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Age determination protocol for tarakihi (*Nemadactylus macropterus*)

New Zealand Fisheries Assessment Report 2016/13

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This report documents the age determination protocol for tarakihi (*Nemadactylus macropterus*), an important New Zealand inshore finfish species, and is a revision of the protocol published in FAR 2014/53. The key amendment concerns the identification of the first annulus, which was previously assumed to be a juvenile check, which resulted in otoliths aged using the previous protocol being under-aged by one year. This specifically affects only tarakihi age data collected from commercial catch samples in 2009–10 and 2010–11 and prepared using the thin section preparation method (Beentjes 2011, Parker & Fu 2011, Beentjes et al. 2012, McKenzie et al. 2015).

This age determination 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 that 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 tarakihi. 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). From this, it was anticipated that age determination protocols would be developed for every species routinely aged through MPI funding.

This report describes the age determination protocol for an important New Zealand inshore finfish species: tarakihi (*Nemadactylus macropterus*), and is an update of FAR 2014/53 (Walsh et al. 2014), the first published protocol for the species in New Zealand. A significant fishstock (TAR 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 tarakihi. 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).

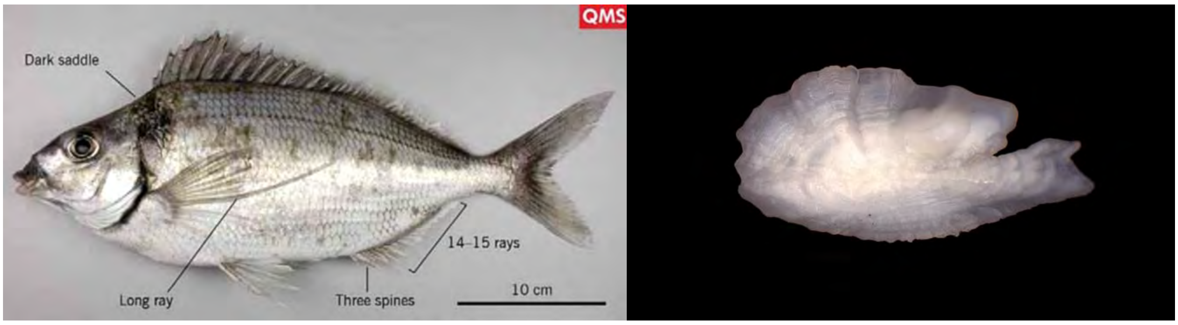
Overall objective

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

Specific objective

1. To develop an age determination protocol for tarakihi (*Nemadactylus macropterus*), including the compilation of otolith reference collections.

2. AGE DETERMINATION PROTOCOL FOR TARAKIHI



2.1 Background

The first ageing study for tarakihi in New Zealand was documented in an unpublished thesis by Marwick (1942), exploring whole ground sagittal otoliths and scales, and validating annual scale ring formation by examining season trends in edge growth. During the late 1950s, McKenzie (1961) used scales from tagged tarakihi before and after recapture, finding one ring to be formed annually, either during the spawning season or during winter, although determining the position of the first ring was found to be problematic.

A comprehensive study on the biology of tarakihi in the late 1960s by Tong & Vooren (1972) investigated age determination using whole untreated otoliths immersed in cedarwood oil. Their aim was to determine whether the zonation patterns in tarakihi otoliths were sufficiently clear and regular to be counted to ascertain the time interval they represent. They found a consistent pattern of alternating wide opaque and narrow hyaline (herein referred to as translucent) zones that were laid down in the spring-summer and autumn-winter periods respectively, for both juvenile and mature fish, and concluded that the age of individual tarakihi could be reliably determined from these zonation patterns. Tong & Vooren (1972) also investigated the reader agreements levels, which were initially low due to minor differences in observation and interpretation of otolith structure, but after discussion improved considerably. Reader agreement was also found to decrease with fish age.

The use of whole otoliths in tarakihi age and growth studies in New Zealand continued for a short period (Vooren & Tong 1973, Vooren 1973a, 1973b, Vooren 1975). However, Vooren (1977) found this method to be unsatisfactory for most large fish and some small ones, because of the poor visibility of narrow zones near the edge of the whole otolith, and investigated the break and burn method (Christensen 1964), as an alternative, as did Annala et al. (1990).

Subsequent research investigating tarakihi growth and age structure from a four year time series of trawl survey collections between 1995 and 2003 trialled bake and embed otoliths for ageing fish greater than 25 cm, continuing with whole otoliths for those 25 cm and below, due to their small size and fragile nature (Stevenson & Horn 2004, see Appendix 2, Figure A2.1). Although a validation of the ageing method was not attempted, zone counts in otoliths from distinct and consecutive juvenile length modes were found to increase by one with increasing mode size. These modes represented consecutive year classes and indicated that one opaque and one translucent zone is laid down annually in otoliths of juvenile tarakihi, after a weak post-larval zone is formed at about 7–9 months (Stevenson & Horn 2004). With high agreement for within- and between-reader trials in ageing tarakihi, indicative of consistent and precise interpretation of otolith structure, Stevenson & Horn (2004) found translucent zones to be clear in bake and embed preparations, and stated that the examination of only whole otoliths in the early studies may have resulted in the under-estimation of some ages.

Baked and embedded otoliths remained the preferred ageing method of Manning et al. (2008) in 2004–05 where year-round random age frequency collections were sampled from the commercial bottom trawl fishery for the first time. However, between-reader agreement was found to be low for this and a subsequent year-round study in 2007–08, and the suitability of the method for accurately ageing tarakihi was questioned. A National Institute of Water and Atmospheric Research (NIWA) ageing workshop (February 2010) reviewed previous ageing methodologies for tarakihi, and trialled thin section preparations as an alternative, investigating magnification required to accurately assess age, as well as the difficulty associated with margin interpretation in year-round collections.

The workshop determined that the inconsistencies observed in the relative year class strengths of tarakihi 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 for ageing tarakihi thin section otolith preparations. 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 with guidance and improve accuracy and precision in age estimations. Between-reader agreement for recent year-round random age frequency sample collections from TAR 1, TAR 2 and TAR 3 (Beentjes 2011, Parker & Fu 2011, Beentjes et al. 2012, McKenzie et al. 2015) has improved considerably, implying that year class strength estimates in catch-at-age comparisons are likely to be more precise than they were in the past, and that the accuracy with which tarakihi is now aged has substantially improved.

A second workshop was convened in December 2014 for the purpose of clarifying the location of the first annulus when using thin otolith sections. Juvenile tarakihi collected from trawl surveys were used for this investigation. The workshop concluded that the first opaque zone (representing the first annulus) had not been correctly identified in thin sections from the trawl survey samples, or in recent collections of commercial catch-at-age samples, resulting in samples being under-aged by 1 year. It is likely that the changes in preparation method (bake and embed to thin section) and the zone type counted (translucent to opaque) were contributing factors to the misinterpretation. Margin interpretation was not influential. The 2014 workshop established that the location of the first annulus could be accurately determined by counting to the outer margin of the first (but indistinct) opaque (dark) zone that lies inside the prominent first translucent (light) zone, following Jordan (2001). This approach produced logical readings and accurate estimates of age which matched the size-at-age for the 0+/1 and 1+/2 year cohorts across the trawl survey length distributions. This report updates the earlier tarakihi age determination report of Walsh et al. (2014) by including the December 2014 ageing workshop's revised methodology for first zone determination.

Tarakihi is a relatively long lived species, although ages over 30 years in New Zealand are relatively uncommon. The oldest recorded age determined for a tarakihi is 44 years, a 47 cm female (estimated 1.9 kg) captured from TAR 7 in 2000 (see Stevenson & Horn (2004)). Although genetic evidence for a second species, "king" tarakihi, exists, found mainly in the subtropical waters of northern New Zealand (Smith et al. 1996), the information presented within this age determination protocol relates only to the commonly caught New Zealand coastal species of tarakihi, *Nemadactylus macropterus*.

Tarakihi are reported to spawn over summer and autumn (Ministry for Primary Industries 2013) with peak spawning occurring mainly from March to May in northern areas (McKenzie 1961, Tong & Vooren 1972, Vooren & Tong 1973, Robertson 1978, Parker & Fu 2011), and earlier with increasing latitude (Tong & Vooren 1972, Robertson 1978, Beentjes 2011). The first published theoretical birthdate used for ageing tarakihi was 1 March (Tong & Vooren 1972), considered to be a reasonable estimate of the early to mid-spawning season for various sites around New Zealand, and was used in subsequent ageing studies (Vooren & Tong 1973, Vooren 1975, Annala et al. 1990). Based on biological data collected from TAR 7, Stevenson & Horn (2004) chose a birthdate of 1 May as being near the end of the spawning season, and this has been adopted as the standard in subsequent tarakihi ageing studies (Manning et al. 2008, Beentjes 2011, Parker & Fu 2011, Beentjes et al. 2012, McKenzie et al. 2015).

In Australia, early ageing studies of tarakihi (referred to as jackass morwong) used counts of translucent zones in scales (Han 1964), and whole otoliths immersed in water (Smith 1982). More recently, age was determined using opaque zones counted from thin sections (Jordan 2001). Growth zone deposition has been validated using the ‘bomb radiocarbon’ technique (Kalish et al. 2002).

2.2 Methods

Sagittal otoliths are acknowledged as the primary structure for ageing tarakihi. 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 tarakihi 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 tarakihi ageing.

2.3 Otolith preparation

Post extraction, tarakihi 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 (see Figure 1). As tarakihi show minor differential growth between the sexes, where females attain a larger average size than males at a given age (Tong & Vooren 1972, Vooren & Tong 1973, Annala et al 1990, Stevenson & Horn 2004), ageing studies should record sex as a mandatory requirement for each fish selected for age. 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 NIWA, Wellington.

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

Appendix 3 outlines the most recent methodology used for thin section otolith preparations in New Zealand (Marriott & Manning 2011). In short, up to five tarakihi 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 were mounted on glass microscope slides using epoxy resin, ground and polished to a thickness of 0.25–0.35 mm.

2.4 Otolith interpretation

Two major zones are formed annually in tarakihi otoliths: a wide opaque zone reflecting rapid calcification is laid down in spring-summer and a narrow translucent zone in autumn-winter (Tong & Vooren 1972), indicative of a period of slow growth (Vooren 1975). The most recent procedure for reading tarakihi otolith thin sections generally follows that described by Jordan (2001). Essentially, when viewed with a compound microscope under transmitted light, the series of alternating opaque and translucent growth zones appear dark and light (Figure 2).

