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Age determination protocol for trevally (*Pseudocaranx dentex*)

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This report documents the age determination protocol for trevally (*Pseudocaranx dentex*), 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 trevally. 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 workshop's 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: trevally (*Pseudocaranx dentex*). Significant fishstocks (TRE 1, TRE 7) for this species fall 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, the sagitta, have been used to age trevally. 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).

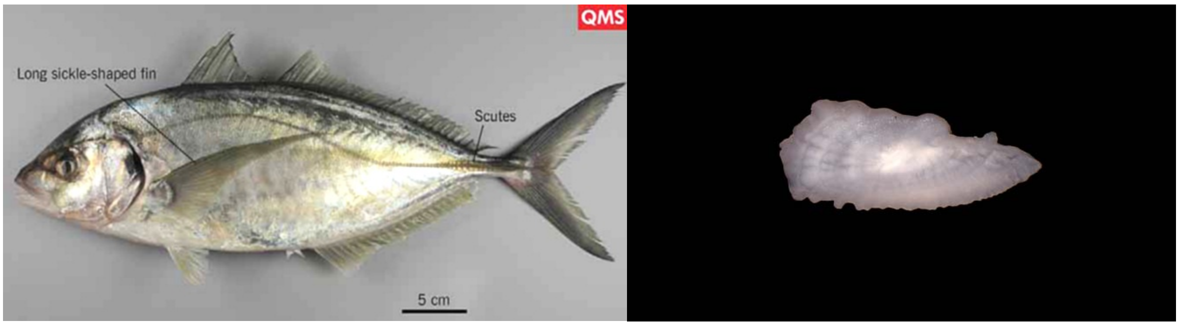
Overall objective

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

Specific objective

1. To develop an age determination protocol for trevally (*Pseudocaranx dentex*), including the compilation of otolith reference collections.

2. AGE DETERMINATION PROTOCOL FOR TREVALLY



2.1 Background

Trevally was first aged by James (1984) who examined scales, fin rays, and whole, and broken and burnt sagittal otoliths from sample collections in the 1970s, in a comprehensive publication on the biology and fishery for trevally. Only the otoliths produced useful results, with a concentric alternating pattern of wide opaque and narrow hyaline (herein referred to as translucent) zones apparent, especially in break and burn preparations. Whole otoliths from older fish proved difficult to read because the outermost zones were crowded and obscured beneath the otolith margin. The formation of annual zones in otoliths was validated in three ways: first, by tracking the progression of strong and weak year classes across several years; second, from an examination of otolith margins, which showed a clear annual cycle in the monthly proportions of otoliths with translucent margins; and third, a confirmation of juvenile growth rates by following the progression of modes in length-frequency distributions and examining otoliths from fish in these modes (James 1984). Determining an accurate estimate of age was nevertheless not straight-forward, with the errors associated with ageing trevally older than about 8–12 years increasing progressively from about ± 1 year for fish up to about 25–30 years to ± 2 years for fish older than this.

With the commencement in 1997–98 of a structured catch sampling programme to establish a time series of length and age composition of the TRE 1 and TRE 7 catch, Walsh et al. (1999) compared three different otolith preparation methods (break and burn, bake and embed, and thin section) to see if trevally could be aged with greater accuracy/consistency than had been achieved by James (1984). Break and burn was rejected as a primary method, as inconsistencies in preparation and interpretation were evident, which were largely a function of the small size of the otoliths (see Appendix 2, Figure A2.1), and difficulties identifying the margin were experienced. The thin section method produced better preparations than the bake and embed, but was more time consuming and therefore costly. Although the bake and embed method was chosen in the first year of the programme, Walsh et al. (1999) concluded that future trevally ageing should be undertaken using thin section preparations only, as zones are more easily resolved, resulting in fewer ageing errors, and this method has been used for ageing trevally ever since.

Despite the initial progress in age determination for trevally, assessments of the TRE 1 and TRE 7 stocks found that the models did not fit well to the time series of catch-at-age observations (McKenzie 2007, 2008). A subsequent review of catch sampling in TRE 1 and TRE 7 (Walsh & McKenzie 2009), determined that inconsistencies observed in the relative year class strengths of trevally catch-at-age data from previous collections were most likely a result of ageing error caused by two main factors: the misinterpretation of growth zones in difficult otolith sections, and the inaccurate determination of the margin. As a result a revised ageing protocol was developed, adopting a more rigorous approach than in previous years, and was first employed in sample collections from 2006–07 (Walsh et al. 2010a). This included the implementation of a forced (or fixed) margin to anticipate the otolith margin type (wide, line, narrow) *a priori* in the month in which the fish was sampled, to provide the reader guidance and improve accuracy and precision in age estimations. Subsequent catch-at-age comparisons have shown more consistency in year class strength estimates between yearly collections (Walsh et al. 2012a, 2012b) as well as considerable similarities with snapper relative year class strengths for the west coast (SNA 8) stock in the same years, meaning that the accuracy with which trevally is now aged has substantially improved (Walsh et al. 2010b, 2012b).

Trevally is a relatively long lived species, although ages over 40 years in New Zealand are uncommon. The oldest recorded age determined for a trevally is 47 years, a 53 cm specimen (estimated 3.1 kg) captured from TRE 7 in 2006.

The theoretical birthdate for ageing trevally is 1 January (James 1984). Although trevally appear to be partial spawners, releasing small batches of eggs over periods of several weeks or months during the summer (Ministry for Primary Industries 2013), 1 January not only provides a useful birthdate, being near the beginning of the trevally spawning season, corresponding with the date chosen by Paul (1976) for the other important demersal inshore spawner, snapper, but is also convenient for collating age data as if it were collected on a calendar year basis (Panfili et al. 2002).

2.2 Methods

Sagittal otoliths are acknowledged as the primary structure for ageing trevally, and all scientific methodologies described in the following sections will be associated with ageing thin sectioned sagittal otoliths, currently the best preparation method. The methodology used for preparing trevally otoliths using the thin section technique initially followed that described by Stevens & Kalish (1998) and Tracey & Horn (1999), 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 trevally ageing.

In Australia, previous ageing studies for trevally (referred to as silver trevally) are restricted to a short study using thin section otolith preparations (Kalish & Johnston 1997) with age estimates validated using the ‘bomb radiocarbon’ technique (Kalish 1995). A comprehensive report on the biology and assessment of the New South Wales fishery for silver trevally (Rowling & Raines 2000) found that thin sectioned otoliths, while still difficult to read, were considered to provide more consistent ages, especially for larger/older fish, than whole otoliths or stained vertebrae. The results of their age validation study (trevally injected with oxytetracycline, OTC), suggest that fish from a range of age classes deposited just one ring annually on their otoliths and support the use of thin sectioned otoliths to accurately determine age.

2.3 Otolith preparation

Post extraction, trevally otoliths are cleaned of adhering tissue, rinsed in water, dried and stored in microcentrifuge (commonly referred to as Eppendorf) tubes within paper envelopes labelled with sample details, including trip code, station number (or landing number for market samples), fish number, date and length (Figure 1). Although collected in most early studies, sex is not a mandatory requirement, as trevally, show no differential growth between sexes (James 1984). The envelopes are stored in labelled box files relating to the project code, fishstock and year of collection, and are archived in the MPI otolith collection, currently housed at the National Institute of Water and Atmospheric Research (NIWA), Wellington.

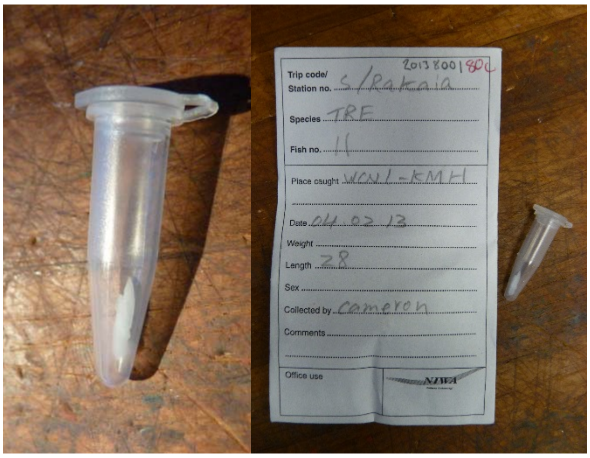


Figure 1: Images of a microcentrifuge tube and envelope used to store small and fragile otoliths like those collected from trevally.

