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Age determination protocol for snapper (*Pagrus auratus*)

New Zealand Fisheries Assessment Report 2014/51

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EXECUTIVE SUMMARY

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This report documents the age determination protocol for snapper (*Pagrus auratus*), 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 that comprehensive ranges 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 snapper. 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” based on the workshop’s results (Ministry of Fisheries Science Group 2011). From this, it was anticipated that age determination protocols would be developed for every species that was routinely aged through MPI funding.

This report describes the age determination protocol for an important New Zealand inshore finfish species: snapper (*Pagrus auratus*). Significant fishstocks (SNA 1, SNA 8) 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, i.e., the sagitta, have been used to age snapper. 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).

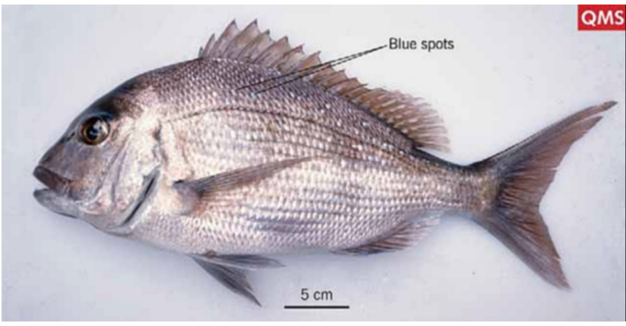
Overall objective

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

Specific objective

1. To develop an age determination protocol for snapper (*Pagrus auratus*), including the compilation of otolith reference collections.

2. AGE DETERMINATION PROTOCOL FOR SNAPPER



2.1 Background

In New Zealand, the first ageing studies for snapper were undertaken during the 1950s (Cassie 1956, Longhurst 1958) using either scales (taken from above the lateral line) and/or length frequency modes. Both methods proved useful in ageing younger fish, although discrepancies in estimating age were relatively common. Age estimates derived from scales were also found to be spatially variable, with west coast snapper more easy to age than east coast fish. Concentric growth rings (annuli) on scales were assumed to be formed annually, and although exhibiting similar patterns, otoliths were overlooked (by Cassie 1956) as they necessitated the killing of fish.

In a comprehensive study on the age, growth and population structure of snapper in the Hauraki Gulf by Paul (1976), both mid-body scales (the more appropriate region for samples), and otoliths (broken, polished and heated, following a modification of the technique described by Christensen (1964)), were examined for age. Both proved useful for ageing juvenile snapper, giving virtually identical results, but for adult snapper, only otoliths with clearly distinguishable alternating opaque (light) and hyaline (dark, and herein referred to as translucent) zones gave ring (herein referred to as zone) counts of the true age. Paul (1976) found scales to be unreliable for ageing fish over 10 years, especially as the marginal annuli were crowded. As a result, ages for older fish in this and previous studies (i.e., Cassie 1956, Longhurst 1958), are likely to have been underestimated. Paul (1976) also determined that scale and otolith annuli (i.e., translucent zones) were formed during winter, becoming visible a few months later only after spring growth resumed.

Subsequent age and growth studies for adult snapper in New Zealand (Vooren & Coombs 1977, Paul & Tarring 1980, Horn 1986, Francis et al. 1992b, McKenzie et al. 1992, Davies et al. 2003, Walsh et al. 2006d, Walsh et al. 2012) and numerous trawl survey and commercial catch sampling programmes to estimate catch-at-age, have focused almost entirely on broken, polished and heated otoliths as the preferred ageing method, colloquially termed and herein referred to as break and burn. Gauldie (1988, 1990) questioned the reliability of otoliths in estimating snapper age using traditional methods, suggesting age to be overestimated. Nevertheless, Francis et al. (1992b) could find little difference in age estimates derived from ageing snapper using the break and burn or thin transverse section (herein referred to as thin section) otolith preparations. Their detailed study validated the annual zone deposition in otoliths from recaptured tagged adult snapper injected with oxytetracycline (OTC). They found that the position of the OTC mark was correctly inferred from age readings in 90% of the 121 samples, concluding that zones are deposited annually. Further support for the ageing methodology was contributed by an analysis of the growth of tagged fish and year-to-year consistency in the relative strength of cohorts.

Francis et al. (1992b) also investigated the accuracy of their ageing, documenting low error rates associated with ring misidentification and edge misinterpretation. They qualified that error rates may differ between readers and for snapper from different areas. Their samples were from SNA 7, known to have the fastest snapper growth rates in New Zealand (Blackwell & Gilbert 2002) and therefore easiest to age (Davies et al. 2003). In a review of methods used to estimate snapper catch-at-age and growth in SNA 1 and SNA 8, Davies et al. (2003) assessed otolith reading precision for New Zealand snapper by carrying out reader comparison tests between each reader and the agreed age, calculating the Average Percentage Error (APE) (Beamish & Fournier 1981) and presenting age-bias plots (Campana et al. 1995). Estimating ageing precision using the mean coefficient of variation (CV) method (Chang 1982) was first used for snapper by Walsh et al. (2012).

The majority of New Zealand snapper age determination has been undertaken on otolith samples collected during the spring and summer months, and the ageing methodology used largely followed that described by Paul (1976) and Francis et al. (1992b). Davies & Walsh (1995) further updated this by documenting a convention for margin interpretation dependent on the position of the outermost ring, with an aim of improving reader zone count agreement when determining ages of snapper in commercial landings. In recent years, emphasis has been placed on sampling commercial snapper fisheries year-round, as opposed to spring–summer, to better reflect the seasonal aspect of the fishery and its fishing operations (Walsh et al. 2006a). This resulted, however, in increased reader error, particularly when ageing fish collected over winter (Walsh et al. 2006b), where some readers had difficulty in correctly interpreting the otolith margin, resulting in one-year age discrepancies. To overcome this, a forced (or fixed) margin (see glossary) was implemented to anticipate the otolith margin type (wide, line, narrow) *a priori* in the month in which the fish was sampled to provide the reader guidance and improve accuracy and precision in age estimations.

Snapper is a relatively long lived species, although ages over 50 years in New Zealand are uncommon. The oldest recorded age determined for a snapper is 65 years, a 67 cm specimen (estimated 5.6 kg) captured from SNA 7 in 2000 (Walsh 2008).

The theoretical birthdate for ageing snapper is 1 January and follows Longhurst (1958) who used scales, and Paul (1976) who used both scales and otoliths. Although snapper are serial spawners, releasing batches of eggs over an extended season during spring and summer (Ministry for Primary Industries 2013), 1 January not only provides a useful birthdate being just after the peak of the spawning period, 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 snapper, and all scientific methodologies described in the following sections will be associated with ageing break and burn sagittal otoliths (Chugunova 1963), currently the preferred preparation method in New Zealand (Appendix 2), and suited to species with large otoliths (Appendix 3). The methodology used for preparing and ageing snapper otoliths was initially described by Paul (1976), and has been further expanded upon by Horn (1986), Francis et al. (1992b) and Davies & Walsh (1995), contributing small but important additions over time. The following sections outline a combination of these and more recent findings.

Although the 'bake and embed' preparation method was trialled in ageing snapper otoliths collected from a Hauraki Gulf trawl survey about 1992 (see Appendix 2), the resulting relative proportion-at-age estimate for 1+ snapper did not match the predicted year class strength determined from a sea surface temperature-recruitment relationship for the 0+ year (Francis 1993). Subsequent re-ageing using the break and burn method (and experienced snapper agers) provided proportions for 1+ snapper that strongly correlated with the expected relative year class strengths of snapper caught during the 1992 survey. Furthermore, the predicted relative year class strengths of recruited year classes for the 1992 age composition and those from the nine other Hauraki Gulf trawl surveys undertaken over consecutive years from 1984 to 1994, strongly correlated with year class strength estimates determined from catch-at-age data sampled from commercial longline landings (Francis et al. 1997). Nevertheless, the bake and embed method provides a robust alternative for long-term storage of archived otoliths, and a comparison with the break and burn method was undertaken as part of this current study to see if the disparities associated with ageing the samples two decades prior (circa 1992) were more related to reader experience than the preparation method alone (see Appendix 2). This trial showed that snapper could be accurately aged with a high degree of confidence using either the break and burn or the bake and embed method.

In Australia, early ageing studies for snapper (often referred to as pink snapper) investigated scales (MacDonald 1982, Jones 1984) but more recently, studies have used thin section otolith preparations that display clear incremental patterns of opaque and translucent zones, counting opaque zones to determine age (McGlennon et al. 2000, McGlennon 2003, Jackson, 2007, Lloyd 2010, Stewart et al. 2010).

2.3 Otolith preparation

Post extraction, snapper otoliths are cleaned of adhering tissue, rinsed in water, dried and stored in paper envelopes labelled with sample details, including trip code and 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 snapper show no differential growth between sexes (Paul 1976). The envelopes are stored in labelled box files relating to the project code, fishstock and year of collection, and archived in the MPI otolith collection at the National Institute of Water and Atmospheric Research (NIWA), Wellington.

One whole left or right otolith (or part thereof if broken from an ikijime spike to the brain, a process used by longline fishermen to kill fish immediately upon capture: see Appendix 2, Figure A2.3) from each envelope is examined under a low power stereomicroscope using transmitted light. The approximate location of the otolith core is marked with a pencil line drawn transversely through this point across the dorso-ventral axis from one edge of the otolith to the other (Figure 1). The otolith is cut slightly to one side of the pencil line using a junior hacksaw on a wooden block, and ground under running water on a Struers LaboPol-21 instrument using 200 grit silicon carbide paper, to a level that bisects the pencil line, with the transverse section remaining perpendicular to the distal surface at all times (Figure 1). Although there is no preference between either the anterior or posterior half of the otolith for preparation, the larger posterior half is chosen more often than not. Polishing of the ground surface is undertaken with 1000 grit silicon carbide paper under running water to remove scored marks left from the grinding stage, and the otolith is then placed into colour-specific plasticine strips annotated with unique sequential numbers (i.e., 1–1000) on a cardboard base, representative of the otolith envelope number in a specific stock or survey collection (Figure 1). The unused portion of the otolith is retained in the envelope and may be prepared subsequently should the initial preparation be considered unsuitable by the reader for deriving an accurate estimate of age.