Tarakihi have a relatively long post-larval pelagic stage, occurring mainly in offshore waters with post-larval metamorphosis occurring inshore during spring or early summer, when the fish are 7–9 cm long and 7–12 (Annala 1987) or 7–9 months old (Stevenson & Horn 2004). The assumption that the first zone, generally a weak post-larval zone in otoliths, reflects the settlement stage in tarakihi was based primarily on trawl length frequency distributions and otolith radii measurements (Jordan 2001, Stevenson & Horn 2004). The post-larval zone is generally visible in over half of thin section otolith preparations, becoming less discernible with increasing age (Jordan 2001).

Initial viewing of the thin sectioned otolith may be undertaken at low magnification (10× objective) to determine which of the preferred sites are the clearest for reading. However, as tarakihi 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 the 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 tarakihi otoliths are:

1. The opaque zone (dark in thin section preparations viewed with transmitted light) first becomes visible in January in young fish and in late summer in old fish, and is completed in early autumn. The formation of the translucent zone takes place in the preceding months, during late autumn to early spring, but may still be apparent in late spring and early summer. The first opaque zone (inside the first prominent translucent zone) is consistent with being the first annual increment.
2. The theoretical 'birthday' for all tarakihi is 1 May.
3. Opaque zones are counted under transmitted light.

Tarakihi ageing workshops held by NIWA staff in 2010 and 2014 led to a revision of reading standards relating to age determination from thin section otolith preparations. Among issues relating to the difficulty associated with ageing tarakihi, 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 age estimation, these issues were addressed with the introduction of the forced margin method, and by 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.

Under transmitted light, the first annulus (opaque zone) appears as an indistinct dark zone inside an obvious light translucent zone and is preceded by a light post-larval zone (marked X) and dark (opaque) core (Figure 2). The deposition of the obvious light translucent zone, reflecting the second winter of the fish's life, and a number of subsequent translucent zones is clarified by a series of small indentations visible on the dorso-ventral distal surface of the otolith, signifying a slowing in growth and the point at which the next opaque zone begins (see Figure 2). A mean radial distance of 754 µm (about 0.75 mm) from the core to the outer edge of the first opaque zone along the ventral axis was determined from forty 1+ and 2+ tarakihi thin section preparations, reflecting one full year of growth, and is most often greater than between successive zones. The often indistinct post-larval (translucent) zone distance was estimated to be approximately 0.50 mm (see Figure 2). Both estimates were similar to those determined from thin section tarakihi otoliths from Australia (Jordan 2001) and from whole otoliths in New Zealand

(Stevenson & Horn 2004). The use of a graticule to accurately determine the position of the first opaque zone, and therefore an accurate count of successive opaque zones for the sectioned otolith, is seen as essential for ageing tarakihi, particularly when the core or zone formation is unclear. The width of the subsequent wide dark (opaque) zones decrease proportionally in size up to about the fourth or fifth zone, at which point zone width becomes relatively uniform between adjacent zones.

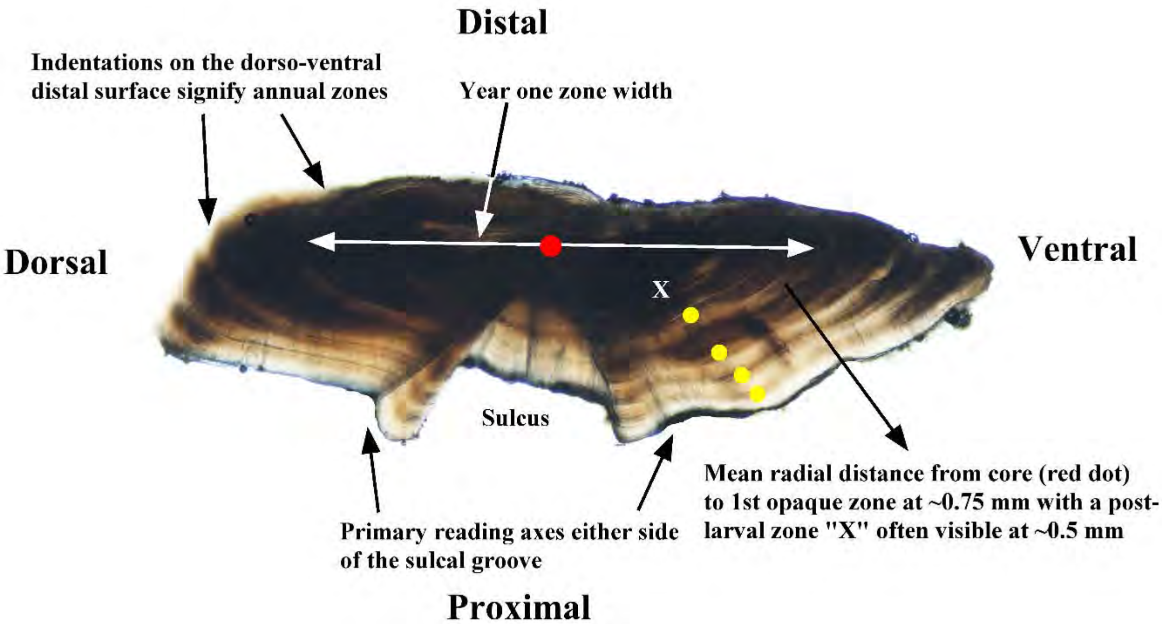


Figure 2: Tarakihi otolith image of a transverse thin section under transmitted light, illustrating otolith terminology. Counts are made primarily along the axes from the core to the proximal margin adjacent to the sulcus, indicated here by the bottom two arrows. This otolith section was interpreted as 4 Wide = age 5 (slide 40-1, 30 cm, January).

The deposition of the first annulus (opaque zone) and its progression between two seasons is examined using thin otolith section image examples from 0+/1 and 1+/2 year old tarakihi in conjunction with the progression of independent cohort length modes from samples taken in east coast South Island trawl surveys during December and May (Figures 3 and 4). Length compositions (under 30 cm) show a partially recruited 0+ cohort first appearing in December at a mean length of about 8.5 cm (about 7 months old), with otoliths showing a weak translucent margin representing what is assumed to be the post-larval settlement zone (Figures 3 and 4). By May, and fortuitously the theoretical 'birthday', these fish had progressed to a mean length of about 12.5 cm and one year of age, their otoliths comprising a fully formed opaque zone inside a narrow translucent margin indicating that the opaque zone had been formed between the two survey periods (Figures 3 and 4). Mean length for the 1+ cohort progressed over the same period from about 14.8 cm (December) to 18.2 cm (May), by which time the fish were 2 years old. The otolith image for 1+ tarakihi (about 1 year 7 months old) from December clearly shows a fully formed annulus (dark opaque zone) inside a wide translucent margin, and by May a second annulus had formed inside a thin translucent margin, (Figure 3).

Two intermittent time series length compositions for tarakihi caught from east coast (1991–2014) and west coast (1992–2015) South Island trawl surveys are presented in Appendices 4a and 4b to provide the reader additional information on the consistency in the size-at-age range for the 0+/1 and 1+/2 cohorts through time. For example, over the March to June period, 0+/1 year old tarakihi usually comprise an independent mode, ranging in size from about 9–16 cm, and seldom overlap with 1+/2 year old tarakihi, which may range from about 14–23 cm.

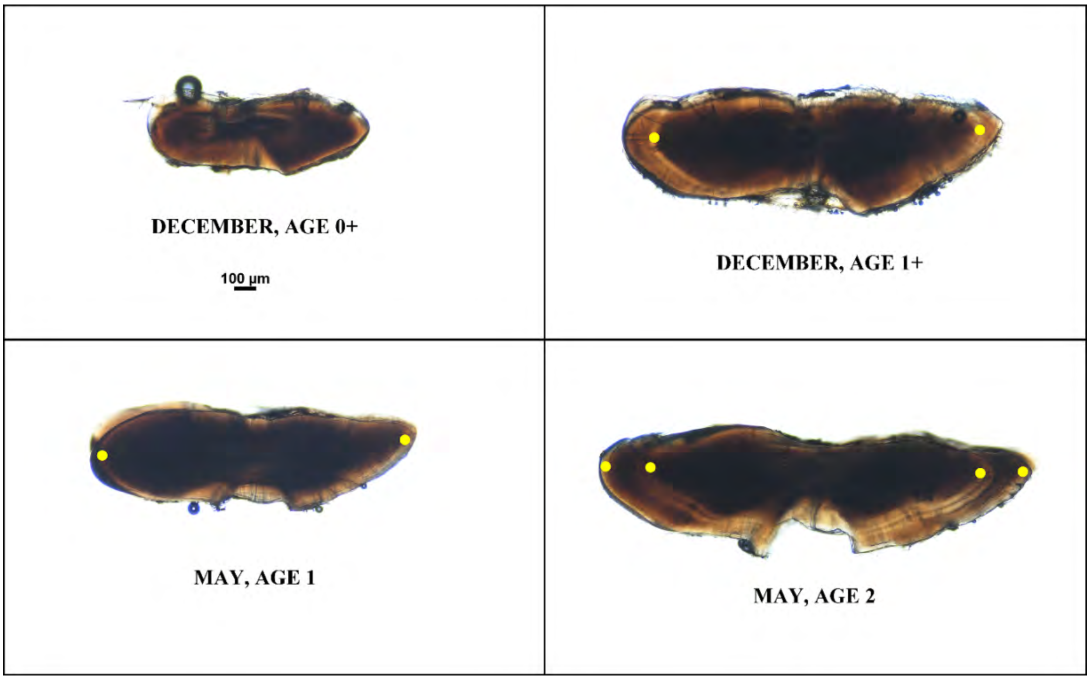


Figure 3: Thin section otolith images of 0+ and 1+ juvenile tarakihi (9 and 15 cm in length) collected in December and 1 and 2 year old juvenile tarakihi (13 and 18 cm in length) collected in May and viewed under transmitted light. The yellow circles mark the positions of the outer edge of annuli (opaque zones). Samples were collected from east coast South Island trawl surveys.

