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Age determination protocol for blue cod (*Parapercis colias*)

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EXECUTIVE SUMMARY

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This report documents the age determination protocol for blue cod (*Parapercis colias*), an important New Zealand inshore finfish species. The protocol describes current scientific methods used for otolith preparation and interpretation, age determination 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 recently prepared archived samples. Agreed readings and ages determined for the reference set are stored in a reference table (*t_reference*) in the *age* database. The reference set sample was a random selection from fishstocks and seasons to account for spatio-temporal variations in otolith readability, also ensuring 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 blue cod. Associated difficulties and idiosyncrasies related to ageing prepared otoliths are also documented.

1. INTRODUCTION

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

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

This report describes the age determination protocol for an important New Zealand inshore finfish species: blue cod (*Parapercis colias*). Blue cod is one of New Zealand's most important commercial and recreational inshore species, with commercial landings exceeding 2000 t in 2013–14. Major commercial fisheries exist off Southland and the Chatham Islands, and smaller but regionally significant fisheries off Otago, Canterbury, the Marlborough Sounds and Wanganui. The Southland fishstock (BCO 5) 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. For others, catch-at-age information is routinely collected via fishery independent potting surveys and is a key input for mortality estimation and for per recruit based stock assessments. The purpose of this 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 blue cod. 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 blue cod (*Parapercis colias*), including the compilation of otolith reference collections.

2. AGE DETERMINATION PROTOCOL FOR BLUE COD

2.1 Background

The blue cod, endemic to New Zealand, was first aged by Rapson (1956) in a comprehensive study on the biology (reproduction, feeding, movement and growth) and commercial fishery during the mid-twentieth century. He examined whole untreated sagittal otoliths, in preference to scales which gave misleading results, from sample collections at three different locations; Marlborough Sounds (1938–39), Foveaux Strait and Stewart Island (1944–45), and the Chatham Islands (1945). Rapson assumed one light and one dark ring (herein referred to as opaque and translucent zones) was laid down in each year; representing periods of fast and slow growth over summer and winter, respectively; but did not document which zone was counted in determining age.

With an apparent decline in Marlborough Sound blue cod stocks over subsequent years, the Ministry of Agriculture and Fisheries initiated a study (although not completed) on blue cod population dynamics between December 1973 and January 1976, principally following on from Rapson's work 30 years earlier. This study was primarily a tag-recapture programme to determine fish movement between the Sounds and adjacent islands (Mace & Johnston 1983), but otolith samples were collected from those fish seen as unfit for tagging (Johnston & Clarke 1982). Although reported by Blackwell (1998) that otoliths from the tag-recapture programme were aged using the break and burn method to estimate growth rates, no documentation could be sourced to verify this.

In a comprehensive study on factors influencing the density and distribution of blue cod in the Leigh marine reserve, Mutch (1983) used the burn and break technique to expose the growth zone patterns on otoliths from 537 specimens caught by spearfishing, where annuli corresponded to dark (translucent) zones, counting these to estimate age. Mutch measured the marginal increment for each monthly otolith sample over one year to determine the timing of the annulus formation; July for 1+ and 2+ individuals and August for 0+, and when used in conjunction with other results (i.e., back calculated lengths using otolith growth markings from previous years that correspond to actual lengths of fish representative of these respective ages) concluded that blue cod otoliths were acceptable for ageing. A decade later, Carter (1992) investigated latitudinal growth patterns in blue cod from three geographically different locations; Leigh, Wellington and Bluff. Carter focused on broken, polished and heated otoliths as the preferred method (a modification of the technique described by Christensen (1964)[1]) for ageing, which enhances the differences between opaque and hyaline (herein referred to as translucent) zones, making them easier to read. Like Mutch (1983), Carter (1992) also counted dark (translucent) zones as annuli to estimate fish age.

Two subsequent potting surveys of Marlborough Sounds blue cod were undertaken in 1995 and 1996 to provide background information for the management of the fishery (Blackwell 1997, 1998). Although otoliths were collected from both surveys, the first sample to be aged, without reference to length and sex, was from 1996, read twice, using thin section preparations viewed under a stereo microscope illuminated with a cold light source (Blackwell 1998). Although not documented, it is likely that Blackwell counted light (opaque) zones in determining ages up to 20 years, noting blue cod

[1] Prepared sagittal otoliths by burning from in a flame and cracking to reveal annuli on the broken face.

age estimation to be unvalidated at that time. The move to using thin section preparations for ageing blue cod was regarded as an improvement over the break and burn method, which may have an error of ± 2 years in comparison (Blackwell 1998). The thin section method was also adopted by Carbines (1998) during the same period, investigating age validation, tagging feasibility and sex change of blue cod in what essentially became a precursor to a thesis (Carbines 2004a). However, unlike Blackwell (1998), Carbines (1998) chose to age transverse thin sections using transmitted light with a compound microscope, counting the number of annuli (dark opaque zones) from the distal to the proximal edge of the otolith section, where light translucent zones were used to define a complete dark zone, i.e., an annulus was counted only if it had a light zone on both sides. Carbines (2000) then used the same methodology to age blue cod samples from the aforementioned 1995 and 1996 potting surveys (Blackwell 1997, 1998) for comparison of age and growth within the Marlborough Sounds to determine the degree of spatial variability in growth rates.

Carbine's thesis (2004a) described age, growth, movement and reproductive biology of blue cod and a subsequent paper (Carbines 2004b) focused on age determination, validation and growth. Carbines examined a number of comparative methodologies for ageing, concluding the use of thin section otoliths to be the best, finding alternative methods to either underestimate age (i.e., scales and broken and burnt, and whole otoliths) or prove unsatisfactory (i.e., otolith length or weight) in comparison with thin section opaque zone counts. As mentioned above, complete opaque zones were counted to estimate age only if they were bound by translucent zones on either side. Incomplete opaque zones on the otolith margin resulted in readings in half years (i.e., 8.5 years) and no birthdate was used to correct for real age differences between sample times. In order to determine the periodicity of alternation between translucent and opaque zones, Carbines (2004a, 2004b) undertook marginal increment analysis, but this proved inconclusive due to uncertainty of the bimonthly measurements for unclear zones and variable zone widths. A far less ambiguous and simpler method was chosen that investigated the proportion of otoliths with opaque margins which showed that nearly all blue cod had opaque (likely to be misinterpreted for translucent) marginal zones in July and September. This is similar to the results of Mutch (1983).

Direct validation that translucent zones were formed annually in otoliths was first achieved by Carter (1992) from recaptured tagged adult blue cod injected, some twice, with oxytetracycline (OTC). He found that the position of the OTC mark visually confirmed the annual periodicity of otolith zone formation and that one year was equal to one opaque (light) and one translucent (dark) zone, the latter visible on the otolith margin during spring (October to December). Carbines (2004a, 2004b) conducted a similar experiment on adult blue cod in Foveaux Strait and Paterson Inlet, and reported that although OTC validation was not complete, as no marked fish were recovered after more than 1.2 years, results were indicative of a single annual winter zone formed in otoliths. Carbines also found that the results of the OTC mark on fin spines and fin rays provided support for annual periodicity of opaque (again, likely to be translucent) zone formation.

Although no validation of the first annulus was attempted in any of the above studies, Mutch (1983) presented plots of mean (standard) length-at-age for 0+ to >4+ blue cod for each season to establish intersexual growth differences and time of year when growth rate was highest (late spring/summer) by comparing marginal increments. In doing so, Mutch's growth curves and temporal plots of length-at-age appear precise for young fish (0+ to 4+ age groups), confirming that his ageing (using translucent zones) was likely to be accurate and that the first annulus was included in the zone count. Nevertheless, Carbines (2004a, 2004b) reported that further validation of age estimates from small blue cod was still required, and questioned the validity of counting opaque zones as annuli in otoliths of adult fish, without supportive data from small or juvenile fish to identify the first annulus, postulating that it may yet be a juvenile check of some form.

Carter (1992) was the first to investigate between-reader comparisons for break and burn aged blue cod, documenting a high level of agreement, while Blackwell (1998) reported agreement to be good using thin section preparations, despite it being below 50%. Carbines (2004a, 2004b) examined the accuracy of ageing by fitting a linear regression to a plot of the variation between sectioned otolith zone count estimates from two independent readers reporting a high level of consistency, no apparent bias, and discrepancies mainly due to ambiguous otoliths. Beentjes & Carbines (2012) were the first to assess otolith reading precision by calculating the mean coefficient of variation (CV) (Chang 1982), Average Percentage Error (APE) (Beamish & Fournier 1981) and presenting age-bias plots (Campana et al. 1995). Finding large differences in using inexperienced readers, they decided to use only ages from the experienced reader in catch-at-age and growth estimates.

Since the mid-1990s, age determination has been undertaken using the thin section method on otolith samples collected from potting surveys that determine the relative abundance, size and age structure, and stock status of blue cod for the main fisheries in the South Island (i.e., Marlborough Sounds, North Canterbury (Motunau and Kaikoura), Banks Peninsula, North Otago, South Otago, Foveaux Strait, Paterson Inlet, Dusky Sound). Although Blackwell (1998) suggested that blue cod recruitment may be variable, comparisons of relative year class strengths, from catch-at-age data, either within (or by sex) or between routine surveys (usually once in three years) were seldom reported, unlike those documented for other New Zealand species, e.g. snapper and trevally (Walsh et al. 2014a,b,c,d). In Rapson's (1956) research, comparisons of blue cod year groups were made, using proportional age compositions derived from sampling the commercial line fisheries from the Chatham Islands, Cook Strait and Foveaux Strait during the late 1930s and early 1940s. He found evidence of variation in recruitment strength, based on strong and weak year classes for some locations, but qualified this by stating that the effect of intensive fishing was that good brood years were quickly reduced. Mutch (1983) postulated that adult blue cod densities in the Leigh marine reserve were fairly stable which might be explained by the relative constancy in recruitment between years. Investigating South Island blue cod data from 2002–2005, Carbines et al. (2008) found it difficult to determine consistent patterns in year class strengths between sexes within a survey, stating that blue cod biology (sex change and growth), sampling and ageing error, and fishing mortality may confound patterns in catch-at-age, with most distributions being smooth and comprising adjacent year classes of similar relative strengths, particularly older age classes. Nevertheless, they did find that year class patterns for most stocks appeared more similar between sexes within a surveyed area than among areas, and therefore indicative of area-specific year class strength variation.

Although a number of tagged blue cod in coastal Foveaux Strait demonstrated counter-current movement trends, migrating somewhat more than those tagged in enclosed waters such as Marlborough and Dusky Sounds (Carbines et al. 2008), most tagging studies show that blue cod in general do not migrate far (Rapson 1956, Mace & Johnston 1983, Carbines 2004a, Carbines & McKenzie 2004) implying very little mixing among areas. Should patterns of relative year class strength exist within a stock or substock fishery, they should be apparent in the recent potting survey time series, although in particular fisheries (i.e., Marlborough Sounds, Motunau and Banks Peninsula (see Carbines et al. 2008), otolith sample sizes often appear too small and mortality too high to make valid comparisons. The existence of year class strength patterns within a fishery would further validate consistency in ageing blue cod.

