Reviewing and refining the method for estimating blue mackerel (*Scomber australasicus*) ages

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EXECUTIVE SUMMARY

Marriott, P.M.; Manning, M.J. (2011). Reviewing and refining the method for estimating blue mackerel (*Scomber australasicus*) ages.

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All steps associated with preparing and interpreting blue mackerel otolith sections were reviewed. The blue mackerel otolith protocol set held in the Ministry of Fisheries otolith collection was expanded and full written protocols on preparing and interpreting blue mackerel otoliths were produced.

1. INTRODUCTION

Blue mackerel (*Scomber australasicus*) is a small- to medium-sized schooling teleost inhabiting epi- and mesopelagic waters throughout the Indo-Pacific including the northern half of the New Zealand Exclusive Economic Zone (EEZ), where it supports moderate volume commercial fisheries. Blue mackerel was introduced into the New Zealand Quota Management System (QMS) at the start of the 2002–03 fishing year and is managed as five separate Quota Management Areas (QMAs): EMA 1–3, 7, & 10 (Figure 1).

The total reported commercial blue mackerel catch in the New Zealand EEZ has ranged from 6700 to 12 700 t in each of the previous five fishing years (2003–04 to 2008–09). The largest and most consistent catches over all fishing years are taken in a target purse-seine fishery in the Bay of Plenty (EMA 1) and as bycatch in a midwater-trawl fishery for jack mackerels (*Trachurus* spp.) in the Taranaki Bight (EMA 7).

Little is known about the status of New Zealand's blue mackerel stocks. No estimates of current or reference biomass or yield are available and it is not known whether recent catches are sustainable or at levels that will allow the stocks to move towards sizes that will support their Maximum Sustainable Yields (Ministry of Fisheries 2010).

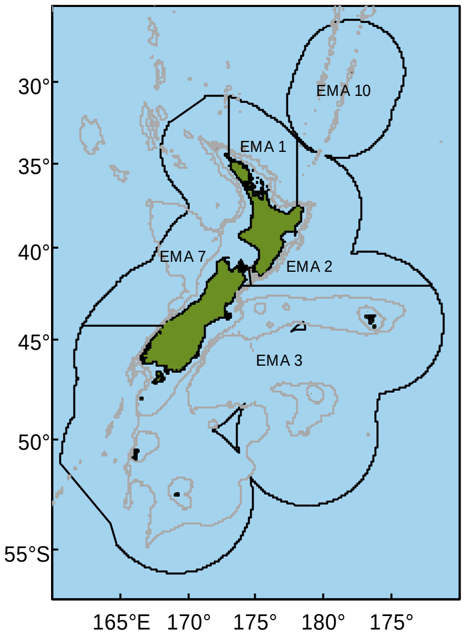


Figure 1: Map of the New Zealand EEZ showing the boundaries of blue mackerel fishstocks during the 2003–04 fishing year. The 250 m and 1000 m isobaths are overlaid in grey.

Manning et al. (2006, 2007) presented the results of catch sampling in EMA 1 and 7 during the 2002–03 and 2003–04 fishing years. In both studies, they found that although blue mackerel otoliths are difficult to interpret, between-reader precision (a between-reader mean coefficient of variation, c.v., of about 14.5%) compared favourably with studies of other species with difficult to interpret otoliths such as cardinalfish (between-reader mean c.v. = 16.7%; Tracey et al. 2000) and giant stargazer (between-reader mean c.v. = 12.4%, Manning & Sutton 2004). Nevertheless, age estimation error may reduce our ability to identify individual year classes in the blue mackerel catch. Furthermore, Manning et al. (2006, 2007) also found some evidence of a slight between-reader difference in interpretation of otoliths from older fish, and the age estimation method they used is unvalidated. Although, Morrison et al. (2001) presented an analysis of data collected from the EMA 1 fishery during the 1997–98 fishing year, they did not carry out any kind of reader accuracy and precision evaluation, and their results cannot be compared with the two more recent studies.