Appendix 3 outlines the most recent methodology used for thin section otolith preparations (Marriott & Manning 2011). In short, up to five trevally 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 trevally otolith thin sections was followed (Walsh et al. 1999). 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 are laid down in trevally otoliths each year (James 1984). 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. However, as trevally are relatively long lived, high magnification (20–40× objectives) is often essential for an 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 core toward the proximal surface, 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 trevally otoliths are:

1. The opaque zones (dark in thin section preparations) first become visible in December–January in young fish, in late summer in old fish, and the formation of the translucent zone takes place in the preceding months, in winter or spring.
2. The theoretical 'birthday' for all trevally is 1 January.
3. Opaque zones are counted.

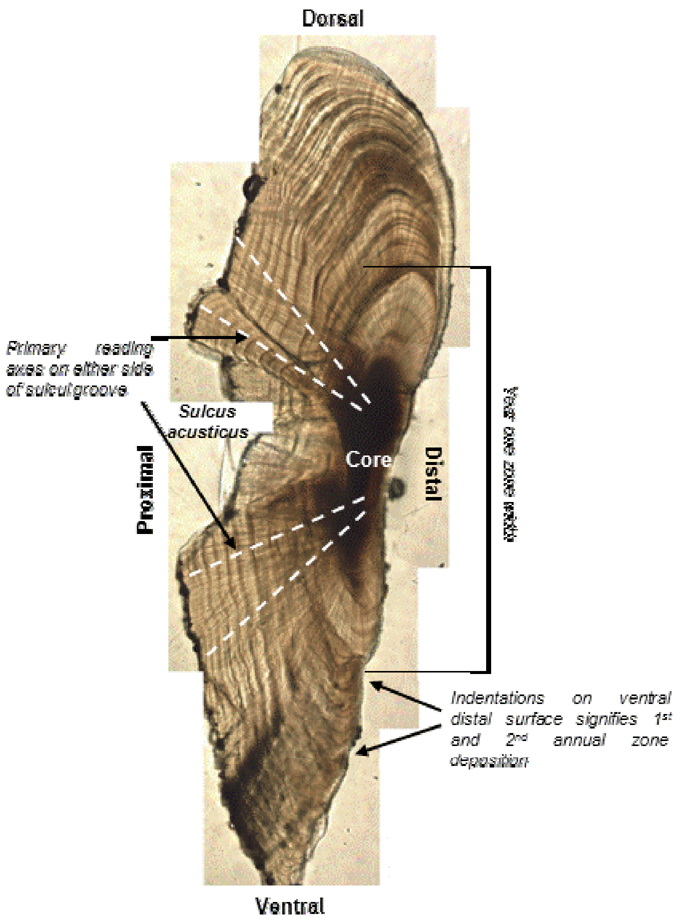


Figure 2: Trevally otolith image of a transverse thin section under transmitted light illustrating otolith terminology. This otolith section was interpreted as 9 wide.

Following the review of catch sampling in TRE 1 and TRE 7 (Walsh & McKenzie 2009), NIWA staff held an ageing workshop in 2007, and revised ageing standards were drafted for trevally. Among other issues relating to the difficulty associated with ageing this species, two main factors were seen to be the most problematic; margin interpretation and location of the first annulus. To improve the level of accuracy in ageing trevally, both issues were addressed with the introduction of the forced margin method and determining the maximum dorsal-ventral width of the year one zone. Readers were also given access to a variety of otolith images from previous collections illustrated with zone counts and otolith terminology to provide additional assistance in age determination.

The first annulus appears as the first most obvious zone after the wide and dark nucleus (core) area, and is further clarified by the first small indentation, of a series, on the ventral distal surface, signifying annual zone deposition (see Figures 2–4). The core to the first zone distance is most often greater than that between successive zones (Figure 2), with the radii along the core–sulcul groove axis usually measuring 0.25–0.35 mm (Figure 3). The importance of a graticule to ascertain the position of the first annulus via this measurement range cannot be overstated, and is essential in determining an accurate zone count of the sectioned otolith. The width of the subsequent wide (light) translucent zones decrease proportionally in size up to about the fourth or fifth dark zone, after which zone width becomes relatively uniform (see Figure 2).

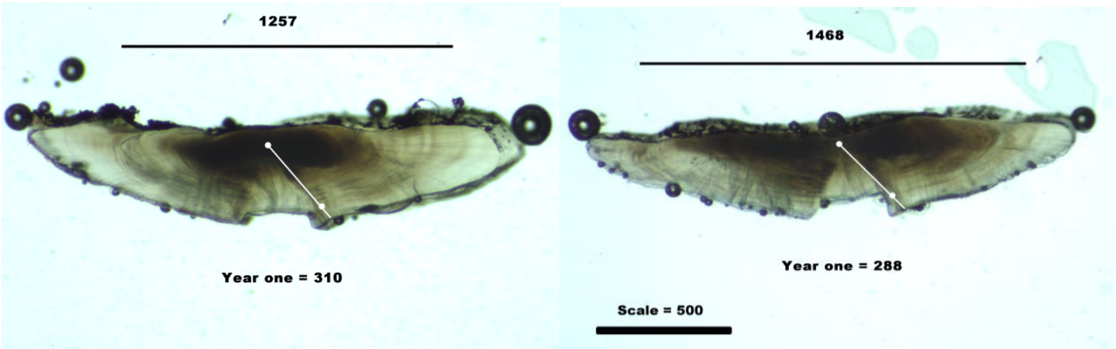


Figure 3: Thin section otolith preparations from 1 year old trevally collected from Porirua Harbour (28 February 2007) showing the core to year one zone distance (white line) along the dorsal sulcus reading axis, and the measured dorsal–ventral maximum width (black line) of the year one zone. All measurements in microns; scale bar = 500 microns (or 0.5mm). Fish sizes ranged 13–16 cm.

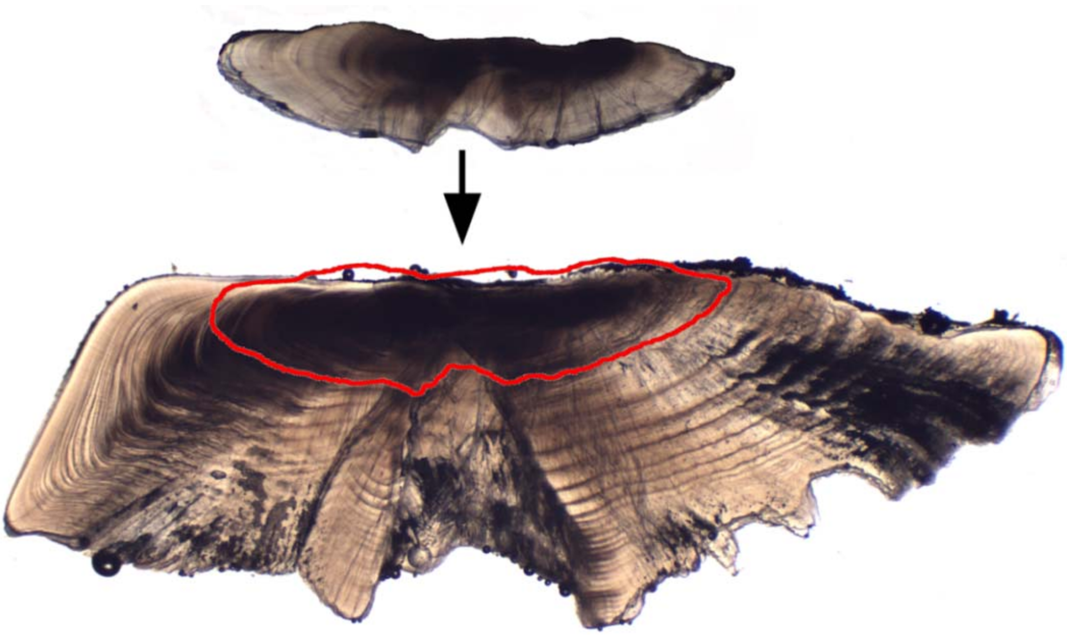


Figure 4: Thin section otolith from a 1 year old trevally (16 cm) with perimeter outline overlaid onto an adult trevally otolith to compare the position of the first annulus (reference set slide #83-4: 64 cm, 26W, agreed age 27).

Juvenile growth and false checks are occasionally present in trevally otoliths and usually lie between the core and the first and second zones, and are most often distinguishable by appearing irregularly spaced and less obvious than the first and second annuli (Figure 5). Generally these are not problematic as the reader has the core to first zone distance measurement and the ventral distal indentation to indicate the location of the true first annual zone.