Blue cod can be considered a moderately long lived species, although ages over 20 years are relatively uncommon in research potting surveys, making up just 2% of the 8700 records currently stored on the MPI *age* database. The oldest recorded age determined for blue cod is 32 years (58 cm male) and was caught from offshore Banks Peninsula in 2002 (Beentjes & Carbines 2003). Two other specimens captured from offshore Banks Peninsula in 2008 (56 cm, 3.0 kg) and South Otago in 2010 (59 cm, 3.5 kg) were aged at 30 years (Beentjes & Carbines 2009, 2011).

Early records of the timing of blue cod spawning were mainly reported around the end of the calendar year but varied slightly between areas, from August to October in the Marlborough Sounds, August to December about Stewart Island, November to January at Chatham Islands (Rapson 1956), and during October in Otago (Thomson & Anderton 1921). More recently, Mutch (1983) reported that most spawning for blue cod at Leigh probably takes place from August to November, while Carbines (2004a) showed that spawning in Southland occurs from November to January. Robertson (1973) established that eggs are pelagic for about five days after spawning and are common in southern waters, deep shelf, in spring and summer; and that larvae are pelagic for about five more days before settling onto the seabed. Young blue cod are first observed on pebbly or shelly bottom near reef edges in water deeper than 15 m during January and February when they are about 5 cm long, and reach 10 cm after 1 year (Francis 1993).

Mutch (1983) reported a birthday for blue cod to equate to that of the annulus formation in July, corresponding to the period of the smallest marginal increment, and just prior to the spawning period. Since this time, no birthdate has been used in determining age in any other blue cod research, although Carbines (2004a) reported an estimate of about 1 January, but did not use it.

The establishment of reference collections of prepared blue cod otoliths and their associated digital images and documented interpretations was recommended by Stephenson et al. (2009) with the first reference library for 100 samples collected from Paterson Inlet in 2010 documented in Carbines & Haist (2014) and stored on the *age* database (administered by National Institute of Water and Atmospheric Research (NIWA) for MPI).

Despite progress in ageing blue cod over many decades, in April 2014, the results for the most recent Marlborough Sounds potting survey from 2013 (BCO2013/01) were presented to the MPI Southern Inshore Working Group (SINSWG-2014/14) and showed unusually high variation in length-at-age, suggesting problems associated with ageing. A blue cod ageing workshop was convened by MPI in July 2014 to investigate the reasons for the high variation in ageing and to make recommendations on relevant aspects to be addressed in a proposed blue cod age determination protocol (this document). In summary, the workshop concluded:

- The first annulus requires validation.
- Annuli (opaque zones) for thin section otoliths were more easily interpreted on a black background using reflected light.
- Otolith sections of 0.4–0.7 mm were most suitable for ageing.
- All ageing should be undertaken by two readers with disagreements resolved jointly to establish an agreed age.
- The use of otolith features (i.e., notches on the distal surface) together with radial measurements to the first annulus should be used to avoid misinterpretation of juvenile checks.
- A theoretical ‘birthday’ for all blue cod should be established.

However, in a following SINSWG meeting (SINSWG-2015/42) where a draft of the age determination protocol for blue cod was presented, further amendments were made, deciding that:

- The average birthdate for blue cod should be 1 October (also the beginning of the New Zealand fishing year) and not 1 January.
- The protocol should be based on reflected light only (with a black background), and should not include the option of transmitted light.
- Translucent (and not opaque) zones should be counted, as they are deposited during winter and completed close to the birth date.
- If two readers cannot agree on a count for a specific otolith, a third reader should be brought in to help decide on the final count (instead of simply accepting the highest count).
- Separate reference collections should be compiled for Marlborough Sounds otoliths (as they are more difficult to read) and all other areas combined if otolith readability is not sufficiently different.
- Reference collections should consist of otoliths spanning the full range of fish length and age encountered in each area.

In order to validate the first annulus, thin sections of the otoliths of blue cod (4–7cm TL) collected from the Marlborough Sounds in March 2002, and assumed to be approximately 6 months old, were prepared and examined (Carbines 2016). As all of the otoliths from these fish contained the check previously assumed to be the first annulus, it was concluded that this was indeed a juvenile check. Radial distances to both the juvenile check and first annulus were subsequently measured to assist with the determination of the first annulus

2.2 Otolith preparation

Sagittal otoliths are acknowledged as the primary structure for ageing blue cod, and all scientific methodologies described in the following sections will be associated with ageing using thin sectioned sagittal otoliths, currently thought to be the best preparation method. The methodology used for preparing blue cod otoliths using the thin section technique generally followed that described by Carbines (2004a, 2004b) with recent minor modifications made to otolith thickness (i.e., 0.4–0.7 mm) for viewing translucent zones with reflected light (MPI BCO ageing workshop July 2014). The following sections present additional information pertinent to blue cod ageing.

Post extraction, blue cod otoliths are cleaned of adhering tissue, rinsed in water, air-dried, and stored in microcentrifuge (commonly referred to as Eppendorf) tubes within in paper envelopes labelled with sample details, including trip code, station number, fish number, date, length and sex (see Figure 1). As blue cod are protogynous hermaphrodites that change growth trajectory with sex change (some females change to males), and where males attain a larger average size than females at a given age (Carbines 2004a), the recording of sex has been mandatory in all MPI ageing studies. The envelopes are stored in labelled box files relating to the project code, fish-stock and year of collection, and are archived in the MPI otolith collection, currently housed at NIWA, Wellington.

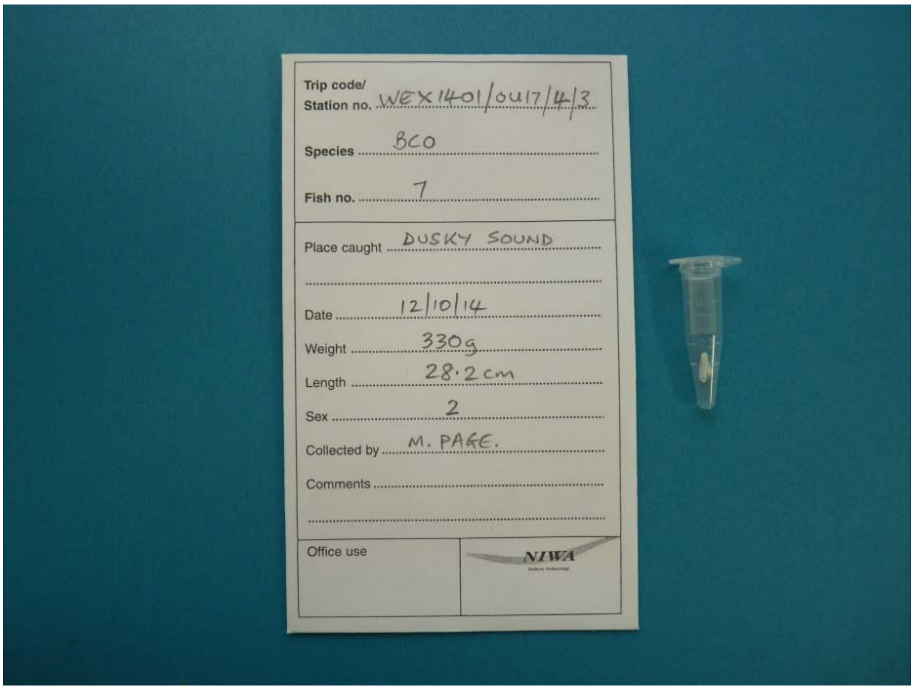

Figure 1: Images of a microcentrifuge tube and envelope used to store small and fragile otoliths like those collected from blue cod.

Appendix 2 outlines the most recent methodology used for thin section otolith preparations (Marriott & Manning 2011), most of which are applicable when preparing blue cod otolith thin sections, with some variations on sectioning machines and curing times.

In short, up to five blue cod otoliths are embedded in an epoxy polymer resin, baked (50° C for at least three hours) to cure, and sectioned transversely about 1 mm either side of the core with a single bladed diamond-tipped cut-off wheel (Carbines 2004a, 2004b). The thin section wafer is then mounted on a glass microscope slide using epoxy resin, sanded with 600-grit sandpaper to about 0.4–0.7 mm thickness. A similar but slightly alternative approach using a Struers Accutom-50 digital sectioning machine results in a section thickness of approximately 400 µm (0.4 mm). The thin wafers are cleaned and embedded directly onto microscope slides using epoxy resin and covered with a coverslip and oven cured at 50ºC.

2.3 Otolith interpretation

One opaque and one translucent zone is laid down in blue cod otoliths each year (Carter 1992, Carbines 2004a, 2004b) which is believed to be reflective of rapid growth over summer through to early autumn and slow growth over mid-autumn through to late spring/early summer, respectively. The most recent procedure for reading blue cod otolith thin sections involves the use of reflected light and a black background and when viewed with a compound microscope, a series of opaque (light) and translucent (dark) growth zones are apparent (Figure 2). Initial viewing may be undertaken at low magnification (10× objective) to determine which of the preferred sites on the section are the clearest for reading, although high magnification (20–40× objectives) is recommended for accurate zone count and margin interpretation, especially for older fish (i.e., those 10 years of age and older). Both ventral and dorsal sides of the otolith should be read from the core toward the proximal surface and the number of complete translucent zones counted. If a discrepancy between counts occurs, the reader rechecks the count until agreement is reached, unless the otolith is given a readability grade of 5 (unreadable), or it is damaged, in which case it is removed from the analysis.

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

1. The translucent zone (dark in thin section preparations using reflected light) may become visible on the margin in mid-autumn and is completed by late spring to early summer. The deposition of the opaque zone takes place in the preceding months, during mid-summer through to early autumn. The first translucent zone is consistent with being the first annual increment.
2. The theoretical ‘birthday’ for all blue cod is 1 October.
3. Translucent zones are counted.