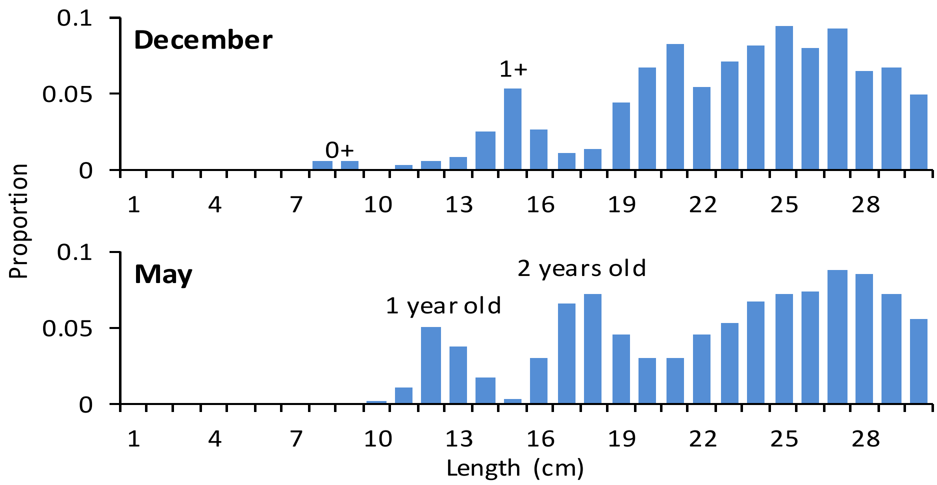


Figure 4: Truncated length (FL) frequency distributions of tarakihi ≤30 cm collected from east coast South Island trawl surveys during December 2000 and May 2007 demonstrating the size at age range for the 0+ and 1+ cohorts and their progression through time.

False checks are occasionally present in tarakihi otoliths and usually lie between the core and the first and second zones. Generally these are not problematic as the reader has the core to first translucent zone distance measurement and the indentations on the otolith dorso-ventral distal surface to indicate the location of the first opaque zone.

To derive an accurate zone count from thin tarakihi otolith sections, readings are typically made along either side of the sulcul groove, designated to be the primary reading axes, where clear, distinctive, and uniform alternating opaque and translucent zones are visible (Figure 2). For ease of reading thin section preparations, the first dark opaque zone (representing the first annulus and lying inside the most prominent first light translucent zone), and all subsequent opaque zones are counted from a point on their outer-edge (see yellow dots in Figure 2) out to the otolith margin. Generally more than one reading from more than one region is required to attain zone count agreement. 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.

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 May is assumed to be the 'birthday' of all tarakihi, and the post-larval metamorphosis translucent zone is apparent on the otolith three to five months before the first birthday, then the first annual translucent zone is formed during the second winter of the fish's life. Complete opaque zones should be visible around March–April with subsequent zones being laid down annually, although for very young tarakihi (i.e. less than four years of age), opaque zone deposition may be complete a month or two earlier. Therefore, an otolith with three opaque zones collected in October will be approximately 3.42 years old, and one with four zones collected in June will be about 4.08 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 tarakihi in relation to each month of the New Zealand fishing year, October–September. The birthdate for tarakihi is 1 May 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	3	3	3	4	4	4	4	4
Age group	3+	3+	3+	3+	3+	3+	3+	4+	4+	4+	4+	4+
Decimalised age	3.42	3.50	3.58	3.67	3.75	3.83	3.92	4.00	4.08	4.17	4.25	4.33
Forced margin	W	W	W	W	W	L	L	N	N	N	N	N
Fishing year age class	4	4	4	4	4	4	4	4	4	4	4	4

In order 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 tarakihi 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 zone in the process of being laid down or fully formed on the margin), March–April; '**N**arrow' (a narrow to moderate light (translucent) zone present on the margin), May–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 allow for minor variations in zone deposition between otoliths in the collection they are reading. This is particularly important for tarakihi of moderate to old age, as the otoliths are small with narrow but regular spacing between zones close to the margin (Figure 5). Although the clarity of the margin appears reasonably clear 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 5: In ageing this otolith collected from an old tarakihi, accurate readings are clearest along either side of the sulcul groove, designated the primary reading axes.

To demonstrate the application of the forced margin to ageing tarakihi, 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 3.75 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 method was not used, 3.75 year old tarakihi 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.

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. This is especially important when ageing a species with a broad age range, such as tarakihi, and where samples are collected over an extended time period (i.e., year-round).

Young, fast growing or pre-recruit tarakihi also pose problems for readers, and are often misinterpreted and over-aged by one year, probably because fewer annual zones are present to compare with and marginal growth appears advanced. The use of the radial distance measurement to the first opaque zone (about 0.75 mm) and the location of the first small indentations on the otolith dorso-ventral distal surface that define the location of the first (and obvious) translucent zone will assist the reader in identifying the correct position to begin their count.

A readability scale ranked 1–5 (Manning et al. 2008) has been used for ageing tarakihi otoliths in the past. However, the scale is not mandatory or 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 are already removed from the collection.

2.5 Ageing procedures

Tong & Vooren's (1972) study in the late 1960s, involved both authors ageing whole tarakihi otolith samples independently, mostly without repeated readings. To investigate a comparison of readings, the authors each aged five subsets of otoliths, and with well-defined criteria for interpretation of the zones after initial readings, attained favourable agreement in second readings, ranging from 72–93%.

Although subsequent studies (Vooren & Tong 1973, Vooren 1973a, 1973b, Vooren 1975, 1977, Annala et al. 1990) usually referred to following the same ageing procedures as described by Tong & Vooren (1972), it remains unclear whether more than one reader was used consistently throughout the sample collections, whether repeated readings were made, and whether disagreements were resolved. This is the same situation for thousands of tarakihi otolith samples collected during the early 1970s in a New Zealand wide multilevel clustered sampling design initiated for age sampling at market for a range of inshore species (West 1978).

Stevenson & Horn (2004) found the interpretation of the zonation patterns in whole and baked and embedded tarakihi otoliths relatively straightforward when ageing 1137 fish sampled from four west coast South Island trawl surveys. A primary reader read the entire otolith collection once, with the primary and a secondary reader used in re-ageing 11% of the collection to assess within- and between-reader variability and estimate reader precision. The readers had no knowledge of fish length or sex at the time of reading. Identical ageing procedures involving the same two readers were used in ageing 260 west coast South Island trawl survey and 1030 market sample tarakihi otoliths in 2004–05 (Manning et al. 2008).

A NIWA in-house ageing workshop was held in February 2010 to improve between-reader agreement levels in ageing year-round collections of baked and embedded tarakihi otoliths sampled in 2007–08 from TAR 1. Recommendations were made for subsequent year-round collections from TAR 1, TAR 2 and TAR 3 in 2009–10 and 2010–11, where two readers were used to age thin otolith sections (Beentjes 2011, Parker & Fu 2011, Beentjes et al. 2012, McKenzie et al. 2015). 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 accepted. Where disagreement occurred, the otolith was jointly reread using images of otoliths projected onto a video screen, with a third experienced otolith reader present to determine the likely source of error and the likely 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. Following this ageing procedure, and using the same experienced readers, produced a noticeable improvement in between-reader agreement levels and consistency in catch-at-age results, the latter point**,** most apparent in the progression of strong and weak year classes over successive years for female tarakihi (Beentjes et al. 2012) (Figures 6 and 7). However, despite this good agreement, the recent finding suggests that all samples had been under-aged by one year. The between-year inconsistency in catch-at-age for male tarakihi may indicate smaller fish recruiting into the fishery, either through growth or migration, spatial movement of fishing effort, or the influence of the temporal distribution of sampling and how sample stratification is undertaken during analysis (Beentjes et al. 2012). Reviewing all reader disagreements is seen as a fundamental step in determining an accurate estimate of the final agreed age for tarakihi.

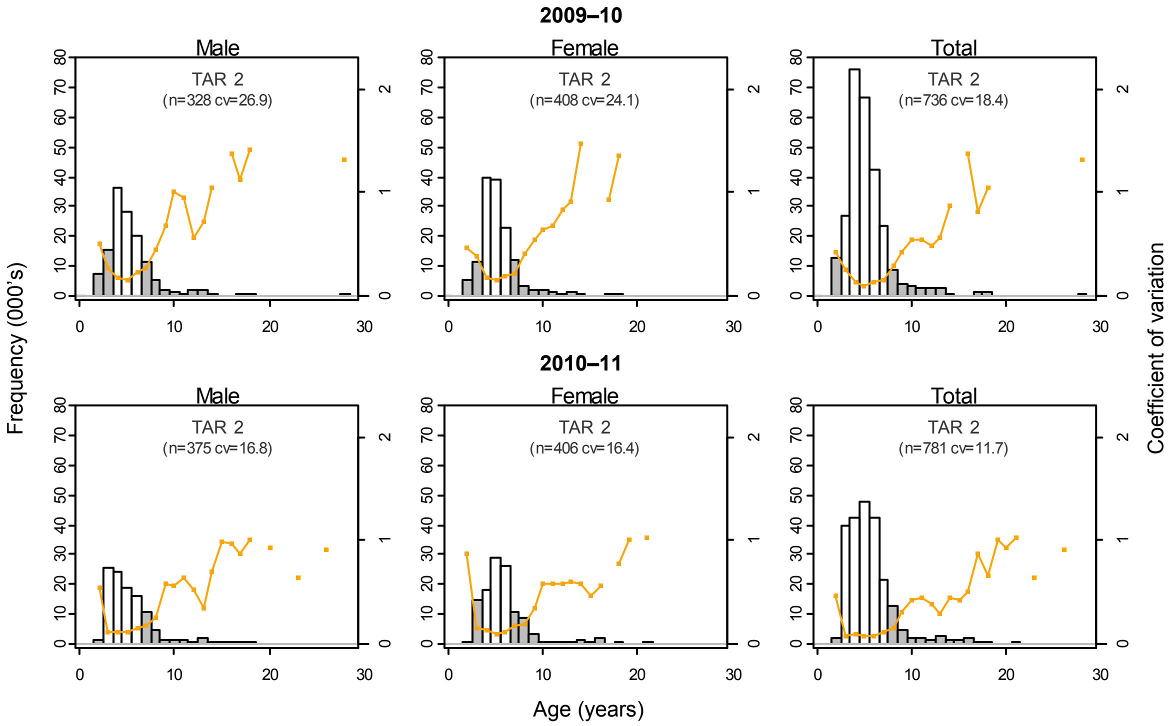


Figure 6: Estimates of annual (seasonally stratified) catch-at-age for tarakihi sampled from the TAR 2 bottom trawl fishery from consecutive fishing years. Reproduced from Beentjes et al. (2012). Note that the ages in these plots are affected by the issue described in paragraph 7 in Section 2.1 and therefore will be under-aged by 1 year.