The overall objective of this project was to investigate the effects of ageing error on commercial catch-at-age estimates before a proposed stock assessment. However, this project addresses two other important issues associated with blue mackerel age estimation; improving between-reader precision, and reducing between-reader differences in interpretation of blue mackerel otoliths, and validating the age estimation method used. This project therefore has three specific objectives: (1) to investigate the effect of ageing error on the development of catch-at-age from the blue mackerel catch sampling programme for the stock assessment (to be reported separately); (2) to review and refine the method used for blue mackerel age estimation; and (3) to validate blue mackerel age estimates (reported separately).

2. REVIEWING AND REFINING THE AGEING METHOD

2.1 Terminology

The terminology we use follows the glossary for otolith studies produced by Kalish et al. (1995). We use the terms “opaque” and “translucent” to refer to presumed winter slow growth and summer fast growth zones respectively. A single year’s growth, an “annulus”, is composed of a single completed opaque zone followed by a single completed translucent zone.

2.2 Introduction

The method used to estimate blue mackerel ages in New Zealand involves counting fully formed opaque zones (sensu Kalish et al. 1995) present in blue mackerel sagittal otolith thin sections viewed under transmitted light. The New Zealand method was first presented by Morrison et al. (2001) and was also used by Manning et al. (2006, 2007). In both of their studies, Manning et al. converted opaque-zone counts to decimalised age estimates using a simple algorithm; Morrison et al. estimated age as integers from the zone counts they obtained.

Any imprecision in the age estimation method will reduce the ability to identify individual year classes in the blue mackerel catch-at-age. Furthermore, studies by Manning et al. (2006, 2007) found some evidence of between-reader differences in interpretation of otoliths from older fish (Figure 2).

Manning et al. (2006, 2007) attributed much of the imprecision and the apparent between-reader differences in interpretation to inherent features of blue mackerel otoliths (i.e., the correct identification of each true, fully formed opaque zone present in the otolith section). These include (a) the relatively diffuse nature of many early opaque zones; (b) the generally poor contrast between successive opaque and translucent zones; (c) the generally large number of presumably false opaque zones present; and (d) the natural interpretative differences that arise between readers due to the presence of these features.

The aim of this specific objective was to review and refine the method used to estimate blue mackerel ages in New Zealand, including both otolith preparation and interpretation, thus improving between-reader precision and eliminating apparent between-reader differences in interpretation, as well as providing for temporal consistency.

2.3 Reviewing and refining the New Zealand method

We itemised and reviewed all steps associated with collection and preparation of blue mackerel otoliths. This was to identify whether each step in the collection and preparation process is necessary or could be improved. Our aim was to produce a collection and preparation protocol that allows the highest quality otolith sections to be produced cost-effectively.

Blue mackerel otoliths are extremely small and fragile. Over the preceding few years a best practice protocol had been developed with this species' otoliths specifically in mind. On review, this protocol was found to be thorough and robust, with a number of steps in place to enhance the quality of subsequent sections made from these small fragile otoliths. This protocol has now been fully documented for the benefit and standardisation of future research on this species (Appendix A). This protocol is also applicable to most other small fragile otolith collections and thin section preparations.