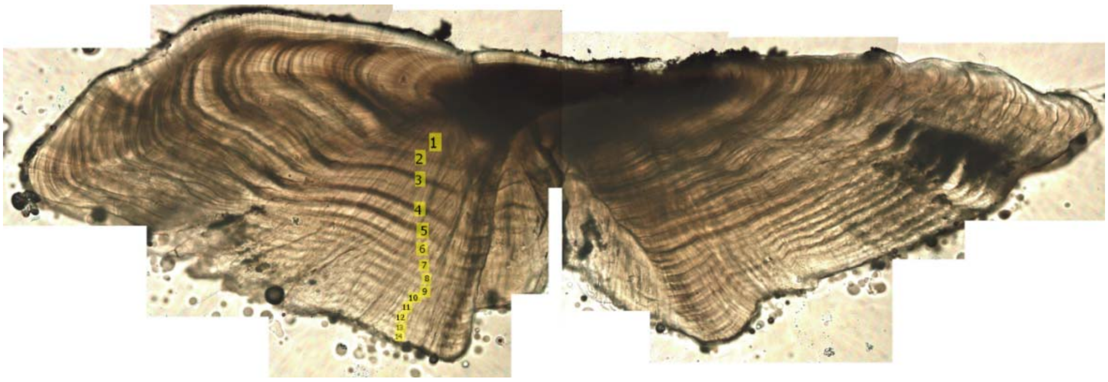


Figure 5: Example where reader has incorrectly interpreted false check as an annual zone for zone count #2.

To derive an accurate zone count from trevally thin otolith sections, readings are typically made along either side of the sulcul groove, designated the primary reading axes, with generally more than one reading from more than one region required to attain zone count agreement (Figure 2). For ease of reading thin section preparations, the opaque dark zones are counted where a fully formed translucent zone precedes it. Zone deposition on the otolith margin, either side of the sulcus, may not always appear to be equal, and if discrepancies occur between counts, the default read is to use the higher estimate, usually that on the ventral side.

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 January is assumed to be the 'birthday' of all trevally, then the first translucent zone is formed in the six to nine months over winter and spring, and the first opaque zone is completed the following autumn (usually March), with all subsequent zones being laid down annually. Therefore, an otolith with three opaque zones collected in October will be approximately 3.75 years old, and one with four zones collected in March will be about 4.17 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 trevally in relation to each month of the New Zealand fishing year, October–September. The birthdate for trevally is 1 January 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	4	4	4	4	4	4	4	4	4
Age group	3+	3+	3+	4+	4+	4+	4+	4+	4+	4+	4+	4+
Decimalised age	3.75	3.83	3.92	4.00	4.08	4.17	4.25	4.33	4.42	4.50	4.58	4.67
Forced margin	W	W	W	W	W	L	N	N	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 trevally 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–February; '**L**ine' (dark opaque line in the process of being laid down or fully formed on the margin), March; '**N**arrow' (a narrow to moderate light (translucent) zone present on the margin), April–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 trevally of moderate to old age, as the otoliths are small with narrow but regular spacing between zones close to the margin. Although the clarity of the margin is reasonably good under low magnification in thin section preparations, viewing under high magnification can 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 (Figure 6).

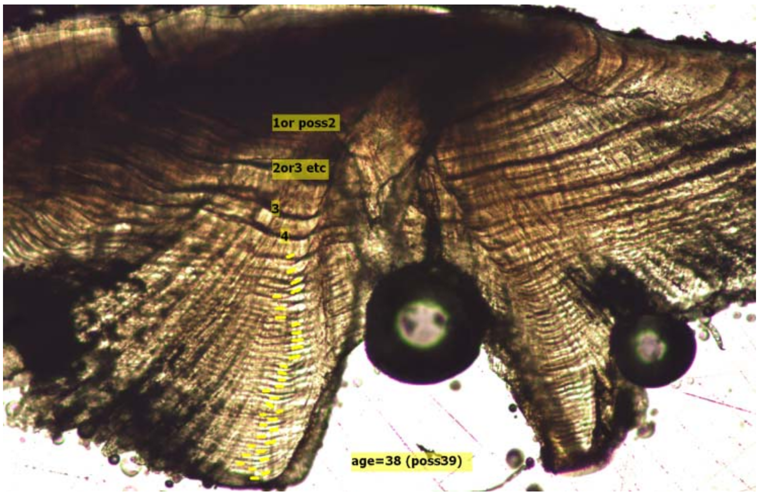


Figure 6: In ageing this otolith collected from an old trevally, accurate readings are only possible along either side of the sulcul groove, designated the primary reading axes. Note, parts of the margin are obstructed due to the otolith being over-ground and containing resin bubbles.

To demonstrate the application of the forced margin to ageing trevally, consider an otolith sampled in February 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 February 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 was not used, 4.08 year old trevally sampled in February could be assigned ages of either 3 or 4, depending on whether an opaque margin was visible, and deemed to be complete.

If the otolith collection time period is protracted (i.e., 12 months), 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. Although otoliths from moderate to old age trevally pose the most difficulty for the reader, fast growing young trevally can also cause problems, often being over-aged by one year (Walsh et al. 2012b). 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). 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. This is especially important when ageing a species with a broad age range, such as trevally, and where samples are collected over an extended time period (i.e., year-round).

Although a readability scale ranked 1–5 has been used for ageing trevally otoliths in the past (Walsh et al. 1999), and demonstrates the level of difficulty associated with ageing the species, the high variability of scores determined by some readers between years (Walsh & McKenzie 2009) questions its utility. Furthermore, the readability scale has not been used in any manner to determine which otoliths are used in the final age selection for catch-at-age analysis other than those ranked 5 (unreadable) which had already been removed from the collection.

2.5 Ageing procedures

In the 1970s a New Zealand wide multilevel clustered sampling design was initiated for age sampling at market for a range of inshore species (West 1978), including trevally. Early ageing procedures only permitted the date of collection to be available to the reader in order to assign age, and although multiple readers were acknowledged for their involvement in ageing thousands of otoliths (James 1984), only one reader was used in ageing each of the sample collections (G. James pers. comm.).

Between 1997–98 and 2005–06, two readers were used in ageing trevally otoliths (Walsh et al. 1999, 2000, Langley 2001, 2002, 2003, 2004, 2009), with each reading made independently, without prior knowledge of counts obtained by the other reader, or of the fish length, knowing only the collection date. Where consensus was reached between readers, 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 to determine the likely source of error and the correct reading. The otolith was only discarded from the dataset if it was unreadable or an obvious outlier with an incorrect length measurement (attributable to less than 1% of samples), and of an age less than 20 years, as samples over 19 years were combined into an aggregate age group (i.e., a plus group) for the analysis (Walsh et al 2010a).

Following the review of catch sampling in TRE 1 and TRE 7 (Walsh & McKenzie 2009), and the trevally ageing workshop in 2007, a revised ageing protocol was developed (see Section 2.1). It was agreed that a third experienced otolith reader be included in all trevally ageing, but only for resolving reader disagreements. This procedure has been followed ever since, with the same experienced readers, and there has been a noticeable improvement in between-reader agreement levels and consistency in relative year class strength estimates between yearly collections (Walsh et al. 2012a, 2012b) (see Figure 7). Furthermore, there have been numerous similarities noted between trevally and

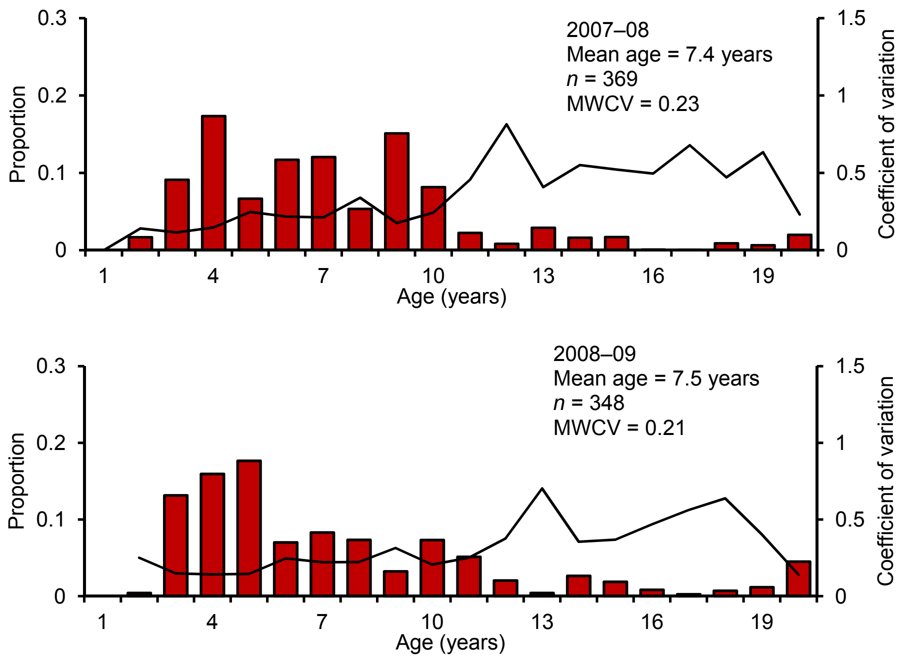


Figure 7: Estimates of proportions at age for trevally sampled from the Bay of Plenty bottom trawl fishery from consecutive fishing years, showing the clear progression of strong and weak year classes, thus demonstrating the consistency of ageing (MWCV, mean weighted coefficient of variation).

