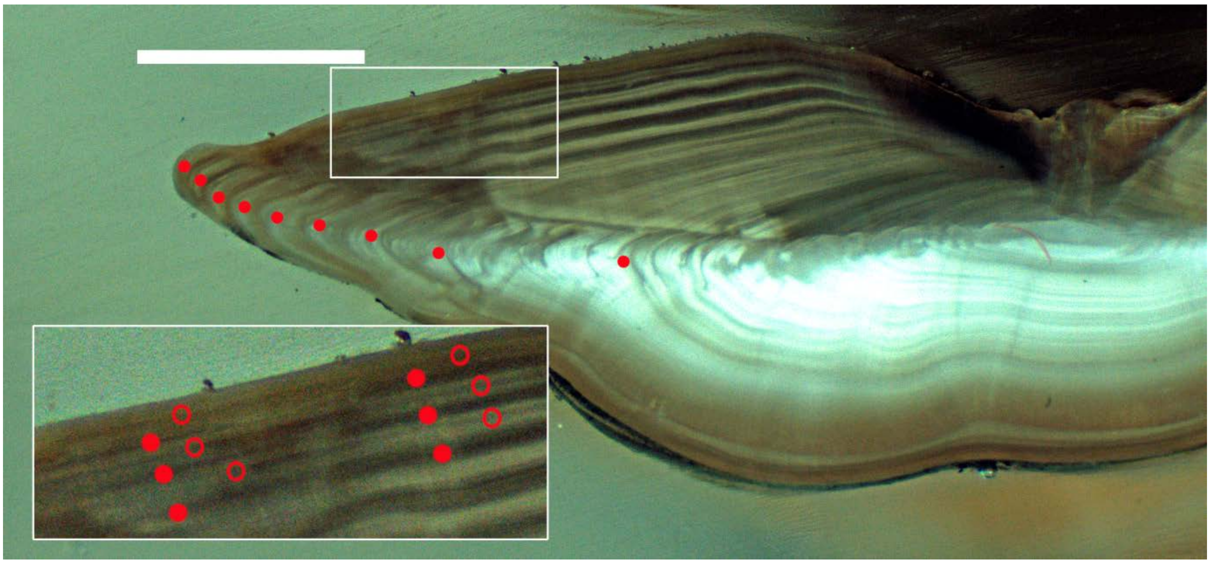

Figure 16: Otolith section image for an 89 cm male hoki aged as 9 years (reference set otolith #249). The counted dark zones are indicated by red dots, which, in this instance, are generally very clear along the long axis. Only the 2nd annual zone lacks clarity. The three zones nearest the margin have a clear paired structure. This is more apparent in the inset, showing the dark counted zones (solid red dots) and the sub-annual zones (open red circles). White bar = 1 mm.

2.6 Estimation of Ageing Precision

Horn & Sullivan (1996) quantified precision and bias in a within-reader comparison. In a sample of 200 otoliths read twice, 66.5% were aged identically, 29% differed by 1 year, and the remaining 4.5% differed by 2 years. There was no apparent bias in the ageing error. Precision declined with age; for fish aged 2–10 years at the first reading, 71% of paired readings were identical, but only 50% were identical for fish older than 10.

A subsequent reader comparison in 2006 compared within-and between-reader precision from a sample of 180 otoliths. Indices of average percentage error (IAPE) were 2.2% for the within-reader test and 3.4% for the between-reader test. Precision was good in both tests up to about age 8, but declined slightly at older ages (Figure 17). IAPE values of less than 5% are still considered to be reasonable for hoki even though otoliths can be moderately difficult to interpret.