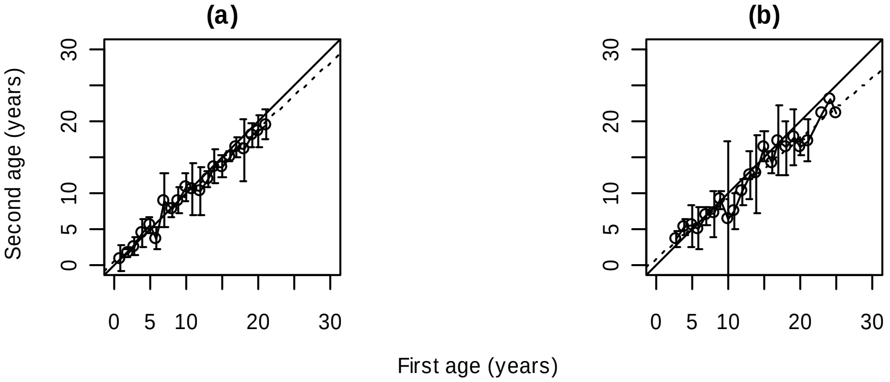


Figure 2: Some results of the between-reader comparison tests carried out by (a) Manning et al. (2006); and (b) Manning (unpublished data). The expected one-to-one (solid line) and actual linear relationship (dashed line) between the mean age assigned by the second reader for a given age assigned by the first reader are overlaid for comparison. The vertical bars are 95% confidence intervals.

We itemised and reviewed all steps in the interpretation of blue mackerel otolith sections. This was to identify what features present in New Zealand otoliths lead to differences in interpretation and how to overcome these. These included the four points discussed in the introduction, but our approach was sequential, e.g., how to identify the location of the first, true, fully formed opaque zone present in a blue mackerel otolith section, how to interpret subsequent opaque zones, and finally how to interpret the outermost, or marginal, fully formed opaque zone.

2.3.1 Quantifying the position of the first and subsequent opaque zones

This involved measuring the radius from the centre of the otolith nucleus to the midpoint of each subsequent opaque zone on a large number of otoliths using digital micrometry. The mean position of each opaque zone was defined and used as a guide in subsequent readings. We applied this to all the otoliths in the extended protocol set (see next point).

2.3.2 Extending the blue mackerel reference set lodged in the MFish otolith collection

This included identifying new specimens for inclusion in the protocol set, digitising these, and producing a between-reader agreed interpretation for each specimen in the extended set. A range of otoliths from those that are easy to those that are hard to interpret was selected for inclusion, but particular attention was given to selecting otoliths that illustrate those features that make interpretation difficult (Figure 3). The extended, digitised protocol set will be the main tool used in the future to illustrate our method for interpreting blue mackerel otolith sections (e.g., for training new readers, monitoring reader performance over time, etc.).