2.6 Estimation of Ageing Precision

Quantification of precision and bias in ageing tarakihi from reader comparisons has been reported for a number of New Zealand fisheries (i.e., TAR 1, TAR 2, TAR 3, TAR 7) in recent years (Stevenson & Horn 2004, Manning et al. 2008, Beentjes 2011, Parker & Fu 2011, Beentjes et al. 2012, McKenzie et al. 2015) (see Figure 7 and Appendix 5, Table A5.1). Although tarakihi can be considered a moderately easy species to age, estimates of Average Percentage Error (APE; Beamish & Fournier, 1981) for initial readings vary considerably, ranging between 0.7% and 4.6% for between-reader comparisons (Appendix 5, Table A5.1). Note that these estimates are initial reading precision estimates and do not represent the final result of independent and repeated readings in determining age as implemented in recent studies (Beentjes 2011, Parker & Fu 2011, Beentjes et al. 2012, McKenzie et al. 2015). Stocks comprising a high proportion of young individuals are generally easier to age and have the lowest APE estimates (i.e., TAR 2 and TAR 3, see Appendix5, Table A5.1). Although not presented for tarakihi in the past, age-bias plots for each reader's initial age compared with the final agreed age have been found to be particularly useful for graphical comparisons between readers ageing other inshore species (i.e., snapper, trevally, kahawai), determining individual reader APE and highlighting where error may vary with fish age.

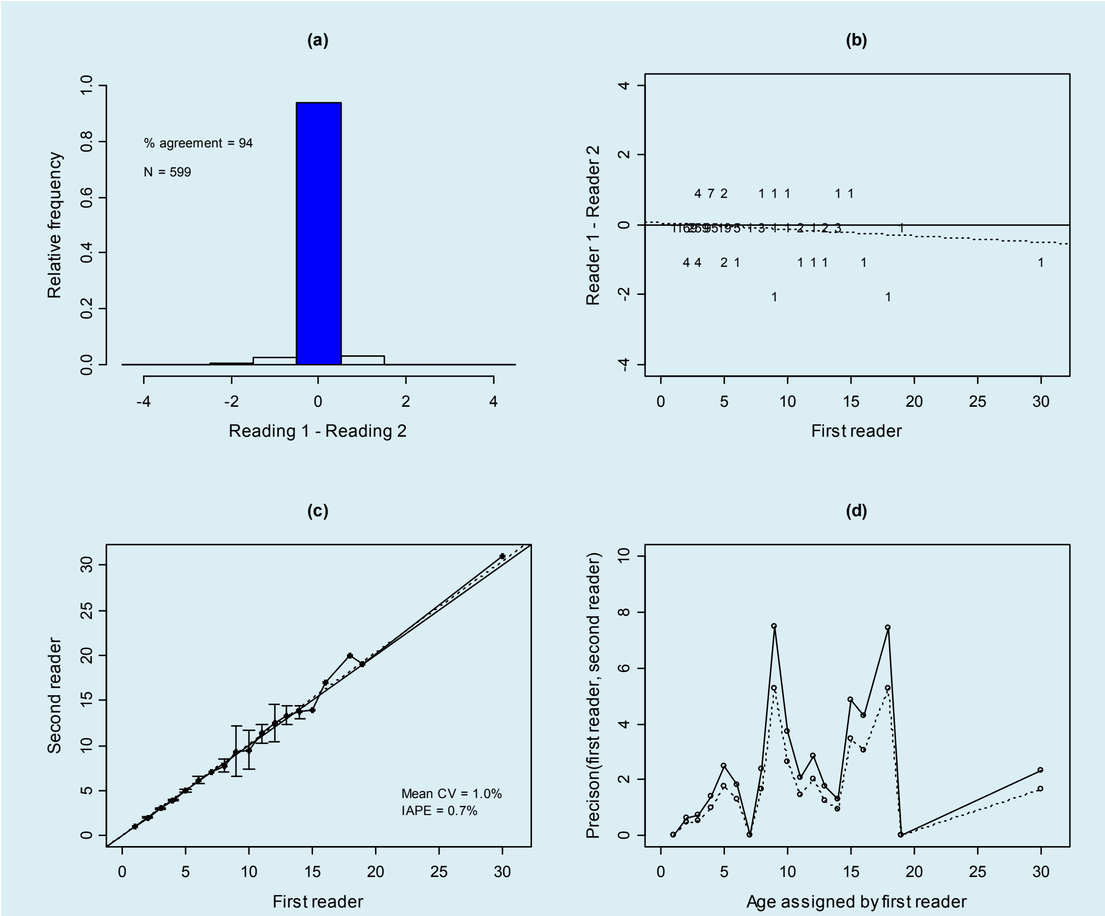


Figure 7: Age reader comparison plots for TAR 3 bottom trawl catch sampling in 2011: (a) histogram of age differences between two readers; (b) Difference between reader 1 and reader 2 as a function of the age assigned by reader 1. The number of fish in each bin is plotted as the plot symbol; (c) Age-bias plot, showing the correspondence of ages between reader 1 and reader 2 for all ages. Error bars indicate the CV of the ages for each age by reader 1; (d) Plot of the CV and the APE for each age as assigned by the first reader. In panels b and c, solid lines show perfect agreement, dashed blue lines show the trend of a linear regression of the data. Reproduced from Beentjes et al. (2012).

2.7 Reference collection

As tarakihi 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, and to ensure consistency among age readers (Campana 2001). The tarakihi reference collection was assembled from about 4000 otolith samples (archived at NIWA Wellington) collected from the TAR 1, TAR 2 and TAR 3 fishstocks over the 2009–10 and 2010–11 fishing years. These years were chosen specifically as the age estimation for tarakihi before 2009–10 was affected by ageing error, the use of only one reader, and different preparation methods, as well as being sampled from point-in-time trawl surveys. The roughly random selection process of the reference set has ensured that the full seasonal distribution of the otolith samples collected from the TAR 1, TAR 2 and TAR 3 fishstocks since 2009–10 were well represented, and that the full length range is covered, while not being strongly dominated by the most abundant length classes in the commercial fishery (Figure 8). Examples of these preparations for a range of fish size and age are presented in Section 2.7.1 (Figures 9–11). As tarakihi 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 for tarakihi between New Zealand fishstocks has been reported for juveniles (Vooren 1975), negligible differences exist between adult populations (Vooren 1977, Stevenson & Horn 2004), and it was agreed that the collation of stock- or sex-specific reference collections was unnecessary.

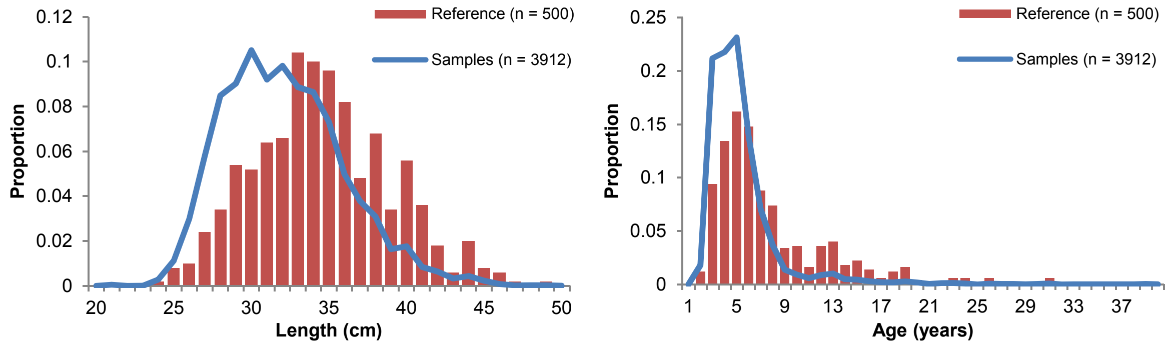


Figure 8: Length and age proportions (lines) of tarakihi sampled for otoliths from the TAR 1, TAR 2 and TAR 3 commercial fisheries from 2009–10 to 2010–11 with a comparison of the selected subsample chosen for the reference set (histograms). Note that the ages in the age plot on the right hand side are affected by the issue described in paragraph 7 in Section 2.1 and therefore will be under-aged by 1 year.

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 tarakihi otoliths with marked opaque zones and agreed reading and age estimates for a range of fish size and age