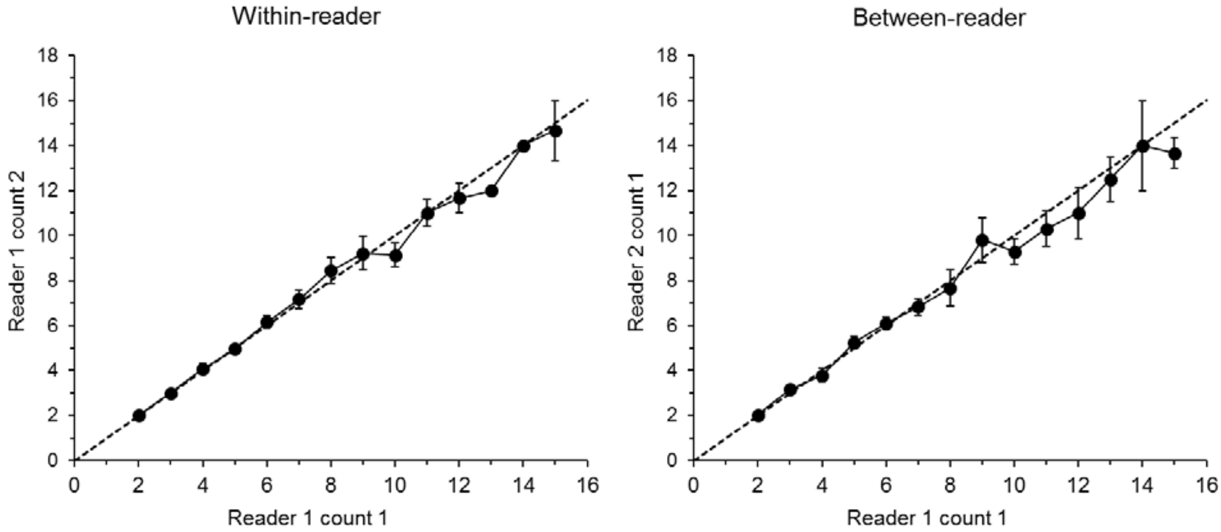

Figure 17: Age reader comparison plots for a sample of 180 hoki otoliths conducted in 2006. The age-bias plots show the correspondence of ages within reader 1, and between reader 1 and reader 2 for all ages. Error bars indicate ± 2 SE. A one-to-one relationship is indicated by the dashed line.

2.7 Reference collection

A collection of 480 hoki otoliths was selected for the reference collection, i.e. eight blocks each containing 60 preparations. This is expected to be sufficient for quality control monitoring in assessing reader performance, but it may be added to over time. The primary role of the reference set is to monitor ageing consistency (and accuracy) over both the short and long term, particularly for testing long-term drift, as well as consistency among age readers (Campana 2001). The hoki reference collection was assembled from the thousands of otoliths (archived at NIWA Wellington) collected from the main hoki fishery areas (west coast South Island, Cook Strait, Chatham Rise, Sub-Antarctic) from June 2012 to February 2013. The roughly random selection process for the reference set has ensured that a seasonal distribution of otolith samples is represented, and that the full length range is covered, while not being strongly dominated by any particular age class. Also, because growth variation between stocks (areas) and sexes was reported for hoki (Horn & Sullivan 1996), the otolith selection process also ensured approximately even sampling of these two categories. Examples of the otolith preparations for a range of fish sizes and ages are presented in Section 2.5 (Figures 7–16). As hoki has only a moderate life-span (i.e., few sampled fish are older than 18 years), a reference collection of 480 otolith preparations is believed to be more than adequate for quality control monitoring purposes.

The agreed ages for otoliths selected for the reference set are stored on the *age* database (administered by NIWA for MPI) in a new table (t_reference) created within this database. As these preparations have already been aged multiple times in the past they may be treated with a reasonable level of confidence. The reference set may also be used for training new readers as well as monitoring their progress as they gain experience in ageing. Any new readings of the reference set collection (e.g., created before embarking on reading a new otolith collection) are stored on a second new table (table t_ref_age) to distinguish each calibration or training reading from those used to estimate catch-at-age distributions or growth parameters.

2.8 Format for data submission to *age* database

NIWA (Wellington) currently undertake the role of Data Manager and Custodian for fisheries research data owned by MPI. This includes storing physical age data (i.e., otolith, spine and vertebral samples) and the management of electronic data in the *age* database. A document guide for users and administrators of the *age* database exists (Mackay & George 1993). This database contains several tables, outlined in an Entity Relationship Diagram (ERD) which physically shows how all tables relate to each other, and to other databases.

When research has been completed, NIWA receives the final age data (usually in an Excel spreadsheet format) from the research provider and performs data audit and validation checks prior to loading these data to the *age* database (Table 2). Additional information that should be recorded include the MPI project code, reader(s) name or number(s), date of reading, preparation method, and a description of how the agreed ages were derived from zone counts.

Table 2: An example of hoki age data submitted for loading onto the *age* database, where the sample contains otoliths originating from a trawl survey (origin denoted by the vessel code THH), the observer programme (SOP), and on-shore catch sampling (SMP). R1, R2, R3 are measurements (mm) from the primordium to the outside of the 1st, 2nd, and 3rd translucent zones on the ventral side of the otolith section. Note that two otoliths in this sample have two possible age interpretations (prep_no 219 and 231).