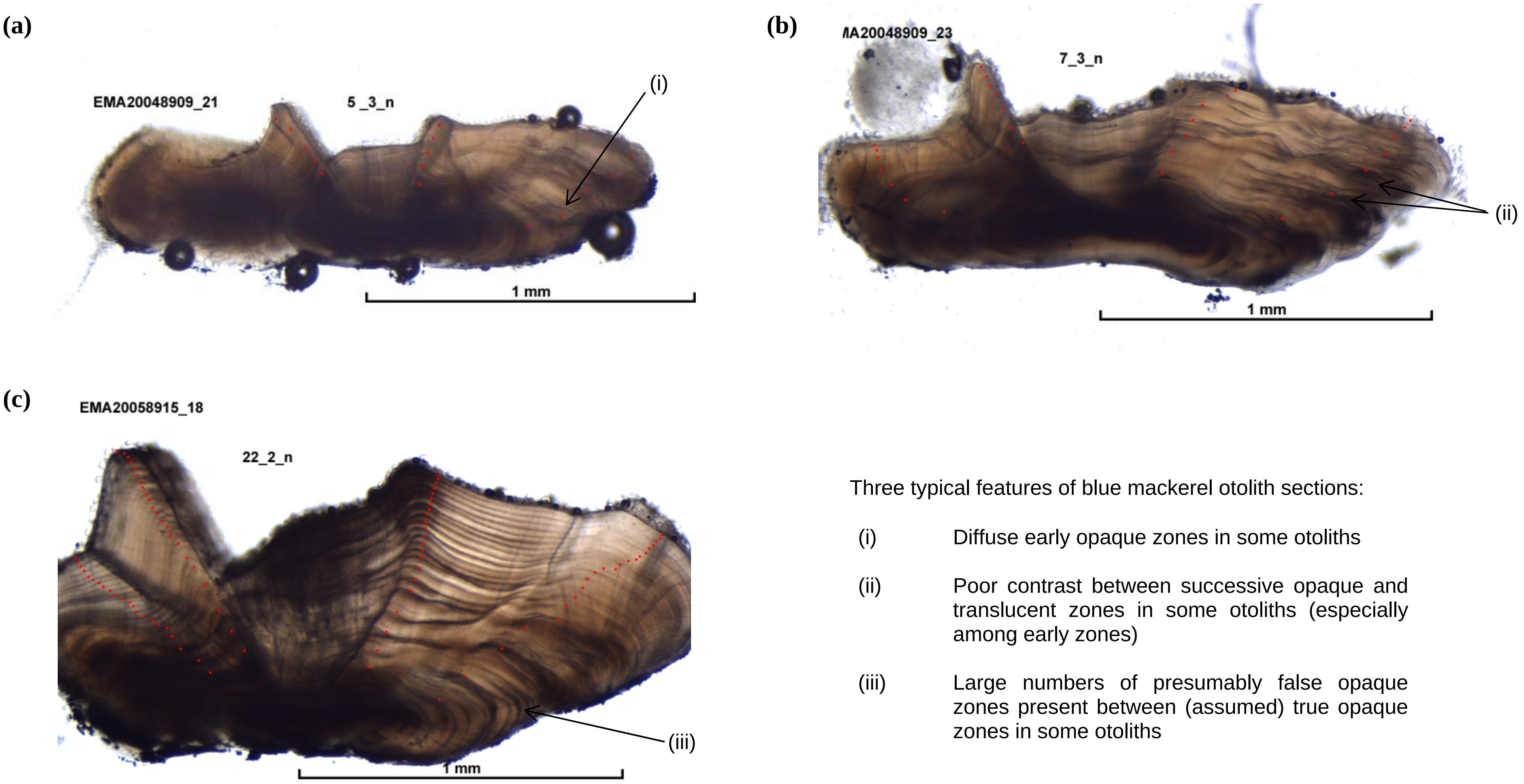
2.3.3 Protocol documentation

We produced a thorough written protocol covering otolith interpretation of blue mackerel (Appendix B). Our aim was to supplement descriptions of the New Zealand method already presented in the scientific literature (e.g., Morrison et al. 2001, Manning et al. 2006, 2007) with a comprehensive, illustrated guide. We intend the written protocol to be a living document.

Before the start of this study, the protocol set held in the MFish otolith collection consisted of 25 otoliths. We have expanded the protocol set to 100 otoliths. For each otolith in the revised protocol set, we quantified the first three opaque zone radii. The means of these first three opaque zone radii now form part of the interpretation protocol they are used as a guide to the placement of the first three opaque zones in all subsequent readings of blue mackerel otoliths.

The images of the 100 otolith sections in the protocol set were marked in Adobe Photoshop with a single, agreed interpretation to show each annual zone (see Figure 3) for examples. The layers function in Adobe Photoshop was used so that the marked zones layer can be turned on and off. This way new readers, and experienced readers who are refreshing their interpretation of blue mackerel otoliths, can make their own interpretations on unmarked images then, by switching on the marked layer, check their interpretation against the agreed interpretation.

This extended blue mackerel otolith protocol set with the added tools of the first three opaque zone mean measurements and layered marked otolith interpretation images will go a long way to reducing within and between reader error and reader drift error.



Three typical features of blue mackerel otolith sections:

(i) Diffuse early opaque zones in some otoliths

(ii) Poor contrast between successive opaque and translucent zones in some otoliths (especially among early zones)

(iii) Large numbers of presumably false opaque zones present between (assumed) true opaque zones in some otoliths

Figure 3: Images of three otolith sections from the revised protocol set illustrating three typical features of blue mackerel otolith sections: (a) an otolith collected from a 35 cm male with an agreed reading of five opaque zones (diffuse early opaque zones); (b) an otolith collected from a 40 cm male with an agreed reading of seven opaque zones (poor contrast between successive opaque and translucent zones); and (c) an otolith collected from a 41 cm female with an agreed reading of 22 opaque zones (large numbers of presumably false opaque zones present). Agreed true opaque zones are indicated by red dots in all images. All lengths are fork lengths.

