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Age determination protocol for kahawai (*Arripis trutta*)

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C. Walsh,
P. Horn,
J. McKenzie,
C. Ó Maolagáin,
D. Buckthought,
C. Sutton,
M. Smith

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Publications Logistics Officer
Ministry for Primary Industries
PO Box 2526
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TABLE OF CONTENTS

EXECUTIVE SUMMARY **1**

1. INTRODUCTION **2**

2. AGE DETERMINATION PROTOCOL FOR KAHAWAI **3**
2.1 Background 3
2.2 Methods 5
2.3 Otolith preparation 5
2.4 Otolith interpretation 6
2.5 Ageing procedures 9
2.6 Estimation of Ageing Precision 10
2.7 Reference collection 13
2.7.1 Examples of thin section preparations of kahawai otoliths with marked opaque zones and agreed reading and age estimates for a range of fish size and age 14
2.8 Format for data submission to *age* database 17

3. ACKNOWLEDGMENTS **18**

4. REFERENCES **18**

APPENDIX 1: Glossary of otolith terminology and ageing definitions. **20**

APPENDIX 2: Diagrammatic thin section drawings of kahawai otoliths. **24**

APPENDIX 3: Protocol for thin section otolith preparation. **26**

APPENDIX 4: Comparison of sagittal otolith size for four commonly aged New Zealand inshore species: snapper, trevally, tarakihi and kahawai. **31**

APPENDIX 5: Summary of between-reader agreement and precision estimates documented in ageing studies for kahawai. **32**

EXECUTIVE SUMMARY

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This report documents the age determination protocol for kahawai (*Arripis trutta*), an important New Zealand inshore finfish species. The protocol describes current scientific methods used for otolith preparation and interpretation, ageing procedures, and the estimation of ageing precision, and also documents the changes in these methodologies over time. In addition, an otolith reference collection numbering approximately 500 preparations has been compiled and documented from previously prepared archived samples. Agreed readings and ages determined for the reference set are stored in a reference table in the *age* database. The reference set sample was generally a random selection from fishstocks and seasons to account for spatio-temporal variations in otolith readability, however the selection process also ensured a comprehensive range of fish size and age were included.

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

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 Ministry for Primary Industries (MPI)) held a fish ageing workshop in Wellington (May 2011), producing a document "Guidelines for the development of fish age determination protocols" (Ministry of Fisheries Science Group, 2011) based on the workshops results. From this, it was anticipated that age determination protocols would be developed for every species that was routinely aged through MPI funding.

This report describes the age determination protocol for an important New Zealand inshore finfish species: kahawai (*Arripis trutta*). A significant fishstock (KAH 1) for this species falls within Group 1 of the Draft National Fisheries Plan for Inshore Finfish, with service strategies that promote regular stock assessment, utilising routinely collected catch-at-age information. The purpose of the protocol is to describe methods used for otolith preparation and age determination to ensure accuracy and consistency over time.

Of the three otolith pairs occurring in bony fishes (asteriscae, lapillae, sagittae), only the largest, i.e., the sagitta, have been used to age kahawai. Therefore, throughout this report, the use of 'otolith' will be synonymous with sagitta. A glossary describing otolith terminologies and ageing definitions outlined in the "Guidelines for the development of fish age determination protocols" has also been included in this report for reference purposes (Appendix 1).

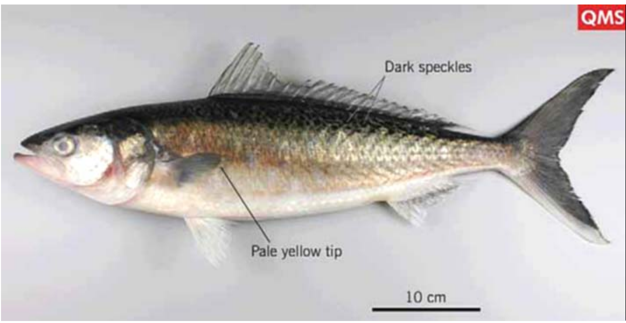
Overall objective

1. To develop age determination protocols for Inshore Finfish species.

Specific objectives

1. To develop an age determination protocol for kahawai (*Arripis trutta*), including the compilation of otolith reference collections.

2. AGE DETERMINATION PROTOCOL FOR KAHAWAI



2.1 Background

The first ageing study for kahawai in New Zealand was undertaken during the early 1970s by Eggleston (1975) with fish caught from many localities. He examined scales and whole sagittal otoliths to determine the suitability of each for ageing. Concentric growth rings (annuli) in scales and a series of opaque and hyaline (herein referred to as translucent) zones in whole untreated otoliths could be counted reliably in young fish only. In older fish, marginal annuli were crowded in scales, and because of the density of the otolith, the outer zones were unclear. Broken and burnt otoliths were examined and the zone counts showed exact correlation in the number of translucent zones in whole burnt and un-burnt otoliths. Whole burnt otoliths were chosen as the preferred method for the remainder of the study, with readability described as being excellent. By examining the otolith marginal state in kahawai over a 12 month period, Eggleston (1975) determined translucent material to be laid down annually, from March to November, and opaque material for the rest of the year.

During a nationwide tagging study on kahawai in the early 1980s (Wood et al. 1990), biological information was collected and age determined using whole otoliths, as described by Eggleston (1975). For otoliths that required burning to read, zones were found to be clearer when prepared using the break and burn method. During the same period, length modes of juvenile kahawai caught by gill nets were tracked over successive months by Jones & Hadfield (1985) with age confirmed from translucent zone counts in broken otoliths.

In a comprehensive study that validated age and growth of kahawai in the Bay of Plenty and Tasman Bay (Stevens & Kalish 1998), three ageing methods were trialled using otoliths: thin section; break and bake (similar to break and burn, but replacing burning with oven baking instead); and break and burn. They found that although both the break and bake and break and burn methods were more cost-effective for ageing kahawai than thin sections (in terms of both labour and materials), the contrast between increments was inferior to that obtained by using thin sections, which they recommended for future ageing. They also stipulated that ageing from whole baked otoliths was not attempted because of the poor correlation between ages from transverse sections and the need for a consistent reading axis. Stevens & Kalish (1998) produced diagrammatic thin section drawings of adult kahawai otoliths to document terminology and reading axes, as well as changes to otolith structure for juvenile fish between 6 months and 3 years of age (Appendix 2).

Although age validation using marginal increment analysis was unsuccessful, Stevens & Kalish (1998) validated the annual zone deposition in otoliths from recaptured tagged adult kahawai injected with oxytetracycline (OTC) in 1991 (see Griggs et al. 1998). They found that the position of the OTC mark was correctly inferred from age readings in 86% of the 139 samples, concluding that zones are deposited annually, with the opaque zone complete in April. Furthermore, the growth of juvenile kahawai was confirmed by following the progression of discrete non-overlapping length-frequency modes collected from three consecutive year Hauraki Gulf trawl surveys, which, based on otolith zone counts, represented 1+, 2+ and 3+ year old fish.

Commercial catch sampling programmes in the early 1990s used either the break and burn (McKenzie et al. 1992) or break and bake (Drummond & Wilson 1993, Drummond 1994) methods for preparing adult kahawai otoliths. Subsequent studies, including numerous recreational (i.e., Armiger et al. 2006, 2009, 2013, Hartill et al. 2007a, 2007b, 2008) and commercial (i.e., Devine 2007, Hartill et al. 2013) catch sampling programmes, used the thin section methodology of Stevens & Kalish (1998). The vast majority of the recreational data were collected over the summer months, but some commercial samples spanned the entire year, and determination in the latter was problematic, particularly in relation to accurately determining the margin state. As a result, a National Institute of Water and Atmospheric Research (NIWA) in-house ageing workshop was held in 2011, with an aim of improving reader zone counts when determining the age of kahawai from year-round samples by documenting a convention for margin interpretation dependent on the position of the outermost zone. To achieve this aim, a forced (or fixed) margin was implemented to anticipate the otolith margin type (wide, line, narrow) *a priori* in the month in which the fish was sampled to provide the reader with guidance and improve accuracy and precision in age estimations.

Stevens & Kalish (1998) also investigated between-reader comparisons for aged kahawai, documenting moderate to high error rates associated with first year zone interpretation and poor specimen preparation. Devine (2007) was the first to assess otolith reading precision for ageing kahawai by calculating the mean coefficient of variation (CV) (Chang 1982), Average Percentage Error (APE) (Beamish & Fournier 1981) and presenting age-bias plots (Campana et al. 1995), which indicated some difficulty in estimating age.

Kahawai is not a long lived species and ages over 20 years have rarely been observed in New Zealand (Eggleston 1978, Hartill et al. 2007a). The oldest recorded age determined for kahawai is reputed to be 26 years (Eggleston, Ministry for Primary Industries, unpublished data), but age estimates determined from whole baked otoliths, particularly from old fish such as this, have been found to be unreliable (Stevens & Kalish 1998). The oldest recorded age in the Ministry for Primary Industries *age* database is currently 21 years, shared by three specimens captured in the Bay of Plenty (2) and West Coast South Island (1) fisheries between 2003 and 2006, and range in size from 54–61 cm (estimated weight 2.3–3.1 kg).

Although a second species "Northern" kahawai (*Arripis xylabion*) exists, it differs from kahawai (*Arripis trutta*) in having a larger caudal fin (Paulin 1993) and growing bigger, attaining a maximum length of at least 94 cm (Ministry for Primary Industries 2013). Northern kahawai are mainly found in subtropical waters north of New Zealand and are rarely encountered in the coastal mainland. The information presented within this age determination protocol relates only to the commonly caught New Zealand coastal species of kahawai, *Arripis trutta*.

Kahawai are reported to spawn from January to April (Eggleston 1975, Drummond & Wilson 1993, Drummond 1994, J. McKenzie NIWA unpublished data). Although a theoretical birthdate for ageing kahawai of 1 April was chosen by Drummond (1994), the majority of kahawai ageing in New Zealand uses 1 February (Stevens & Kalish 1998), selected out of convenience as being near the peak of the kahawai spawning season, and this is used as the standard in this document.

2.2 Methods

Sagittal otoliths are acknowledged as the primary structure for ageing kahawai, and all scientific methodologies described in the following sections will be associated with ageing using thin sectioned sagittal otoliths, currently the best preparation method. The methodology used for preparing kahawai otoliths using the thin section technique initially followed that described by Stevens & Kalish (1998), was fully documented by Marriott & Manning (2011) for blue mackerel, and is included here in Appendix 3. The following sections present additional information pertinent to kahawai ageing.