Before burning the otolith in the flame of a spirit burner, the section surface is wiped clean to remove residual dust particles left over from the grinding stage. Otoliths are held with forceps with the sectioned surface perpendicular to the flame and the proximal (sulcul) side facing downwards, allowing the progression of the burn to be monitored (Figure 1). A distance of approximately 2 mm away from the blue part of the flame ensures that a rich caramel colour is obtained across the entire ground surface without unnecessary over-burning of the otolith. After burning, the otolith is immediately placed into immersion oil to end the burning process and then back into its allotted space in the plasticine strip. Most readers prefer the otolith orientation to be positioned with the dorsal side closest to the reader, to allow viewing through the dorsal lobe to enhance well defined translucent zones that lie three-dimensionally within the otolith. The sectioning and burning stages of otolith preparation for the break and burn method are usually undertaken in batches (i.e., one or more strips) with each plasticine strip comprising 25 otolith preparations in total.

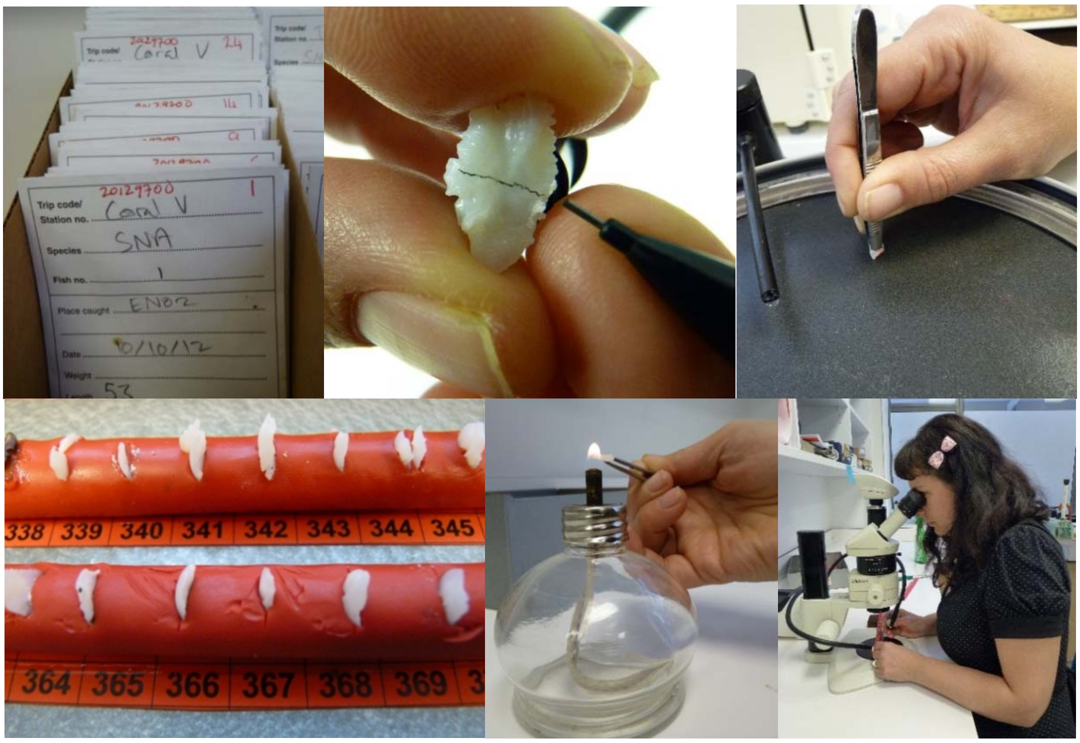


Figure 1: Montage of the various stages in snapper otolith preparation of a transverse break and burn section: Images depict envelope storage, marking the section, grinding and polishing, sequentially numbered otolith samples in plasticine strips, burning the section, ageing.

2.4 Otolith interpretation

Before viewing under a low power stereomicroscope, otoliths are recoated with immersion oil to enhance the series of alternating light and dark zones discernible in the burnt section. Initial viewing may be undertaken at low to medium magnifications (6–16× objectives) with illumination from a reflected cold light source to determine which of the preferred sites on the sectioned surface are the clearest for reading and to identify any visible ambiguities or secondary growth zones such as false checks. However, as snapper are relatively long lived, high magnification (25–50× objectives) is generally recommended for an accurate zone count of an otolith from old fish (i.e., those 10 years of age and older) and for fish from stocks with slow growth rates (i.e., SNA 1), as these are more difficult to age and lead to increased reader error (Davies et al. 2003). Although most snapper ageing is undertaken using reflected light to count zones on the sectioned surface, transmitted side lighting of the otolith, particularly on the dorsal side, is commonly used to enhance annual zones and confirm false checks.

The main assumptions made when interpreting zones in break and burn snapper otoliths are:

1. The dark translucent zone is laid down in winter during a period of slow growth and the wide light opaque zone is laid down throughout the summer period of rapid growth.
2. The theoretical ‘birthday’ for all snapper is 1 January.
3. Translucent zones are counted as being annual.

The first annulus in a sectioned otolith appears as the most obvious dark zone when counting from the core to the otolith margin (Figure 2). The sagittal-subcupular meshwork fibre zone (SMF) located on the ventral side of the otolith has a marked inflection point formed during the first winter (Francis et al. 1992a) and further signifies the position of the first annulus (Figure 2). Less obvious, but also a useful indicator of the annulus position, is the slight indentation present on the otolith’s ventral distal surface. The core to first zone distance is most often greater than that between successive zones, and although no measurements have been documented in adult snapper, they are likely to be unreliable due to significant growth variations both within and between stocks and in off-centre preparations (Figure 3).

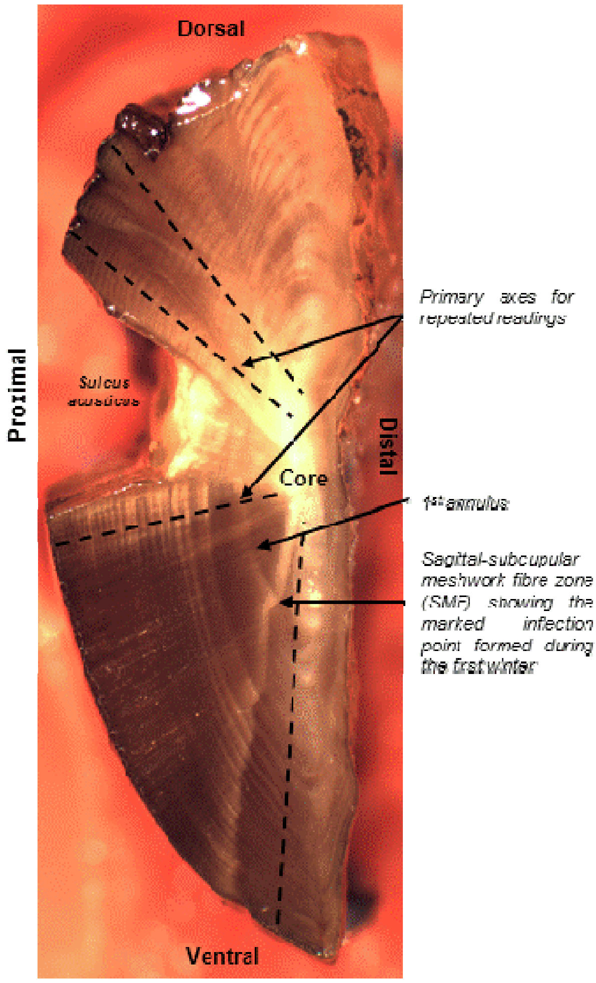


Figure 2: Snapper otolith image of a transverse break and burn section under reflected light illustrating otolith terminology. Counts are made primarily along the axes adjacent to the sulcus, indicated here by broken lines. Two other frequently used counting axes are also shown as broken lines. This otolith section was interpreted as 23 wide.

The identification of non-seasonal secondary zones (i.e., false checks and split rings) can be a major cause of age-reading errors (Panfili et al. 2002). If present, false checks usually lie between the core and the first translucent zone, or the first and second translucent zones, and are most often distinguishable by appearing irregularly spaced and less obvious than the first and second annuli, and usually diminish in strength with transmitted side lighting (Figure 3). Split rings are rare in snapper otoliths, and are usually identifiable by their irregularity in zone width and spacing, and commonly seen toward the ventral tip. On occasions, small dominant concentric juvenile checks close to the core may be present, but these can be disregarded from being annual as they do not match the regularity and spacing associated with annual zone deposition, or the zone size relative to that expected from a fish of the one year old cohort size range (Figure 3).

Translucent zones (or the inside of light opaque zones when ageing either side of the sulcul groove) may be counted from the core to the otolith margin on both ventral and dorsal axes toward the proximal surface (see Figure 4). Otolith readers vary in their preference for the location of zone counts, and many choose to read solely on the ventral side. However, the uniformity of zone deposition is particularly clear along either side of the sulcul groove, and although compressed, usually results in the most accurate counts. Counts made toward the ventral or dorsal tips can also be useful, especially for ageing young snapper, and for confirming counts made along other axes, but for old fish, zone deposition, especially toward the ventral tip, can be irregular and difficult to read accurately (see Figure 3 for examples). Otolith readability depends upon a number of factors, including the age of the fish, growth rate, collection date, clarity of the deposited structure, and the quality of the preparation.

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). Although translucent zones in snapper are laid down during winter, the newly formed zone may not become fully apparent on the otolith margin until late spring (juveniles) or early to mid-summer (adults) when the formation of the next opaque zone has begun (Paul 1976, Horn 1986, Francis et al. 1992b). The visibility of the newly formed translucent zone (annulus formation) in otoliths appears to be age related, similar to that observed for scales (Paul 1976), being apparent earlier in young age classes, and later with increasing fish age. Assuming the 'birthday' of all snapper to be 1 January, then the first translucent zone is deposited after six to nine months of life, with subsequent zones being laid down annually (Horn 1986). Therefore, an otolith with three translucent 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 snapper in relation to each month of the New Zealand fishing year, October–September. The birthdate for snapper 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 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 snapper in New Zealand, this is dependent upon the position of the outermost translucent zone and is as follows: '**W**ide' (a moderate to wide opaque zone present on the margin), October–February; '**L**ine' (translucent zone in the process of being laid down or fully formed on the margin), March; '**N**arrow' (a narrow to moderate opaque zone present on the margin), April–September.