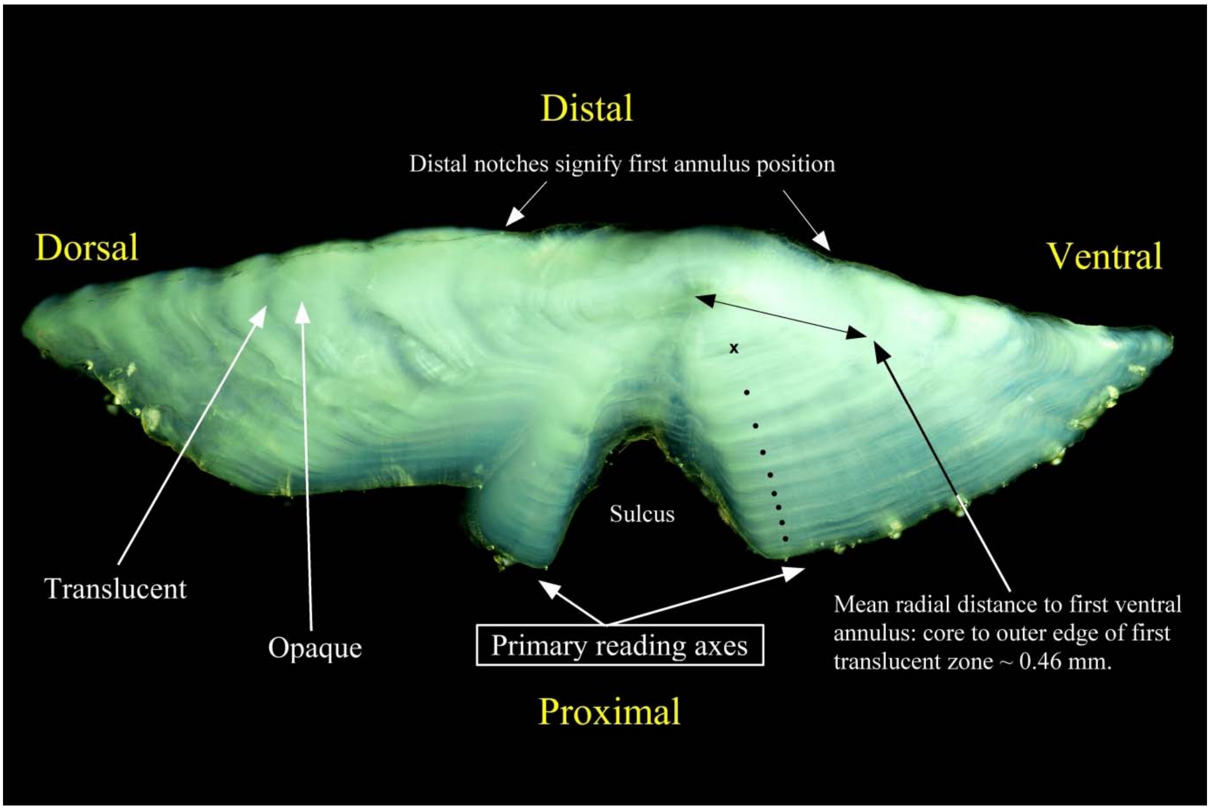

Figure 2: Blue cod otolith (Dusky Sound, October 2014, slide 22e) image of a transverse thin section under reflected light illustrating otolith terminology. This otolith section was interpreted as 8 wide. '×' indicates a juvenile check.

In the MPI blue cod ageing workshop in July 2014, identification of the first annulus was seen to be one of the main problematic issues. The first annulus in a sectioned otolith viewed with reflected light appears as the most obvious dark zone when counting from the core to the otolith margin, and is associated with a defined notch visible on the distal surface (see Figure 2). A mean radial distance of about 460 µm (0.46 mm) from the core to the outer edge of the first translucent zone along the ventral axis was determined from forty-five 2+ and 3+ blue cod thin section preparations (15 each from Dusky Sound, October 2014; Kaikoura, December 2015; Motunau, January 2016) reflecting one full year of growth (see Figure 2). In well prepared transverse sectioned blue cod otoliths, the radial distance to the first annulus is almost always greater than that between successive zones. In late recruits and poor sections the first annulus may be closer to the core but can be easily distinguished based on the otolith shape at age one. The use of a graticule to accurately determine the position of the first translucent zone, and therefore an accurate count of successive translucent zones for the sectioned otolith, is seen as essential for ageing blue cod, particularly when the core or zone formation is unclear. The width of the subsequent narrow dark (translucent) zones decrease proportionally in size up to about the fourth dark zone, and then zone width becomes relatively uniform.

The length distributions of juvenile blue cod sampled from a Motunau potting survey for an age-length key collection and aged 1+, 2+ or 3+ years as of January 2016 are presented in Figure 3 which shows relatively minor overlap between the adjacent modes. As the number of translucent zones visible in otoliths of fish from these consecutive modes increments by one for each age group, this confirms that translucent zones are formed annually in blue cod otoliths and provide a suitable structure from which to determine an accurate estimate of fish age (Figure 4).

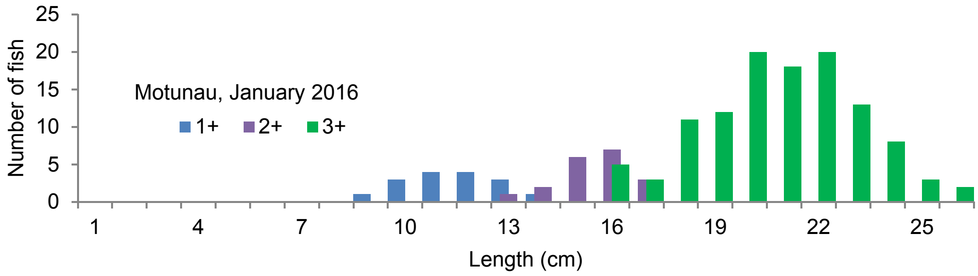

Figure 3: Length (FL) distributions demonstrating the size at age range for the 1+, 2+ and 3+ cohorts of juvenile blue cod collected from a Motunau potting survey during January 2016.

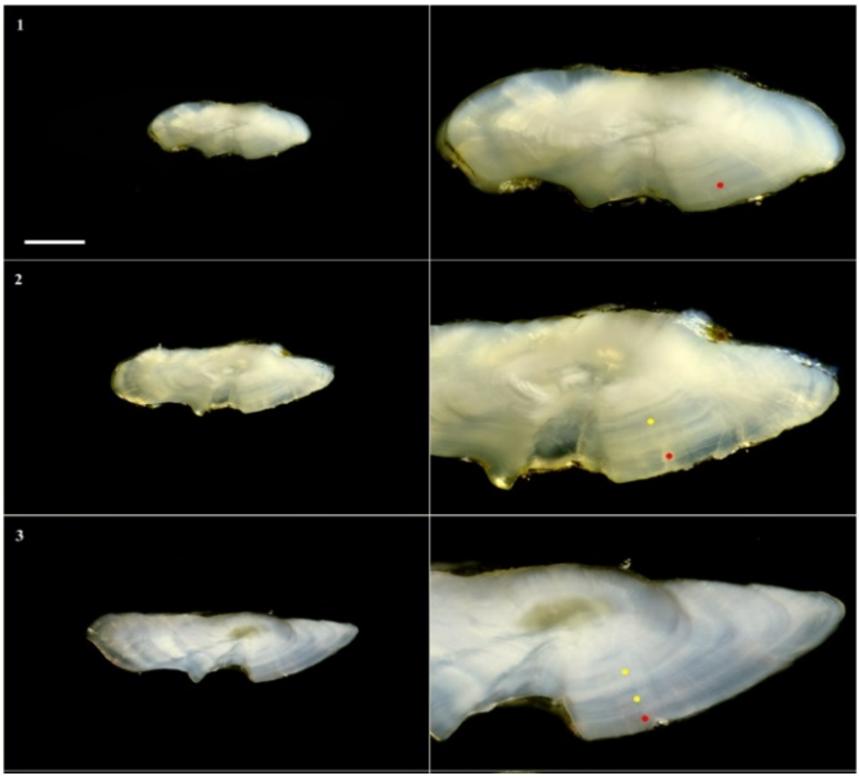

Figure 4: Thin section otolith images (whole section and enlarged sub-sections) of 1+, 2+ and 3+ juvenile blue cod (10, 16 and 19 cm in length) collected from a Motunau potting survey during January 2016 and viewed under reflected light. (*Yellow and red dots indicate the translucent zone count and margin state respectively; White scale bar = 500 µm for all whole sections.*)

The identification of non-seasonal secondary zones (i.e., false checks) can be a major cause of age-reading errors (Panfili et al. 2002). Juvenile growth (marked 'x') and false checks are occasionally present in blue cod otoliths, particularly from those fish that are fast growing, and usually lie between the core and the first and second zones (Figure 2). Although sometimes problematic, the spacing and line definition (too strong or weak) of false checks compared to annual zones are usually indicative of their presence.

To derive an accurate zone count from ageing blue cod thin section otoliths, readings can be made anywhere from the core out to the proximal edge, although the uniformity of opaque and translucent zone deposition tends to be clearest close to the ventral sulcul region, than toward the ventral and dorsal tips, particularly with increasing fish age (Figure 2). For ease of reading thin section preparations, only fully formed translucent zones are counted where an opaque zone precedes it, the only exception being if a translucent zone is present on the otolith margin, it is not included in the count. Comparison readings should be made along the ventral and dorsal sides to confirm initial reads, although multiple readings may be required for otoliths without clear zone definition. If discrepancies occur between an individual reader's counts, the default read is to use the higher estimate. Discrepancies generally occur where readers are unable to accurately identify poorly defined zones, often the first or second, but can also be attributed to 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). However, compared to many MPI catch-at-age studies (i.e., snapper, trevally, kahawai, tarakihi) undertaken over extended periods (i.e., New Zealand fishing year, October to September), blue cod potting surveys are normally completed within a discrete fixed time period (i.e., one month) and there appears to have been no requirement in the past to determine the otolith margin type (i.e., wide, line, narrow) in the month in which the fish was sampled (Carbines & Haist 2014). Instead, all blue cod age estimates within a survey have been taken to be equal to that of the opaque zone count agreement of the readers, as long as each opaque zone had translucent zones on both sides (Carbines 2004a, 2004b). Given the narrow window of time, the otolith margin type for recruited blue cod was likely to be similar for all fish captured within the survey period, although very young fish may display zone deposition slightly earlier than adults. However, where independent area surveys had been conducted several months apart within a fishing year period, for example, Kaikoura in December and Banks Peninsula in April–May, then fish from the same age class within a stock (i.e., BCO 3) could theoretically differ by 1 year using this methodology, as an opaque zone would have been deposited on the otolith during the interim period between the surveys. Therefore, knowing increment formation is fundamental in determining fish age and making valid comparisons in age compositions both within and between potting surveys and for blue cod catch sampling programmes conducted over extended periods (e.g., 6–12 months for the BCO 5 Seafood Innovation project 2012). It is proposed that margin state become a requirement for all MPI blue cod ageing studies which will allow for alignment of age with the 1 October birthdate and in relation to the fishing year of capture. It should however be noted by the reader that the nomenclature (readings and margins) used here for ageing blue cod are not exactly aligned with protocols used for ageing other inshore species (i.e., snapper, trevally, kahawai and tarakihi) due to the complexity of determining age from translucent zone counts within a fishing year and in relation to a birthdate of 1 October (see "application of forced margin" paragraph on the following page).

As blue cod release batches of eggs over an extended season during late winter and spring (MPI 2015), a theoretical birthdate for ageing blue cod of 1 October provides a useful birthdate for a number of reasons. Firstly, it is close to the peak of the spawning period (August to December) for most blue cod fisheries in New Zealand. Secondly, it is convenient for collating age data as it marks the beginning of the MPI fishing year (October to September) meaning that the age of a blue cod will remain the same over all months, with only the margin state changing through time. Lastly, it is aligned with the timing of increment formation of translucent zones which are counted to determine fish age. If 1 October is assumed to be the 'birthday' of all blue cod, then the first translucent zone is formed at seven to ten months of life (May–June (authors findings[2]); July–August, Mutch (1983[3])) over winter and present on the margin throughout spring (October–December, Carter (1992)) with all subsequent zones being laid down annually. Opaque zones are thought to be formed from mid-summer through to autumn. Therefore, an otolith with three complete translucent zones and one translucent zone depositing on the margin collected in October will be approximately 4.00 years old, one with four complete translucent zones and an opaque margin collected in March will be about 4.42 years old, and one with four complete translucent zones and a narrow translucent margin collected in July will be about 4.75 years old. Based on a calendar year, these fish will belong to the age class (age group) 4, and for the New Zealand fishing year which begins 1 October, they will all belong to fishing year age class 4 (Table 1).