2.4 Converting opaque-zone counts to age estimates

Zone counts are routinely converted to decimalised age estimates for subsequent data analysis. Following Manning et al. (2006), opaque-zone counts are converted to estimated ages by treating estimated fish age as the sum of three time components. The estimated age of the *i*th fish, $\hat{a}_i$, is

$$\hat{a}_i = t_{i,1} + t_{i,2} + t_{i,3}$$

where $t_{i,\,1}$ is the elapsed time from spawning to the end of the first opaque zone present, $t_{i,\,2}$ is the elapsed time from the end of the first opaque zone present to the end of the outermost fully formed opaque zone, and $t_{i,\,3}$ is the elapsed time from the end of the outermost fully formed opaque zone to the date when the *i*th fish was captured. Hence,

$$\begin{aligned} t_{i,1} &= t_{i,\text{ end first opaque zone}} - t_{i,\text{ spawning date}} \\ t_{i,2} &= (n_i + w) - 1 \\ t_{i,3} &= t_{i,\text{ end last opaque zone}} \end{aligned}$$

where n_i is the total number of opaque zones present for fish *i*, and *w* is an edge interpretation correction after Francis et al. (1992) applied to n_i: $w = 1$ if the recorded margin state = “wide” *and* fish *i* was collected *after* the date when opaque zones are assumed to be fully formed; $w = -1$ if the recorded margin state = “narrow” *and* fish *i* was collected *before* the date when opaque zones are assumed to be fully formed, otherwise $w = 0$.

For New Zealand blue mackerel, a standardised “birth-date” of 1 January and a standardised opaque zone completion date of 1 November are used for all fish. Stewart et al. (1999) found that opaque zones in Australian blue mackerel, although formed during winter, were not always visible until spring or summer on the edge of the otolith. The matching landing date is substituted for the capture date of each fish. Using this method, a fish with four completed opaque zones counted, a “narrow” otolith margin recorded, and caught during a fishing trip that was landed on 19 November 2003, would be estimated to be 3.88 years of age.

2.5 Testing the validity of our reader protocols

To test the within– and between–reader variability a test set of just over 100 prepared otoliths was selected for comparison tests. As this was a trial to specifically test the validity of our revised reader protocols, an honest attempt was made to ascribe an age estimate to every otolith, even those classed with a readability score of 5 (i.e., those that are considered routinely unreadable).

The primary reader made two independent readings of each otolith; the second reading made a few days after the first. The second reader read the entire dataset once. As three of the otoliths read by the first reader were deemed unreadable by the second, these three otoliths were excluded from the analysis, resulting in 111 valid between reader comparisons.

Age bias plots have been shown to be better at detecting bias than other commonly used techniques (Campana et al. 1995). We use age bias plots to assess whether there is any evidence of between– and within–reader bias. Vertical lines are 95% confidence intervals for the mean age by the “y” reader for all otoliths aged to be x by the “x” reader. The points on the graphs which have large 95% confidence intervals all had few comparison observations at that given age. Plots show little evidence of ageing bias (Figure 4).

We then wanted to test the degree of precision of the age estimates. Campana (2001) advocated using the