To demonstrate the application of the forced margin to ageing snapper, consider an otolith sampled in January that has three completed translucent zones and a translucent margin. Using the forced margin method (Table 1), the translucent margin is ignored, and the otolith interpreted as 3W (wide referring to a wide opaque margin). When determining age, however, the sampling date and assumed birth date are taken into account to assign an age of 4.00 years. Ignoring the translucent margin, which may be present in January 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, 4.00 year old snapper sampled in January could be assigned ages of either 3 or 4, depending on whether a translucent margin was visible, and deemed to be complete.

Although the timing of newly formed zone deposition is influenced temporally, and may vary slightly between individual fish, stocks and years, readers are able to anticipate the expected temporal change

2.4.1 Examples of break and burn preparations of snapper otoliths for a range of fish size and age illustrating areas of difficulty faced by readers

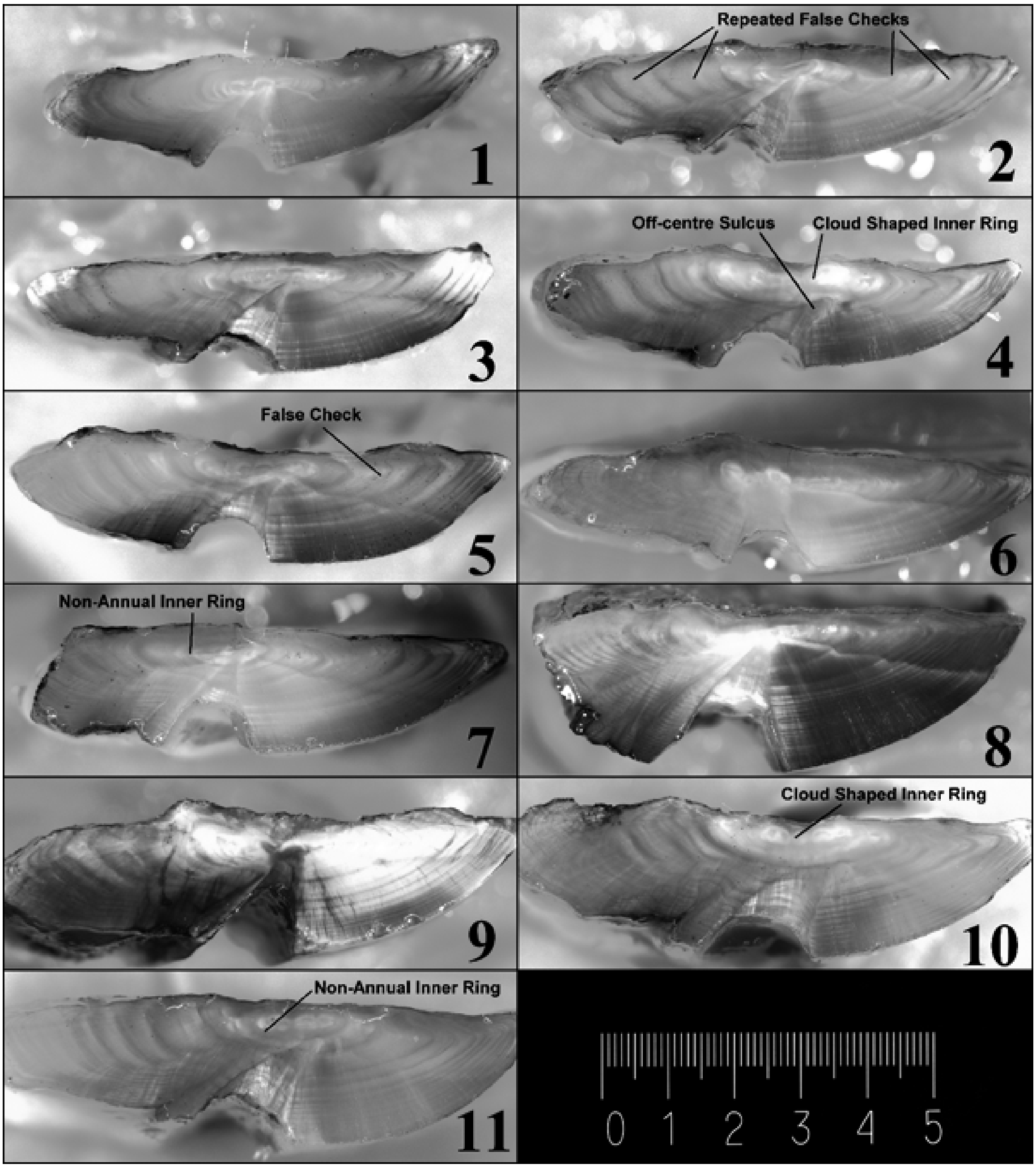


Figure 3: Snapper otolith transverse sections showing variant preparations: fish#1, 28 cm (under-burnt centre, over-burnt dorsal and ventral tips); fish#2, 29 cm (false check); fish#3, 31 cm (false check); fish#4, 33 cm (over-ground); fish#5, 33 cm (false check); fish#6, 34 cm (under-burnt); fish#7, 34 cm (centre ring); fish#8, 38 cm (slow growing); fish#9, 39 cm (over-burnt); fish#10, 41 cm (over-ground) and fish#11, 48 cm (over-ground, under-burnt dorsally). (*Scale in millimetres.*)

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. The otoliths of pre-recruit snapper in particular may pose the most problems for readers because fewer annual zones are present to compare with and initial zone margins are often more diffuse than those formed later in life. Under high magnification, the width of the last opaque zone may appear to the reader to be too large not to have another translucent zone present, and consequently can be misinterpreted and over-aged by one year. The clarity of the otolith margin can also create problems for the reader being affected by over- or under-burning, burnt-on debris, and growth variability in different axes of the otolith (i.e., faster dorsal or ventral growth apparent on proximal or distal surfaces compared to the sulcul region). Obviously, if the time period of the otolith collection is considerable (i.e., 12 months), it is prudent that otolith preparation is undertaken and presented to the reader in the same chronological order in which the otoliths were sampled, making interpretation of the margin much easier, thus reducing the potential for reader 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 state vary. This is especially important when ageing a species with a broad age range, such as snapper, and where samples are collected over an extended time period (i.e., year-round).

No official readability scale has been implemented for ageing snapper in New Zealand, as the growth zones are clear and reader agreement is high.

2.4.2 Examples of break and burn preparations of snapper otoliths with marked translucent zones and agreed reading and age estimates

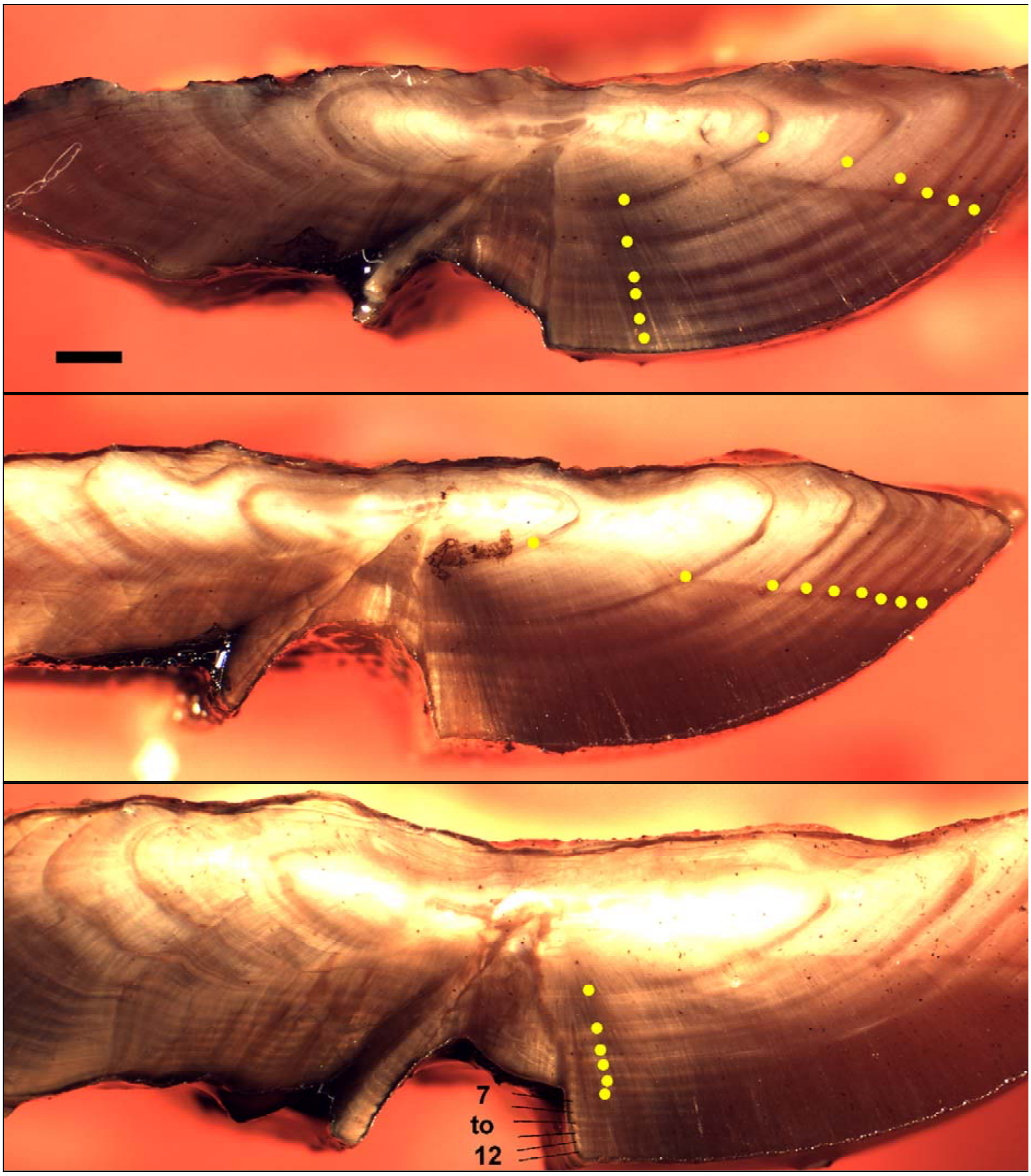


Figure 4: Aged snapper otolith transverse sections showing zone interpretation: Top (48 cm, agreed reading 6W, agreed age 7); Middle (57 cm, agreed reading 9W, agreed age 10); Bottom (62 cm, agreed reading 12W, agreed age 13). (*Scale bar = 500 µm*).