snapper relative year class strengths for the west coast fisheries – i.e., TRE 7 and SNA 8 - despite selectivity differences between the species and the relative exploitation status of the respective populations (Walsh et al. 2010a, 2012b) (see Figure 8). These similarities suggest that an environmental variable (e.g., water temperature) strongly influences spawning success and/or larval survival. The accuracy with which trevally is now aged has substantially improved, and reviewing all reader disagreements has become a fundamental step in ensuring that a high level of accuracy is maintained.

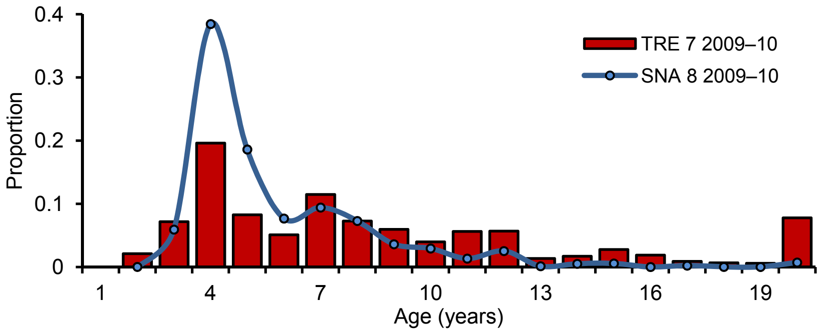


Figure 8: Comparison of the TRE 7 (histogram) and SNA 8 (line) proportion-at-age distributions sampled from the single trawl fishery in 2009–10. Note similarities in recruitment strength between these two species off the west coast of the North Island.

2.6 Estimation of Ageing Precision

Trevally have small otoliths, with those from adult fish of average size (40–45 cm) measuring between 7 and 10 mm long (James 1984, see Appendix 2, Figure A2.1), making them difficult to age. James (1984) established that the error associated with trevally ages increases with age; from about ± 1 year for fish between 8–12 years to about 25–30 years, to ± 2 years for fish older than this. Nevertheless he did not record between-reader comparisons, or precision or bias in determining age.

Between-reader comparisons of trevally age determination for the TRE 1 and TRE 7 stocks were first undertaken on otoliths collected in 1998–99 (Walsh et al. 2000) and between 1997–98 and 2002–03 (Walsh & McKenzie 2009), with agreement ranging between 34–71%, decreasing slightly with age. It was deemed that misinterpretation of growth zones and misinterpretation of the margin, were the main contributing factors leading to ageing error (Walsh & McKenzie 2009). However, between-reader agreement levels (44–55%) did not appear to improve dramatically in 2006–07 (Walsh et al. 2010a). Comparison of ages determined by each reader and the final agreed reading were considerably higher (64–79%), showing that using at least two independent readers improves the accuracy and precision of the final agreed age estimates. Average Percentage Error (APE; Beamish & Fournier, 1981) was calculated for initial readings and ranged between 3.7% and 5.1% (Appendix 4, Table A4.1), and individual reader bias was presented using age-bias plots.

The consistency evident in year class strength estimates in recent catch-at-age comparisons for the TRE 1 and TRE 7 fisheries (Walsh et al. 2012a, 2012b) is indicative of the recent advances in the methodology used for ageing trevally. The APE determined from ageing TRE 7 samples in 2009–10 was 3.8% for initial readings (see Appendix 4, Table A4.1), and individual reader bias, presented in age-bias plots, showed considerable improvements compared to previous years (Figure 9).

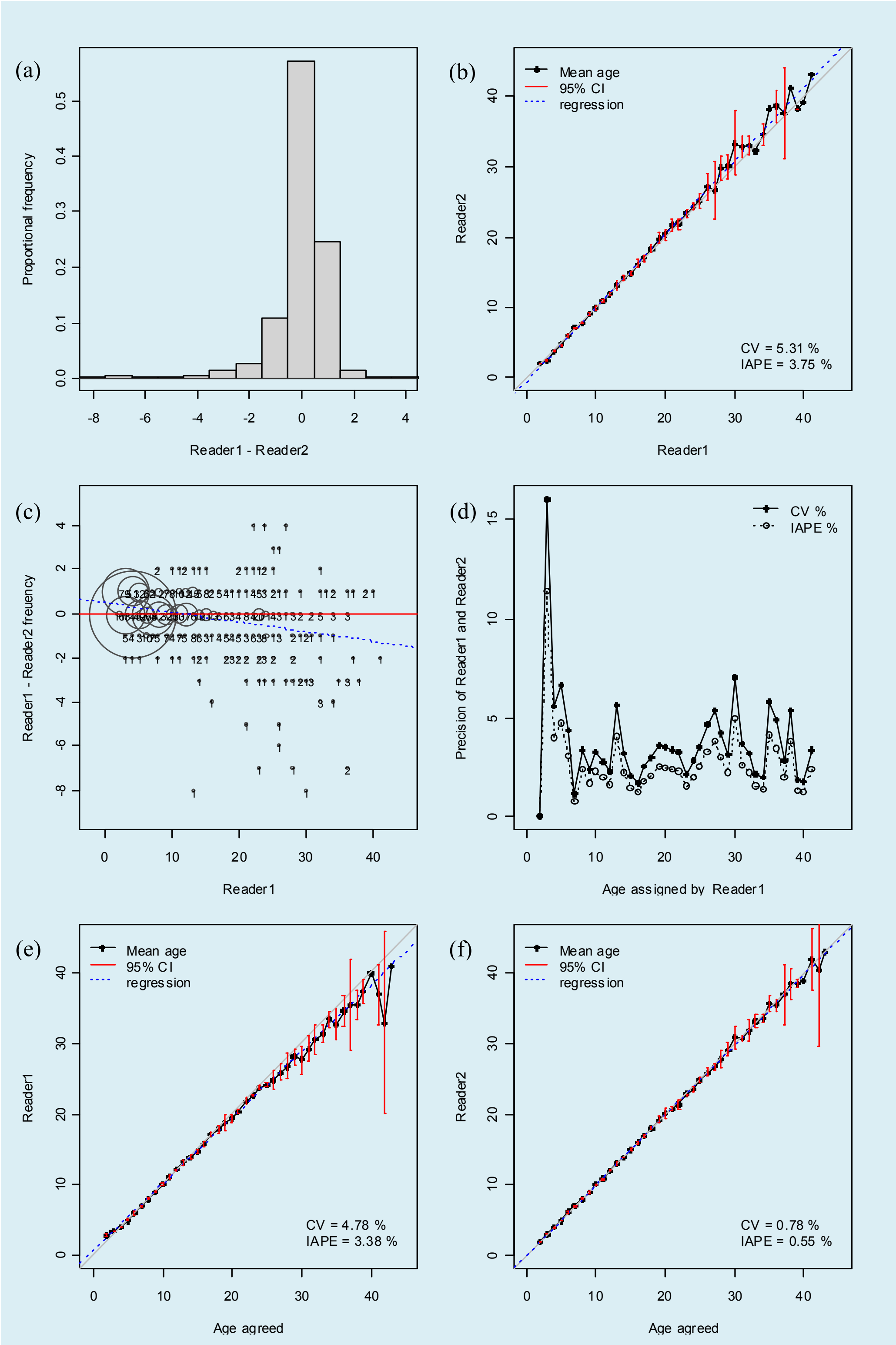


Figure 9: Results of between-reader comparison test (reader 1 and 2) for TRE 7 otoliths collected in 2009–10 (*n* = 1158): (a) histogram of differences between readings for the same otolith; (b) bias plot between readers; (c) differences between readers for a given age assigned by reader 1 (d) CV and IAPE profiles (precision) relative to the age assigned by reader 1; (e) bias plot between reader 1 ((f) reader 2) and agreed age. The expected one-to-one (solid line) and actual relationship (dashed line) between readers are overlaid on (b) and (c), and between reader 1 and 2 and the agreed age on (e) and (f). Reproduced from Walsh et al. (2012b).