[2] Partially deposited translucent zone visible on otolith margin (South Otago).
[3] Annulus formation (translucent zone) in 0+ to 2+ juveniles (Leigh).

Table 1: Diagrammatic representation of the age assignment for blue cod in relation to each month of the New Zealand fishing year, October–September. The birthdate for blue cod is 1 October 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	4	4	4	4	4	4	4	4	4	4	4	4
Age group	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
Decimalised age	4.00	4.08	4.17	4.25	4.33	4.42	4.50	4.58	4.67	4.75	4.83	4.92
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

To provide the reader with guidance and improve accuracy and precision in age estimations, a forced margin was implemented to anticipate the otolith margin relative to the month in which the otolith was collected (Table 1). For ageing blue cod in New Zealand, this is dependent upon the position of the outermost zone and is as follows: '**W**ide' (a moderate to wide translucent zone present on the margin), October–February; '**L**ine' (an opaque zone in the process of being laid down or fully formed on the margin), March–April; '**N**arrow' (a narrow to moderate translucent zone present on the margin), May–September. Although the timing of the deposition of the newly formed zones may vary slightly between individual fish, stocks and years, the forced margin method allows readers to anticipate the expected temporal change to the otolith margin in comparison to what they see whilst allowing for minor variations in zone deposition between otoliths in the collection they are reading. This is particularly important for old blue cod, as the zones close to the otolith margin, although most often regularly spaced, can be narrow, not well defined and therefore difficult to interpret. Although the clarity of the margin appears reasonably clear under low magnification in thin section preparations, viewing under high magnification is often necessary for an accurate zone count, but can be problematic due to focal depth through the section, poor preparation (i.e., over- or under-ground or off-axis) or the presence of resin bubbles and residual endolymphatic sac tissue resulting in reader uncertainty. Using the forced margin method, the three otolith examples collected in October, March and July (outlined in the previous paragraph) would be interpreted as 3W, 4L and 4N, respectively.

To demonstrate the application of the forced margin, consider an otolith sampled in February that has four completed translucent zones and an opaque margin. Using the forced margin method (Table 1), the opaque margin is ignored and the otolith interpreted as 3W (referring to a wide translucent margin). When determining age, however, the sampling date and assumed birth date are taken into account to assign an age of 4.33 years (Table 1). Ignoring the opaque margin in this instance, which may be present 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, a 4.33 year old blue cod sampled in February could be assigned ages of either 3 or 4, depending on whether an opaque margin was visible, and the last translucent zone was deemed to be complete.

It is prudent that prepared otoliths are 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 count of fully formed translucent zones (e.g., 4L or 4N are aged as a 4 year old) (see Table 1). Using the forced margin method should obviate the need for algorithms that convert a reader zone count to an age estimate, which may increase unnecessary error in age should reader interpretation of the margin states vary, especially important when ageing a species collected over an extended time period (i.e., year-round) or when making age composition or year class strength comparisons between surveys conducted 4–6 months apart within the same stock (e.g., North Canterbury (December 2007 to January 2008) and Banks Peninsula (April to May 2008) that lie within BCO 3).

Fast growing young blue cod (i.e., 1–3 year olds) may pose problems for readers, and may be misinterpreted and over-aged by one year because fewer annual zones are present to establish zone patterning, and marginal growth appears advanced. The use of the radial distance measurement (about 0.46 mm) to the first translucent zone on the ventral lobe and the location of the first small indentations or “notches” on the otolith dorso-ventral distal surface that further define the first translucent zone position will assist the reader in identifying the correct place to begin their count (see Figure 2).

A readability scale ranked 1–5 has been used for ageing blue cod otoliths from potting surveys dating back to 1995 (Carbines 2000). 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 (very poor/unreadable) which are already removed from the collection.

2.4 Ageing procedures

Despite not being recorded in initial ageing studies on blue cod, information suggests that only a single reader was used to age whole (Rapson 1956), burn and break (Mutch 1983), and break and burn (Carter 1992) otoliths, although the latter did use an independent reader to investigate reader agreement in ageing a small number of fish.

Although no age information for blue cod is recorded on the *age* database prior to 2001, it appears that ageing of Marlborough Sounds blue cod from potting surveys conducted during the mid-1990s (i.e., Blackwell 1998, Carbines 2000) used two readers to age thin section otolith preparations. Subsequent surveys that estimated the age structure (many surveys focused only on relative abundance, size structure and stock status) for other South Island blue cod fisheries tended to use the thin section method description of Carbines (2004a, 2004b) reporting two independent readers (except Beentjes & Carbines (2012) who initially used three readers, but ended up using the results from one experienced reader) in making comparisons between readings when counting complete opaque zones. Where counts differed, readers consulted to resolve the final age estimate and otoliths given a readability grade of 5 (unreadable) or damaged were removed from the analysis (Carbines & Beentjes 2009, Carbines & Haist 2014). The discarding of unreadable otoliths appears relatively uncommon across blue cod ageing studies, although Rapson (1956) reported the otoliths from several large blue cod to be impossible to read due to the structure being crystalline (composed of calcite or vaterite; refer Panfili et al. 2002) suggesting that this was an effect of senescence.

Although considered common practise in determining fish age, it was only a recent publication (Carbines & Haist 2014) in an Appendix titled “Otolith reference library for Paterson Inlet blue cod potting surveys” that first noted that interpretations (of opaque zone counts) are to be made without knowledge of fish length or sex at the time of reading, and that as the date of capture is a discrete fixed period for potting surveys, edge classification is not recorded (as it would be in a catch sampling programme). With the initiation of a MPI ageing workshop in July 2014 to improve between-reader agreement levels in ageing blue cod thin section otoliths, recommendations were made for ageing subsequent collections, many of these already listed in the previous paragraph. In summary, age determination of all otoliths should be undertaken independently by two experienced readers without prior knowledge of counts obtained by the other reader or of the fish length or sex, knowing only the collection date. Where sections have differing counts, they should be viewed jointly in an effort to establish an agreed age (see Carbines & Haist 2014) as is the current practise for other MPI studies where catch-at-age is regularly determined (i.e., snapper, tarakihi, trevally and kahawai).

2.5 Estimation of Ageing Precision

The first between-reader comparison test undertaken on blue cod resulted in 87% agreement from ageing a semi-random subsample of 23 break and burn otoliths (Carter 1992). Using two readers to age 237 thin section otoliths, Blackwell (1998) reported agreement to be good (46%; 84% differing by 1 year) with a trend in underestimation apparent, particularly for older fish, and high variability in age estimates for males. In ageing a larger sample of 425 thin section otoliths, Carbines (2004a,b) plotted the variation between opaque zone counts from two independent readers but did not determine between-reader percentage agreement or precision estimates, reporting instead a high level of consistency and no apparent bias, but nonetheless found differences for the same otolith of up to three years. By the late 2000s between-reader comparisons (percent agreement, plots of between-reader agreement, and between each reader and the agreed age) for ageing blue cod thin section otoliths collected from numerous potting surveys (Beentjes & Carbines 2009, Carbines & Beentjes 2009, 2011a, 2011b, 2012, Carbines & Haist 2014) were commonly reported for MPI research (Figure 5). However, Beentjes & Carbines (2012) were the first to quantify precision and bias between readers (three) ageing a subset of 99 thin section otoliths, documenting very low percent agreement (<20%) and very high CV (>15%) and APE (>10%) estimates due to reader inexperience (Figure 6), deciding to use only ages from the experienced reader in catch-at-age and growth estimates.

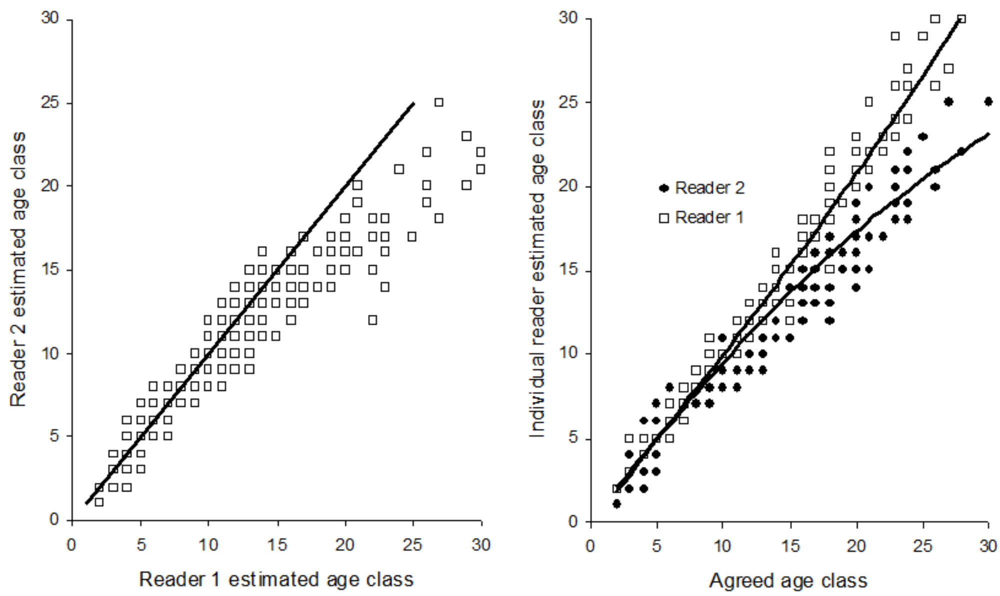

Figure 5: Between-reader comparison plots for aged blue cod sampled from the South Otago potting survey in 2010. Reproduced from Beentjes & Carbines (2011).

Although not entirely clear, it would appear that those blue cod populations that comprise a broad age range and/or have a high proportion of old fish may result in low percentage agreement (Appendix 3, Table A3.1) and if determined, would have high CV and APE estimates. Recommendations for quantifying precision and bias in future blue cod ageing studies have been summarised in Appendix 3.