2.5 Ageing procedures

A range of procedures have been used in the past for ageing snapper in New Zealand although documentation relating to number of readers and techniques used to resolve reader disagreements is generally scarce. During the 1960s, otolith collections were aged once by one reader and the age of each fish determined (L. Paul pers comm). In the early 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 snapper. Most often, only a single reader was used for ageing each otolith sample, knowing only the date of collection in order to assign age, and this became the standard for ageing snapper over subsequent years (Francis et al. 1992b). Although thousands of otolith samples were aged during this period, it remains unclear if one or more readers were involved, and, if there were multiple readers, exactly how disagreements were resolved.

In the 1988–89 fishing year, a catch-at-age sampling programme was reinstated in the Auckland Fisheries Management Area sampling landings from both the SNA 1 and SNA 8 commercial fisheries (Davies & Walsh 1995). Otoliths were aged "blind" by three readers, i.e., without prior knowledge of counts obtained by other readers or of the fish length, knowing only the collection date. Where all readers agreed, the age of the fish was determined from their reading. Where two of the three readers agreed, the disagreeing reader reread the otolith to determine the likely source of error. When all readers disagreed, the otolith was reread jointly to reach a consensus, or the otolith discarded from the collection as unreadable. The latter situation was uncommon and usually related to abnormal crystalline otoliths composed of calcite or vaterite (refer Panfili et al. 2002) and estimated to occur in about 1 in every 500 snapper (Figure 5). Since this time, reviewing all reader disagreements has been a fundamental step in determining an accurate estimate of the final agreed age for snapper. The preparation of the second otolith is regularly undertaken to resolve initial reader disagreements which may be affected by poor initial preparation (see Section 2.4.1).

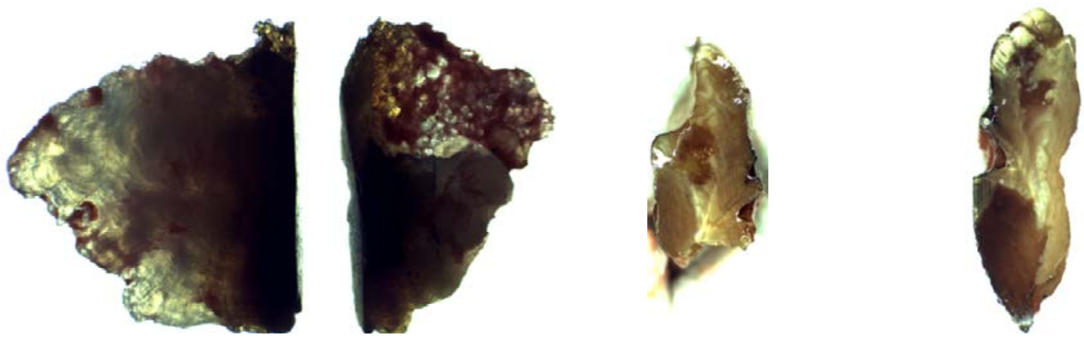


Figure 5: Example of a sectioned vaterite snapper otolith in lateral (left two images) and transverse view (right two images).

With SNA 8 otolith collections based predominantly on faster growing fish that are generally easier to age than those from the SNA 1 fishery, a move to two readers was initiated in 2005–06 with the constraint that at least one reader had substantial experience and expertise in ageing snapper. Based on this and through discussion at a Ministry of Fisheries workshop titled "Guidelines for New Zealand fish age determination protocols" in May 2011, it was agreed that future ageing of snapper, given that the species is reasonably easy to age, will be undertaken with only two readers, which will save on costs. It was stated that the best readers with the most experience should be those ageing the samples. For SNA 2 and SNA 7 collections, only two readers have ever been used. Using two readers and similar ageing techniques to those outlined in this report, Davies & Walsh (2000) demonstrated that considerable improvement in the age estimation of snapper has been achieved in recent years compared to ageing undertaken in the 1970s.

2.6 Estimation of Ageing Precision

Quantifying precision and bias (within- and between-reader) in ageing snapper in New Zealand has been reported on two previous occasions, firstly by Davies et al. (2003) for the SNA 1 substocks (East Northland, Hauraki Gulf, Bay of Plenty) and SNA 8 in 1995–96, and secondly by Walsh et al. (2012) for SNA 2 in 2007–08 and 2008–09 (see Figure 6). As snapper are considered a relatively easy species to age, precision in APE estimates for initial readings was relatively high, ranging from 0.1% for samples from SNA 2, to 3.5% for samples from East Northland (Appendix 4, Table A4.1). Note that these are initial reading precision estimates (Davies et al. 2003), and may not truly reflect the consistency present in final annual catch-at-age estimates (see Figure 7). Although growth within stocks has been shown to vary considerably (Figure 8), those stocks that comprise fast growing snapper with otoliths that have broad growth zones, and/or a high proportion of young individuals (i.e., SNA 2 and SNA 8) are generally easier to age and result in the lowest APE estimates (see Appendix 4, Table A4.1). Those stocks with slow growing snapper having otoliths with narrow growth zones, and/or a high proportion of old individuals (i.e., East Northland and Hauraki Gulf) are often much harder to age and result in the highest APE estimates (see Appendix 4, Table A4.1). An age-bias plot for each reader's initial age estimate compared with the final agreed age has been found to be particularly useful for graphical comparisons between readers, determining individual reader APE and highlighting where error may vary with fish age (see Figure 6).

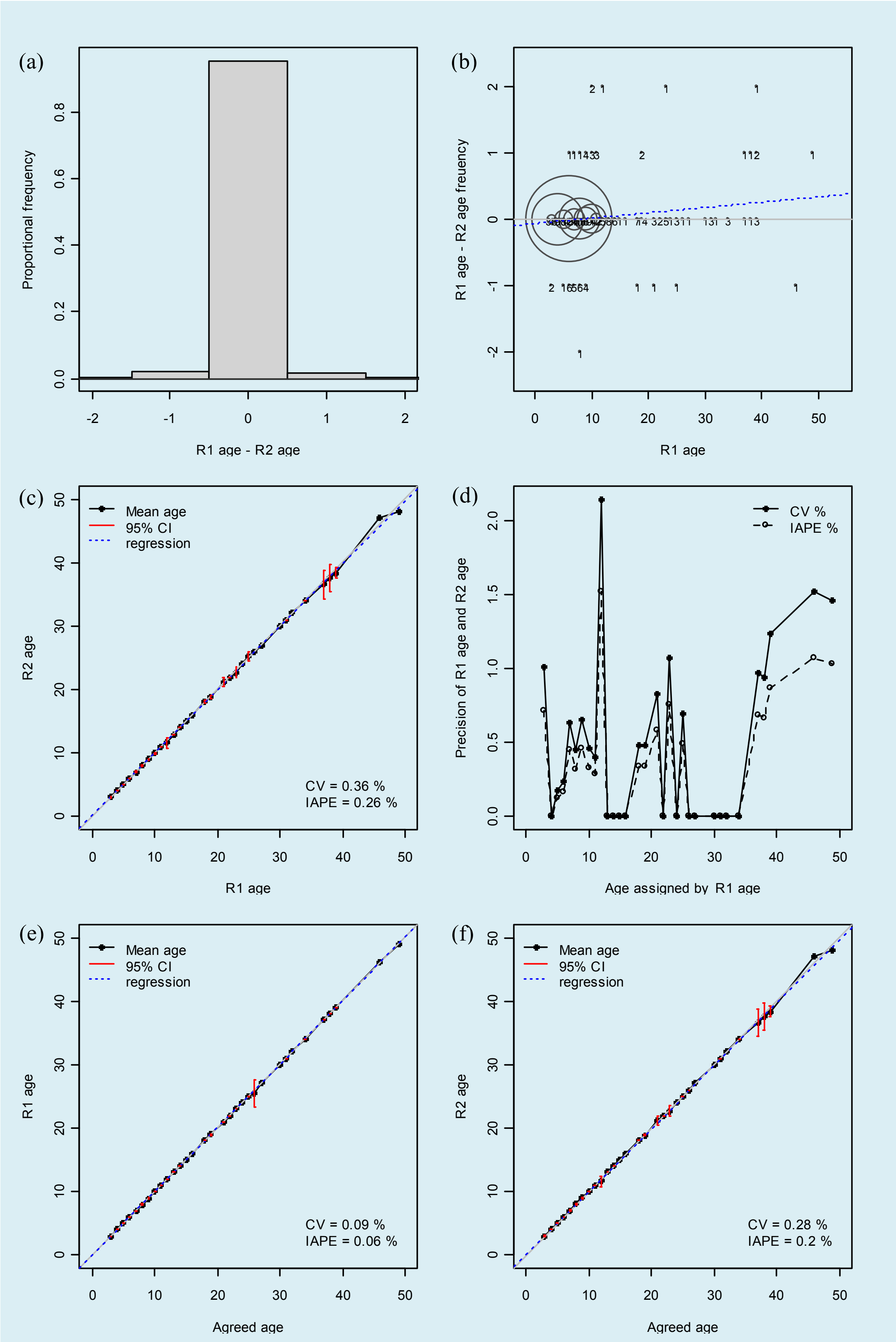


Figure 6: Results of a between-reader comparison test (reader 1 and 2) for SNA 2 otoliths collected in 2008–09 (*n* = 1250): (a) histogram of differences between readings for the same otolith; (b) bias plot between readers; (c) differences between readers for a given age assigned by reader 1 (d) CV and 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. (2012).