In Australia, all historical ageing of kahawai (referred to as Australian salmon) during the 1960–70s was undertaken using annuli counts in scales (Nicholls 1973, Stanley 1978), but more recently, thin section otolith preparations have been used (Stewart et al. 2011).

2.3 Otolith preparation

Post extraction, kahawai otoliths are cleaned of adhering tissue, rinsed in water, dried and stored in paper envelopes labelled with sample details, including trip code, station number (or landing number for market samples), fish number, date and length (see Figure 1). Although kahawai show minor differential growth between the sexes, where females attain a larger average size than males at a given age (McKenzie et al. 1992), the difference is small and most recent ageing studies do not record sex. The envelopes are stored in labelled box files relating to the project code, fish-stock and year of collection, and are archived in the MPI otolith collection, currently housed at NIWA, Wellington. Although kahawai otoliths sampled from commercial and recreational landings appear large enough to be stored only in paper envelopes (see Appendix 4, Figure A4.1), samples from small and medium sized fish may require storage in microcentrifuge (commonly referred to as Eppendorf) tubes due to their fragility (see Figure 1).

Figure 1: Images of an envelope used to store otoliths like those collected from kahawai. Small kahawai otoliths are best stored in microcentrifuge tubes inside labelled envelopes.

Appendix 3 outlines the most recent methodology used for thin section otolith preparations (Marriott & Manning 2011). In short, up to five kahawai otoliths are embedded in epoxy resin and sectioned along a dorsal-ventral line directly through the core with a twin-blade sectioning saw to produce thin wafers of about 0.5 mm thick. The wafers are mounted on glass microscope slides using epoxy resin, ground and polished to a thickness of 0.25–0.35 mm.

2.4 Otolith interpretation

A standardised procedure for reading thin kahawai otolith sections generally follows Stevens & Kalish (1998), but also includes some recent findings/additions. Essentially, when viewed with a compound microscope, a series of opaque (dark) and translucent (light) growth zones are apparent under transmitted light (Figure 2). One opaque and one translucent zone is laid down in kahawai otoliths each year (Eggleston 1975, Stevens & Kalish 1998); which we believe to be reflective of rapid growth over spring-summer and slow growth over autumn-winter, respectively. Initial viewing may be undertaken at low magnification (10× objective) to determine which of the preferred sites on the section are the clearest for reading, although high magnification (20–40× objectives) is recommended for accurate zone count and margin interpretation, especially for older fish (i.e., those 10 years of age and older). Both ventral and dorsal sides of the otolith should be read from the core toward the proximal surface close to the sulcus, and the number of complete opaque zones counted. If a discrepancy between counts occurs, the reader rechecks the count until agreement is reached.

The main assumptions made when interpreting zones in thin section kahawai otoliths are:

1. The opaque zone (dark in thin section preparations) may become visible in late summer in young fish and in autumn in old fish and is completed by late autumn. The formation of the translucent zone takes place in the preceding months, during winter, spring and early summer. The first opaque zone is consistent with being the first annual increment.
2. The theoretical ‘birthday’ for all kahawai is 1 February.
3. Opaque zones are counted.

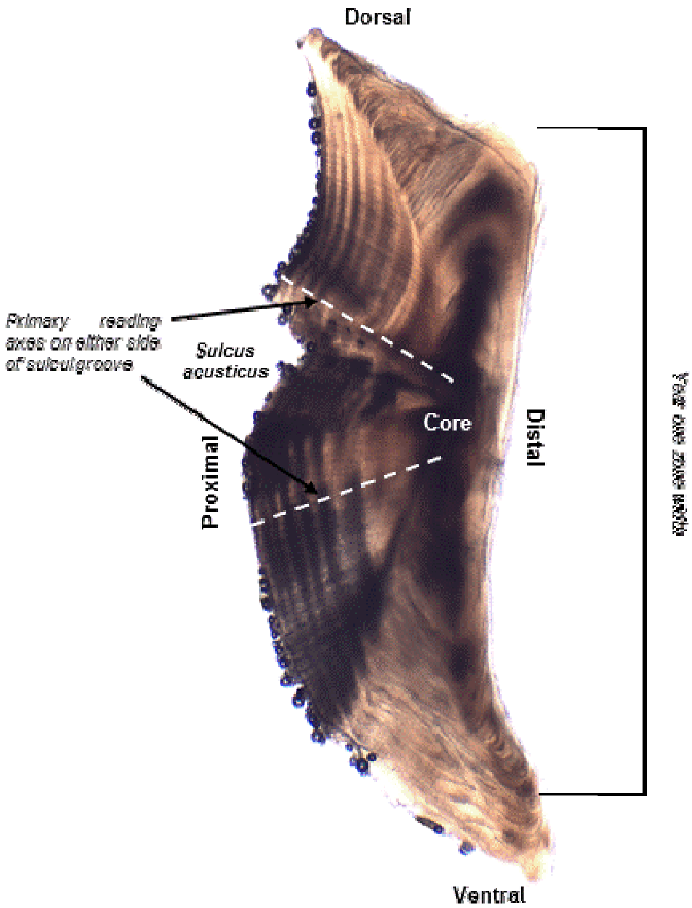


Figure 2: Kahawai otolith image of a transverse thin section under transmitted light illustrating otolith terminology. This otolith section was interpreted as 7 narrow.

Dedicated sampling of recreational landings of kahawai in northern New Zealand first began in the summer of 2000–01 (Hartill et al. 2007a), as part of a Ministry of Fisheries directive to provide better insight into changes in population age composition than was likely to be attainable through commercial catch sampling. As sampling was mainly conducted over summer and autumn, the margin of sampled otoliths was found to be relatively similar during these periods, and only few additional changes to the reading standards were required over time, with between-reader agreement considered to be generally high (Hartill et al. 2007a). Readers were given access to kahawai otolith images from previous collections, illustrated with zone counts and otolith terminology to provide additional assistance in age determination. Furthermore, diagrammatic thin section drawings of otoliths from Stevens & Kalish (1998), and presented here in Appendix 2, were also provided to readers. With a recent emphasis of sampling commercial landings year-round (Devine 2007, Hartill et al. 2013), the use of the forced margin method was instigated in 2011 to provide readers with additional assistance in relation to temporal changes of the kahawai otolith margin, thereby ensuring that ageing precision and accuracy was maintained (Hartill et al. 2013, Armiger et al. 2013).

The first annulus was described by Stevens & Kalish (1998) using the following criteria: being distinguished by generally being the first broad (and broadest) opaque zone beyond the core; dorsally flattened and meeting or nearly meeting the otolith edge; ventrally tapering to a blunt point (see Figures 2 and 3, Appendix 2). They noted that in late recruits and poor sections the first annulus may be closer to the core but can be easily distinguished based on the otolith shape at age one, but did not determine a core to first annulus measurement range.

Stevens & Kalish (1998) otolith documentation is comprehensive, and provides a useful guide to kahawai readers for determining the location of the first annulus and subsequent zones, as well as describing other structures, including the “transition zone”, a straw brown region which ends in an abrupt check, inside the first annulus, and often a cause of confusion for readers (see Appendix 2, Figures A2.1 and A2.3). They found that after the first year, otolith growth develops asymmetrically and is largely confined to the proximal face, with the dorsal lobe flattening and the ventral lobe becoming more elongate and tapered. With age, proximal growth is mostly uniform and proportionality is maintained between increments either side of the sulcul groove even in otoliths of older fish, with the ventral side considered the primary reading axis. Despite this, some care is required when ageing older fish to avoid error, as growth is not always uniform along the same (reading) axis.

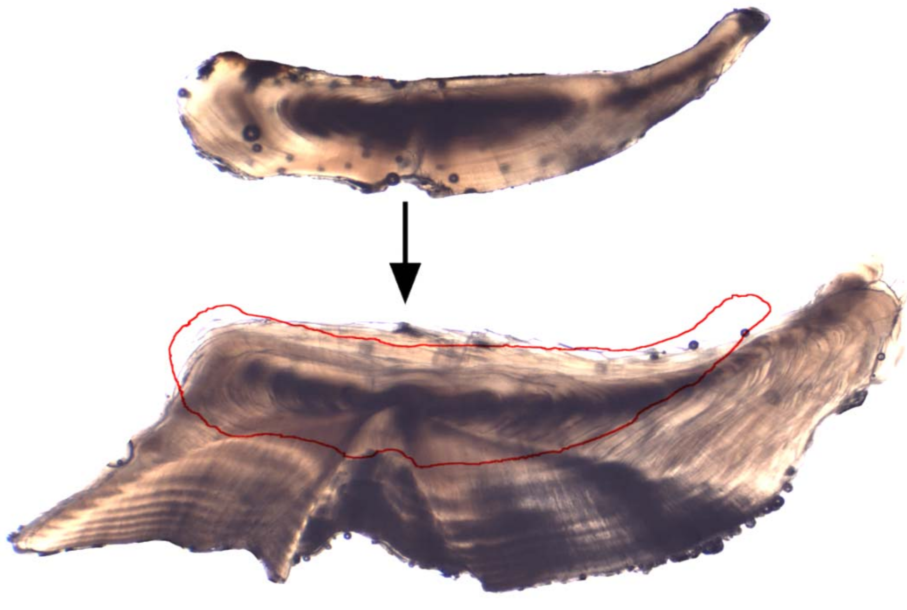


Figure 3: Thin section otolith from a 1+ pre-recruit kahawai (13 cm) with perimeter outline overlaid onto an adult kahawai otolith to compare the position of the first annulus (reference set slide #112-3: 53 cm, 9N, agreed age 9).

False checks are occasionally present in kahawai otoliths and usually lie between the core and the first and second zones. Although occasionally problematic, the spacing and line definition (too strong or weak) of false checks compared to annual zones are usually indicative of their presence.

To derive an accurate zone count from ageing kahawai thin section otoliths, readings are typically made along the ventral side close to the sulcus, designated the primary reading axis, where clear, distinctive, and uniform alternating opaque and translucent zones are generally visible (Figure 2). For ease of reading thin section preparations, opaque zones are counted where a fully formed translucent zone precedes it. However, comparison readings may be undertaken on the dorsal sulcus and the dorsal ridge to confirm initial reads, although some confusion can arise between counting dark opaque zones and those between these that are "straw brown" in colour. Generally, multiple readings are required. Furthermore, zone deposition on the otolith margin either side of the sulcus is typically non-uniform in kahawai with more dark zones often present on the ventral lobe (see Appendix 2, Figure A2.1). It is ambiguous whether the structures on either side of the sulcus that are counted are always comparable. If discrepancies occur between counts, the default read is to use the higher estimate, usually that from the ventral side. Discrepancies also occur where readers are unable to accurately identify poorly defined zones, often the first or second.