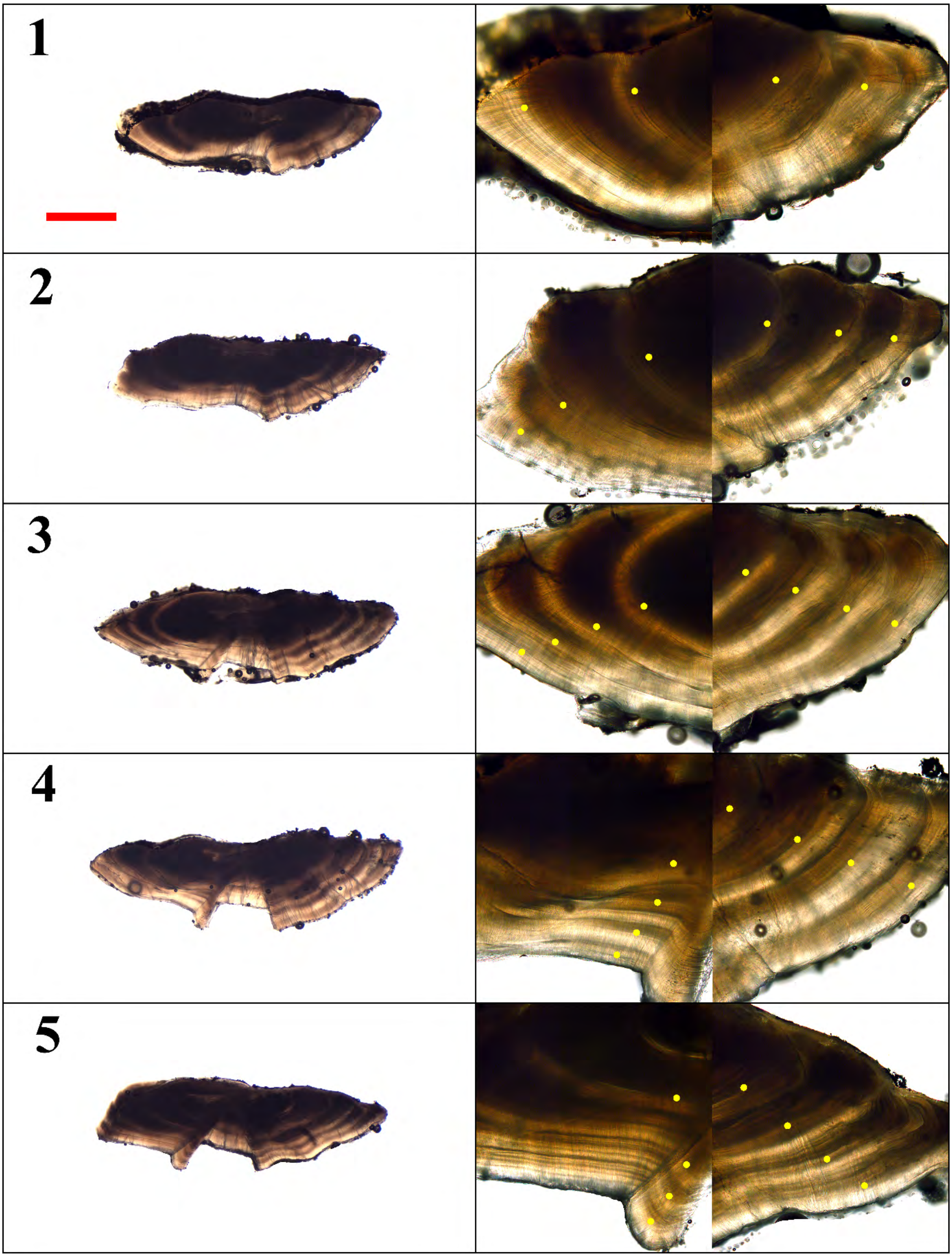


Figure 9: Aged tarakihi otoliths (whole section and enlarged sub-sections) from the reference set for fish lengths up to 30cm: fish#1 March (slide 81-4, 25 cm, agreed reading 2W, agreed age 3); fish#2 Jan (slide 80-4, 26 cm, 3W, 4); fish#3 Aug (slide 84-5, 29 cm, 4N, 4); fish#4 May (slide 45-1, 29 cm, 4N, 4) and fish#5 Jan (slide 40-1, 30 cm, 4W, 5). (*Red scale bar = 500 µm for all whole sections.*)

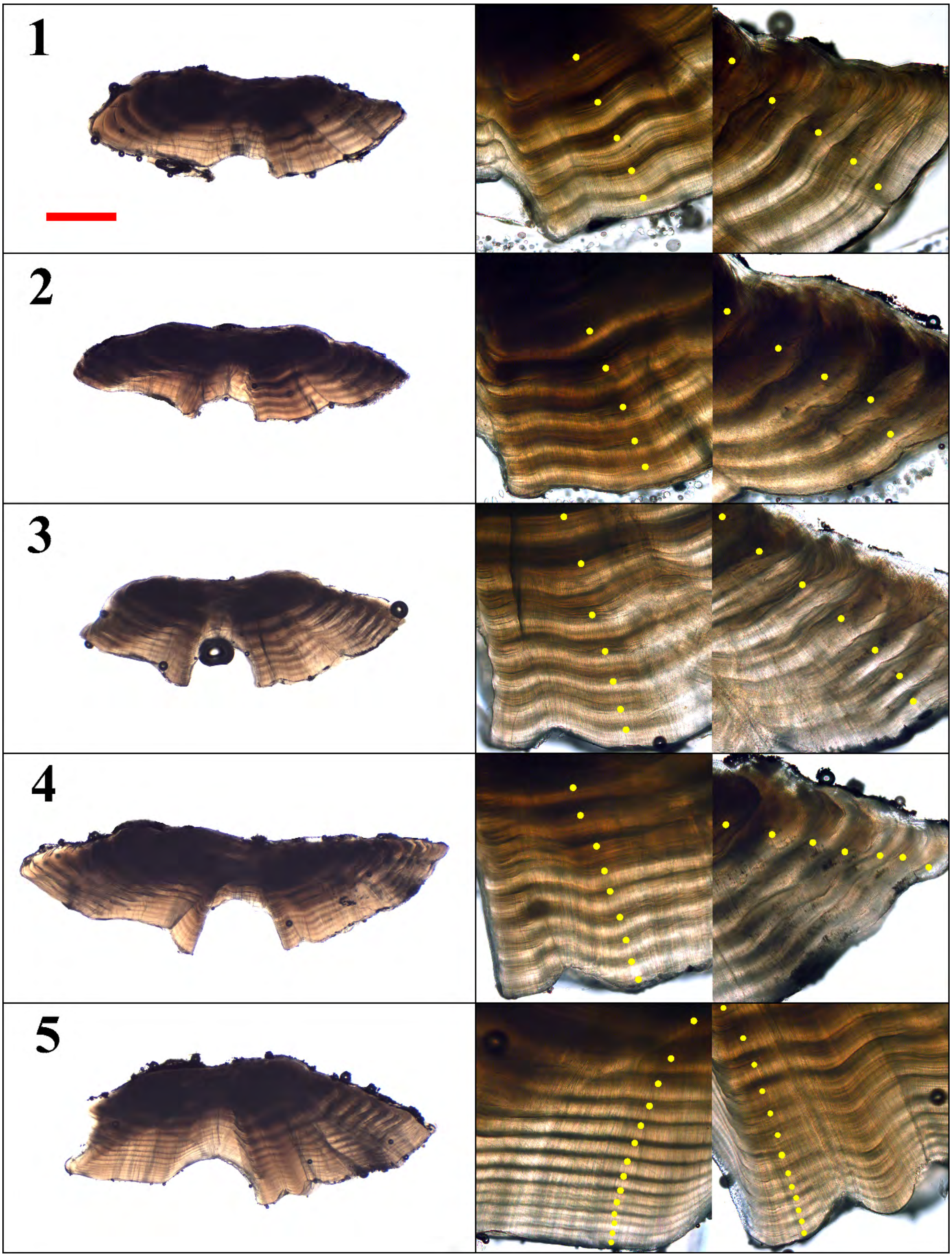


Figure 10: Aged tarakihi otoliths (whole section and enlarged sub-sections) from the reference set for fish lengths 31–40 cm: fish#1 August (slide 84-2, 32 cm, agreed reading 5N, agreed age 5); fish#2 Jan (slide 86-4, 33 cm, 5W, 6); fish#3 Dec (slide 32-3, 35 cm, 7W, 8); fish#4 May (slide 48-5, 37 cm, 9N, 9) and fish#5 Apr (slide 54-1, 39 cm, 14L, 14). (*Red scale bar = 500 µm for all whole sections.*)

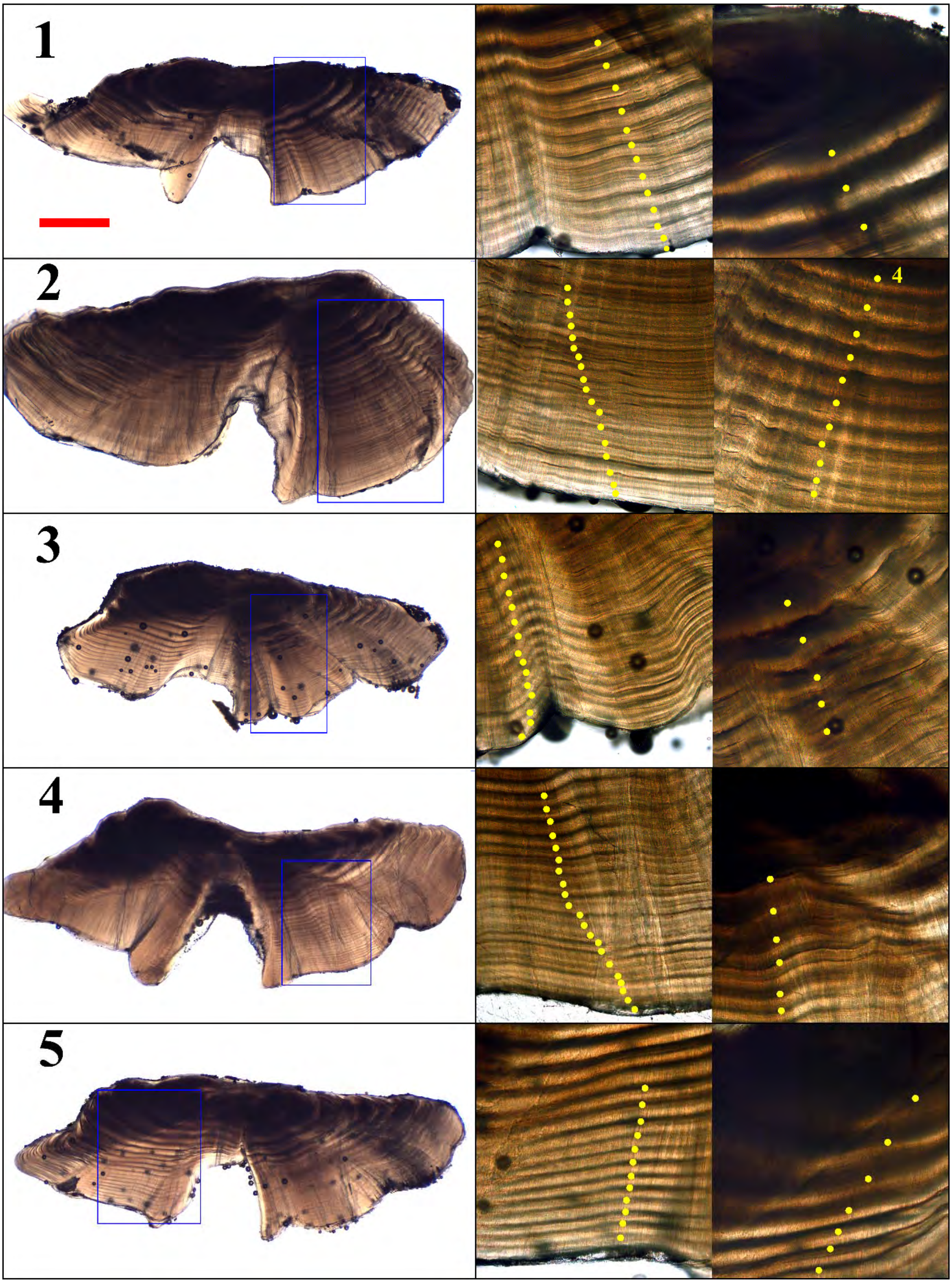


Figure 11: Aged tarakihi otoliths (whole section and enlarged sub-sections) from the reference set for fish lengths at least 41cm: fish#1 March (slide 46-5, 41 cm, agreed reading 16L, agreed age 16); fish#2 Aug (slide 15-2, 42 cm, 32N, 32); fish#3 Jun (slide 67-2, 44 cm, 20N, 20); fish#4 Nov (slide 1-4, 45 cm, 27W, 28) and fish#5 Feb slide 59-2, 49 cm, 19W, 20). (*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 tarakihi age data submitted for loading onto the *age* database.