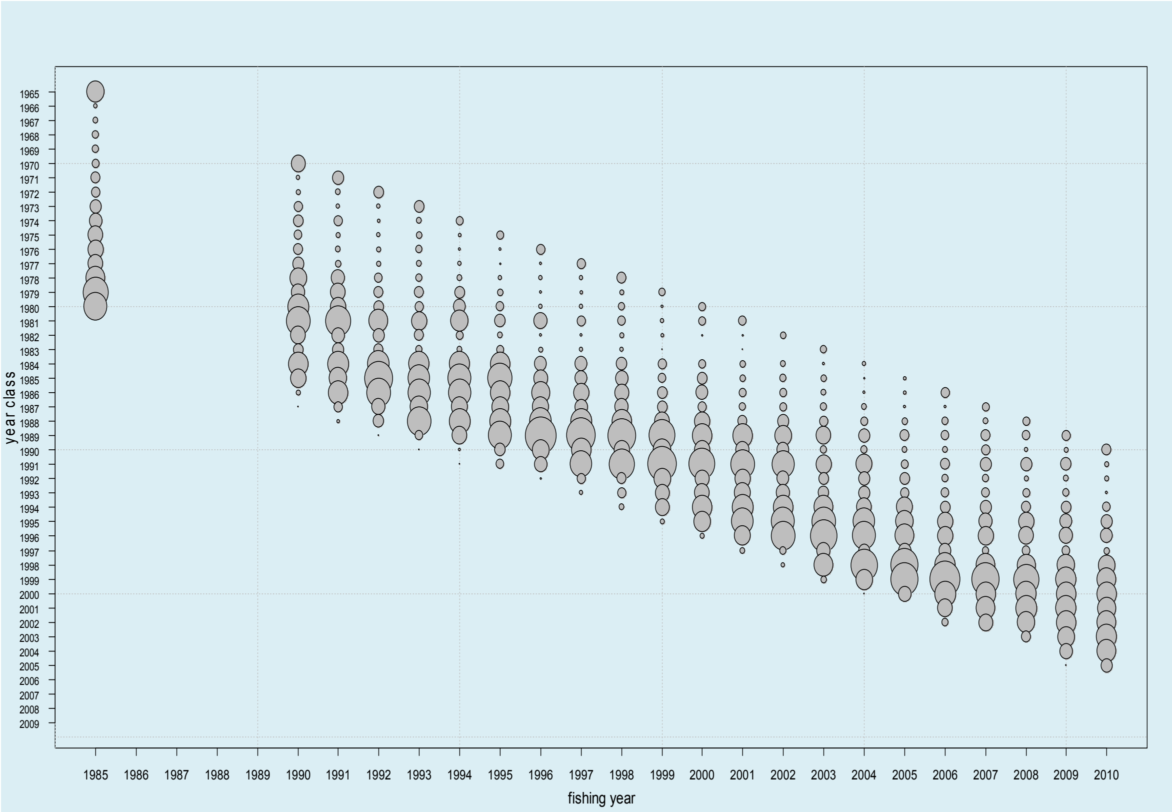


Figure 7: Age frequency distributions by year class and year from the Hauraki Gulf longline spring-summer fishery from 1984–85 to 2009–10. Symbol area is proportional to the proportion-at-age. The proportion of the oldest year class in each year is represented by an aggregate (over 19) age group. Reproduced from Walsh et al. (2011).

Figure 8: Comparison of the high variability in growth rates present within a year class for snapper captured from the SNA 1 bottom longline fishery. These 28 and 58 cm (0.49 and 3.76 kg) Hauraki Gulf snapper represented the broad size range of the 1994 year class (16 years of age) in 2009–10. Reproduced from Walsh et al. (2011).

2.7 Reference collection

As snapper is a long-lived species, a reference collection numbering 500 otolith preparations is believed to be necessary 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 to test for long-term drift, as well as consistency among age readers (Campana 2001). The snapper reference collection assembled in this study was selected from more than 10 000 otolith samples (archived at NIWA Wellington) collected from the SNA 1 and SNA 8 commercial fisheries over the 2007–08 to 2009–10 fishing years. The reference otoliths were chosen roughly randomly to ensure that the full seasonal distribution of the SNA 1 and SNA 8 fishstock samples, and all length and age ranges were well represented (Figure 9), and that neither year- or region-specific anomalies were given undue weight. A minor deviation from the roughly random selection procedure ensured that fish from particularly abundant year classes in the respective fisheries were not over represented in the reference collection. Despite the considerable growth variation that exists within and between the SNA 1 and SNA 8 stocks (Davies et al. 2003, Walsh et al. 2006a,c,d), it was agreed that as snapper are a moderately easy species to age, the collation of stock-specific reference collections was unnecessary. Comparable examples of break and burn snapper otolith preparations to those of the reference collection for a range of fish size and age, preparation quality, and readability are presented in Section 2.4.1.

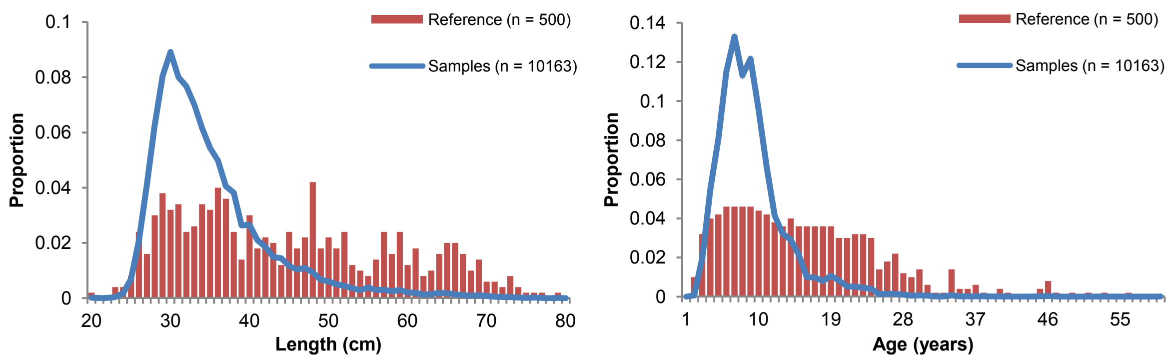


Figure 9: Length and age proportions (lines) of snapper sampled for otoliths from the SNA 1 and SNA 8 commercial fisheries from 2007–08 to 2009–10 with a comparison of the selected subsample chosen for the reference set (histograms).

The agreed ages for otoliths selected for the reference set already exist on the *age* database (also 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 high level of confidence, despite some preparations being less than optimal in quality and clarity. The reference set may also be used for training new readers as well as monitoring their progress as they gain experience in ageing.

Storage of the reference collection of break and burn otoliths may not be as ideal as that of other preparation methods (i.e., bake and embed, thin section), and may decay over time (Chilton & Beamish 1982). A comparison of the utility of break and burn otolith preparations compared to bake and embed has been undertaken within this study (see Appendix 2).

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

Species = SNA
Stock = SNA 8 (West Coast North Island)
Material = Otolith
Method = 22 (Break and burn)
Readers = 22, 113
Project code = SNA2012-02
Sampling period = October 2012 to February 2013

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	20129001	902	1	WCNI	SNA	1	1	23/10/12	27	4	22	2	25/10/12	113	2	28/10/12	2	W	3	SNA2012-02	
SMP	20129001	902	1	WCNI	SNA	2	2	23/10/12	25	4	22	2	25/10/12	113	2	28/10/12	2	W	3	SNA2012-02	
SMP	20129001	902	1	WCNI	SNA	3	3	23/10/12	47	4	22	6	25/10/12	113	6	28/10/12	6	W	7	SNA2012-02	
SMP	20129001	902	1	WCNI	SNA	4	4	23/10/12	43	4	22	5	25/10/12	113	5	28/10/12	5	W	6	SNA2012-02	

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) and McMillan et al. (2011). We thank Peter Marriott for reviewing this document.

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APPENDIX 1: Glossary of otolith terminology and ageing definitions.

Reprinted from the MPI "Guidelines for the development of fish age determination protocols". These were based on Kalish et al. (1995) "Glossary for otolith studies", with modifications and addition of items including definitions for "fishing year age class" and "forced margin" to describe New Zealand practice.

Accuracy – the closeness of a measured or computed value to its true value.
Age estimation, age determination – these terms are preferred when discussing the process of assigning ages to fish. The term ageing should not be used as it refers to time-related processes and the alteration of an organism's composition, structure, and function over time. The term age estimation is preferred.
Age group – the cohort of fish that have a given age (e.g., the 5 year old age group). The term is not synonymous with year class or day class.
Age class – same as age group, but see "Fishing year age class".
Annulus (pl. annuli) – one of a series of concentric zones on a structure that may be interpreted in terms of age. The annulus is defined as either a continuous translucent or opaque zone that can be seen along the entire structure or as a ridge or a groove in or on the structure. In some cases, an annulus may not be continuous nor obviously concentric. The optical appearance of these marks depends on the otolith structure and the species and should be defined in terms of specific characteristics on the structure. This term has traditionally been used to designate year marks even though the term is derived from the Latin "anus" meaning ring, not from "annus", which means year. The variations in microstructure that make an annulus a distinctive region of an otolith are not well understood.
Antirostrum – anterior and dorsal projection of the sagitta. Generally shorter than the rostrum (see Figure A1.1).
Asteriscus (pl. asteriscii) – one of three otolith pairs found in the membranous labyrinth of osteichthyan fishes.
Bias – The systematic over- or underestimation of age.
Birth date – A nominal date at which age class increases, generally based on spawning season.
Check – a discontinuity (e.g., a stress induced mark) in a zone, or in a pattern of opaque and translucent zones, sometimes referred to as a false check.
Cohort – group of fish of a similar age that were spawned during the same time interval. Used with both age group, year class and day class.
Core – the area or areas surrounding one or more primordia and bounded by the first prominent D-zone. Some fishes (e.g., salmonids) possess multiple primordial and multiple cores.
Corroboration – a measure of the consistency or repeatability of an age determination method. For example, if two different readers agree on the number of zones present in a hard part, or if two different age estimation structures are interpreted as having the same number of zones, corroboration (but not validation) has been accomplished. The term verification has been used in a similar sense; however, the term corroboration is preferred as verification implies that the age estimates were confirmed as true.
D-zone – that portion of a microincrement that appears dark when viewed with transmitted light, and appears as a depressed region when acid-etched and viewed with a scanning electron microscope. This component of a microincrement contains a greater amount of organic matrix and a lesser amount of calcium carbonate than the L-zone. Referred to as discontinuous zone in earlier works on daily increments; D-zone is the preferred term. See L-zone.
Daily increment – an increment formed over a 24 hour period. In its general form, a daily increment consists of a D-zone and an L-zone. The term is synonymous with "daily growth increment" and "daily ring". The term daily ring is misleading and inaccurate and should not be used. The term daily increment is preferred. See increment.
Drift – Shift with time in the interpretation of otolith macrostructure for the purposes of age determination.