2.7 Reference collection

As trevally 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 trevally reference collection was assembled from about 4500 otolith samples (MPI otolith collection, archived at NIWA, Wellington) collected from the TRE 1 and TRE 7 commercial fisheries over the 2006–07 to 2009–10 fishing years. These fishing year samples were chosen specifically as the age estimation for trevally before this was affected by both ageing error and inadequate sampling (see Section 2.1). Despite this, the roughly random selection process of the reference set has ensured that the full seasonal distribution of the otolith samples collected since 2006–07 from the two main fishstocks, TRE 1 and TRE 7, 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 fishery (Figure 10). Examples of these preparations for a range of fish size and age are presented in Section 2.7.1 (Figures 11–14). As trevally is a long-lived species, a reference collection of 500 otolith preparations is believed to be necessary for quality control monitoring purposes. Although growth variation within and between the TRE 1 and TRE 7 fishstocks has been documented (James 1984, Langley 2002, Walsh et al. 1999, 2000, 2010a, 2010b, 2012a, 2012b, Walsh & McKenzie 2009) it was agreed that the collation of stock-specific reference collections was unnecessary.

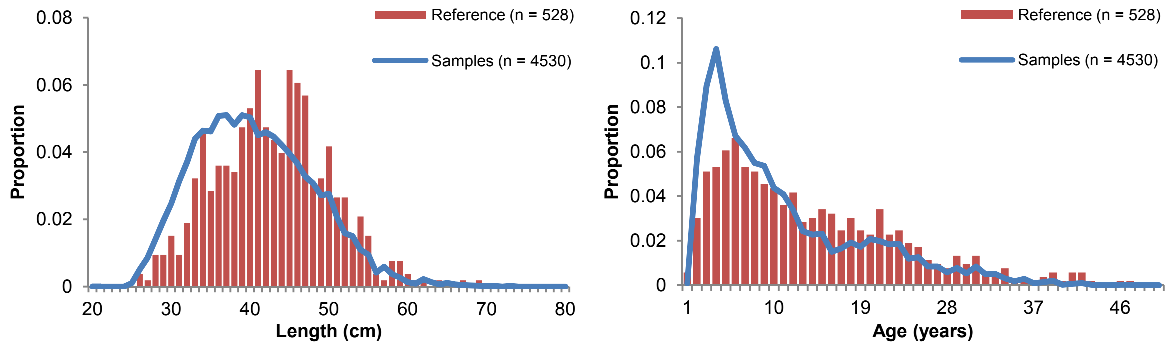


Figure 10: Length and age proportions (lines) of trevally sampled for otoliths from the TRE 1 and TRE 7 commercial fisheries from 2006–07 to 2009–10 with a comparison of the selected subsample chosen for the reference set (histograms).

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 not 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 trevally otoliths with marked opaque zones and agreed reading and age estimates for a range of fish size and age

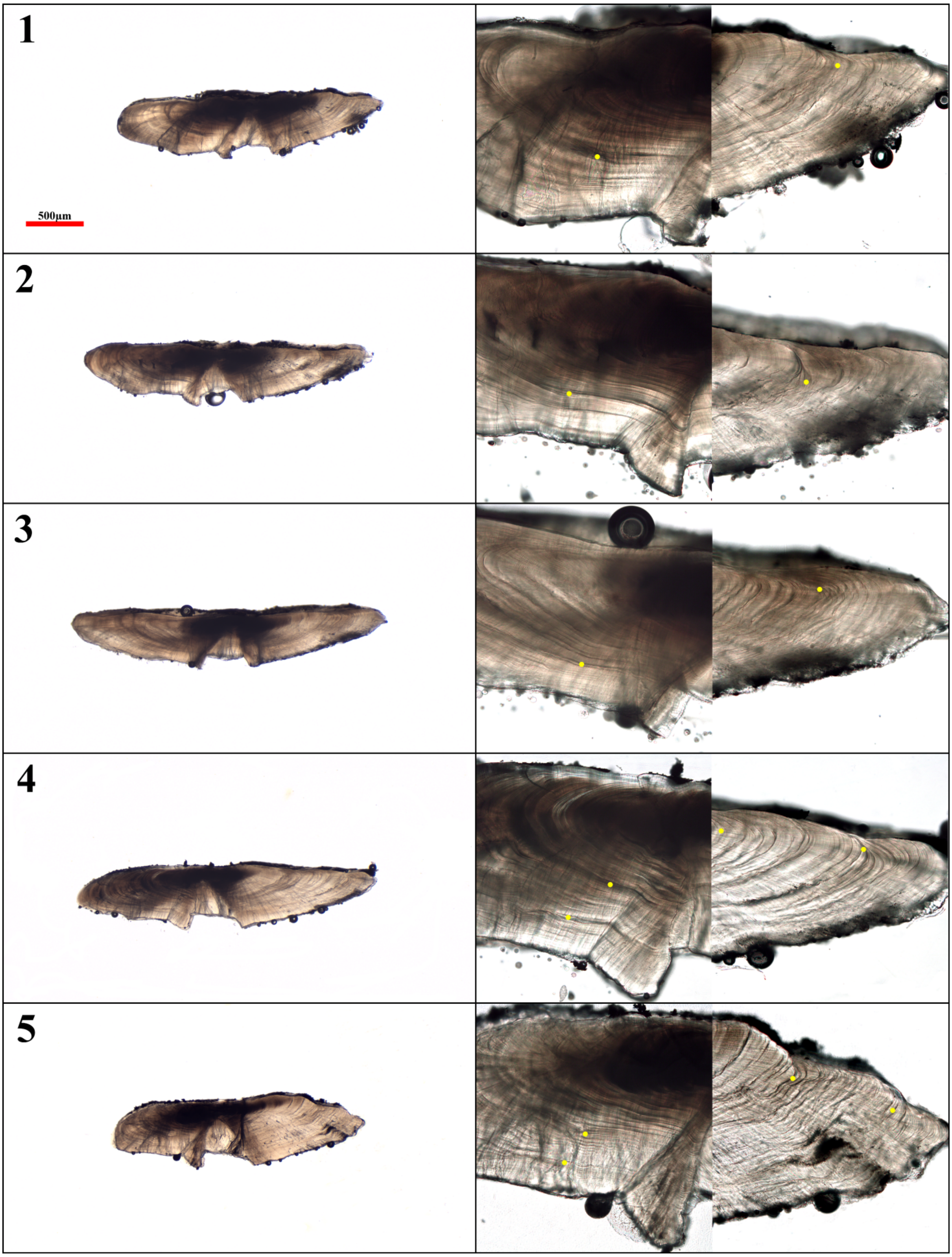


Figure 11: Aged trevally otoliths (whole section and enlarged sub-sections) from the reference set for fish lengths ≤30 cm: fish#1 (slide 27-5, 28 cm, agreed reading 1W, agreed age 2), fish#2 (slide 28-2, 26 cm, 1W, 2), fish#3 (slide 46-4, 29 cm, 1N, 1), fish#4 (slide 59-1, 30 cm, 2W, 3) and fish#5 (slide 87-3, 30 cm, 2N, 2).