																	First choice interpretation				Alternative interpretation					
origin	yr	trip_code	sample_no	sub_sample_no	area	species	fish_no	prep_no	block_no	reading_no	reading_date	material	method	reader	length	sex	count	R1	R2	R3	count	R1	R2	R3	age	proj_code
THH	2015	thh1501	8	-1	CKST	HOK	11	59	1E	1	18-Nov-15	1	14	124	86	2	9	2.53	3.17	3.47					9	MID2015-01
THH	2015	thh1501	8	-1	CKST	HOK	13	60	1E	1	18-Nov-15	1	14	124	67	2	4	2.50	3.17	3.47					4	MID2015-01
THH	2015	thh1501	8	-1	CKST	HOK	16	61	2A	1	18-Nov-15	1	14	124	87	2	9	2.57	3.27	3.53					9	MID2015-01
THH	2015	thh1501	8	-1	CKST	HOK	19	62	2A	1	18-Nov-15	1	14	124	31	2	1	2.33							1	MID2015-01
THH	2015	thh1501	9	-1	CKST	HOK	2	63	2A	1	18-Nov-15	1	14	124	76	1	6	2.37	3.13	3.53					6	MID2015-01
THH	2015	thh1501	9	-1	CKST	HOK	5	64	2A	1	18-Nov-15	1	14	124	97	2	10	2.57	3.17	3.53					10	MID2015-01
SOP	2015	4343	344	-1	CEE	HOK	5	218	4D	1	18-Nov-15	1	14	124	111	2	16	2.57	3.13	3.53					16	MID2015-01
SOP	2015	4343	344	-1	CEE	HOK	7	219	4D	1	18-Nov-15	1	14	124	91	2	10	2.57	3.27	3.53	11	2.57	3.00	3.27	10	MID2015-01
SOP	2015	4343	344	-1	CEE	HOK	10	220	4D	1	18-Nov-15	1	14	124	81	1	9	2.40	3.23	3.50					9	MID2015-01
SOP	2015	4343	346	-1	CEE	HOK	8	221	4D	1	18-Nov-15	1	14	124	69	1	4	2.17	3.27	3.47					4	MID2015-01
SOP	2015	4343	346	-1	CEE	HOK	10	222	4D	1	18-Nov-15	1	14	124	105	1	20	2.57	3.17	3.47					20	MID2015-01
SOP	2015	4343	353	-1	CEE	HOK	1	223	4D	1	18-Nov-15	1	14	124	72	1	5	2.33	3.20	3.47					5	MID2015-01
SMP	2015	20150001	1	-1	CKST	HOK	3	227	4D	1	18-Nov-15	1	14	124	88	2	10	2.50	3.17	3.53					10	MID2015-01
SMP	2015	20150001	1	-1	CKST	HOK	11	228	4D	1	18-Nov-15	1	14	124	77	1	6	2.47	3.27	3.83					6	MID2015-01
SMP	2015	20150001	1	-1	CKST	HOK	16	229	4E	1	18-Nov-15	1	14	124	69	1	4	2.33	3.27	3.53					4	MID2015-01
SMP	2015	20150001	1	-1	CKST	HOK	19	230	4E	1	18-Nov-15	1	14	124	78	1	8	2.50	3.27	3.53					8	MID2015-01
SMP	2015	20150001	1	-1	CKST	HOK	22	231	4E	1	18-Nov-15	1	14	124	79	1	8	2.50	3.27	3.60	9	2.50	3.03	3.27	8	MID2015-01
SMP	2015	20150001	1	-1	CKST	HOK	24	232	4E	1	18-Nov-15	1	14	124	68	2	4	2.07	3.27	3.80					4	MID2015-01

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APPENDIX 1: Glossary of otolith terminology and ageing definitions.

Based on Kalish et al. (1995) "Glossary for otolith studies" but with some added items including definitions for "fishing year age-class" and "forced margin" to describe New Zealand practice.