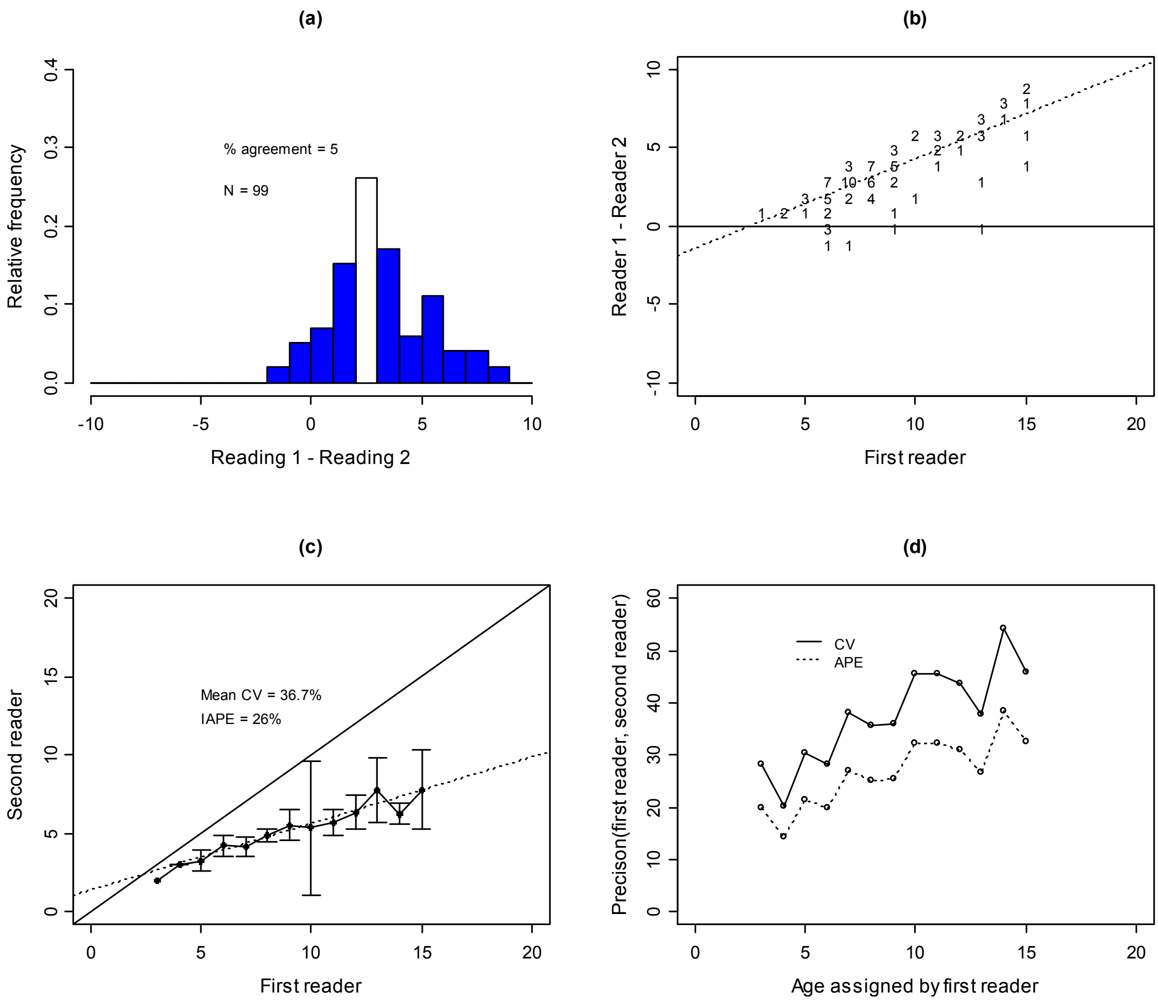

Figure 6: Age-bias diagnostic plots for blue cod sampled from the Marlborough Sounds potting survey 2010. Reproduced from Beentjes & Carbines (2012).

2.6 Reference collection

As blue cod otolith sections are most often mounted in sets of 5 on each microscope slide, it is proposed that 100 slides will be selected for the reference collection, rather than 500 individual preparations. This is expected to be sufficient for quality control monitoring in assessing reader performance, and may be added to over time. The primary role of the reference set is to monitor ageing consistency (and accuracy) over both the short and long term, particularly for testing long-term drift, as well as consistency among age readers (Campana 2001). The initial blue cod reference collection ($n = 479$) assembled in this study was selected from over 1800 otolith samples (archived at NIWA Wellington) collected from BCO 3 and BCO 5 fishstock potting surveys conducted between 2014 and 2016 (Figure 7). These samples were chosen specifically as the age estimation for blue cod from earlier collections may be affected by the following: ageing error, the use of only one reader, different preparation methods and zone interpretation, without recognition of the first annulus, margin state or birthdate. Over time, the selection process for the blue cod otolith reference collection will ensure that the full seasonal distribution of samples from potting surveys or commercial catch sampling of the main fisheries (i.e., BCO 3, BCO 5, BCO 7) and all length and age ranges across both sexes are well represented, while not being strongly dominated by those length and age classes most abundant (Figure 7). Examples of these preparations for a range of fish size and age are presented in Section 2.6.1 (Figures 8–10). As blue cod are considered a species of moderate longevity, a reference collection of about 500 otolith preparations is expected to be more than sufficient for quality control monitoring purposes. Although spatial differences in growth for blue cod stocks were reported in previous studies (Rapson 1953, Carter 1992, Carbines 2004a, MPI 2015), it is unlikely that the interpretation of blue cod otoliths will be affected by where the samples came from, and therefore the collation of stock-specific reference collections is deemed unnecessary. However, when new age samples for blue cod become available from other fishstocks such as BCO 4 (Chatham Islands) and BCO 8 (southwest North Island), and are aged following those protocols outlined within this document, then subsamples of these should be included within the current reference collection.

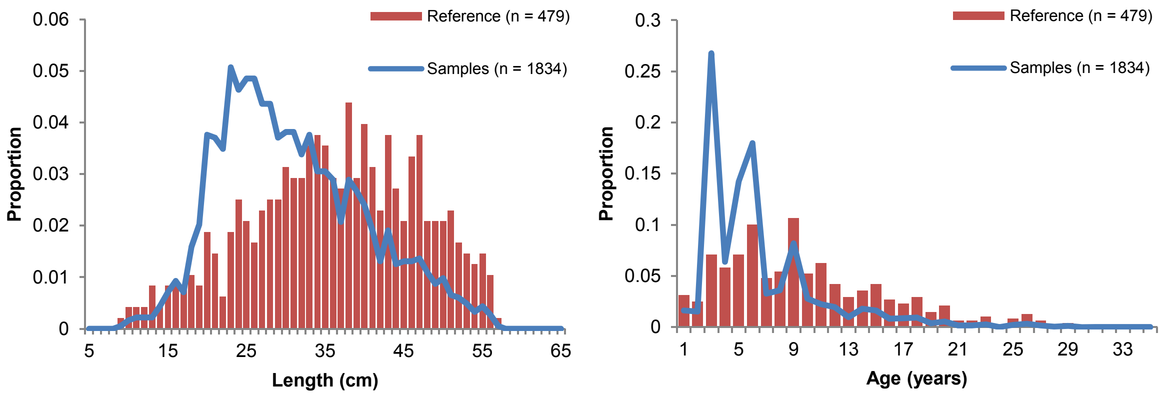

Figure 7: Length and age proportions (lines) of blue cod sampled for otoliths from the BCO 3 and BCO 5 fishstocks from 2014 to 2016 and selected for the reference set (histograms).

The agreed ages for otoliths selected for the reference set already exist on the *age* database (administered by NIWA for MPI), and have been stored in a new table, *t_reference*, created within this database along with another table, *t_reference_reading*, comprising any new readings of the reference set collection. As these preparations have been aged following the guidelines of the MPI blue cod ageing workshop (July 2014) and subsequent SINSWG meeting (November 2015), outlined in this age determination protocol, they may be treated with a reasonable level of confidence, given that the species is moderately 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.

2.6.1 Examples of thin section preparations of blue cod otoliths with marked translucent zones and agreed reading and age estimates for a range of fish size and age

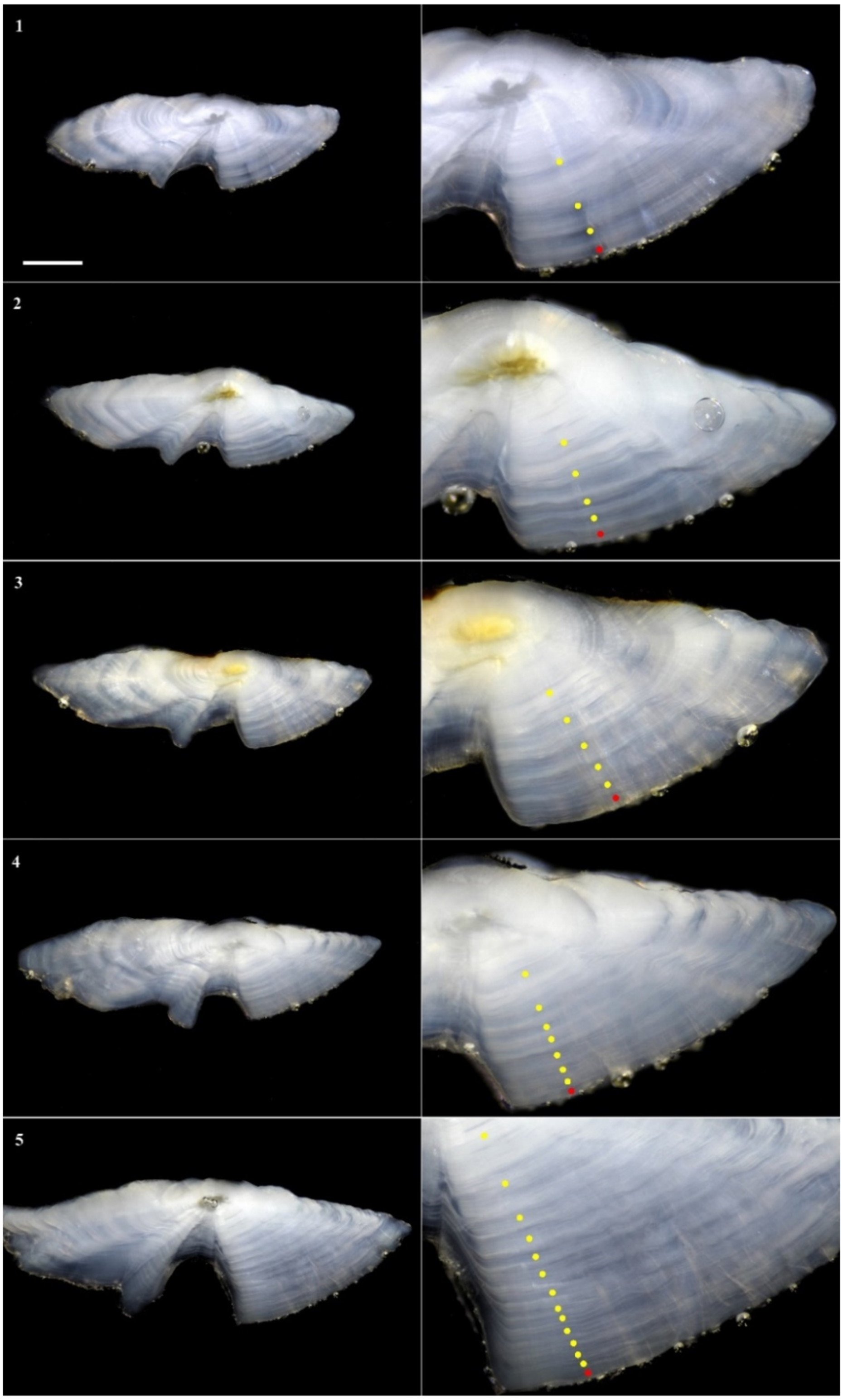

Figure 8: Aged blue cod otoliths (whole section and enlarged sub-sections) from Dusky Sound, October 2014: fish#1 (slide 40d, 25 cm female, agreed reading 3W, agreed age 4); fish#2 (slide 24a, 27 cm male, 4W, 5); fish#3 (slide 28e, 32 cm female, 5W, 6); fish#4 (slide 24e, 35 cm male, 7W, 8) and fish#5 (slide 34d, 45 cm male, 13W, 14). (*Yellow and red dots indicate the translucent zone count and margin state respectively; White scale bar = 500 µm for all whole sections.*)