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). If 1 February is assumed to be the 'birthday' of all kahawai, then the first translucent zone is formed from the five to eight months of life during winter and complete opaque zones are visible around April–May, with all subsequent zones being laid down annually. Therefore, an otolith with three opaque zones collected in October will be approximately 3.67 years old, and one with four zones collected in May will be about 4.25 years old. Based on a calendar year, these fish will belong to the age classes (age groups) 3 and 4 respectively, and for the New Zealand fishing year which begins 1 October, they will both belong to fishing year age class 4 (Table 1).

Table 1: Diagrammatic representation of the age assignment for kahawai in relation to each month of the New Zealand fishing year, October–September. The birthdate for kahawai is 1 February and the forced margin states used are: W = wide, L = line, N = narrow.

				←Spawning→								
Month	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
Age class	3	3	3	3	4	4	4	4	4	4	4	4
Age group	3+	3+	3+	3+	4+	4+	4+	4+	4+	4+	4+	4+
Decimalised age	3.67	3.75	3.83	3.92	4.00	4.08	4.17	4.25	4.33	4.42	4.50	4.58
Forced margin	W	W	W	W	W	W	L	L	N	N	N	N
Fishing year age class	4	4	4	4	4	4	4	4	4	4	4	4

To provide the reader with guidance and improve accuracy and precision in age estimations in year-round collections, a forced margin was implemented to anticipate the otolith margin relative to the month in which the otolith was collected (Table 1). For ageing kahawai in New Zealand, this is dependent upon the position of the outermost opaque zone and is as follows: '**W**ide' (a moderate to wide light (translucent) zone present on the margin), October–March; '**L**ine' (dark opaque zone in the process of being laid down or fully formed on the margin), April–May; '**N**arrow' (a narrow to moderate light (translucent) zone present on the margin), June–September. Although the timing of the deposition of the newly formed zones is influenced temporally and may vary slightly between individual fish, stocks and years, readers are able to anticipate the expected temporal change to the otolith margin in comparison to what they visually see by using the forced margin method, and at the same time allowing for minor variations in zone deposition between otoliths in the collection they are reading. This is particularly important for old kahawai, as the zones close to the otolith margin, although most often regularly spaced, can be narrow and difficult to interpret, occasionally showing variation in incremental deposition (see Appendix 2, Figure A2.2). Although the clarity of the margin appears reasonably clear under low magnification in thin section preparations, viewing under high magnification can also be problematic with poor preparation (i.e., over- or under-ground), or the presence of resin bubbles and residual endolymphatic sac tissue resulting in reader uncertainty.

To demonstrate the application of the forced margin to ageing kahawai, consider an otolith sampled in March that has three completed opaque zones and an opaque margin. Using the forced margin method (Table 1), the opaque margin is ignored, and the otolith interpreted as 3W (wide referring to a wide translucent margin). When determining age, however, the sampling date and assumed birth date are taken into account to assign an age of 4.08 years (Table 1). Ignoring the opaque margin, which may be present in March in some but not all otoliths of fish from a particular cohort, does not compromise the age determination. In fact the forced margin method results in consistent ageing of fish in a given cohort. By way of example, if the forced margin method was not used, 4.08 year old kahawai sampled in March could be assigned ages of either 3 or 4, depending on whether an opaque margin was visible, and deemed to be complete.

It is prudent that otolith preparation is undertaken and presented to the reader in the same chronological order that the otoliths were sampled in, making interpretation of the margin much easier, and reducing the potential for error. To determine the "fishing year age class" of fish using the forced margin, 'wide' readings are increased by 1 year (e.g., 3W is aged as a 4 year old) and 'line' and 'narrow' readings remain the same as the zone count (e.g., 4L or 4N are aged as a 4 year old) (see Table 1). We believe that using the forced margin method obviates the need for algorithms that convert a reader zone count to an age estimate, which may increase unnecessary error in age should reader interpretation of the margin states vary, especially important when ageing a species collected over an extended time period (i.e., year-round).

Fast growing young kahawai may pose problems for readers, and can be misinterpreted and over-aged by one year probably because fewer annual zones are present to compare with. The diagrammatic thin section drawings of juvenile kahawai otoliths in Stevens & Kalish (1998) will assist the reader in identifying between one, two and three year old fish.

A readability scale ranked 1–5 has been used for ageing kahawai otoliths on only one occasion (Devine 2007). However, the scale is not mandatory or generally used to determine which otoliths are used in the final age selection for catch-at-age analysis, other than those ranked 5 (unreadable) which are already removed from the collection.

2.5 Ageing procedures

Although not recorded in the initial ageing studies on kahawai in New Zealand (Eggleston 1975, Wood et al. 1990, Jones & Hadfield 1985), information suggests that only a single reader was used to age burnt whole or break and burn otolith collections. No age information for kahawai is recorded on the *age* database prior to 1992. In the Stevens & Kalish (1998) study, a single reader read the thin section samples twice (four years apart) to determine the within-reader comparison, and a subsample of Eggleston's break and burn otoliths were re-read for the between-reader comparison with Eggleston's original readings, but no attempt was made to resolve differences where disagreement occurred.

During the early 1990s, a single reader generally aged the break and bake kahawai otoliths (Drummond & Wilson 1993, Drummond 1994), and when two independent readers were used, and could not differentiate between two ages, 0.5 observations were assigned to each age (Drummond 1994). During the same period, McKenzie et al. (1992) documented the first occasion where two independent readers were used to age break and burn otoliths and where a final age was agreed upon for each. With the commencement of a recreational catch sampling programme in KAH 1 in 2000–01 to provide length and age information for stock assessments (Hartill et al. 2007a), three readers were used to interpret thin section otoliths and disagreements were resolved using a similar method to that used for snapper (Davies & Walsh 1995). In 2011, ageing procedures changed again and since then, two experienced readers have been used (Hartill et al. 2013, Armiger et al. 2013). Each reading was made independently without prior knowledge of counts obtained by other readers or of the fish length, with only the collection date given. Where both readers agreed, the age of the fish was determined from their reading. Where disagreement occurred the otolith was jointly reread using images of otoliths projected onto a video screen and with a third experienced otolith reader present to determine the likely source of error, and the correct reading. If no consensus could be reached (most often because the otolith was unreadable), the otolith was discarded from the dataset; less than 1% of samples were discarded. Reviewing all reader disagreements was seen as a fundamental step in determining an accurate estimate of the final agreed age for kahawai for this long-term programme.

Nevertheless, not all recent kahawai ageing studies have followed the same multiple reader design. Determining the age composition of commercial kahawai landings in KAH 1, 2, 3 and 8 during the 2005–06 fishing year, a single reader was used to read all otoliths, and another reader aged a subsample, determining between-reader variability (Devine 2007). For those otoliths with high readability scores (4, zones unclear; 5, unreadable), these were re-examined jointly to achieve consensus or discarded from the analysis.

2.6 Estimation of Ageing Precision

The first within-reader comparison tests were undertaken on recaptured kahawai that had been tagged in Tasman Bay and the Bay of Plenty in 1991, and resulted in 74.1% agreement from ageing 328 thin section otoliths (Stevens & Kalish 1998). The samples had been read four years apart and the primary reason for differences were put down to problems interpreting the first annulus. Stevens & Kalish (1998) also read some of Eggleston's (1975) break and bake otolith preparations but recorded poor between-reader correlation and cited differences in specimen preparation and interpretation of the first year zone as being the most likely reasons for the low 28% agreement.

In the early 2000s, Hartill et al. (2007a) used three independent readers to reduce the probability of reader error in ageing the first of many kahawai collections sampled from the KAH 1 recreational fisheries, but qualified this by stating that although the magnitude of ageing error remains uncertain and is unlikely to be totally eliminated, consistency in relative year class strengths suggest that it is not excessive (see Figure 4). They found that readers had difficulty in ageing kahawai otoliths collected in late April, and avoided ageing samples later than this when sufficient otoliths were available, but gave no indication as to the level of reader disagreement or precision associated with ageing the thin section preparations. With an increase in estimates of mean length-at-age over time evident in these early collections, and inconsistencies in the relative proportions of catch-at-age data, the potential for progressive ageing error was explored (Hartill et al. 2008). A total of 300 randomly selected otolith samples from five survey years, previously aged at 2–8 years of age, were re-aged by an experienced kahawai reader to test for bias. Although a detectable difference was evident in one survey year sample, age-bias plots showed the reader to be generally consistent over time, meaning that the trend observed in the time series was not an artefact of progressive ageing error (Hartill et al. 2008).

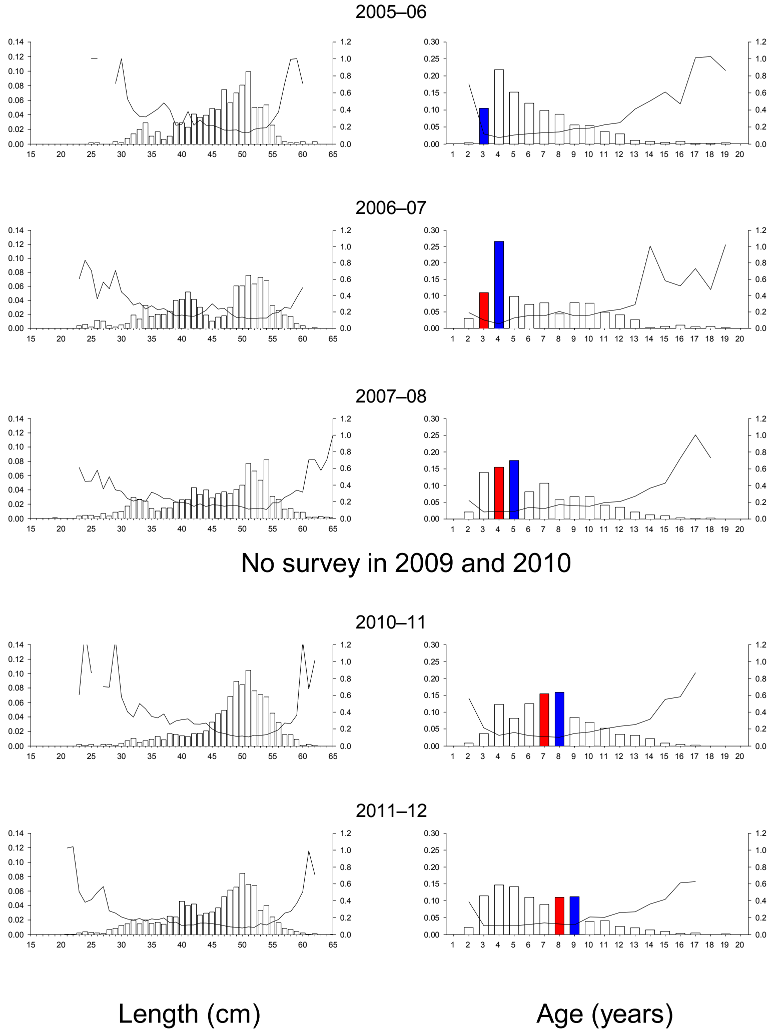


Figure 4: Example of consistency in estimating catch-at-age for kahawai sampled from the Bay of Plenty recreational fishery over consecutive fishing years (Reproduced from Armiger et al. 2013).