Accuracy – the closeness of a measured or computed value to its true value.
Age estimation, age determination – these terms are preferred when discussing the process of assigning ages to fish. The term 'ageing' should not be used as it refers to time-related processes and the alteration of an organism's composition, structure, and function over time. The term 'age estimation' is preferred.
Age-group – the cohort of fish that have a given age (e.g., the 5 year old age-group). The term is not synonymous with year-class or day-class.
Age-class – same as age-group, but see "Fishing year age-class".
Annulus (pl. Annuli) – one of a series of concentric zones on a structure that may be interpreted in terms of age. The annulus is defined as either a continuous translucent or opaque zone that can be seen along the entire structure or as a ridge or a groove in or on the structure. In some cases, an annulus may neither be continuous nor obviously concentric. The optical appearance of these marks depends on the otolith structure and the species and should be defined in terms of specific characteristics on the structure. This term has traditionally been used to designate year marks even though the term is derived from the Latin "anus" meaning ring, not from "annus", which means year. The variations in microstructure that make an annulus a distinctive region of an otolith are not well understood.
Antirostrum – anterior and dorsal projection of the sagitta. Generally shorter than the rostrum.
Asteriscus (pl. Asteriscii) – one of three otolith pairs found in the membranous labyrinth of osteichthyan fishes.
Bias – The systematic over or under estimation of age.
Birth Date – A nominal date at which age class increases, generally based on spawning season.
Check – a discontinuity (e.g., a stress-induced mark) in a zone, or in a pattern of opaque and translucent zones. Sometimes referred to as a false check.
Cohort – group of fish of a similar age that were spawned during the same time interval. Used with age-group, year-class and day-class.
Core – the area or areas surrounding one or more primordia and bounded by the first prominent D-zone. Some fishes (e.g., salmonids) possess multiple primordial and multiple cores.
Corroboration – a measure of the consistency or repeatability of an age determination method. For example, if two different readers agree on the number of zones present in a hard part, or if two different age estimation structures are interpreted as having the same number of zones, corroboration (but not validation) has been accomplished. The term verification has been used in a similar sense; however, the term corroboration is preferred as verification implies that the age estimates were confirmed as true.
D-zone – that portion of a micro-increment that appears dark when viewed with transmitted light, and appears as a depressed region when acid-etched and viewed with a scanning electron microscope. This component of a micro-increment contains a greater amount of organic matrix and a lesser amount of calcium carbonate than the L-zone. Referred to as a discontinuous zone in earlier works on daily increments; D-zone is the preferred term. See L-zone.
Daily increment – an increment formed over a 24-hour period. In its general form, a daily increment consists of a D-zone and an L-zone. The term is synonymous with "daily growth increment" and "daily ring". The term daily ring is misleading and inaccurate and should not be used. The term daily increment is preferred. See increment.
Drift – Shift with time in the interpretation of otolith macrostructure for the purposes of age determination.
Forced Margin or Fixed Margin – Otolith margin description (Line, Narrow, Medium, Wide) is determined according to the margin type anticipated *a priori* for the season/month in which the fish was sampled. The otolith is then interpreted and the age determined based on the forced margin. The forced margin method is usually used in situations where fish are sampled throughout the year and otolith readers have difficulty correctly interpreting otolith margins.

Fishing Year Age-class – The age of an age group at the beginning of the New Zealand fishing year (1 October). It does not change if the fish have a birthday during the fishing season. This is not the same as Age Group/Age Class.

Hatch date – the date a fish hatched; typically ascertained by counting daily increments from a presumed hatching check (see check) to the otolith edge.

Hyaline zone – a zone that allows the passage of greater quantities of light than an opaque zone. The term hyaline zone should be avoided; the preferred term is translucent zone.

Increment – a reference to the region between similar zones on a structure used for age estimation. The term refers to a structure, but it may be qualified to refer to portions of the otolith formed over a specified time interval (e.g., sub-daily, daily, annual). Depending on the portion of the otolith considered, the dimensions, chemistry, and period of formation can vary widely. A daily increment consists of a D-zone and an L-zone, whereas an annual increment comprises an opaque zone and a translucent zone. Both daily and annual increments can be complex structures, comprising multiple D-zones and L-zones or opaque and translucent zones, respectively.

L-zone – that portion of a micro-increment that appears light when viewed with transmitted light, and appears and as an elevated region when acid etched and viewed with a scanning electron microscope. The component of a micro-increment that contains a lesser amount of organic matrix and a greater amount of calcium carbonate than the D-zone. Referred to as an incremental zone in earlier works on daily increments; L-zone is the preferred term. See D-zone.

Lapillus (pl. Lapilli) – one of three otolith pairs found in the membranous labyrinth of osteichthyan fishes. The most dorsal of the otoliths, it lies within the utriculus (“little pouch”) of the pars superior. In most fishes, this otolith is shaped like an oblate sphere and it is smaller than the sagitta.