Species = TAR
Stock = TAR 2 (Central East)
Material = Otolith
Method = 30 (Thin section)
Readers = 109, 113
Project code = TAR2010-02
Sampling period = October 2010 to September 2011

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	20101230	901	1	CEE	TAR	2	1-1	13/12/10	37	2	109	6	12/10/11	113	5	12/10/11	5	W	6	TAR2010-02	
SMP	20101230	901	1	CEE	TAR	3	1-2	13/12/10	36	2	109	5	12/10/11	113	5	12/10/11	5	W	6	TAR2010-02	
SMP	20101230	901	1	CEE	TAR	4	1-3	13/12/10	42	1	109	16	12/10/11	113	17	12/10/11	17	W	18	TAR2010-02	
SMP	20101230	901	1	CEE	TAR	5	1-4	13/12/10	35	2	109	4	12/10/11	113	4	12/10/11	4	W	5	TAR2010-02	

For reference sets, new tables have been developed within the *age* database to include record counts and accepted ages (*t_reference, t_ref_reading*). 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

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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 µm 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.

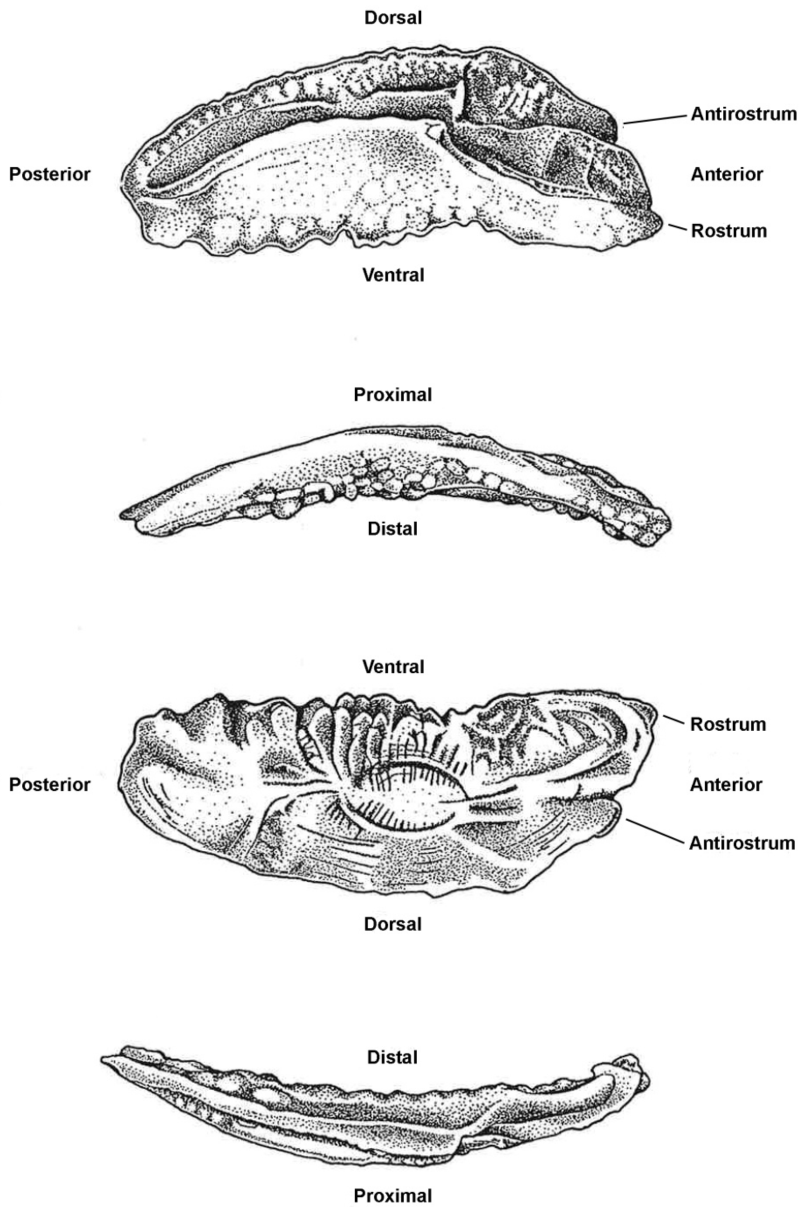


Figure A1.1: Views of a left sagittal otolith from *Arripis trutta* illustrating orientation and basic structure. A) the proximal surface, B) the ventral edge, C) the dorsal edge. (Drawing by Darren Stevens, NIWA).

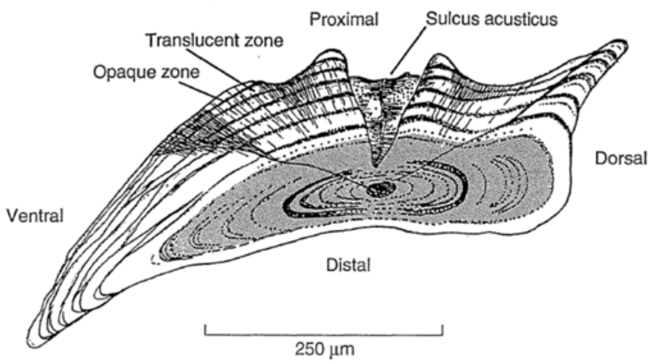


Figure A1.2: Transverse thin section through a sagittal otolith from *Arripis trutta* viewed with transmitted light illumination. The section is taken through the core. (Drawing by Darren Stevens, NIWA).

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).

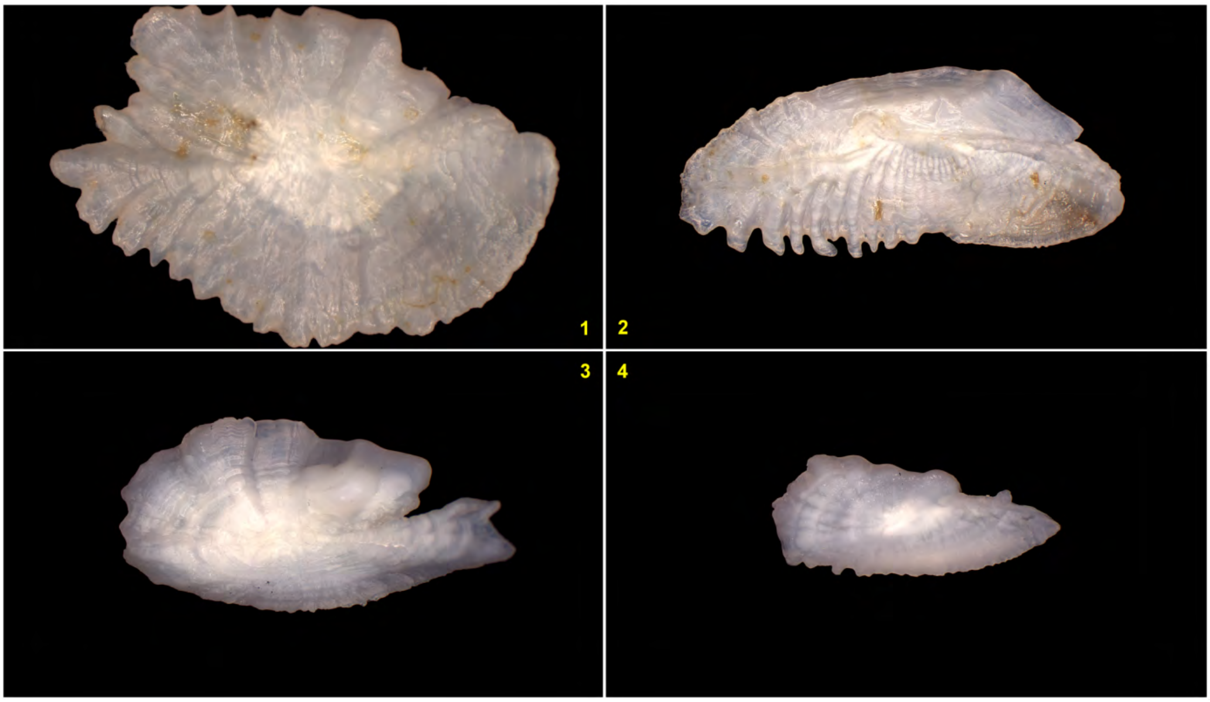


Figure A2.1: Whole right hand side otoliths in lateral view under reflected light at the same magnification demonstrating the differences in otolith size and shape for four important New Zealand inshore species (Image 1, snapper; 2, kahawai; 3, tarakihi; 4, trevally) extracted from fish of equivalent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

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

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

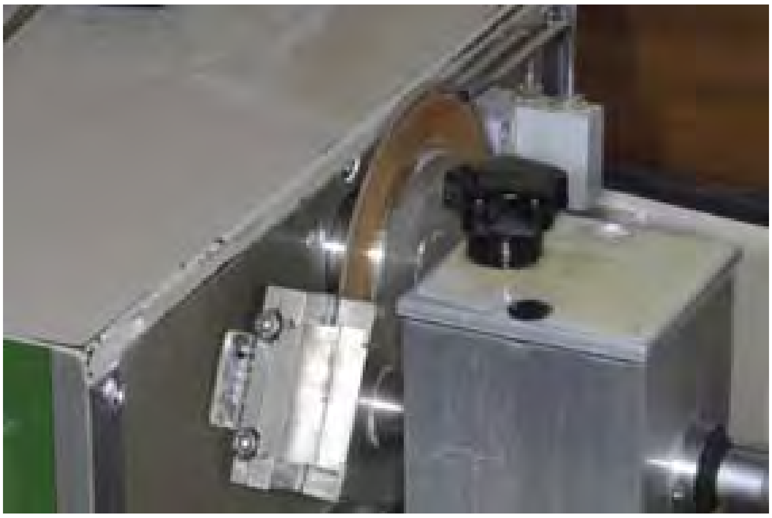
We cut our thin sections on a Struers Accutom-2 high-speed saw, or our new Struers Secotom-10 high-speed saw. The blades are ‘EXTEC’ Diamond wafering blades, part number 12205. They are 102 mm in diameter 0.3 mm thick with a 12.7 mm axle diameter.

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.