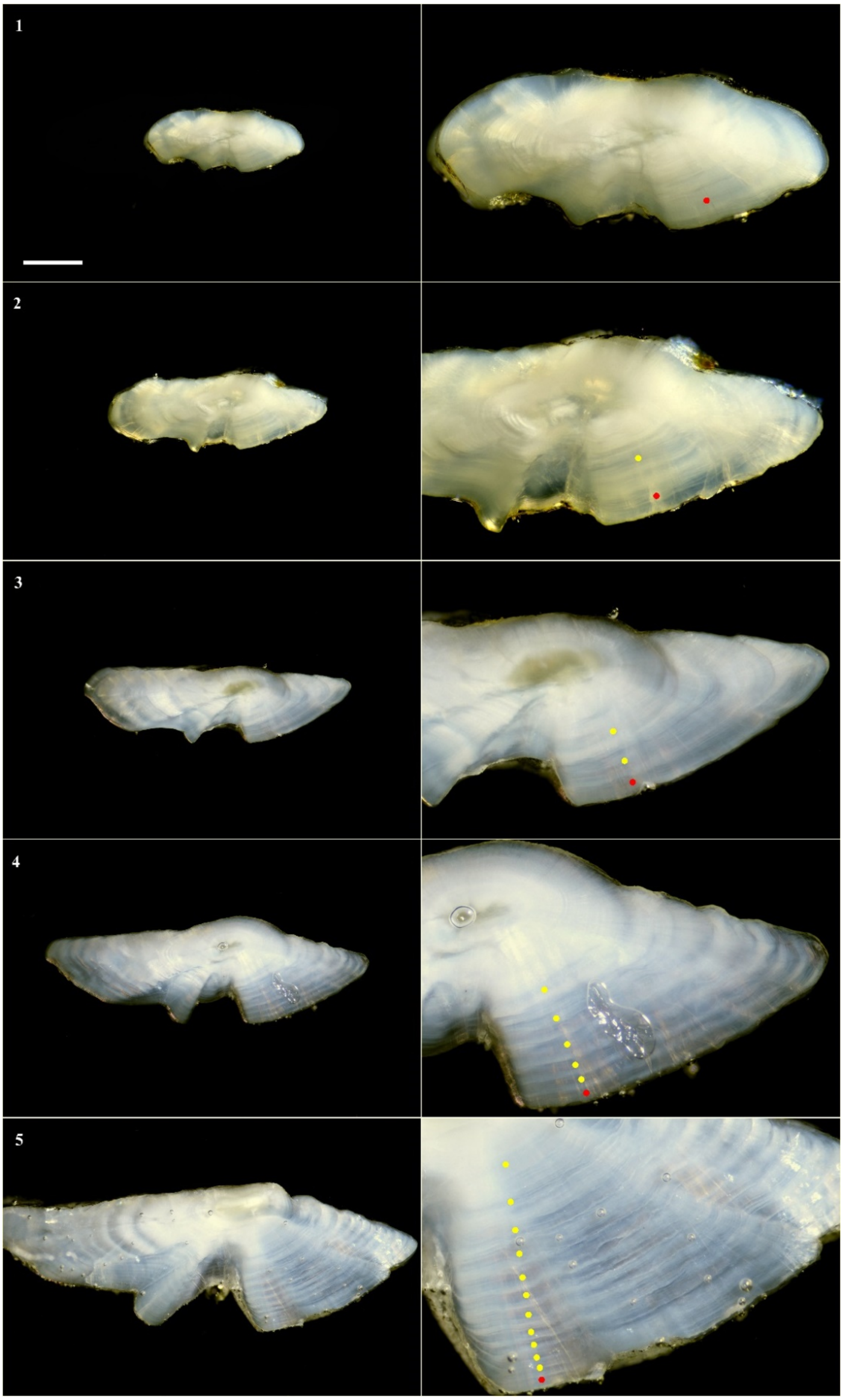

Figure 9: Aged blue cod otoliths (whole section and enlarged sub-sections) from Motunau, January 2016: fish#1 (slide 1027b, 10 cm unsexed, agreed reading 0W, agreed age 1); fish#2 (slide 1022e, 16 cm male, 1W, 2); fish#3 (slide 1021e, 19 cm female, 2W, 3); fish#4 (slide 1009a, 29 cm male, 5W, 6) and from Kaikoura, December 2015: fish#5 (slide 74b, 46 cm male, 11W, 12). (*Yellow and red dots indicate the translucent zone count and margin state respectively; White scale bar = 500 µm for all whole sections*.)

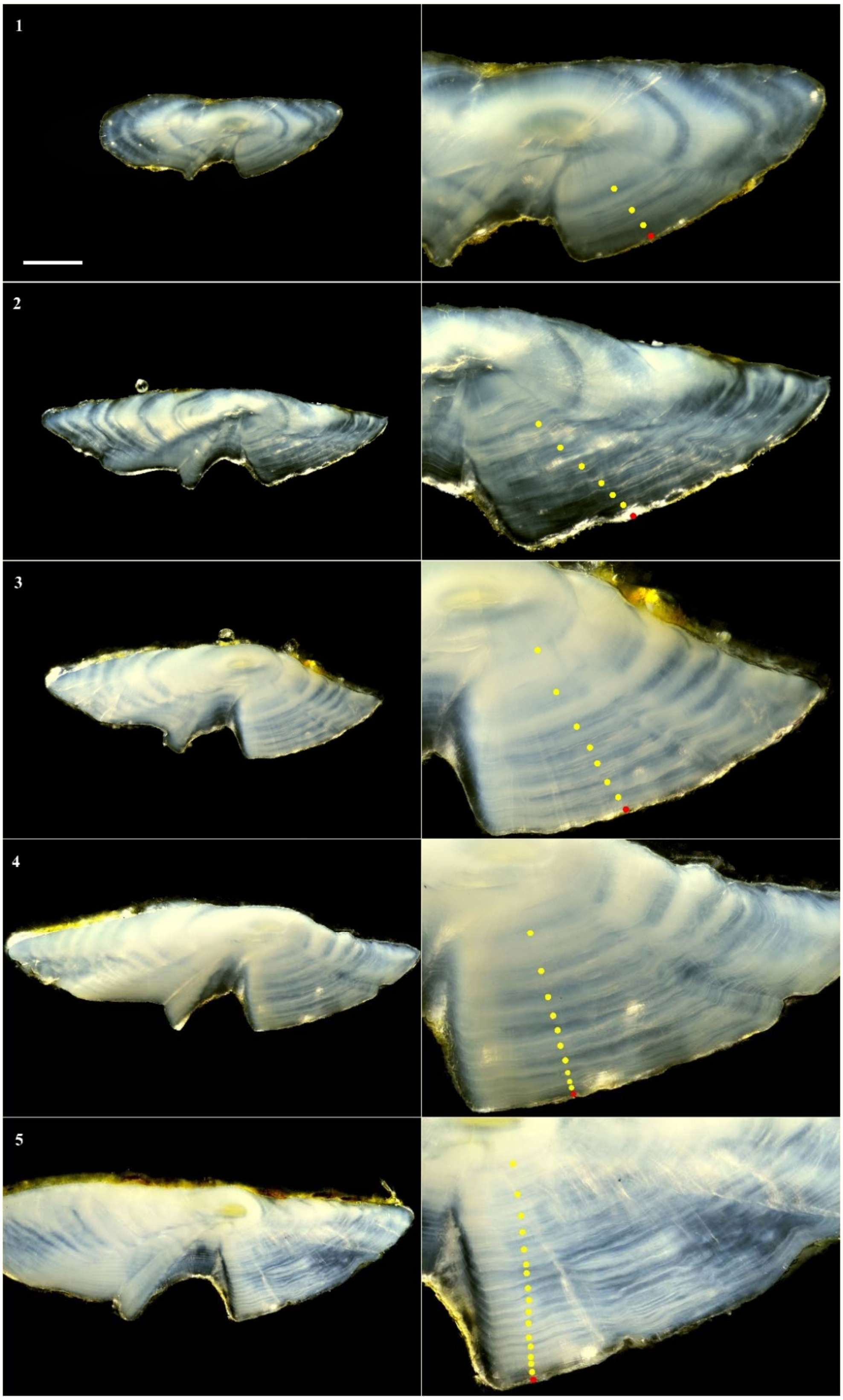

Figure 10: Aged blue cod otoliths (whole section and enlarged sub-sections) from South Otago, April–May 2010: fish#1 (slide 12e, 20 cm male, agreed reading 3L, agreed age 3); fish#2 (slide 9b, 30 cm male, 6L, 6); fish#3 (slide 44d, 32 cm female, 7N, 7); fish#4 (slide 37d, 46 cm male, 10N, 10) and fish#5 (slide 37b, 44 cm female, 16N, 16). (*Yellow and red dots indicate the translucent zone count and margin state respectively; White scale bar = 500 µm for all whole sections.*)

2.7 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, revised January 2015; https://marlin.niwa.co.nz/files/dataHoldings/scientificResearchDbs/age.pdf). 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 onto the *t_reading* and *t_age* tables in the *age* database (Tables 2 and 3). A readability score from 1 (excellent) to 5 (unreadable), although not mandatory, has previously been recorded for most blue cod readings on the *age* database.

Table 2: A potting survey example of blue cod age data submitted for loading onto the *t_reading* table in the *age* database.

origin	yr	trip_code	sample_no	sub_sample_no	area	species	fish_no	prep_no	block_no	reading_no	reading_date	material	method	reader	result1	result2	age	proj_code	comments
CHJ	2016	chj1601	13	1	BNKS	BCO	6	2a		1	05/09/2016	1	29	22	3	L	3	BCO201502	
CHJ	2016	chj1601	14	1	BNKS	BCO	7	2b		1	05/09/2016	1	29	22	3	L	3	BCO201502	
CHJ	2016	chj1601	14	1	BNKS	BCO	8	2c		1	05/09/2016	1	29	22	3	L	3	BCO201502	
CHJ	2016	chj1601	15	1	BNKS	BCO	9	2d		1	05/09/2016	1	29	22	5	L	5	BCO201502	
CHJ	2016	chj1601	15	1	BNKS	BCO	10	2e		1	05/09/2016	1	29	22	4	L	4	BCO201502	
CHJ	2016	chj1601	13	1	BNKS	BCO	6	2a		2	05/09/2016	1	29	113	3	L	3	BCO201502	
CHJ	2016	chj1601	14	1	BNKS	BCO	7	2b		2	05/09/2016	1	29	113	3	L	3	BCO201502	
CHJ	2016	chj1601	14	1	BNKS	BCO	8	2c		2	05/09/2016	1	29	113	3	L	3	BCO201502	
CHJ	2016	chj1601	15	1	BNKS	BCO	9	2d		2	05/09/2016	1	29	113	4	L	4	BCO201502	
CHJ	2016	chj1601	15	1	BNKS	BCO	10	2e		2	05/09/2016	1	29	113	4	L	4	BCO201502	

Table 3: A potting survey example of blue cod age data submitted for loading onto the *t_age* table in the *age* database.

origin	yr	trip_code	sample_no	sub_sample_no	area	species	fish_no	prep_no	block_no	agreed_result1	agreed_result2	method	agreed_age	proj_code	comments
CHJ	2016	chj1601	13	1	BNKS	BCO	6	2a		3	L	29	3	BCO201502	
CHJ	2016	chj1601	14	1	BNKS	BCO	7	2b		3	L	29	3	BCO201502	
CHJ	2016	chj1601	14	1	BNKS	BCO	8	2c		3	L	29	3	BCO201502	
CHJ	2016	chj1601	15	1	BNKS	BCO	9	2d		5	L	29	5	BCO201502	
CHJ	2016	chj1601	15	1	BNKS	BCO	10	2e		4	L	29	4	BCO201502	

For reference sets, a new table, *t_reference*, 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, *t_reference_reading*, 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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4. REFERENCES

Beamish, R.J.; Fournier, D.A. (1981). A method for comparing the precision of a set of age determinations. *Canadian Journal of Fisheries and Aquatic Sciences 38*: 982–983.