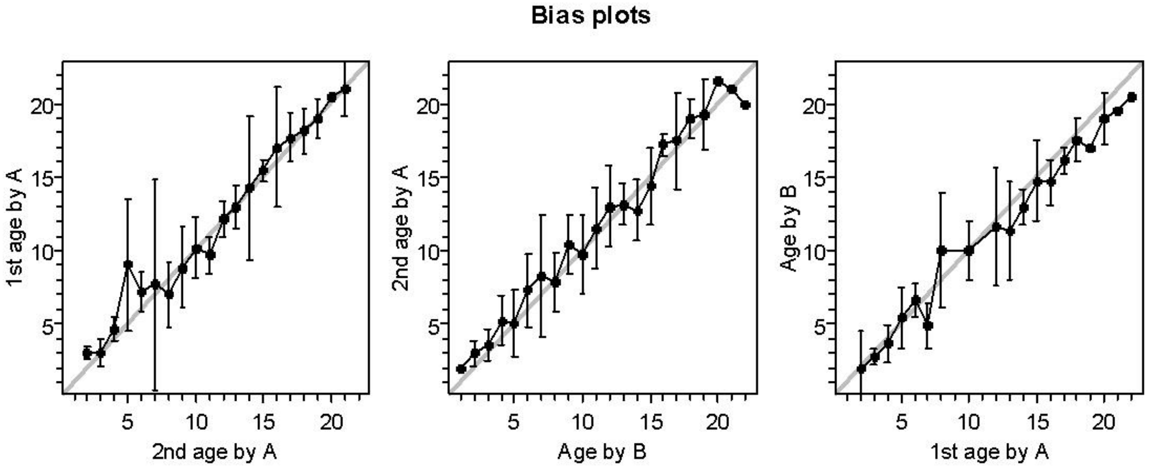


Figure 4: **Results of the within and between-reader comparison tests. The observed reading comparisons are the black line, the vertical bars are 95% confidence intervals, and the expected one-to-one comparisons are the background grey line.**

mean coefficient of variation as a measure of imprecision of ageing. We determined the overall mean c.v. for the three ageings and also the mean c.v. for the two ageings by reader A (Table 1). The index of average percent error (IAPE), which has often been used as a measure of ageing imprecision, has been included for comparison.

The 111 otoliths (with ages that have no missing values) were split into two groups representing the easier and hard to read otoliths. The split was done by placing otoliths for which all three ageings had an ease of reading index less than or equal to 3. The rest of the otoliths (any otolith for which at least one ease of index was 4 or 5) were placed in the hard to read group. The groups comprised 53 and 58 otoliths respectively. The measures of imprecision were calculated for each group (Table 1). Not surprisingly, all imprecision measures were larger for the hard to read group. To determine if the difference is significant, a test of the null hypothesis that the mean c.v.s for both groups are the same against the one-sided alternative that the mean c.v. of the harder to read group is larger was carried out. To do this a permutation test (Edgington 1995) using the ratio of the mean c.v. for the hard group to the mean c.v. of the easy group as the test statistic was carried out. In the permutation test the c.v. of the three readings for each otolith was calculated.

Under the null hypothesis these c.v.s are a sample from a distribution with a common c.v. To obtain the p-value associated the one-tailed test of the hypotheses, 10,000 permutations of the c.v.s were drawn with 53 otoliths assigned at random to the easy group and the remainder to the hard group. The value of the ratio of mean c.v.s is then calculated giving a sample of 10,000 values of the test statistic from the null permutation distribution (Figure 5). The p-value is 0.0286, which is the proportion of the sampled values that exceed the observed value of the ratio of mean c.v.s, which is 1.38. Therefore, the larger imprecision of the hard to read group compared with the easy to read group is significant at the 5% level but not at the 1% level.

Table 1: Coefficients of variance, IAPE scores and permutation test results.

	All otoliths	Easy to read	Hard to read
Number of otoliths	111	53	58
Mean cv Both readers(%)	13.4	11.2	15.4
Mean cv for Reader A (%)	11.2	9.0	13.2
IAPE (%)	9.9	8.3	11.4

Permutation test (one-sided) 10000 permutations

	Easy to read	Hard to read	Ratio of mean c.v.'s	p-value
Mean cv (%)	11.16	15.40	1.3803	0.0286