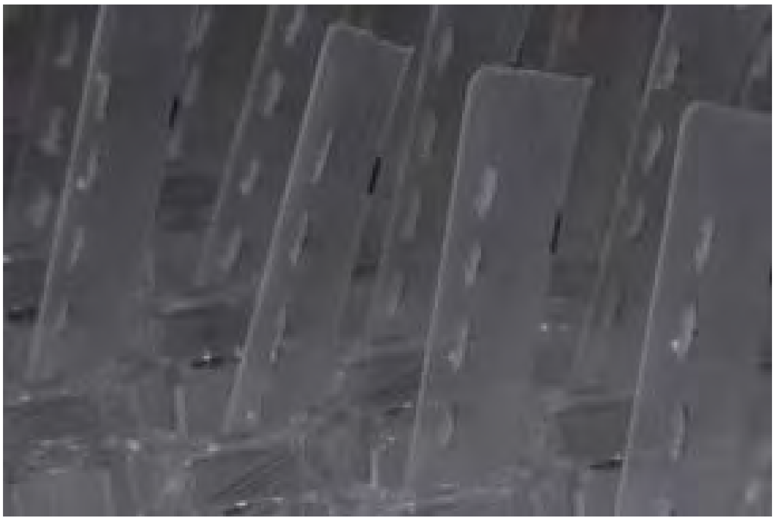
Section blocks at a slow regular speed to ensure even cutting. If one end of the cut wafer is a different thickness to the other end of the cut wafer, slow down the advance speed of the block into the saw, this may produce a more regular section. Utilising clean cutting lubricant should also help to 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: Time series length compositions for tarakihi caught from east coast (1991–2014) and west coast (1992–2015) South Island trawl surveys.

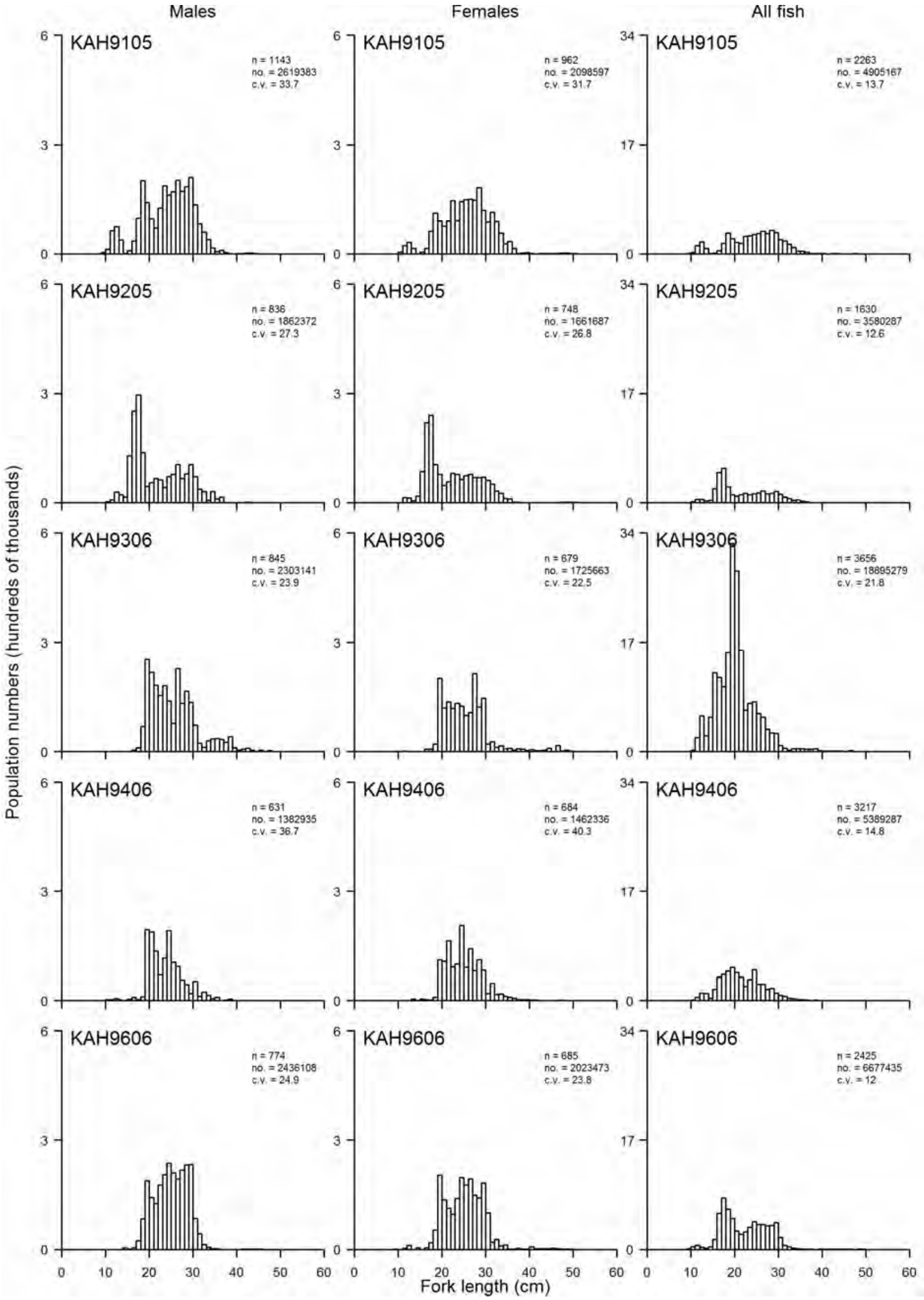


Figure A4.1: Intermittent time series length compositions for tarakihi caught from east coast South Island trawl surveys (April–June 1991–2014). Reproduced from Beentjes et al. (2015).

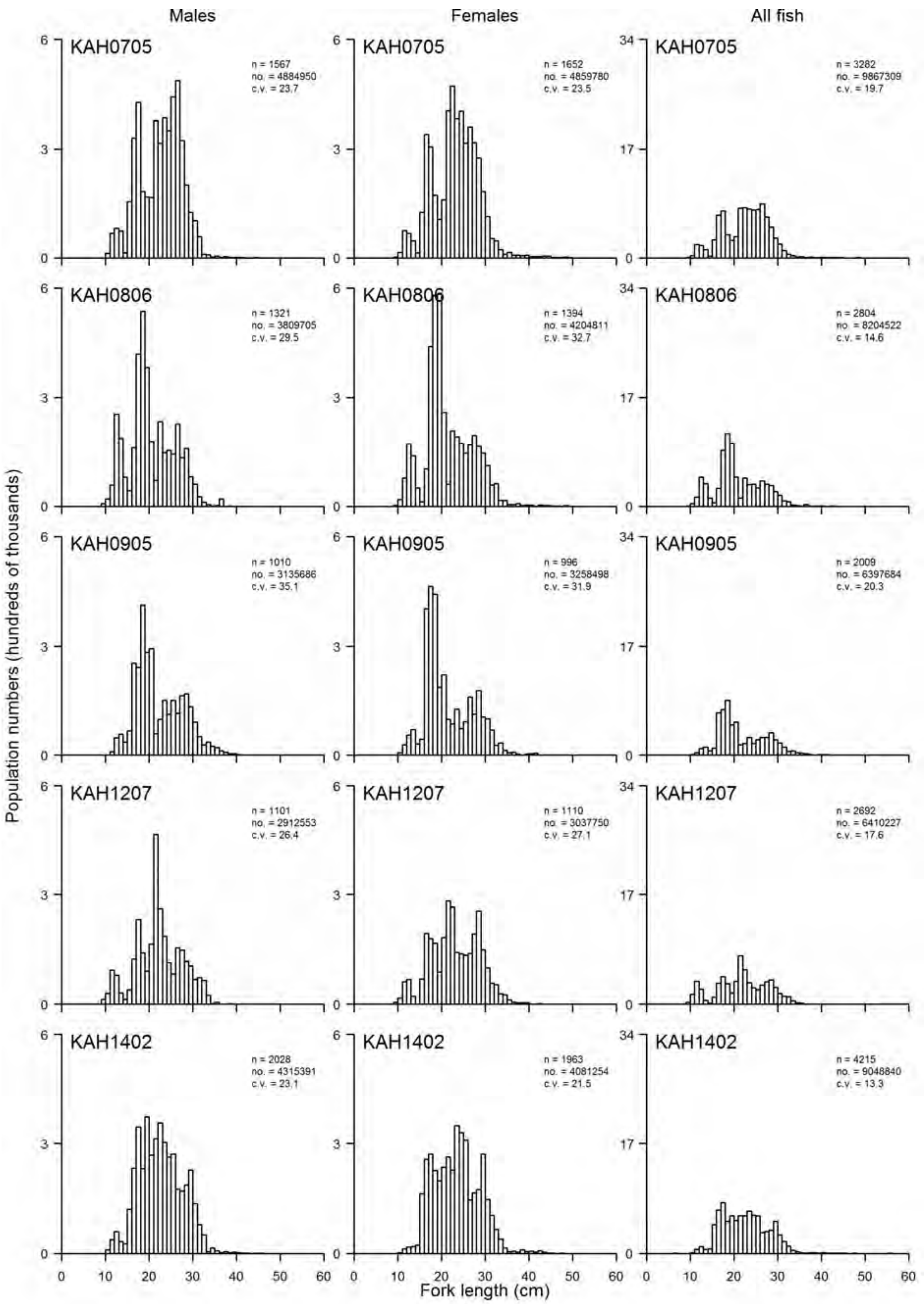


Figure A4.1–continued.