Devine (2007) was the first to quantify precision and bias in ageing kahawai in New Zealand documenting between-reader mean CV (11.72%) and APE (8.29%), showing that considerable bias existed between readers in estimating age for the subsample of 497 thin section otoliths (Appendix 5, Table A5.1). With a recent move to using two experienced readers to age kahawai otoliths, as well as implementing the forced margin method and reviewing all reader disagreements with a third experienced reader, considerable improvement has been made in the precision with which kahawai is now aged (Hartill et al. 2013, Armiger et al. 2013). Estimates of between-reader CV and APE regularly now lie below 2% (see Figure 5, Appendix 5, Table A5.1), and those compared to the final agreed age are most often lower than this (Hartill et al. 2013, Armiger et al. 2013).

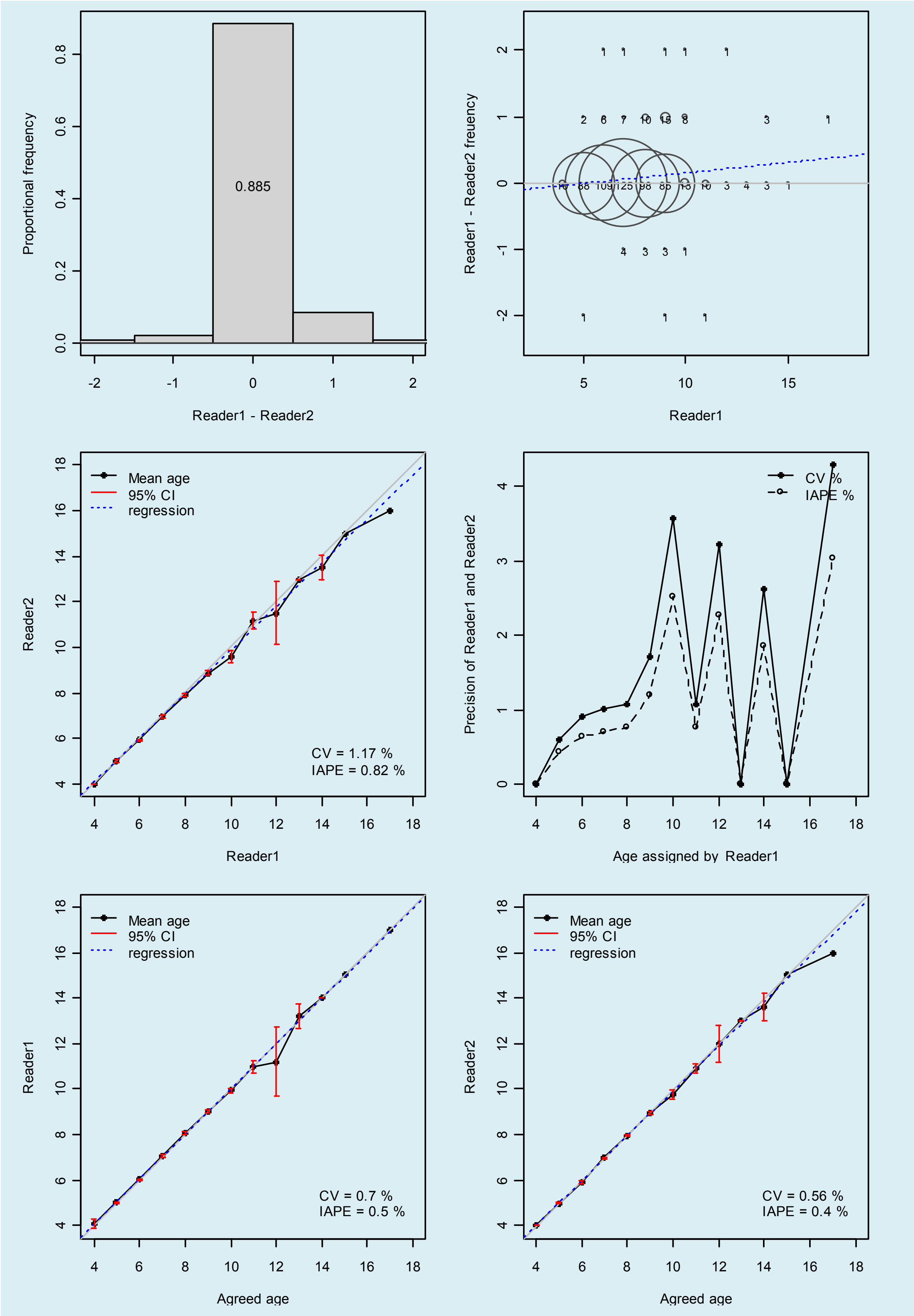


Figure 5: Age-bias diagnostic plots for kahawai sampled from the Bay of Plenty purse seine fishery in 2012. Reproduced from Hartill et al. (2013).

2.7 Reference collection

As kahawai otolith sections are most often mounted in sets of 5 on each microscope slide, 100 slides have been selected for the reference collection, rather than 500 individual preparations. This is expected to be sufficient for quality control monitoring in assessing reader performance, and 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 kahawai reference collection was assembled from about 4000 otolith samples (archived at NIWA Wellington) collected from the KAH 1 commercial and recreational fisheries over the 2006–07, 2007–08, 2010–11 and 2011–12 fishing years. These fishing year samples were chosen specifically as the age estimation for kahawai from some earlier collections may be affected by ageing error, the use of only one reader, and different preparation methods. Despite this, the roughly random selection process of the reference set has ensured that the full seasonal distribution of the otolith samples collected from the KAH 1 fish-stock since 2006–07 were well represented, and that the full length range is covered, while not being strongly dominated by those most abundant length classes in the commercial or recreational fisheries (Figure 6). Examples of these preparations for a range of fish size and age are presented in Section 2.7.1 (Figures 7–9). As kahawai are considered a species of moderate longevity, a reference collection of 500 otolith preparations is expected to be more than sufficient for quality control monitoring purposes. Although negligible spatial differences in growth parameters for kahawai were reported in two previous studies (McKenzie et al. 1992, Drummond & Wilson 1993), the differences in growth rates between studies appears more obvious (Drummond 1994). Despite this, it was agreed that as the interpretation of kahawai otoliths was unlikely to be affected by where the samples came from, the collation of stock-specific reference collections was unnecessary. However, when new age samples for kahawai become available from fishstocks other than KAH 1, and are aged following those protocols outlined within this document, then subsamples of these should be included within the current reference collection.

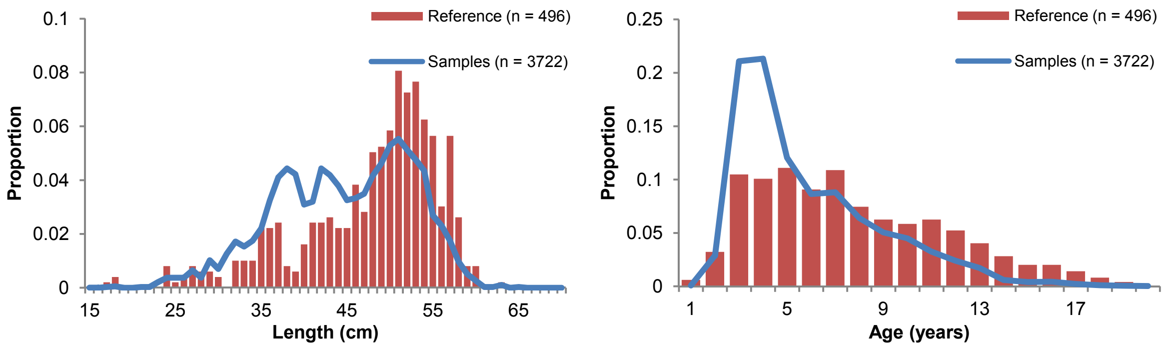


Figure 6: Length and age proportions (lines) of kahawai sampled for otoliths from the KAH 1 commercial fishery from 2006–07, 2007–08, 2010–11 and 2011–12 with a comparison of the selected subsample chosen for the reference (histograms).

The agreed ages for otoliths selected for the reference set already exist on the *age* database (administered by NIWA for MPI), and have been stored in a new table created within this database along with any new readings of the reference set collection. As these preparations have already been aged in the past as accurately as possible, they may be treated with a reasonable level of confidence, given that the species is reasonably easy to age. The reference set may also be used for training new readers as well as monitoring their progress as they gain experience in ageing.