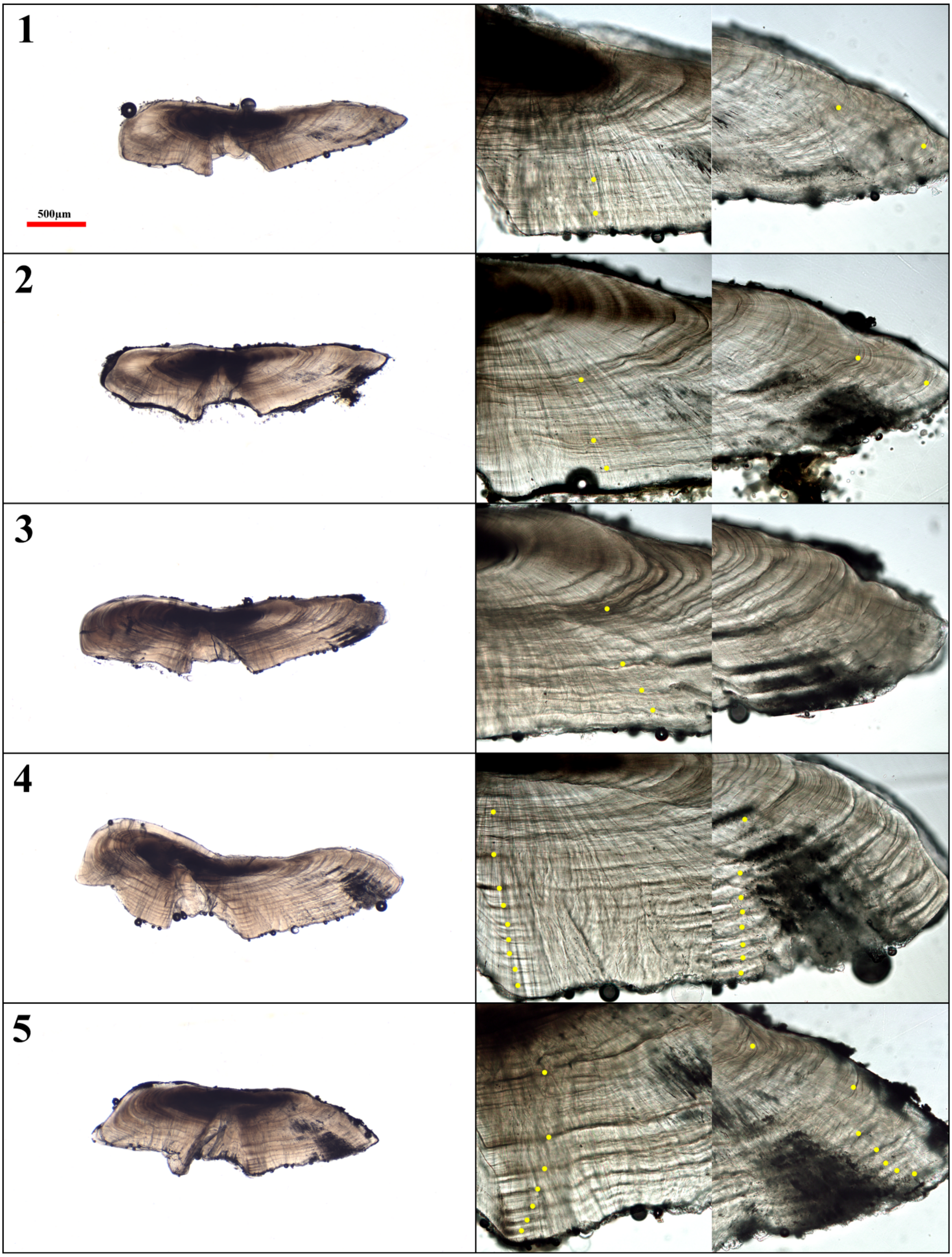


Figure 12: Aged trevally otoliths (whole section and enlarged sub-sections) from the reference set for fish lengths 31–40 cm: fish#1 (slide 86-2, 32 cm, agreed reading 2W, agreed age 3), fish#2 (slide 59-4, 34 cm, 3W, 4), fish#3 (slide 82-4, 36 cm, 4W, 5), fish#4 (slide 86-6, 37 cm, 9W, 10) and fish#5 (slide 68-2, 40 cm, 7N, 7).

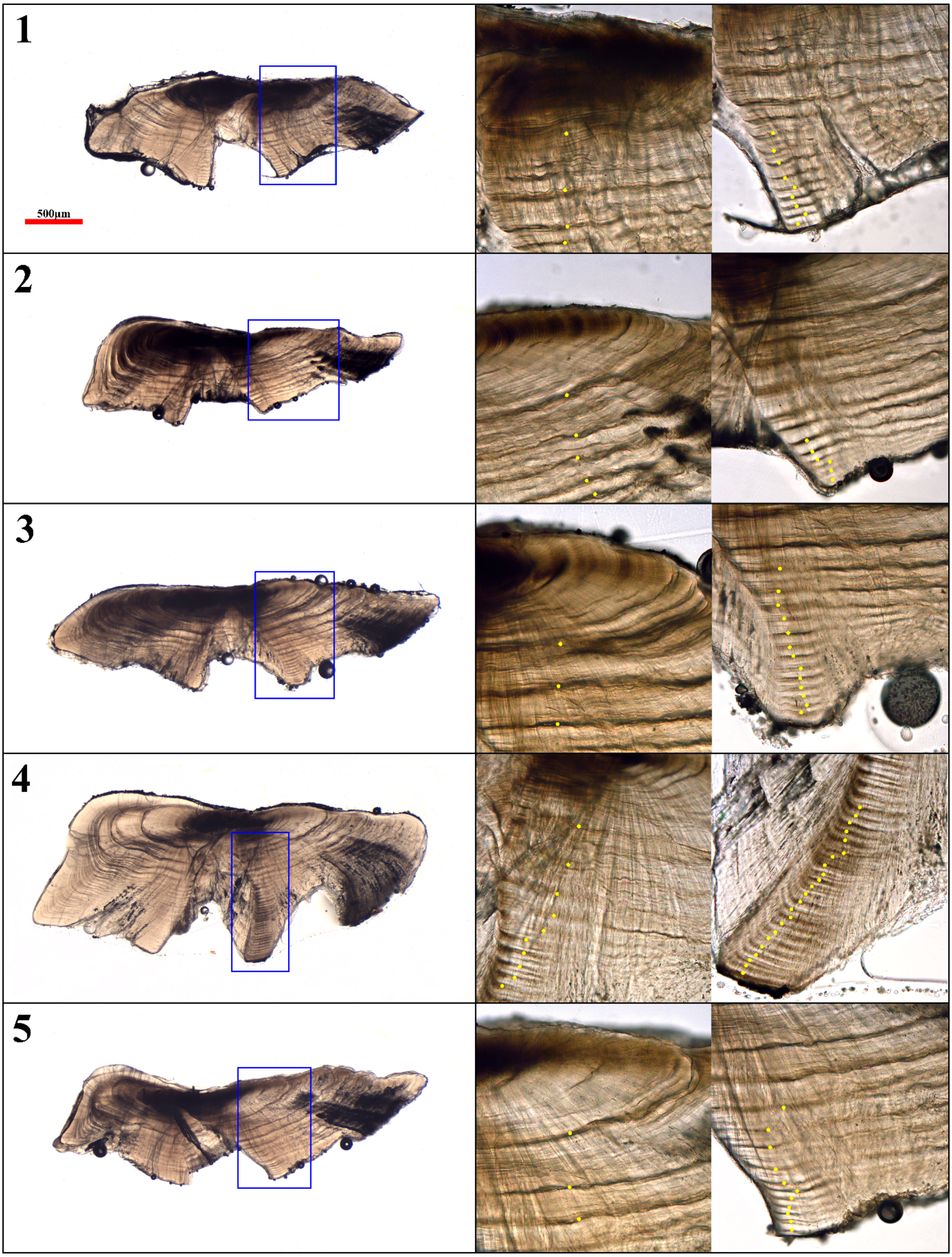


Figure 13: Aged trevally otoliths (whole section and enlarged sub-sections) from the reference set for fish lengths 41–50 cm: fish#1 (slide 10-1, 41 cm, agreed reading 13W, agreed age 14), fish#2 (slide 23-5, 44 cm, 11W, 12), fish#3 (slide 39-5, 46 cm, 16W, 17), fish#4 (slide 41-1, 50 cm, 33W, 34) and fish#5 (slide 83-2, 48 cm, 14W, 15).