Beentjes, M.P.; Carbines, G.D. (2003). Abundance of blue cod off Banks Peninsula in 2002. *New Zealand Fisheries Assessment Report 2003/16*. 25 p.

Beentjes, M.P.; Carbines, G.D. (2009). Abundance, size and age composition, and mortality of blue cod off Banks Peninsula in 2008. *New Zealand Fisheries Assessment Report 2009/25*. 46 p.

Beentjes, M.P.; Carbines, G.D. (2011). Relative abundance, size and age structure, and stock status of blue cod off South Otago in 2010. *New Zealand Fisheries Assessment Report 2011/42*. 60 p.

Beentjes, M.P.; Carbines, G.D. (2012) Relative abundance, size and age structure, and stock status of blue cod from the 2010 survey in Marlborough Sounds, and review of historical surveys. *New Zealand Fisheries Assessment Report 2012/43*. 137 p.

Blackwell, R.G. (1997). Abundance, size composition, and sex ratio of blue cod in the Marlborough Sounds, September 1995. *New Zealand Fisheries Data Report No.* 88. 17 p.

Blackwell, R.G. (1998). Abundance, size and age composition, and yield per recruit of blue cod in the Marlborough Sounds, September 1996. *NIWA Technical Report 30*. 47 p.

Campana, S.E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology 59*, 197–242.

Campana, S.E.; Annand, M.C.; McMillan, J.I. (1995). Graphical and statistical methods for determining the consistency of age determinations. *Transactions of the American Fisheries Society 124*: 131–138.

Carbines, G.D. (1998). Blue cod age validation, tagging feasibility and sex-inversion. Final Research Report for Ministry of Fisheries Project SOBC04. September 1998. 74 p. (Unpublished report held by Ministry for Primary Industries, Wellington.)

Carbines, G. (2000). Comparisons of age and growth of blue cod within the Marlborough Sounds (BCO 7). Final Research Report for Ministry of Fisheries Research Project BCO9801. April 2000. 28 p. (Unpublished report held by Ministry for Primary Industries, Wellington.)

Carbines, G. (2004a). Age, growth, movement and reproductive biology of blue cod (*Parapercis colias* – Pinguipedidae): Implications for fisheries management in the South Island of New Zealand. Unpublished Ph.D. thesis, University of Otago, New Zealand. 225 p.

Carbines, G. (2004b). Age determination, validation, and growth of blue cod, *Parapercis colias*, in Foveaux Strait, New Zealand. *New Zealand Journal of Marine and Freshwater Research 38*: 201–214.

Carbines, G. (2016). Validation of first annulus in blue cod (*Parapercis colias*) otolith. Final Research Report for Ministry for Primary Industries Project SEA201417.14 p. (Unpublished report held by Ministry for Primary Industries.)

Carbines, G.; Dunn, A.; Walsh, C. (2008). Age composition and derived estimates of total mortality for blue cod taken in South Island potting surveys, 2002–2005. *New Zealand Fisheries Assessment Report 2008/68*. 74 p.

Carbines, G.; McKenzie, J. (2004). Movement patterns and stock mixing of blue cod in Dusky Sound in 2002. *New Zealand Fisheries Assessment Report 2004/36*. 28 p.

Carbines, G.D.; Beentjes, M.P. (2009). Relative abundance, size and age structure, and mortality of blue cod in north Canterbury (BCO 3) in 2007–08. *New Zealand Fisheries Assessment Report 2009/37*. 56 p.

Carbines, G.D.; Beentjes, M.P. (2011a). Relative abundance, size and age structure, and stock status of blue cod in Dusky Sound, Fiordland, in 2008. *New Zealand Fisheries Assessment Report 2011/35*. 56 p.

Carbines, G.D.; Beentjes, M.P. (2011b). Relative abundance, size and age structure, and stock status of blue cod off north Otago in 2009. *New Zealand Fisheries Assessment Report 2011/36*. 57 p.

Carbines, G.D.; Beentjes, M.P. (2012). Relative abundance, size and age structure, and stock status of blue cod in Foveaux Strait in 2010. *New Zealand Fisheries Assessment Report 2012/39.* 66 p.

Carbines G.D.; Haist, V. (2014). Relative abundance, size and age structure, and stock status of blue cod in Paterson Inlet of BCO 5 in 2010. *New Zealand Fisheries Assessment Report 2014/14*. 84 p.

Carter, S.E. (1992). Latitudinal changes in growth patterns of two teleost fishes within New Zealand. Unpublished MSc thesis, University of Auckland, New Zealand. 72 p.

Chang, W.Y.B. (1982). A statistical method for evaluating the reproducibility of age determination. *Canadian Journal of Fisheries and Aquatic Sciences 39*: 1208–1210.

Christensen, J.M. (1964). Burning of otoliths, a technique for age determination of soles and other fish. *Journal du Conseil Permanent International pour L'Exploration de la Mer 29*: 73–81.

Davies, N.M.; Hartill, B.; Walsh, C. (2003). A review of methods used to estimate snapper catch-at-age and growth in SNA 1 and SNA 8. *New Zealand Fisheries Assessment Report 2003/10.* 63 p.

Francis, M. (1993). Coastal fishes of New Zealand. A diver's identification guide. Reed Publishers Group (NZ) Ltd. Auckland. 63 p.

Johnston, A.D.; Clarke, G.H. (1982). Data from a study of blue cod (*Parapercis colias*) (Pisces: Mugiloidae) in the Marlborough Sounds, N.Z., between December 1973 and January 1976. (Unpublished report held in NIWA library, Wellington.)

Kalish, J.M.; Beamish, R.J.; Brothers, E.B.; Casselman, J.M.; Francis, R.I.C.C; Mosegaard, H.; Panfili, J.; Prince, E.D.; Thresher, R.E.; Wilson, C.A.; Wright, P.J. (1995). Glossary for Otolith Studies, pp. 723–729. *In* D.H. Secor, J.M. Dean, S.E. Campana (eds.), *Recent Developments in Fish Otolith Research*, University of South Carolina Press, Columbia, South Carolina.

Mace, J.T.; Johnston, A.D. (1983). Tagging experiments on blue cod (*Parapercis colias*) in the Marlborough Sounds, New Zealand. *New Zealand Journal of Marine and Freshwater Research 17*: 207–211.

Mackay, K.A.; George, K. (1993). Research database documentation. 8. Age. Internal Report, MAF Fisheries Greta Point, No. 214. 28 p. (Unpublished report held by NIWA library, Wellington.)

Marriott, P.M.; Manning, M.J. (2011). Reviewing and refining the method for estimating blue mackerel (*Scomber australasicus*) ages. *New Zealand Fisheries Assessment Report 2011/11*. 25 p.

McMillan, P.J.; Francis, M.P.; James, G.D.; Paul, L.J.; Marriott, P.J; Mackay, E.; Wood, B.A.; Griggs, L.H.; Sui, H.; Wei, F. (2011). New Zealand fishes. Volume 1: A field guide to common species caught by bottom and midwater fishing. *New Zealand Aquatic Environment and Biodiversity Report No. 68.*

Ministry for Primary Industries (2015). Report for the Fisheries Assessment Plenary, May 2015: stock assessments and yield estimates. Ministry for Primary Industries.

Ministry of Fisheries Science Group (2011). Guidelines for the development of fish age determination protocols. 20 p. (Unpublished report held by Ministry for Primary Industries, Wellington.) http://www.fish.govt.nz/NR/rdonlyres/A76B8528-9324-465B-BC83-0836D89383D3/0/GuidelinesforfishagedeterminationprotocolsAugust2011.pdf

Mutch, P.G. (1983). Factors influencing the density and distribution of the blue cod (*Parapercis colias*) (Pisces: Mugilodae). Unpublished MSc thesis, University of Auckland, New Zealand. 76 p.

Panfili, J.; de Pontual, H.; Troadec, H.; Wright, P.J. (eds). (2002). Manual of fish sclerochronology. Brest, France: Ifremer-IRD coedition, 464 p.

Rapson, A.M. (1956). Biology of the Blue Cod (*Parapercis colias* Forster) of New Zealand. Unpublished DSc. thesis, VictoriaUniversity, Wellington, New Zealand. 53 p.

Robertson, D.A. (1973). Planktonic Eggs and Larvae of some New Zealand Marine Teleosts. Unpublished Ph.D. thesis, University of Otago, Dunedin, New Zealand.

Stephenson, P.; Sedberry, G.; Haist, V. (2009). Review of blue cod potting surveys in New Zealand. Draft May 14, 2009. BCOREV-2009-22, 14 p. (Unpublished report held by Ministry for Primary Industries, Wellington.)

Thomson, G.M.; Anderton, T. (1921). History of the Portobello Marine Fish Hatchery and Biological Station. *Bulletin (New Zealand Board of Science and Art), 2: 1–131.*

Walsh, C.; McKenzie, J.; Bian, R.; Armiger, H.; Rush, N.; Smith, M.; Spong, K.; Buckthought, D. (2014a). Age composition of commercial snapper landings in SNA 1, 2012–13. *New Zealand Fisheries Assessment Report 2014/55*. 62 p.

Walsh, C.; McKenzie, J.M.; Bian, R.; Buckthought, D.; Armiger, H.; Ó Maolagáin, C. (2014b). Length and age composition of commercial trevally landings in TRE 7, 2012–13. *New Zealand Fisheries Assessment Report 2014/66*. 51 p.

Walsh, C.; McKenzie, J.R.; Bian, R.; Buckthought, D.; Armiger, H.; Ó Maolagáin, C.; Spong, K. (2014c). Length and age composition of commercial trevally landings in TRE 1, 2012–13. *New Zealand Fisheries Assessment Report 2014/65*. 53 p.

Walsh, C.; McKenzie, J.; Buckthought, D.; Bian, R.; Armiger, H. (2014d). Length and age composition of commercial snapper landings in SNA 8, 2012–13. *New Zealand Fisheries Assessment Report 2014/63*. 34 p.