2.7.1 Examples of thin section preparations of kahawai otoliths with marked opaque zones and agreed reading and age estimates for a range of fish size and age

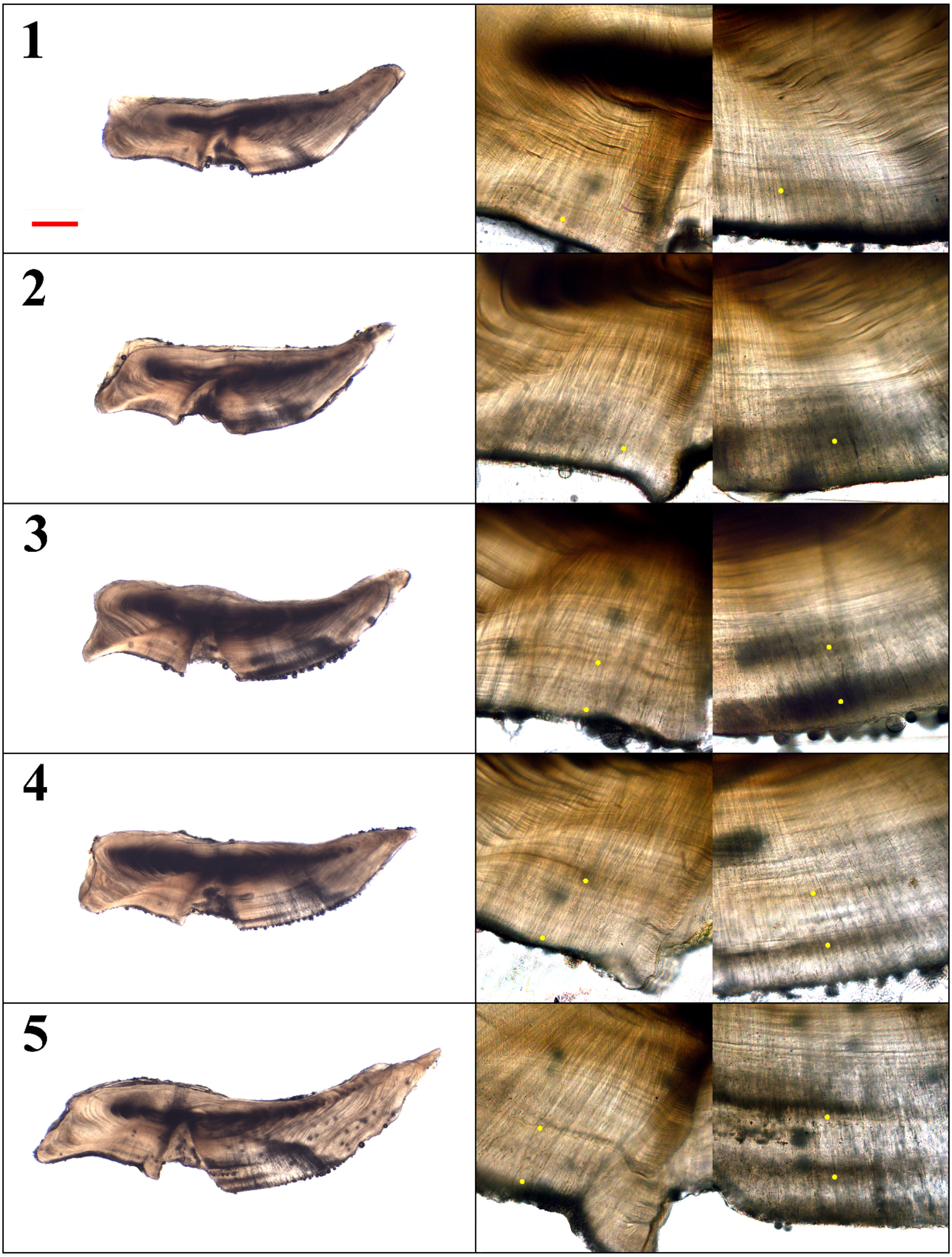


Figure 7: Aged kahawai otoliths (whole section and enlarged sub-sections) from the reference set for fish lengths <35 cm: fish#1 (slide 57-2 , 24 cm, agreed reading 1W, agreed age 2); fish#2 (slide 3-3, 27 cm, 1W, 2); fish#3 (slide 107-2, 30 cm, 2N, 2); fish#4 (slide 19-4, 32 cm, 2W, 3) and fish#5 (slide 49-1, 34 cm, 2W, 3). (*Red scale bar = 500 µm for all whole sections.*)

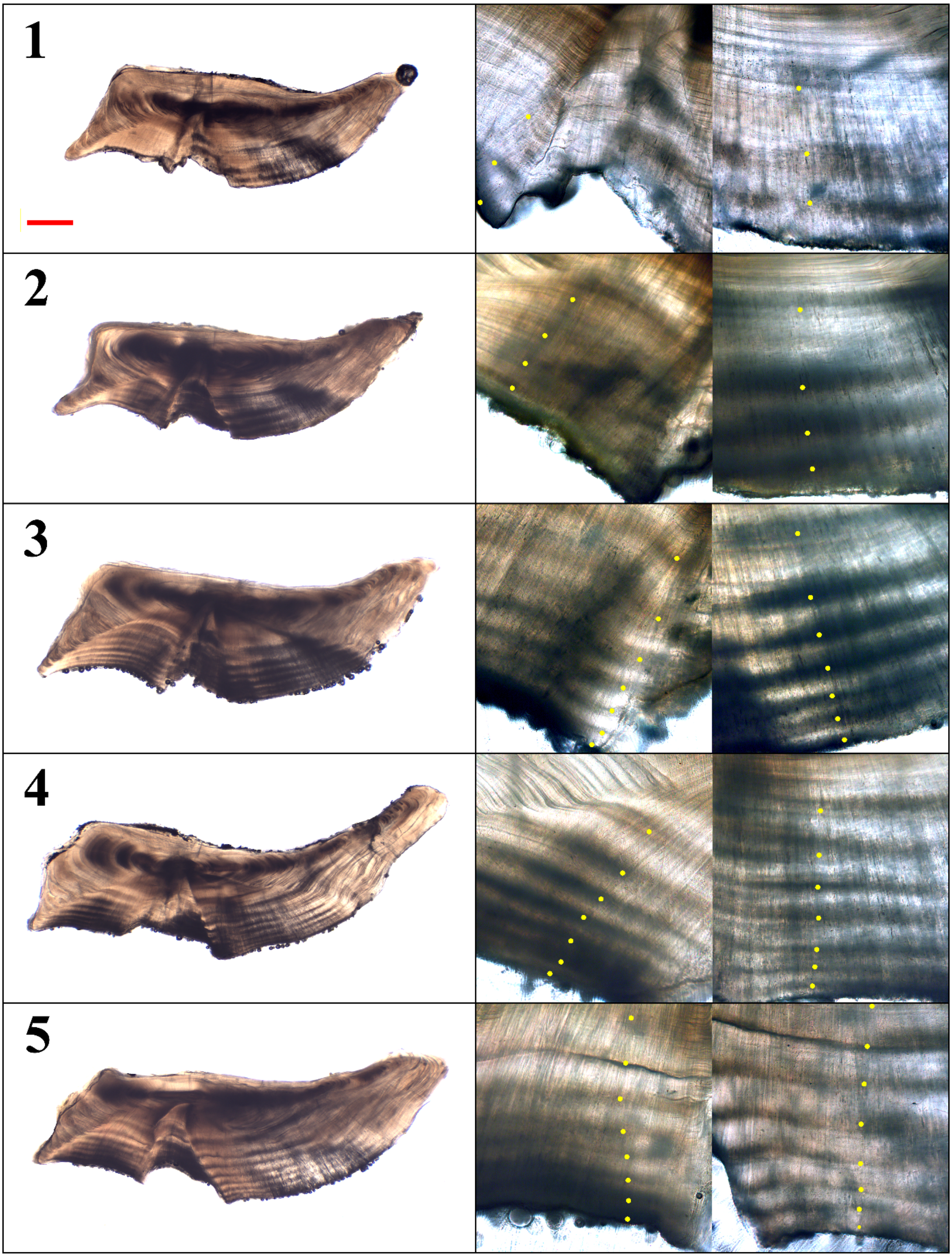


Figure 8: Aged kahawai otoliths (whole section and enlarged sub-sections) from the reference set for fish lengths 36–50 cm: fish#1 (slide 36-3, 39 cm, agreed reading 3W, agreed age 4); fish#2 (slide 76-2, 43 cm, 4W, 5); fish#3 (slide 114-2, 46 cm, 7N, 7); fish#4 (slide 12-3, 48 cm, 7W, 8) and fish#5 (slide 72-3, 50 cm, 8W, 9). (*Red scale bar = 500 µm for all whole sections.*)

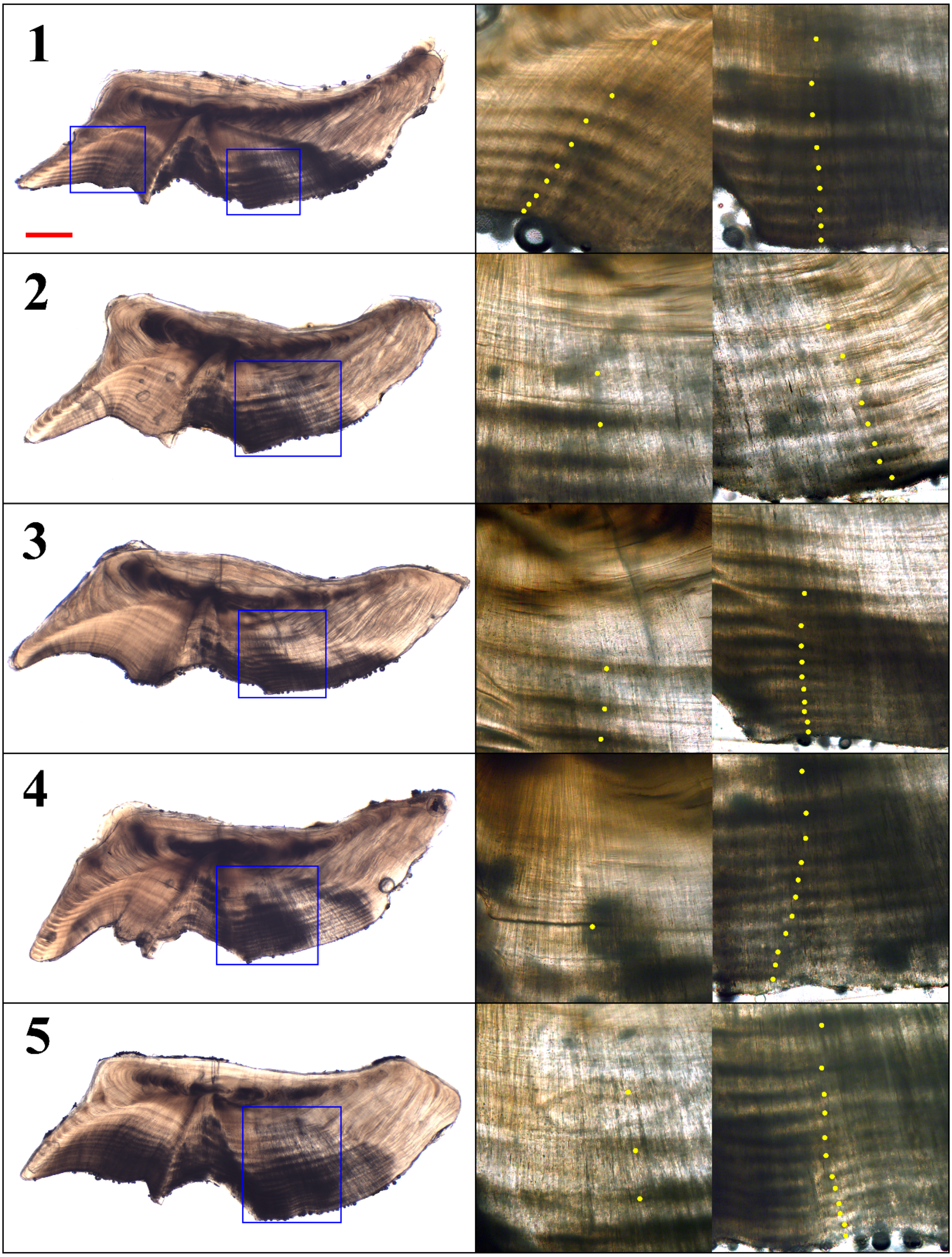


Figure 9: Aged kahawai otoliths (whole section and enlarged sub-sections) from the reference set for fish lengths ≥ 51 cm: fish#1 (slide 112-3, 51 cm, agreed reading 9N, agreed age 9); fish#2 (slide 66-1, 53 cm, 10W, 11); fish#3 (slide 84-2, 55 cm, 13W, 14); fish#4 (slide 37-2, 58 cm, 12W, 13) and fish#5 (slide 74-2, 60 cm, 15W, 16). (*Red scale bar = 500 µm for all whole sections.*)

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 documented 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. A readability score, although not mandatory, is also sometimes included.