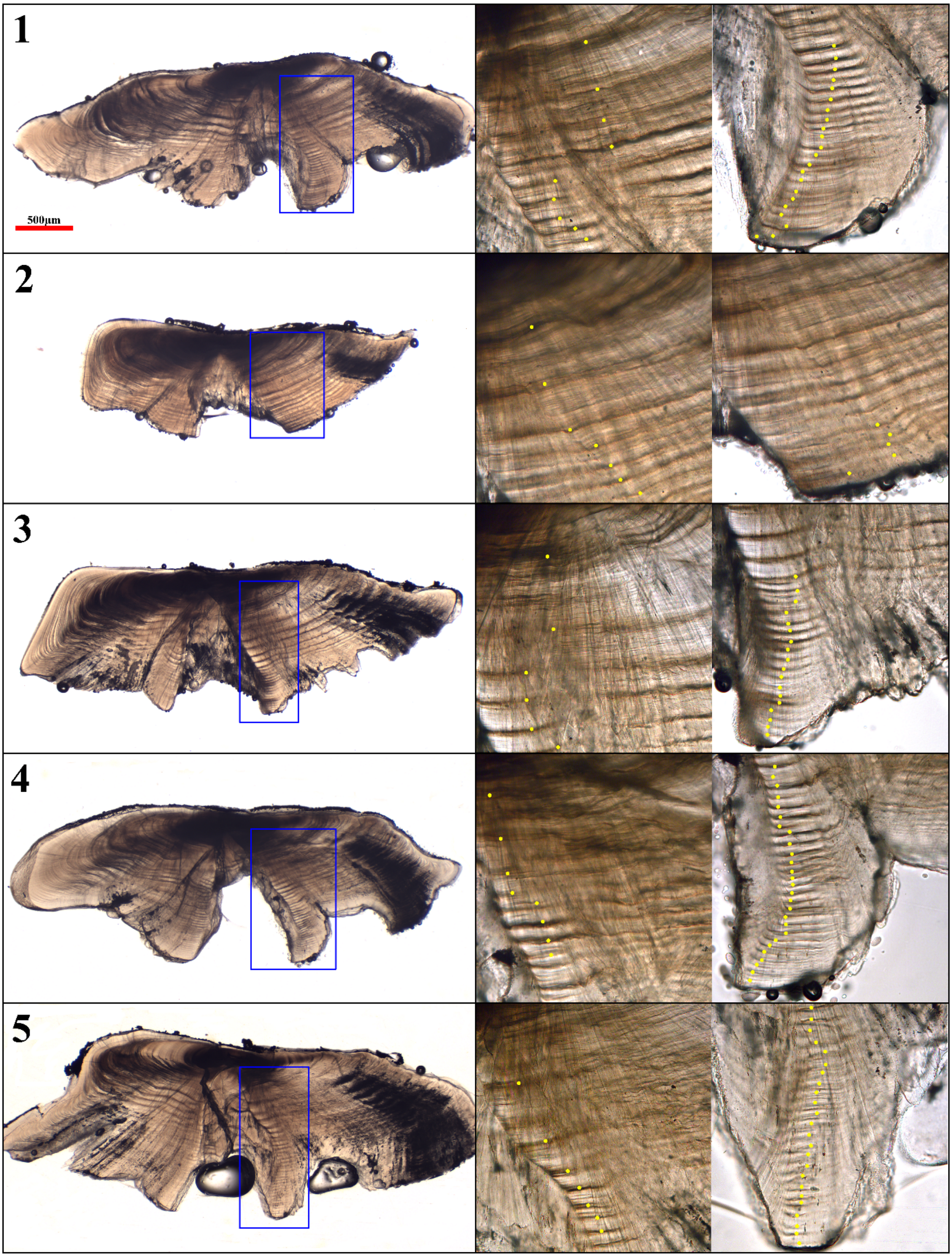


Figure 14: Aged trevally otoliths (whole section and enlarged sub-sections) from the reference set for fish lengths ≥51cm: fish#1 (slide 8-1, 54 cm, agreed reading 31W, agreed age 32), fish#2 (slide 48-1, 51 cm, 12W, 13), fish#3 (slide 83-4, 64 cm, 26W, 27), fish#4 (slide 44-1, 52 cm, 35W, 36) and fish#5 (slide 62-4, 60 cm, 31L, 31).

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 trevally age data submitted for loading onto the *age* database.

Species = TRE
Stock = TRE 1 (Auckland East)
Material = Otolith
Method = 30 (Thin section)
Readers = 77, 113
Project code = PEL2008-03
Sampling period = October 2008 to September 2009

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	20098150	901	1	AKE	TRE	1	1-1	13/01/09	44	2	77	14	12/10/09	113	13	12/10/09	13	W	14	PEL2008-03	
SMP	20098150	901	1	AKE	TRE	2	1-2	13/01/09	41	1	77	13	12/10/09	113	13	12/10/09	13	W	14	PEL2008-03	
SMP	20098150	901	1	AKE	TRE	3	1-3	13/01/09	42	2	77	9	12/10/09	113	9	12/10/09	9	W	10	PEL2008-03	
SMP	20098150	901	1	AKE	TRE	4	1-4	13/01/09	41	2	77	11	12/10/09	113	11	12/10/09	11	W	12	PEL2008-03	

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), 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 <u>dark</u> when viewed with transmitted light, and appears as a <u>depressed</u> 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.

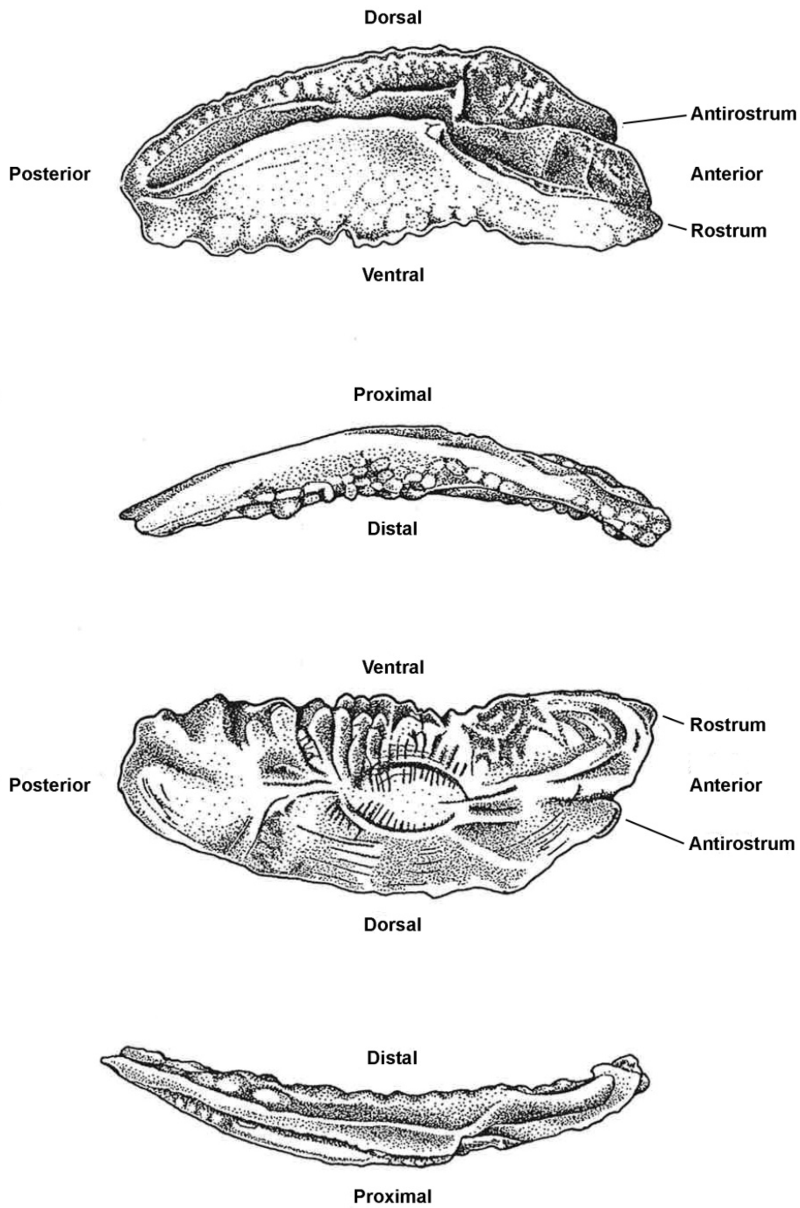


Figure A1.1: Views of a left sagittal otolith from *Arripis trutta* illustrating orientation and basic structure. A) the proximal surface, B) the ventral edge, C) the dorsal edge. (Drawing by Darren Stevens, NIWA).

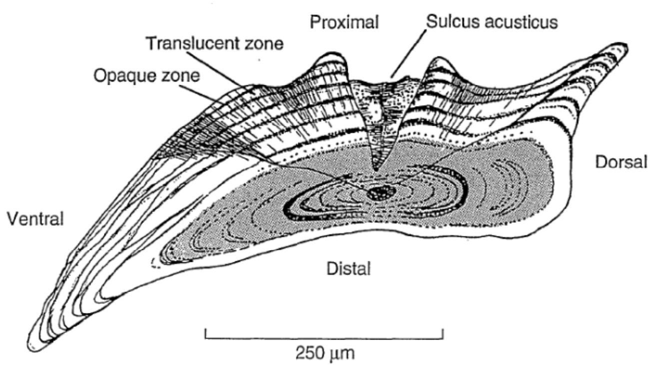


Figure A1.2: Transverse thin section through a sagittal otolith from *Arripis trutta* viewed with transmitted light illumination. The section is taken through the core. (Drawing by Darren Stevens, NIWA).