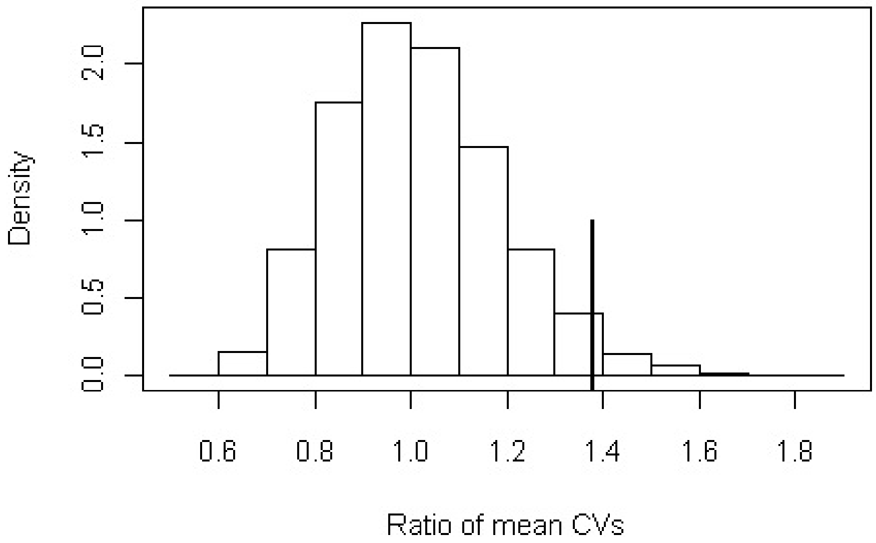


Figure 5: **The null permutation distribution for the ratio of mean c.v.s . The vertical black bar is the observed ratio of c.v.s.**

These tests show that there was no observable bias within or between the two readers' observations. The results of the precision tests show that in order to minimise the c.v. of any age data generated, all observations with a readability score of 4 (difficult, possibly more than two zones out) or 5; (unreadable) should be discarded in our test case this produced a mean c.v. of 11.2% for the three readings. Even if all observation data are retained, we suggest that the mean c.v., in this case 13.4% for three readings, is acceptable given the nature of this difficult to read species. In routine ageing studies, otoliths with a readability score of 5 are considered unreadable, so no age estimate would be given to them. This would have the effect of further reducing the total c.v.

3. CONCLUSIONS

Revising the collection, preparation, and interpretation techniques has been a valuable process. The otolith collection and preparation techniques were found to be robust and appropriate for blue mackerel. They have now been accurately documented to ensure high quality and standardisation in future work.

We extended the blue mackerel otolith protocol set, took images of them, and produced additional image layers demarcating each annual zone for all 100 images.

We developed tools, such as mean measurements to the first three opaque zones and protocols for the identification of complete zones and reading techniques, to assist in the interpretation of otolith sections.

Otolith interpretation protocols and techniques were all thoroughly documented for the benefit of future ageing studies. This will go a long way to reducing within– and between–reader error and reader drift error.

Within and between reader comparison tests on readings made using the revised protocols showed no evidence of bias. Reader precision tests also showed that the overall percentage c.v. has reduced.

4. ACKNOWLEDGMENTS

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APPENDIX A: A protocol for preparing blue mackerel otoliths

Otolith storage

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

Marking otoliths

Mark the sectioning plane on 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.

Embedding otoliths

Embed otoliths in blocks of clear epoxy resin (Araldite K142), ratio 5:1 resin to hardener, and cure at 50 °C 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. Moulds are prepared with an initial layer of resin 1–2mm thick, so that when embedded otoliths sit off the bottom surface of the block. Otoliths are placed 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. To prepare the resin, heat it to 50°C 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 use reusable latex moulds each with 10 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 mounting.

Calibrating the saw

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

Twin blades are mounted on the axle with spacers to achieve the desired section thickness. The spacers need to be the same diameter as the mounting plates which sit on the outside of the blades, so that the entire set-up is held rigid. The spacers need to be cut from non-compressible material so the distance between the blades remains constant. An array of spacers of various thicknesses 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 before any otoliths are 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 280–300 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.