Table 2: A market sample example of kahawai age data submitted for loading onto the *age* database.

Species = KAH
Stock = KAH 1 (Bay of Plenty)
Material = Otolith
Method = 30 (Thin section)
Readers = 76, 113
Project code = KAH2011-01
Sampling period = January 2012 to December 2012

origin	trip_code	sample_no	sub_sample_no	area	species	fish_no	prep_no	collection_date	lgth	sex	reader1	count1	reading_date	reader2	count2	reading_date	agreed_count	margin	agreed_age	proj_code	comments
SMP	20128801	901	1	BPLE	KAH	1	1/A	06/06/12	51	4	76	8	06/11/12	113	8	06/11/12	8	N	8	KAH2011-01	
SMP	20128801	901	1	BPLE	KAH	2	1/B	06/06/12	49	4	76	7	06/11/12	113	7	06/11/12	7	N	7	KAH2011-01	
SMP	20128801	901	1	BPLE	KAH	3	1/C	06/06/12	51	4	76	8	06/11/12	113	8	06/11/12	8	N	8	KAH2011-01	
SMP	20128801	901	1	BPLE	KAH	4	1/D	06/06/12	48	4	76	7	06/11/12	113	7	06/11/12	7	N	7	KAH2011-01	

For reference sets, a new table has been developed within the *age* database to include record counts and accepted ages. Readings of the reference set, prior to embarking on reading a new otolith collection, are stored on a second new table to distinguish each calibration or training reading from those used to estimate catch-at-age distributions or growth parameters.

3. ACKNOWLEDGMENTS

This work was funded by the Ministry for Primary Industries under project INS201201. We acknowledge the inclusion of a glossary of otolith terminology and additional figures and images that have been reproduced within this report from a number of fish ageing publications, and are most grateful to those authors for this, particularly Kalish et al. (1995), Stevens & Kalish (1998), Marriott & Manning (2011) and McMillan et al. (2011). We thank Peter Marriott for reviewing this document.

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APPENDIX 1: Glossary of otolith terminology and ageing definitions.

Reprinted from the MPI "Guidelines for the development of fish age determination protocols". These were based on Kalish et al. (1995) "Glossary for otolith studies", with modifications and addition of 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 not 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 (see Figure A1.1).
Asteriscus (pl. asteriscii) – one of three otolith pairs found in the membranous labyrinth of osteichthyan fishes.
Bias – The systematic over- or underestimation 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 both 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 microincrement 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 microincrement contains a greater amount of organic matrix and a lesser amount of calcium carbonate than the L-zone. Referred to as 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 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., subdaily, 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 microincrement that appears light when viewed with transmitted light, and appears as an elevated region when acid etched and viewed with a scanning electron microscope. The component of a microincrement 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.

Microincrement – increments that are typically less than 50 um in width; with the prefix "micro" serving 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 subdaily 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 fibrillar 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 (Figure A1.1).

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 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 (Figure A1.2). A thickened portion of the otolithic 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.

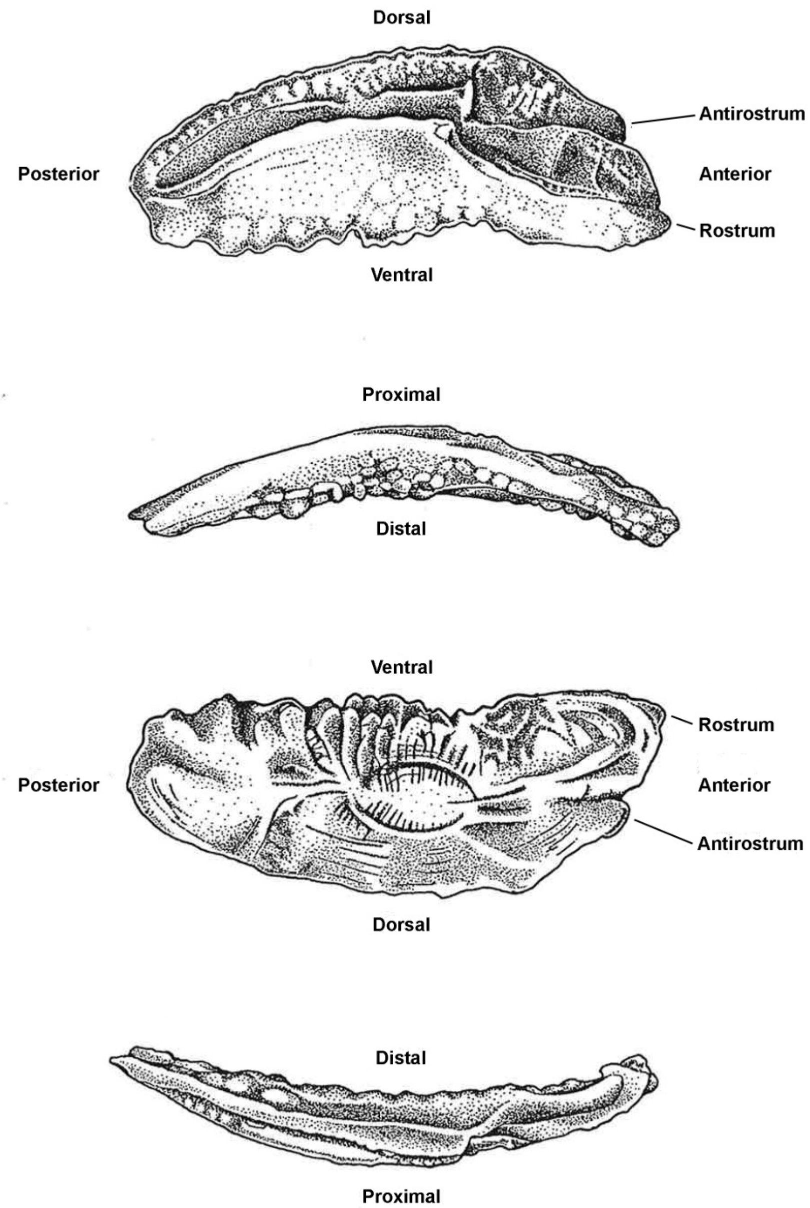


Figure A1.1: Views of a left sagittal otolith from *Arripis trutta* illustrating orientation and basic structure. A) the proximal surface, B) the ventral edge, C) the dorsal edge. (Drawing by Darren Stevens, NIWA).

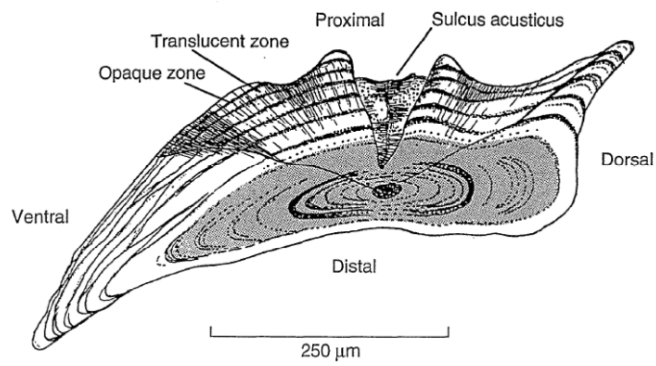


Figure A1.2: Transverse thin section through a sagittal otolith from *Arripis trutta* viewed with transmitted light illumination. The section is taken through the core. (Drawing by Darren Stevens, NIWA).

APPENDIX 2: Diagrammatic thin section drawings of kahawai otoliths.

Figures reproduced from Stevens & Kalish (1998).

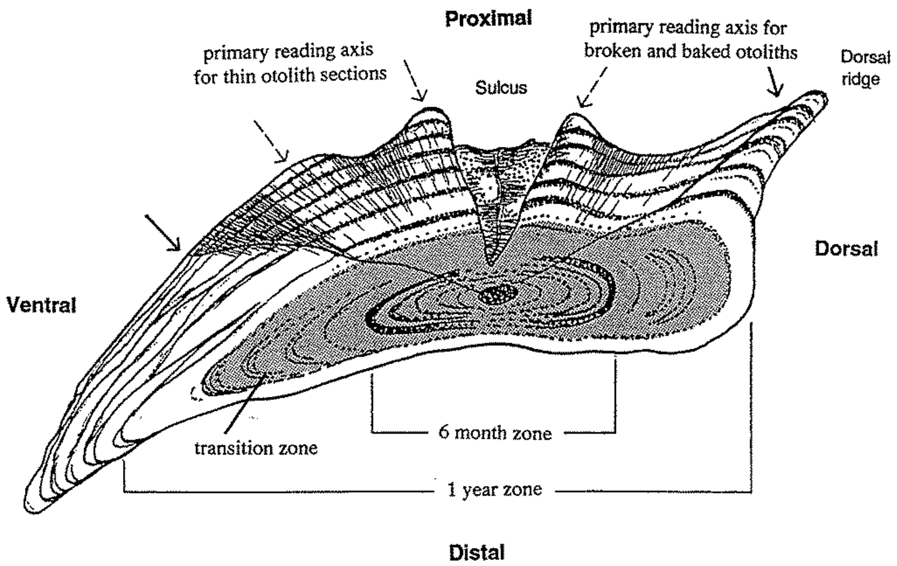


Figure A2.1: Schematic diagram of a thin section of a kahawai otolith showing terminology and reading axes mentioned in the text.