Margin/Marginal increment – the region beyond the last identifiable mark at the margin of a structure used for age estimation. Quantitatively, this increment is usually expressed in relative terms, that is, as a fraction or proportion of the last complete annual or daily increment.

Micro-increment – increments that are typically less than 50 µm in width; with the prefix “micro” serves to indicate that the object denoted is of relatively small size and that it may be observed only with a microscope. Often used to describe daily and sub-daily increments. See increment.

Microstructural growth interruption – a discontinuity in crystallite growth marked by the deposition of an organic zone. It may be localized or a complete concentric feature. See check.

Nucleus, Kernel – collective terms originally used to indicate the primordia and core of the otolith. These collective terms are considered ambiguous and should not be used. The preferred terms are primordium and core (see definitions).

Opaque zone – a zone that restricts the passage of light when compared with a translucent zone. The term is a relative one because a zone is determined to be opaque on the basis of the appearance of adjacent zones in the otolith (see translucent zone). In untreated otoliths under transmitted light, the opaque zone appears dark and the translucent zone appears bright. Under reflected light the opaque zone appears bright and the translucent zone appears dark. An absolute value for the optical density of such a zone is not implied. See translucent zone.

Precision – the closeness of repeated measurements of the same quantity. For a measurement technique that is free of bias, precision implies accuracy.

Primordial granule – the primary or initial components of the primordium. There may be one or more primordial granules in each primordium. In sagittae the granules may be composed of vaterite, whereas the rest of the primordium is typically aragonite.

Primordium (pl. Primordia) – the initial complex structure of an otolith, it consists of granular or fibrillary material surrounding one or more optically dense nuclei from 0.5 um to 1.0 um in diameter. In the early stages of otolith growth, if several primordia are present, they generally fuse to form the otolith core.

Rostrum – anterior and ventral projection of the sagitta. Generally longer than the antirostrum.

Sagitta (pl. Sagittae) – one of the three otolith pairs found in the membranous labyrinth of osteichthyan fishes. It lies within the sacculus (“little sack”) of the pars inferior. It is usually compressed laterally and is elliptical in shape; however, the shape of the sagitta varies considerably among species. In non-ostariophysan fishes, the sagitta is much larger than the asteriscus and lapillus. The sagitta is the otolith used most frequently in otolith age estimation studies.

Subdaily increment – an increment formed over a period of less than 24 hours. See increment.

Sulcus acusticus (commonly shortened to 'sulcus') – a groove along the medial surface of the sagitta. A thickened portion of the otolith membrane lies within the sulcus acusticus. The sulcus acusticus is frequently referred to in otolith studies because of the clarity of increments near the sulcus in transverse sections of sagittae.

Transition zone – a region of change in otolith structure between two similar or dissimilar regions. In some cases, a transition zone is recognised due to its lack of structure or increments, or it may be recognised as a region of abrupt change in the form (e.g., width or contrast) of the increments. Transition zones are often formed in otoliths during metamorphosis from larval to juvenile stages or during significant habitat changes such as the movement from a pelagic to a demersal habitat or a marine to freshwater habitat. If the term is used, it requires precise definition.

Translucent zone – a zone that allows the passage of greater quantities of light than an opaque zone. The term is a relative one because a zone is determined to be translucent on the basis of the appearance of adjacent zones in the otolith (see opaque zone). An absolute value for the optical density of such a zone is not implied. In untreated otoliths under transmitted light, the translucent zone appears bright and the opaque zone appears dark. Under reflected light the translucent zone appears dark and the opaque zone appears bright. The term hyaline has been used, but translucent is the preferred term.

Validation – the process of estimating the accuracy of an age estimation method. The concept of validation is one of degree and should not be considered in absolute terms. If the method involves counting zones, then part of the validation process involves confirming the temporal meaning of the zones being counted. Validation of an age estimation procedure indicates that the method is sound and based on fact.

Vaterite – a polymorph of calcium carbonate that is glassy in appearance. Most asteriscii are made of vaterite, and vaterite is also the principal component of many aberrant 'crystalline' sagittal otoliths.

Verification – the process of establishing that something is true. Individual age estimates can be verified if a validated age estimation method has been employed. Verification implies the testing of something, such as a hypothesis, that can be determined in absolute terms to be either true or false.

Year-class – the cohort of fish that were spawned or hatched in a given year (e.g., the 1990 year-class). Whether this term is used to refer to the date of spawning or hatching must be specified as some high-latitude fish species have long developmental times prior to hatching.

Zone – region of similar structure or optical density. Synonymous with ring, band and mark. The term zone is preferred.