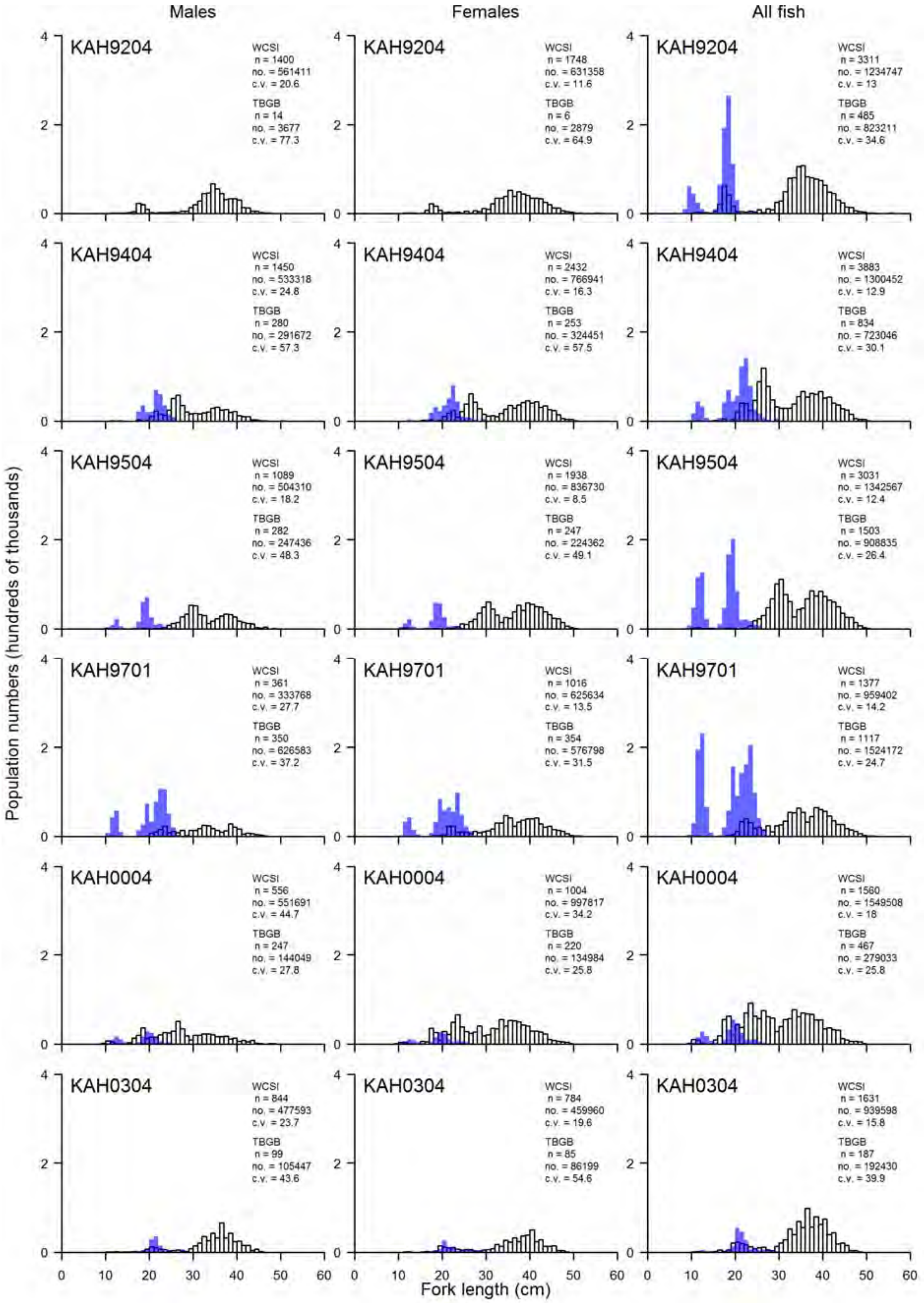


Figure A4.2: Intermittent time series length compositions for tarakihi caught from west coast South Island trawl surveys (March-April 1992–2015). Blue bars = Tasman and Golden Bays, white bars = west coast South Island. Reproduced from Stevenson & MacGibbon (2015).

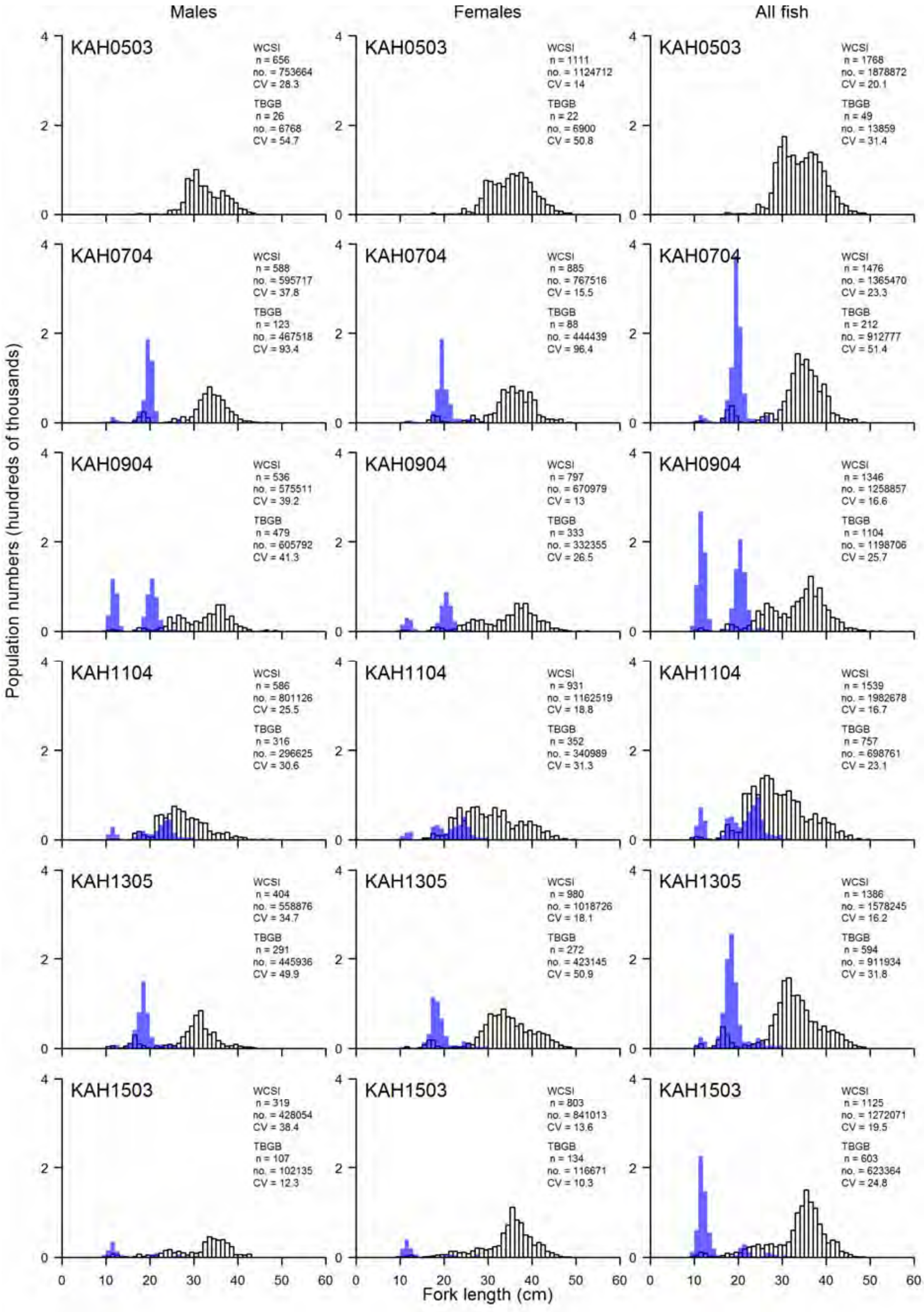


Figure A4.2–continued.

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

Previously reported between-reader agreement and precision estimates (APE) determined from ageing tarakihi in New Zealand are presented in Table A5.1. Although a reasonable level of consistency in reader agreement and precision is apparent in ageing tarakihi, some estimates are low relative to other inshore species that are routinely aged e.g., snapper, kahawai (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 tarakihi in New Zealand (BT = Bottom trawl; RT = Research trawl; SN = Set net; * = a subsample of otoliths used). Note that the data from 2009–10 and 2010–11 in this table are affected by the issue described in paragraph 7 in Section 2.1 and therefore will be under-aged by 1 year.

Stock	Method	Fishing Year	No. of readers	Percent agreement	APE	CV	No. aged	Age range	Publication
TAR 1	BT (at-sea)	2010–11	2	68%	3.06	4.33	~600	2–20+	McKenzie et al. (2015)
TAR 2	BT	2009–10	2	79%	2.68	3.79	736	2–28	Parker & Fu (2011)
		2010–11		85%	1.60	2.27	781	2–26	Beentjes et al. (2012)
TAR 3	SN	2009–10	2	67%	4.38	6.20	345	3–39	Beentjes (2011)
		2010–11		90%	1.09	1.54	353	3–24	Beentjes et al. (2012)
TAR 3	BT	2009–10	2	92%	1.15	1.62	502	2–19	Beentjes (2011)
		2010–11		94%	0.70	1.00	599	2–31	Beentjes et al. (2012)
TAR 7	BT	2004–05	2	~50%	4.57	6.46	200*	2–38	Manning et al. (2008)
	RT	2006–07	2	78%	1.09	2.70	124*	1–40	Stevenson & Horn (2004)

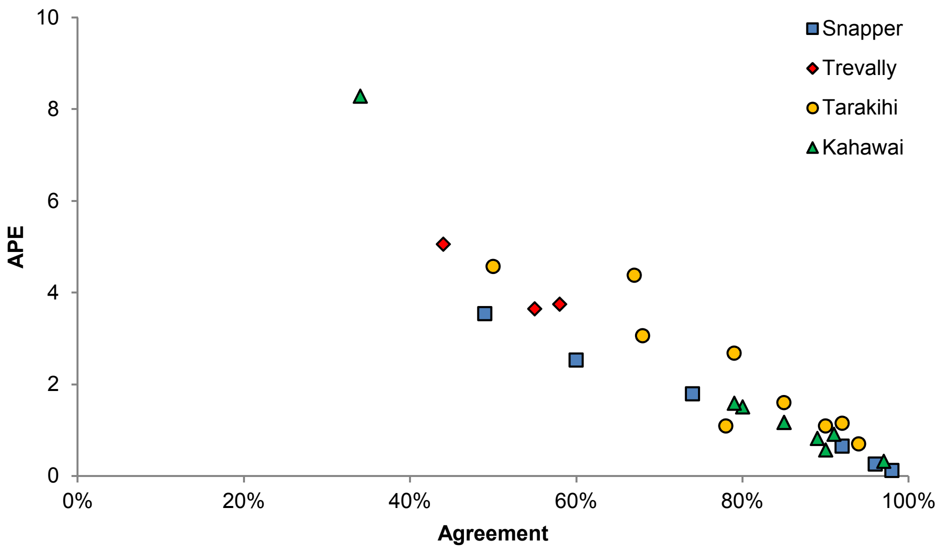


Figure A5.1: Visualised comparison of between-reader agreement and APE scores documented in ageing studies for snapper, trevally, tarakihi and kahawai in New Zealand.

Although percent agreement is considered an inferior method of determining ageing precision compared to APE and 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 tarakihi, 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 tarakihi, 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 tarakihi that demonstrate 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 tarakihi 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.