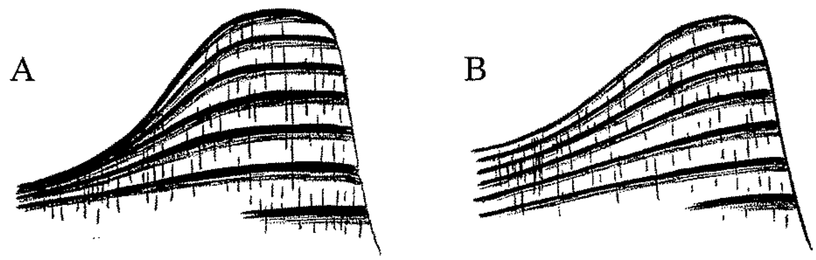


Figure A2.2: Variation in increment growth in the dorsal ridge of a kahawai thin otolith section.
A: uneven deposition of otolith material; B: more 'typical' growth in the dorsal ridge.

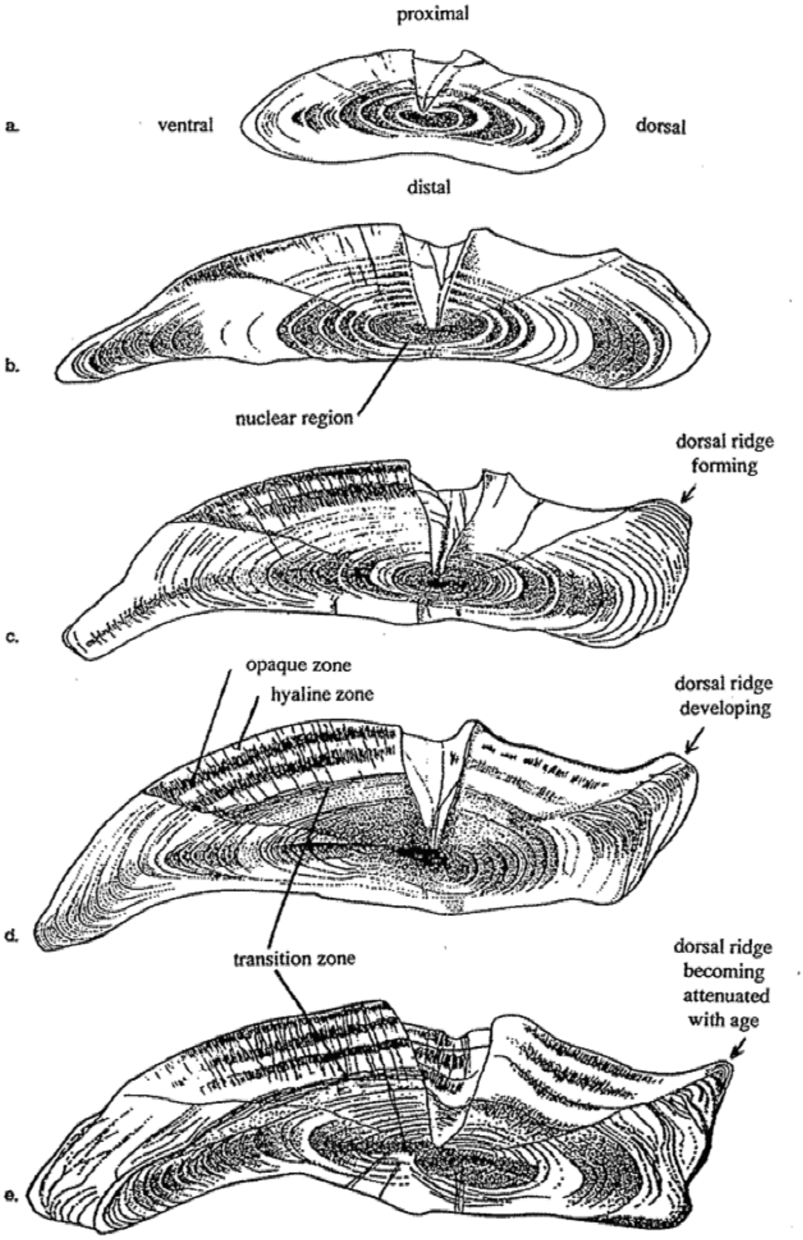


Figure A2.3: Drawings of thin transverse sections of kahawai otoliths. a: thin otolith section of a 5–6 month old kahawai; b: thin otolith section of a 1 year old kahawai; c: thin otolith section of a 2 year old kahawai; d: thin otolith section of a 2+ year old kahawai with a complete hyaline edge; e: thin otolith section of a 3 year old kahawai.

APPENDIX 3: Protocol for thin section otolith preparation.

A protocol for preparing blue mackerel otoliths from Marriott & Manning (2011).

Otolith storage
When collected, all blue mackerel otoliths need to be stored in 1 ml plastic microcentrifuge tubes to protect them as they are very small and fragile. These can then be placed in standard otolith collection packets which are appropriately labelled.

Mark otoliths
Mark the sectioning plane on the cleaned and dried otoliths with a fine pencil along the transverse axis through the nucleus on the distal side. Use the left sagittal otolith where possible, if this is missing or damaged then use the right sagittal otolith. Using otoliths from the same side of the fish makes interpretation during the reading phase easier, as the otolith sections will all be aligned in the same orientation.

Embed otoliths
Otoliths are embedded in blocks of clear epoxy resin (Araldite K142), ratio 5:1 resin to hardener, and cured at 50ºC overnight. The moulds are pretreated by smearing a thin veneer of modelling release wax on the surface of the wells. This facilitates removal of the cured blocks and prolongs the life of the moulds. The moulds are prepared with an initial layer of resin 1–2 mm thick so that when embedded, otoliths sit off the bottom surface of the block. Place the otoliths on the initial layer while the resin is still just soft so they stick in place while the rest of the resin is poured into place. When preparing the resin heat it to 50ºC for a few minutes as this reduces the viscosity aiding mixing, and encourages bubbles of air formed during the mixing process to rise and separate from the resin.

For blue mackerel we utilise reusable latex moulds each with ten wells. Each well has a vertical black line drawn on the base to facilitate aligning the sectioning plane of the otoliths. Five otoliths are placed in each well in a single layer along the line in the base of the well.

Embedded otolith blocks are labelled with a preparation number and are marked with a black line on the upper top surface of the block in the region of the sectioning plane. This enables the cut otolith wafers to be readily oriented on the microscope slide during the mounting procedure.

Calibrating the saw

We cut our thin sections on a Struers Accutom-2 high-speed saw, or our new Struers Secotom-10 high-speed saw. The blades are ‘EXTEC’ Diamond wafering blades, part number 12205. They are 102 mm in diameter 0.3 mm thick with a 12.7 mm axle diameter.

Twin blades are mounted on the axle with spacers to achieve the desired section thickness. The spacers need to be the same diameter as the mounting plates which sit on the outside of the blades, so that the entire set-up is held rigid. The spacers need to be cut from uncompressible material so the distance between the blades remains constant. An array of spacers of varying thickness should be produced so a range of final section thicknesses can be obtained.

Great care needs to be taken with blades used in this manner as the slightest deformation or bend will greatly affect the section thickness. Even with new blades the orientation (Blades mounted with the label side out or in) can affect section thickness by 100–200 microns.

Rotating the blades clockwise or counter-clockwise in relation to each other can fine-tune the sectioning thickness. Use old stubs of blocks to make sure the set-up is reliably cutting at the desired thickness prior to any otoliths being sectioned.

Mounting plates, blades and an array of spacers.

Struers Accutom-2 saw with twin wafering blade set up.

Sectioning

Sections are cut from the blocks at a thickness of 280 to 300 microns. In Blue mackerel this thickness provides the best resolution in the finished mounted sections. If they are thicker the central region of the otolith sections becomes too dark to readily observe zone structure. If they are thinner the marginal zones on the otolith are too faint and are difficult to discern.

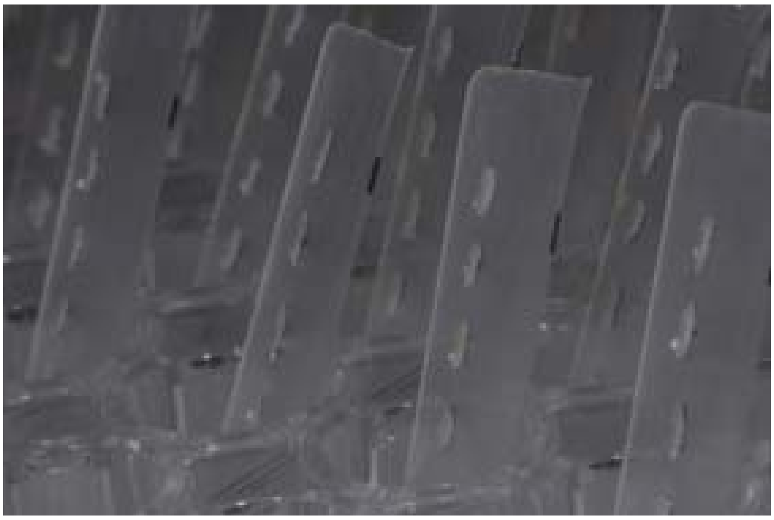
Section blocks at a slow regular speed to ensure even cutting. If one end of the cut wafer is a different thickness to the other end of the cut wafer, slow down the advance speed of the block into the saw, this may produce a more regular section. Utilising clean cutting lubricant should also help to ensure clean regular cuts. Our saw is run at 1800 rpm.

Stop the saw before it cuts right through the block. If the saw is allowed to cut right through the block the cut wafer will fly off at high speed with fractures occurring in the otolith section. Then twist off one half of the block and carefully cut the otolith wafer from the other half where it is attached by a tag of araldite resin. Cut off the whole connecting tag of resin from the wafer, as this raised tag of resin would hinder the mounting procedure.

Carefully wash the wafer in soapy water to remove any cutting detritus and cutting lubricant. It is very important not to bend the wafer at all as this will cause fractures in the otolith section.

Sectioned block showing wafer still held in place by a small tag of connecting resin on the near edge.

Cleaned wafers stored in a tray prior to mounting on glass slides.



Note the black reference mark on the edge of the wafer; this is used for orientation during the embedding procedure.

Mounting the wafers

Standard microscope slides are ideal for these types of preparation. Clean the slides in alcohol to remove any dust and label the bottom with the preparation block number. Then prepare resin as for the embedding process and spread some onto the slide to cover the region to be cover-slipped.

Place the otolith wafer on the middle of the resin and tamp down carefully with a toothpick to squeeze out any air bubbles and settle the wafer onto the surface of the slide. Place a small amount more of the resin on top of the wafer and ensure the whole top surface of the wafer has been wetted with resin. Then float a cover-slip on top of the wafer and carefully tamp it down with a toothpick to remove air bubbles.