Forced margin or fixed margin – Otolith margin description (Line, Narrow, Medium, Wide) is determined according to the margin type anticipated *a priori* for the season/month in which the fish was sampled. The otolith is then interpreted and age determined based on the forced margin. The forced margin method is usually used in situations where fish are sampled throughout the year and otolith readers have difficulty correctly interpreting otolith margins.

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

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

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

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

L-zone – that portion of a microincrement that appears <u>light</u> when viewed with transmitted light, and appears as an <u>elevated</u> 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.

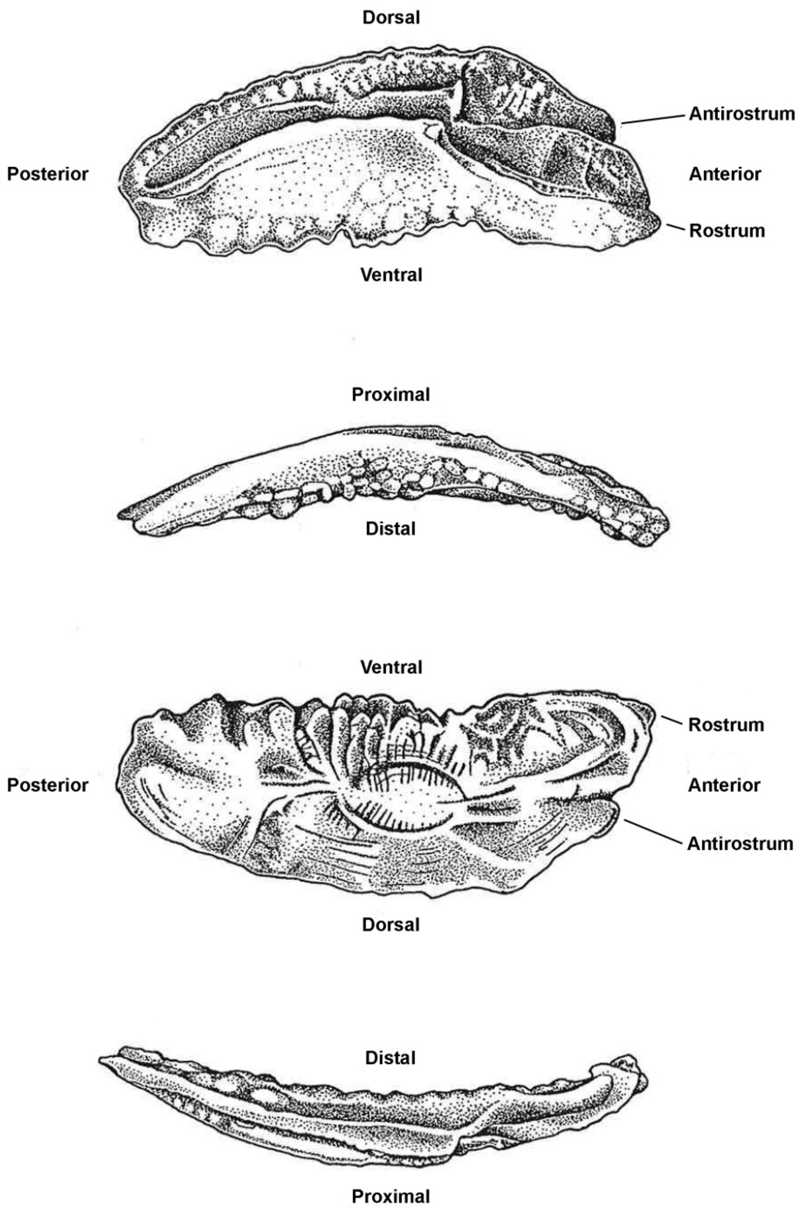

Figure A1.1: Views of a left sagittal otolith from *Arripis trutta* illustrating orientation and basic structure. A) the proximal surface, B) the ventral edge, C) the dorsal edge. (Drawing by Darren Stevens, NIWA).

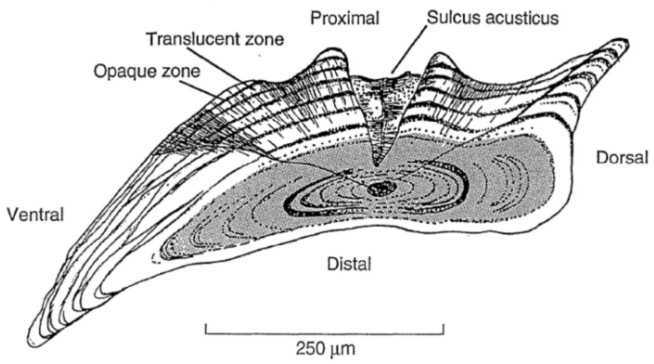

Figure A1.2: Transverse thin section through a sagittal otolith from *Arripis trutta* viewed with transmitted light illumination. The section is taken through the core. (Drawing by Darren Stevens, NIWA).

APPENDIX 2: Comparison between two preparation methods for ageing snapper otoliths: break and burn, and bake and embed.

Background

The break and burn technique (Chugunova 1963, Figure A2.1) has been the main preparation method used for ageing snapper otoliths in New Zealand since the late 1960s and early 1970s, a period when catch-at-age sampling of commercial fisheries (West 1978) and population studies on snapper stocks (Paul 1976, Vooren & Coombs 1977) were first initiated.

Preparation method comparisons in 1992

Around 1992, an alternative preparation method, bake and embed (Figure A2.1), was used for the first time to determine the catch-at-age of snapper from a Hauraki Gulf trawl survey, although it was reported to be commonly used on a number of other commercial species (Stevens & Kalish 1998). Essentially, otoliths are baked whole in an oven at 280 ºC for about 5 minutes until amber coloured, and then batch-processed and embedded in resin blocks such that a single saw cut sections multiple otoliths. This method also produces a robust and permanent otolith preparation that can be easily labelled and stored, as opposed to that for break and burn.

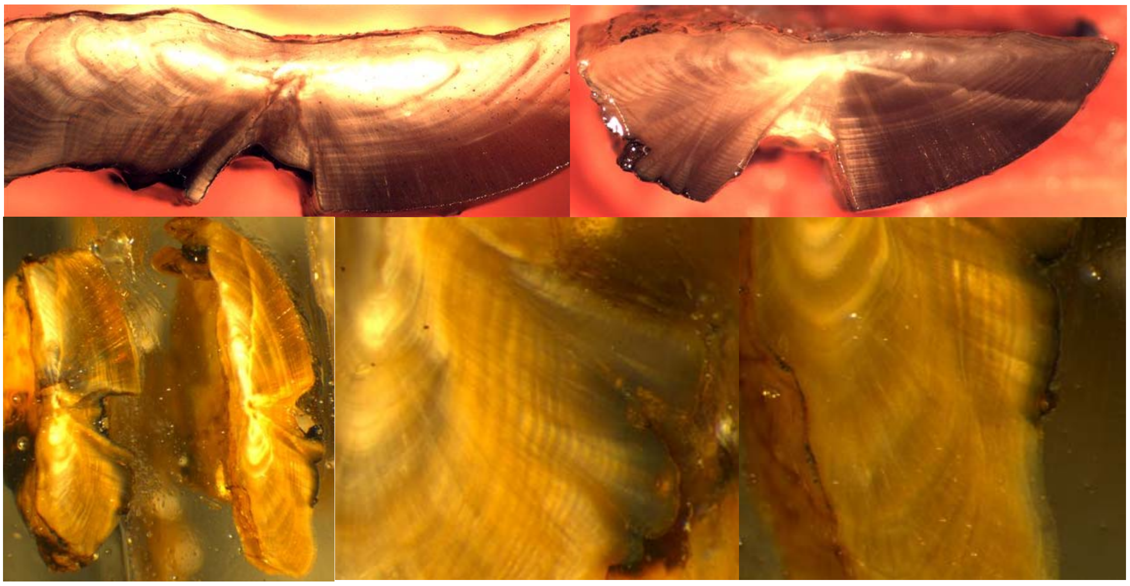

Figure A2.1: Examples of snapper otoliths prepared using the break and burn (top two images), and bake and embed (bottom three images) methods.

During the late 1980s and early 1990s trawl surveys were regularly conducted in the Hauraki Gulf to estimate the year class strength of 1+ snapper in order to develop a recruitment prediction model, where variation in year class strength was found to be positively correlated to sea surface temperature (Francis 1993). However, the 1992 catch-at-age proportion for 1+ snapper using the bake and embed method did not match the predicted year class strength estimated from the mean sea surface temperature during February–June of the 0+ year, nor did other year classes in the sample of known relative strength correlate well with estimates previously determined.