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

Forced margin or Fixed margin – Otolith margin description (Line, Narrow, Medium, Wide) is determined according to the margin type anticipated *a priori* for the season/month in which the fish was sampled. The otolith is then interpreted and age determined based on the forced margin. The forced margin method is usually used in situations where fish are sampled throughout the year and otolith readers have difficulty correctly interpreting otolith margins.
Fishing year age class – The age of an age group at the beginning of the New Zealand fishing year (1 October). It does not change if the fish have a birthday during the fishing season. This is not the same as age group/age class.
Hatch date – the date a fish hatched; typically ascertained by counting daily increments from a presumed hatching check (see check) to the otolith edge.
Hyaline zone – a zone that allows the passage of greater quantities of light than an opaque zone. The term hyaline zone should be avoided; the preferred term is translucent zone.
Increment – a reference to the region between similar zones on a structure used for age estimation. The term refers to a structure, but it may be qualified to refer to portions of the otolith formed over a specified time interval (e.g., subdaily, daily, annual). Depending on the portion of the otolith considered, the dimensions, chemistry, and period of formation can vary widely. A daily increment consists of a D-zone and an L-zone, whereas an annual increment comprises an opaque zone and a translucent zone. Both daily and annual increments can be complex structures, comprising multiple D-zones and L-zones or opaque and translucent zones, respectively.
L-zone – that portion of a microincrement that appears light when viewed with transmitted light, and appears as an elevated region when acid etched and viewed with a scanning electron microscope. The component of a microincrement that contains a lesser amount of organic matrix and a greater amount of calcium carbonate than the D-zone. Referred to as an incremental zone in earlier works on daily increments; L-zone is the preferred term. See D-zone.
Lapillus (pl. Lapilli) – one of three otolith pairs found in the membranous labyrinth of osteichthyan fishes. The most dorsal of the otoliths, it lies within the utriculus ("little pouch") of the pars superior. In most fishes, this otolith is shaped like an oblate sphere and it is smaller than the sagitta.
Margin/marginal increment – the region beyond the last identifiable mark at the margin of a structure used for age estimation. Quantitatively, this increment is usually expressed in relative terms, that is, as a fraction or proportion of the last complete annual or daily increment.
Microincrement – increments that are typically less than 50 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 µm to 1.0 µm 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.

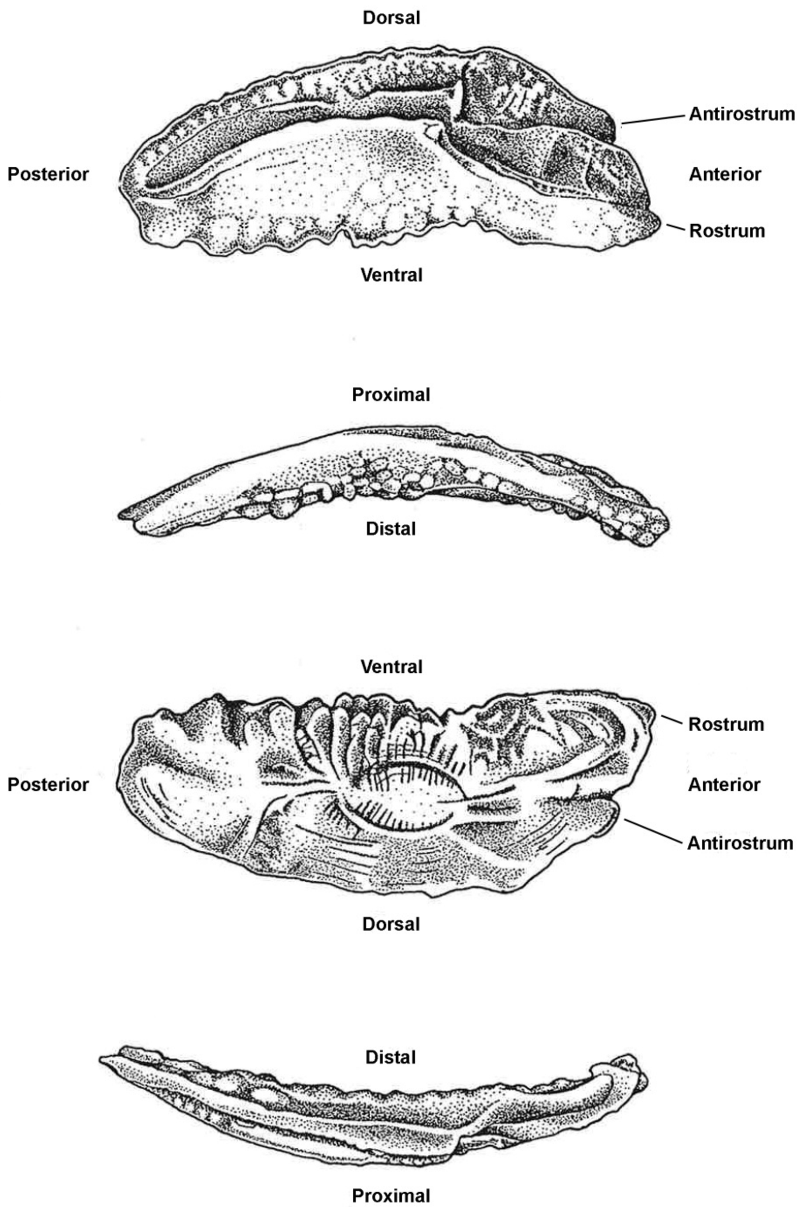


Figure A1.1: Views of a left sagittal otolith from *Arripis trutta* illustrating orientation and basic structure. A) the proximal surface, B) the ventral edge, C) the dorsal edge. (Drawing by Darren Stevens, NIWA).

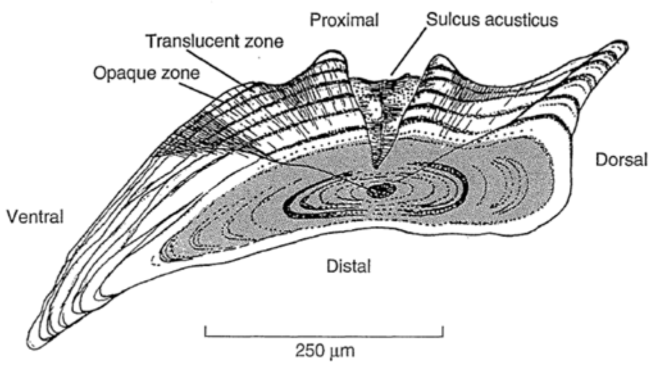


Figure A1.2: Transverse thin section through a sagittal otolith from *Arripis trutta* viewed with transmitted light illumination. The section is taken through the core. (Drawing by Darren Stevens, NIWA).

APPENDIX 2: Protocol for thin section otolith preparation.

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

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

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

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

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

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

Calibrating the saw

We cut our thin sections on a Struers Accutom-2 high-speed saw or 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.

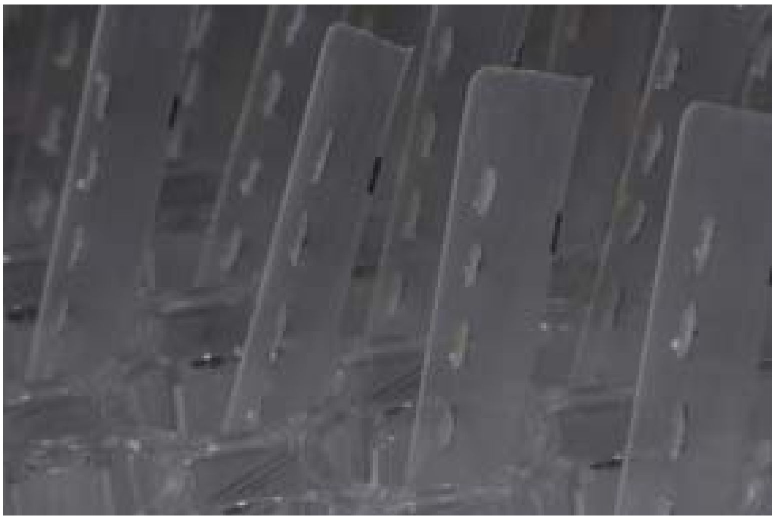
Section blocks at a slow regular speed to ensure even cutting. If one end of the cut wafer is a different thickness to the other end of the cut wafer, slow down the advance speed of the block into the saw, this may produce a more regular section. Utilising clean cutting lubricant should also help to 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 3: Summary of between-reader agreement and precision estimates documented in ageing studies for blue cod.

Previously reported between-reader agreement and precision estimates (APE) determined from ageing blue cod in New Zealand are presented in Table A3.1. Although two main readers are acknowledged with ageing approximately 80% of all samples on the *age* database, overall, only a moderate level of consistency is apparent between readers in most recent blue cod ageing studies (Figure A3.1) 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 A3.1: Between-reader agreement and precision estimates documented in ageing studies for blue cod in New Zealand (HAGU = Hauraki Gulf; SNDS = Marlborough Sounds; KAIK = Kaikoura; MOTN = Motunau; BNKS = Banks Peninsula; DUSK = Dusky Sound; OTAG = North Otago; DUNE = South Otago; FOVE = Foveaux Strait; PATE = Paterson Inlet; DI = Diving; CP = Cod potting, HL = Hand lining).

Stock	Subarea	Method	Calendar year	No. of readers	Percent agreement	APE	CV	No. aged	Age range	Publication
BCO 1	HAGU	DI	1990	2	87%	–	–	23	3–7	Carter (1992)
BCO 7	SNDS	CP, HL	1996	2	46%	–	–	237	1–20	Blackwell (1998)
BCO 3	KAIK	CP	2007	2	62%	–	–	276	3–24	Carbines & Beentjes (2009)
BCO 3	MOTN	CP	2008	2	73%	–	–	256	3–12	Carbines & Beentjes (2009)
BCO 3	BNKS	CP	2008	2	36%	–	–	315	2–30	Beentjes & Carbines (2009)
BCO 5	DUSK	CP	2008	2	50%	–	–	337	3–25	Carbines & Beentjes (2011a)
BCO 3	OTAG	CP	2009	2	67%	–	–	293	2–25	Carbines & Beentjes (2011b)
BCO 3	DUNE	CP	2010	2	48%	–	–	567	2–30	Beentjes & Carbines (2011)
BCO 5	FOVE	CP	2010	2	51%	–	–	291	1–27	Carbines & Beentjes (2012)
BCO 5	PATE	CP	2010	2	72%	–	–	282	1–25	Carbines & Haist (2014)
BCO 7	SNDS	CP	2010	2	5%	26.0	36.7	99	3–25	Beentjes & Carbines (2012)
BCO 7	SNDS	CP	2010	2	17%	10.9	15.4	99	3–15	Beentjes & Carbines (2012)

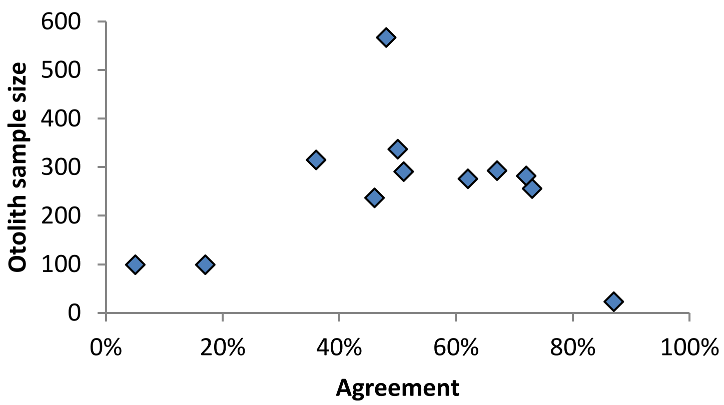


Figure A3.1: Between-reader agreement and otolith sample size documented in ageing studies for blue cod.

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 blue cod, but 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 now implemented in ageing blue cod, of independent identification and re-reading of otoliths where disagreements occur (when two readers are used), almost always resolves disagreements. 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. A minimum of two readers should always read all otoliths once and resolve all disagreements to ensure that accuracy in age estimation is maintained. 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 blue cod 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.