Take care not to press directly onto the otolith when tamping down the wafer onto the slide, as this can cause fractures in the resultant section. Air bubbles away from the wafer won't affect the reading of otoliths. Ensure any bubbles on top or underneath the wafer are teased away from the section by careful tamping with the toothpick, as these bubbles can migrate on top of the critical viewing area as the resin cures.

Take note of the orientation mark on the edge of the wafer when the wafer is placed on the slide to ensure that all otoliths are presented in the same orientation, as this will aid the subsequent reading of the otolith.

Leave the prepared sections to cure overnight at 50°C and label with an adhesive sticker at the top of the slide, stating Species and otolith identification information.

The wafer section is correctly oriented on the slide and has been gently tamped down to remove air bubbles.

Half mounted slides showing the resin spread over the cover-slip area of the slide.

Finished slides labelled with the relevant information on adhesive labels

Note all wafers are oriented the same way for the reader's benefit.

APPENDIX 4: Comparison of sagittal otolith size for four commonly aged New Zealand inshore species: snapper, trevally, tarakihi and kahawai.

Although the size of a fish's otolith increases with increasing somatic growth, the relative difference in otolith size and shape for different fish species of the same size can be considerable. For these four important New Zealand inshore species, snapper has the largest sagittal otoliths (Figure A4.1, image 1). Kahawai, tarakihi and trevally have elongated sagittal otoliths of smaller size and considerably greater fragility than otoliths of snapper (Figure A4.1, images 2–4).

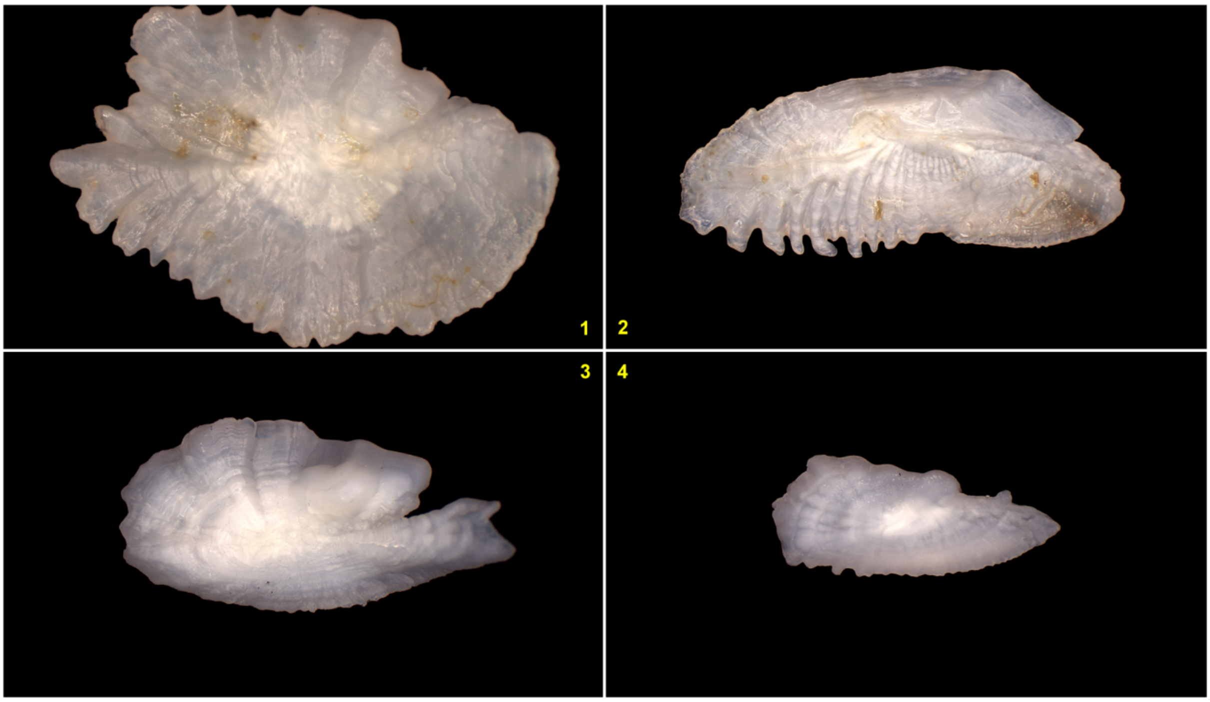


Figure A4.1: Whole right hand side otoliths in lateral view under reflected light at the same magnification demonstrating the differences in otolith size and shape for four important New Zealand inshore species (Image 1, snapper; 2, kahawai; 3, tarakihi; 4, trevally) extracted from fish of equivalent length (42 cm).

Table A4.1: Otolith dimension data for the four species outlined in Figure A4.1.

Species	Otolith bounding box dimensions (mm)		Perimeter (mm)	Surface area (mm²)	Weight (mg)	Age (years)
	Width	Height				
Snapper	13.4	9.0	45.7	81.5	252	8
Kahawai	11.7	5.1	35.8	43.7	74	5
Tarakihi	10.5	5.2	30.7	37.3	47	14
Trevally	7.6	3.2	20.4	17.1	22	9

APPENDIX 5: Summary of between-reader agreement and precision estimates documented in ageing studies for kahawai.

Previously reported between-reader agreement and precision estimates (APE) determined from ageing kahawai in New Zealand are presented in Table A5.1. Aside the one exception in 2005–06, a high level of consistency in reader agreement and precision is apparent in most recent kahawai ageing studies (Figure A5.1). Uncertainty in age estimation arises when independent readers do not initially agree on their interpretation of otolith structures, and these may vary greatly between fishstocks due to specific growth characteristics and differences in population age structure (Davies et al. 2003).

Table A5.1: Between-reader agreement and precision estimates documented in ageing studies for kahawai in New Zealand (ENLD = East Northland; BPLE = Bay of Plenty; HAGU = Hauraki Gulf; PS = Purse seine; REC = Recreational; SN = Set net; * = a subsample of otoliths used; ^ = calendar year).

Stock	Subarea	Method	Fishing Year	No. of readers	Percent agreement	APE	CV	No. aged	Age range	Publication
KAH 1, 2, 3, 8		PS Trawl	2005–06	2	~34%	8.29	11.72	497*	2–21	Devine (2007)
KAH 1	ENLD	REC	2010–11 2011–12	2	85% 80%	1.17 1.51	1.65 2.14	497 485	2–17 2–17	Armiger et al. (2013)
KAH 1	BPLE	REC	2010–11 2011–12	2	85% 79%	1.17 1.59	1.66 2.25	499 492	2–17 2–17	Armiger et al. (2013)
KAH 1	BPLE	PS	2011^ 2012^	2	90% 89%	0.57 0.82	0.81 1.17	449 619	3–17 4–16	Hartill et al. (2013)
KAH 1	HAGU	SN	2011^ 2012^	2	91% 97%	0.91 0.32	1.29 0.45	615 444	1–19 2–13	Hartill et al. (2013)

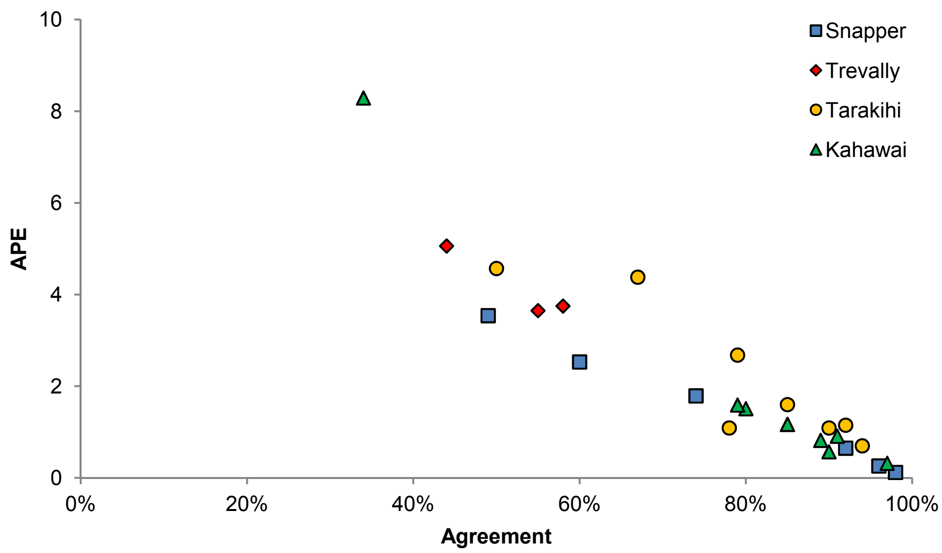


Figure A5.1: Visualised comparison of between-reader agreement and APE scores documented in ageing studies for snapper, trevally, tarakihi and kahawai in New Zealand.

Although percent agreement is considered an inferior method of determining ageing precision compared to APE and CV as it varies so widely among species and among ages within a species, all measures of precision may be artificially inflated by any bias which exists between readers (Campana 2001). It is therefore difficult to make firm conclusions when comparing between-reader precision estimates for a particular species as reader experience and ageing ability may vary. A CV estimate of

5% (APE 3.5%) may serve as a reference point for fishes of moderate longevity and reading complexity (Campana 2001), such as kahawai, but we suggest that with a high level of reader competency and the guidance of the revised age determination protocol in this document, a CV of below 5% should always be attainable.

Furthermore, although error associated with initial readings may imply uncertainty in final age estimates, the process that we now implement in ageing kahawai, of independent identification and re-reading of otoliths where disagreements occur (when at least two readers are used), almost always resolves disagreements. We feel that individual reader age-bias plots and precision estimates (APE and CV) between each reader and the agreed age should become the mandatory requirement for reporting ageing results for new otolith collections, and will provide an additional quality control measure by identifying individual reader consistency and accuracy in ageing over time. We suggest that a minimum of two readers always read all otoliths once and resolve all disagreements to ensure accuracy in age estimation is maintained. This is particularly important for species such as kahawai that demonstrate considerable inter-annual year class strength variability. Individual reader age-bias plots and precision estimates should also be used in setting target reference points and evaluating reader competencies against the reference collection, therefore making reader selection relatively straightforward and unequivocal. The target reference APE and CV estimates for individual readers in the ageing of kahawai in future studies that require fish age to be determined have been set at 1.50% and 2.12% respectively. No comparison should be made with target reference APE and CV estimates for individual readers and those determined from ageing complete otolith collections, as target reference readings are likely to comprise a higher proportion of old fish, making them more difficult to accurately age, therefore resulting in inflated reader APE and CV estimates. Note: When two sets of readings are being compared (e.g., initial age from readings for reader 1 and the final agreed age), the relationship between APE and CV is an exact one, where the CV equals the APE multiplied by the square root of two.