To determine whether the preparation method or reader error were the cause for the marked and unanticipated differences in the resulting proportion-at-age estimates, the entire sample collection was re-aged by experienced snapper readers using the second otolith (of the pair) and the break and burn preparation method. The resulting proportion-at-age for 1+ snapper correlated well to the predicted year class strength expected from the sea surface temperature–recruitment relationship model. Furthermore, other year classes of known relative strength determined in previous surveys closely matched the relative proportions of samples aged in 1992. An ageing workshop was conducted with expert snapper agers present to determine the reasons for the difference between the preparation methods. Although reader error and experience were likely contributing factors to the differing results, it was determined (in 1992) that aside from the fact that the bake and embed method allows for a robust and permanent long-term storage of snapper otoliths, most other factors favoured the break and burn method for ageing snapper otoliths. These were:

1. The burning and baking processes appear to result in different levels of darkening of the otolith. It is believed that there is a finite level to which the baking process can darken the otolith structure, as opposed to burning, which under intense heat produces higher resolution and definition of zones on the sectioned surface. As a result, translucent zones in particular, which are counted when ageing snapper, appear darker and more obvious in break and burn preparations.
2. The otolith margin is considerably clearer in break and burn preparations, and particularly transparent in bake and embed preparations, meaning that zones close to the margin cannot be easily identified, therefore determining an accurate estimate of age more difficult, especially in older fish (This problem was also apparent when ageing bake and embed red gurnard otoliths in 2011).
3. The technique of using transmitted light shone sideways through the otolith preparation to highlight zones is more advantageous for singularly aligned break and burn otoliths. Light appears to be more diffused by the resin block and the number of otoliths present within a bake and embed preparation, therefore reducing translucent zone recognition.
4. As live snapper taken in the SNA 1 bottom longline fishery are “iki”-ed (pithed) upon capture, a proportion of these fish may not provide a complete pair of whole otoliths, instead having a single whole and a partial otolith, or two partial otoliths (see Figures A2.2 and A2.3). This effectively would make preparation more difficult using the bake and embed method in terms of accurate alignment and sectioning as the otolith core, or part thereof, is often incomplete. It is highly probable that partial otoliths prepared using the bake and embed method would increase the imprecision associated with ageing snapper. As the break and burn method involves the individual manipulation of each otolith, small partial pieces of otoliths are nevertheless able to be visually aligned along the correct axis through the core, or part thereof, and accurately ground, polished and burnt accordingly to provide the reader with a preparation almost as readable as that when prepared using whole otoliths.
5. When a break and burn preparation is considered unreadable due to aspects that may be unclear (i.e., first annulus or margin, or just poorly prepared), subsequent preparations may easily be undertaken by the reader using the second otolith of the pair, or parts thereof. The bake and embed method does not allow for additional otolith preparations.
6. Most importantly, reader accuracy in ageing snapper was initially found to be considerably higher in break and burn preparations than bake and embed preparations, although the level to which reader experience may have influenced this result was not determined.

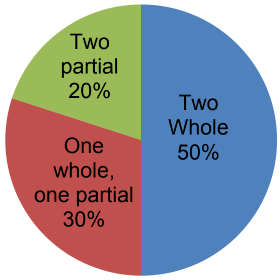

Figure A2.2: Unweighted percentage of snapper sampled from the SNA 1 bottom longline fishery in 2012–13 (sample from 23 different vessels, 567 otoliths) that have otolith pairs in three possible states (whole, whole and partial, partial) due to the ikijime process used by fishermen to kill fish immediately upon capture.

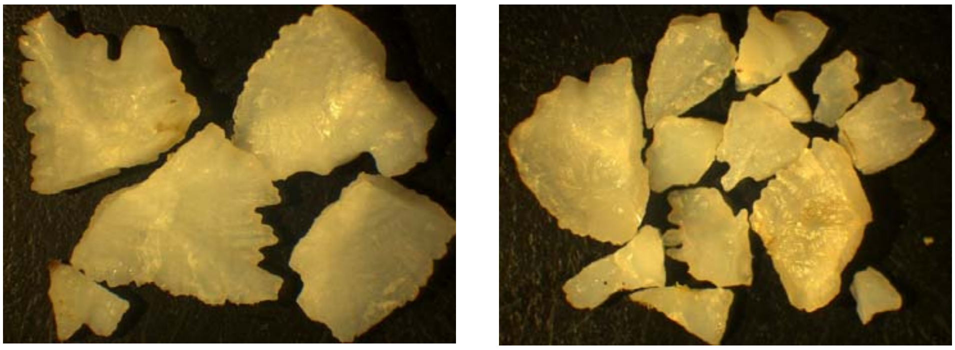

Figure A2.3: Two examples of partial snapper otoliths broken through the ikijime process.

Preparation method comparisons in 2013

A new comparison of the break and burn and bake and embed preparation methods to age snapper is presented here to enable consideration of which method should be used for future ageing. A random subsample of 50 break and burn otoliths was selected from the semi-random set of 500 otolith preparations making up the reference collection (see Section 2.7), and a set of 'sister' otoliths from the selected subsample were prepared using the bake and embed method. The otolith sample was selected from a broad range of fish size and age (see Figure A2.4). Bake and embed samples were batch-processed with 20 otoliths embedded into each resin block, sectioned into six individual blocks, each comprising four otoliths (i.e., 2 deep × 2 wide). A total of 49 preparations (one otolith was vateritic and unable to be accurately aged) were successfully aged by two experienced otolith readers to determine if any obvious differences exist in the age determination of snapper using either of the two preparation methods described.

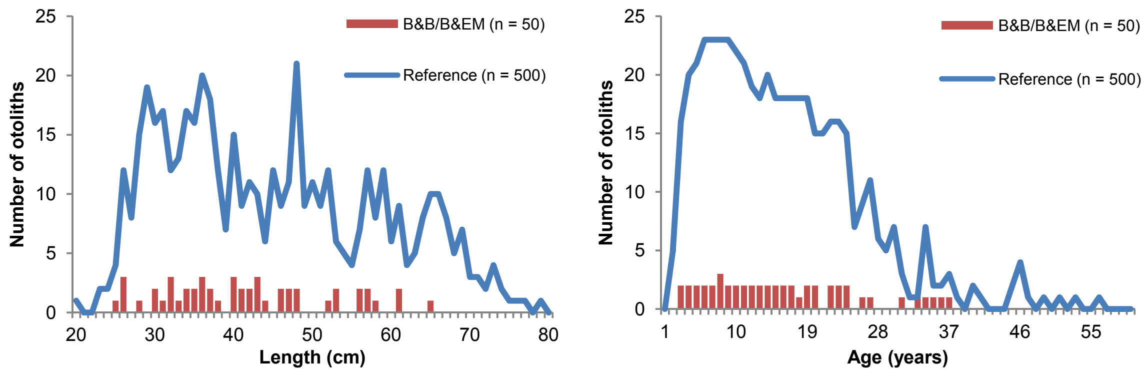

Figure A2.4: Length and age frequencies of snapper comprising the semi-random reference set (*n* = 500) and the subsample of otoliths chosen (*n* = 50) for the comparison of two ageing methods, break and burn, and, bake and embed.

Reader 1 had eight years experience specialising in ageing snapper break and burn otoliths (as well as a number of other inshore species). Reader 2 had more than 25 years experience in ageing a range of marine fish species from inshore, middle depth, and deep water environments, primarily using the bake and embed methodology, but limited snapper ageing experience. Reader 2 had not read snapper otoliths since the late 1980s, and did not familiarise himself with literature on how they should be read or how the juvenile area should be interpreted, but aimed simply to interpret consistently between the two preparation methods. Reader comparisons were determined for each reader and preparation method (Figures A2.5 and A2.6). Although only marginal within-reader differences occurred in the ageing of snapper otoliths using the break and burn or bake and embed preparation methods, the between-reader differences were more obvious, with reader 1 consistently having a higher level of agreement (82–86%) with the predetermined agreed age than reader 2 (61–63%). Reader 1 attained the highest agreement and precision overall when ageing break and burn otoliths (86%), but still produced 82% agreement with bake and embed otoliths. Reader 2 did marginally better when ageing the bake and embed otoliths (63%) than he did with break and burn (61%). The symmetry of the disagreements was better for reader 1 than reader 2, with reader 2 demonstrating a slight bias to produce higher ages for otoliths prepared with the bake and embed method. Ageing error for reader 1 was limited mainly to older snapper, particularly those 20 or more years of age, whereas the error for reader 2 encompassed a broader range of age classes.

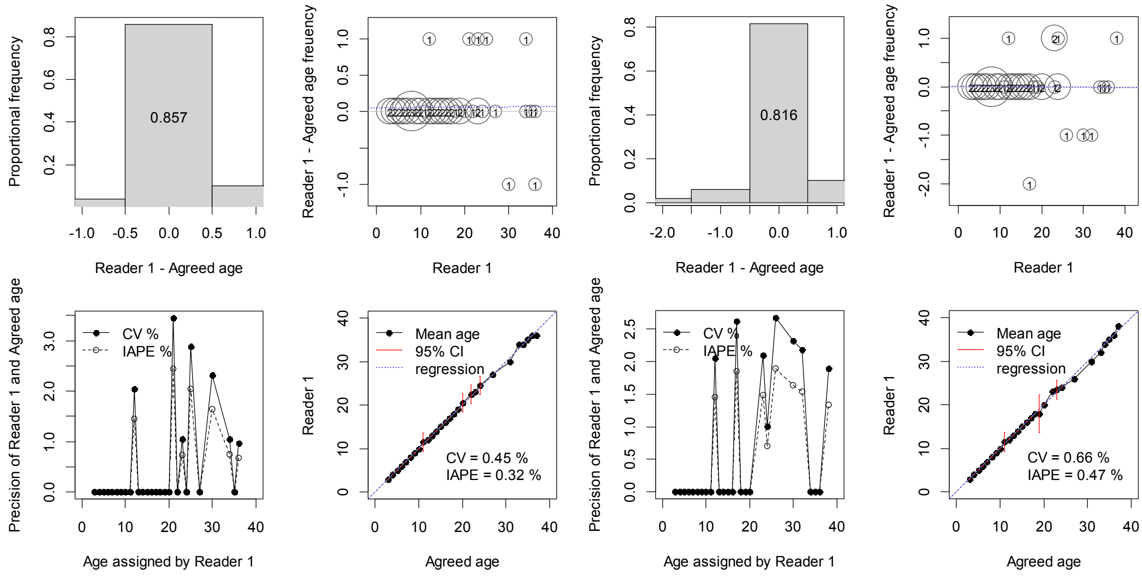

Figure A2.5: Reader 1 comparison tests for break and burn (left) and bake and embed (right).