APPENDIX 2: 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 A2.1, image 1). Kahawai, tarakihi and trevally have elongated sagittal otoliths of smaller size and considerably greater fragility than otoliths of snapper (Figure A2.1, images 2–4).

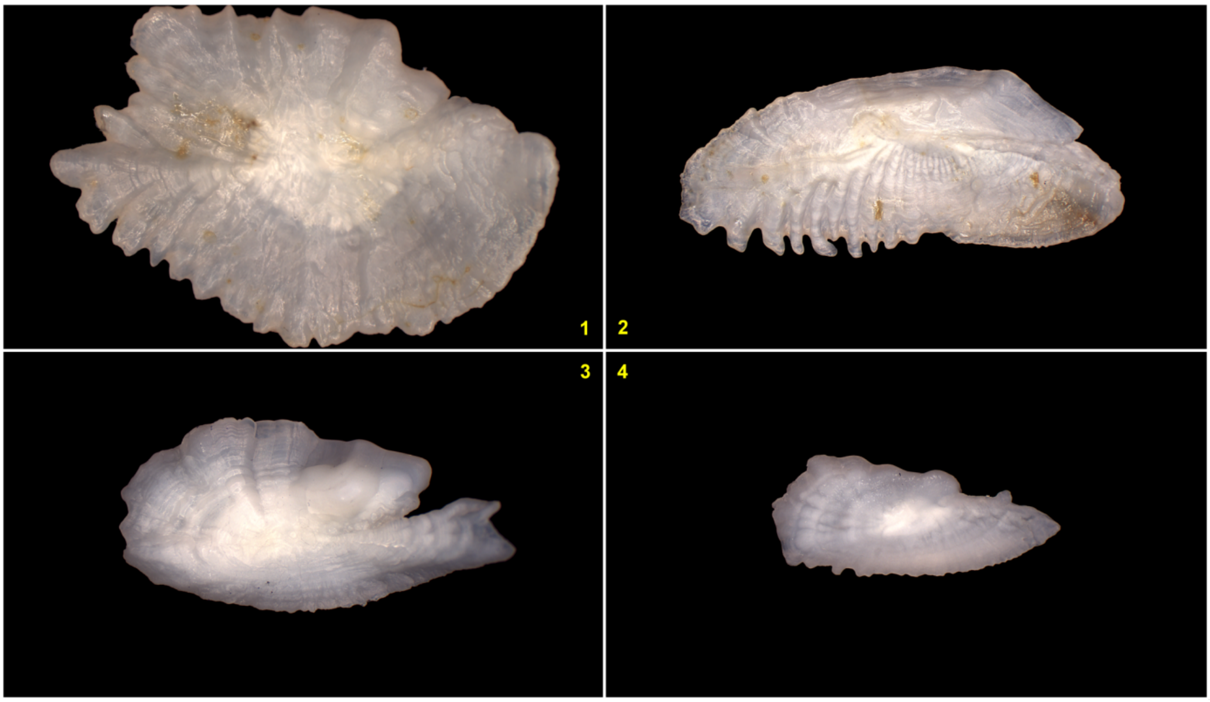


Figure A2.1: Whole right hand side otoliths in lateral view under reflected light at the same magnification demonstrating the differences in otolith size and shape for four important New Zealand inshore species (Image 1, snapper; 2, kahawai; 3, tarakihi; 4, trevally) extracted from fish of equivalent length (42 cm).

Table A2.1: Otolith dimension data for the four species outlined in Figure A2.1.

Species	Otolith bounding box dimensions (mm)		Perimeter (mm)	Surface area (mm^2)	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 3: Protocol for thin section otolith preparation.

A protocol for preparing blue mackerel otoliths from Marriott & Manning (2011). The same methodology is followed for trevally.

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.

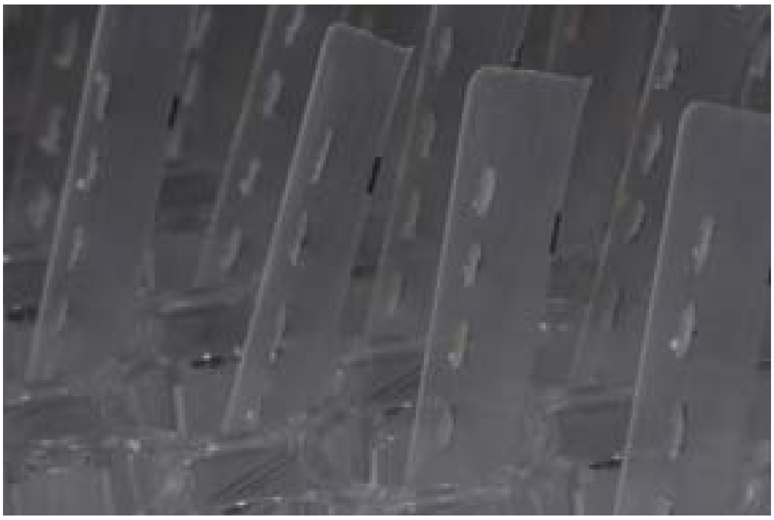
Section blocks at a slow regular speed to ensure even cutting. If one end of the cut wafer is a different thickness to the other end of the cut wafer, slow down the advance speed of the block into the saw, this may produce a more regular section. Utilising clean cutting lubricant should also help to 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: Summary of between-reader agreement and precision estimates documented in ageing studies for trevally.

Previously reported between-reader agreement and precision estimates (APE) determined from ageing trevally in New Zealand are presented in Table A4.1. Although a reasonable level of consistency in reader agreement and precision is apparent in ageing trevally, most estimates are low relative to other inshore species that are routinely aged e.g., snapper, tarakihi, kahawai (Figure A4.1). 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 A4.1: Between-reader agreement and precision estimates documented in ageing studies for trevally in New Zealand (BT = Bottom trawl).

Stock	Method	Fishing Year	No. of readers	Percent agreement	APE	CV	No. aged	Age range	Publication
TRE 1	BT	2006–07	2	44%	5.06	–	338	2–32	Walsh et al. (2010)
TRE 7	BT	2006–07	2	55%	3.65	–	920	3–47	Walsh et al. (2010)
		2009–10	2	58%	3.75	5.31	1158	2–43	Walsh et al. (2012)

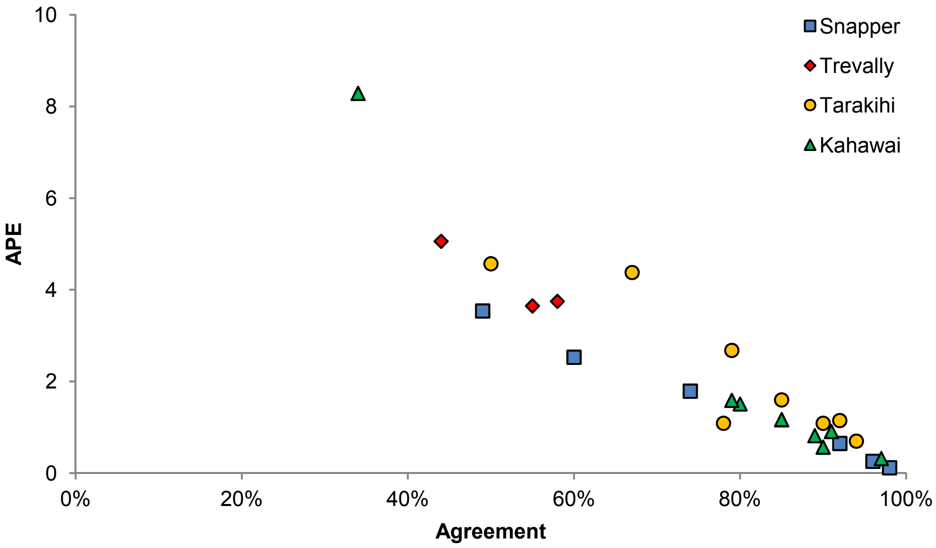


Figure A4.1: Visualised comparison of between-reader agreement and APE scores documented in ageing studies for snapper, trevally, tarakihi and kahawai in New Zealand.

Although percent agreement is considered an inferior method of determining ageing precision compared to APE and the mean coefficient of variation (CV) method (Chang 1982) 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 trevally, 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 trevally, 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 trevally 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 trevally in future studies that require fish age to be determined have been set at 2.50% and 3.54% 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.