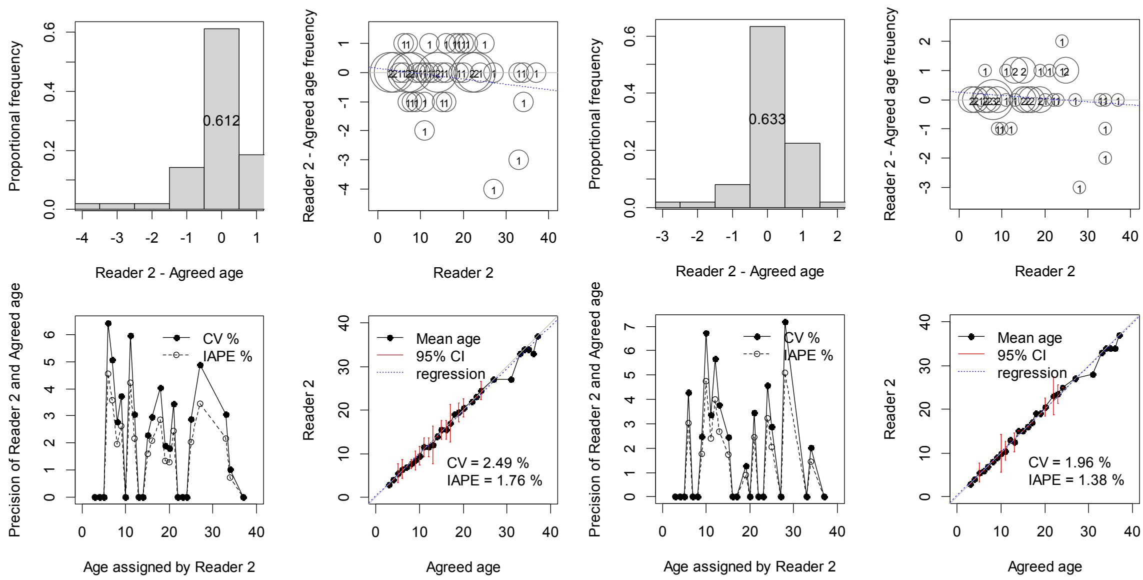

Figure A2.6: Reader 2 comparison tests for break and burn (left) and bake and embed (right).

These results show that only slight within-reader differences occur when snapper are aged using either the break and burn or bake and embed methods, but that reader experience with snapper otoliths was an important factor in terms of ageing precision and determining accurate estimates of age. Reader 1 concurred with the first three of the six points of difference, outlined in the preparation comparisons in 1992 for the break and burn and bake and embed methods, although reader 2 found neither preparation method to be consistently better in regards to these three points. Reader 1 estimated that it took at least twice as long to age the bake and embed otoliths and was not as confident in his ageing as he was with break and burn preparations (although his ageing results from both methods were similar), while reader 2 took almost twice as long to read the break and burn preparations. Clearly, familiarity with the preparation method influenced reading times in this test. Reader 2 found that the extra time needed to read the break and burn otoliths was not due to differences in clarity between the preparation methods, but to the extra time taken to extract the otolith from the packet, level it up in the plasticine lump, coat it with oil, and adjust the light source to provide optimal illumination, and then clean and return it to the packet after reading. The embedded otoliths are multiple in one section, are already levelled when the block is put on the microscope stage, can all be quickly oiled together, and once the light source was directed optimally it is not necessary to change its position again for that block. An advantage of the bake and embed method is its production of a robust and permanent prepared product. A stated advantage of the break and burn method is that when the otolith has been shattered due to the fish having been pithed, small pieces of otolith are better able to be ground and polished along the correct axis through the core using this method (although between-method comparisons of shattered otolith were not tested here). However, this trial showed that snapper could be accurately aged with a high degree of confidence using the break and burn or the bake and embed method.

APPENDIX 3: 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 A3.1, image 1). Kahawai, tarakihi and trevally have elongated sagittal otoliths of smaller size and considerably greater fragility than otoliths of snapper (Figure A3.1, images 2–4).

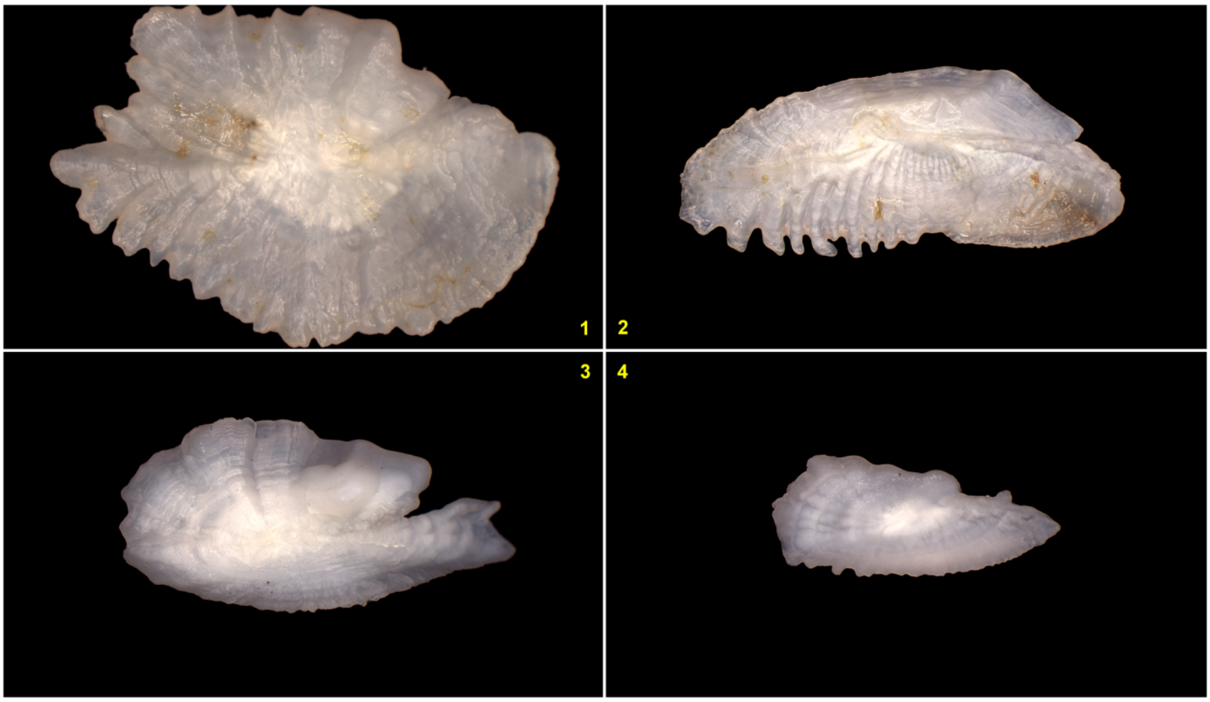

Figure A3.1: Whole right hand side otoliths in lateral view under reflected light at the same magnification demonstrating the differences in otolith size and shape for four important New Zealand inshore species (Image 1, snapper; 2, kahawai; 3, tarakihi; 4, trevally) extracted from fish of equivalent length (42 cm).

Table A3.1: Otolith dimension data for the four species outlined in Figure A3.1.

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 4: Summary of between-reader agreement and precision estimates documented in ageing studies for snapper.

Previously reported between-reader agreement and precision estimates (APE) determined from ageing snapper in New Zealand are presented in Table A4.1. Although a reasonable level of consistency in reader agreement and precision is apparent in ageing snapper, some estimates are low relative to other inshore species that are routinely aged e.g., 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 snapper in New Zealand (ENLD = East Northland; HAGU = Hauraki Gulf; BPLE = Bay of Plenty; BLL = Bottom longline; BT = Bottom trawl).

Stock	Subarea	Method	Fishing Year	No. of readers	Percent agreement	APE	CV	No. aged	Age range	Publication
SNA 1	ENLD	BLL	1995–96	3	49%	3.54	–	535	4–46	Davies et al.
	HAGU				60%	2.53		803	4–43	(2003)
	BPLE				74%	1.79		620	3–42	
SNA 2		BT	2007–08	2	98%	0.12	0.17	350	3–52	Walsh et al.
			2008–09		96%	0.26	0.36	1250	3–49	(2012)
SNA 8		BT	1995–96	3	92%	0.65	–	634	3–34	Davies et al. (2003)

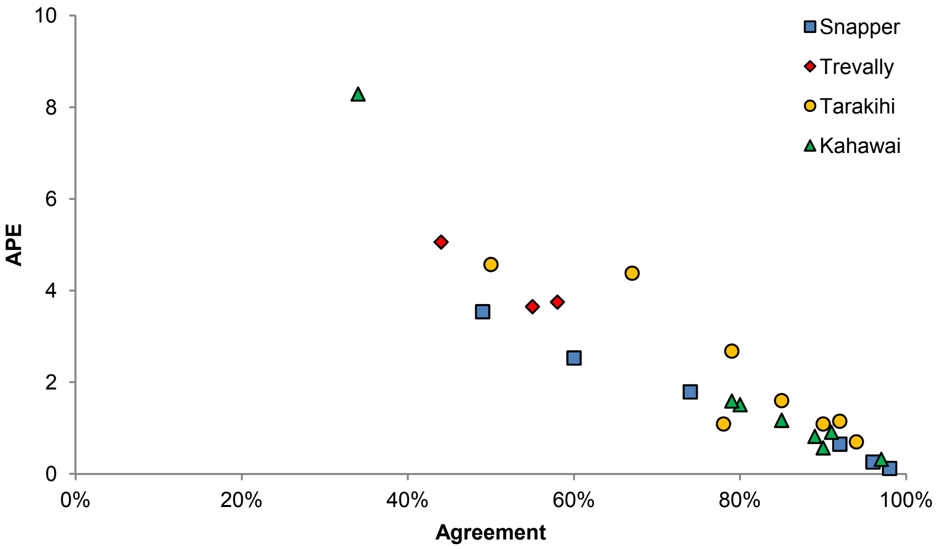

Figure A4.1: Visualised comparison of between-reader agreement and APE scores documented in ageing studies for snapper, trevally, tarakihi and kahawai in New Zealand.

Although percent agreement is considered an inferior method of determining ageing precision compared to APE and CV as it varies so widely among species and among ages within a species, all measures of precision may be artificially inflated by any bias which exists between readers (Campana 2001). It is therefore difficult to make firm conclusions when comparing between-reader precision estimates for a particular species as reader experience and ageing ability may vary. A CV estimate of 5% (APE 3.5%) may serve as a reference point for fishes of moderate longevity and reading complexity (Campana 2001), such as snapper, 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 snapper, 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 that accuracy in age estimation is maintained. This is particularly important for species such as snapper 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 snapper 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.