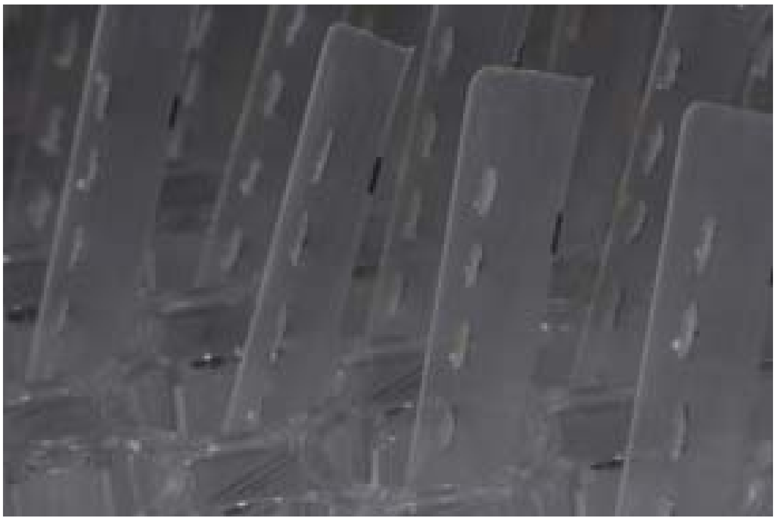
Section blocks at a slow regular speed to ensure even cutting. If one end of the cut wafer is a different thickness from the other end, slow down the advance speed of the block into the saw as this may produce a more regular section. Using clean cutting lubricant should also help to ensure clean regular 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 will 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 before 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 on to 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 on to 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 on the otolith when tamping down the wafer on to 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, giving 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 B: Interpretation of blue mackerel (EMA) thin sections

Reading protocol

First, generally view the entire section under a lowish magnification. At this stage you are trying to get an overall impression of the otolith. Assess which axes are possible to generate zone counts along, and whether discrete zones can be observed along the entire axis. Generally the axis just to the dorsal side of the sulcus (Figure B1) will show the clearest zone structure throughout its length from the primordium to the otolith margin. Other useful axes are along the dorsal aspect, usually just on the sulcal side, and axes on the ventral side of the sulcus (Figure B1). The distal aspect of the otolith generally exhibits poor zone structure as very little material accretes on this aspect as the otolith grows.

At this stage you also want to assess roughly how old the otolith is (juvenile, adolescent, or old), as this will help with later interpretation of the zone structure in difficult to read fish where there is a high degree of split or poorly resolved zones.

Split zones generally appear as two distinct zones in some regions of the otolith, but in closely adjacent areas they converge into a single zone. This splitting and re-converging of zones should be observable in regions of the otolith where single zones would normally be clearly viewed for it to be classified as a split zone. False checks are usually portrayed as one or more additional bands within a zone. They can be very difficult to differentiate from the band that is determined as being the edge of the zone. Commonly they are evident in only a small region of the otolith and if you follow them around the otolith they quickly disappear. Sometimes they can be viewed across large regions of an otolith. They are often less coloured and not as strongly contrasting as the band that is determined as being the edge of the zone. As a guide when trying to differentiate split bands and false checks from ‘real’ zones take into consideration the width of the adjacent zones. In most cases zone width will decrease reasonably regularly from primordium to edge. There are always exceptions to the above.

The observed zone structure in an otolith will generally go through three phases of growth; these phases are not usually discrete but tend to merge from one to the next. First, the juvenile growth years up to zone three which are characterised by wide zones with many split zones (Figure B3), false checks (Figure B4), and varied morphology. These zones are often not very clearly defined and show poor contrast between the opaque and translucent bands. View the otolith at about x80 magnification and use the measurements from Figure B2 and Table B1 to ‘guide’ your placement of the first three annual zones. It is important that the measurements are only used as a guide as this species has a long spawning season. Consequently juveniles will be exhibiting a highly variable amount of growth in that first year, and this will mean that the radii of the juvenile zones will also be quite variable. This is confounded by the variable morphology exhibited by the otoliths; some otoliths exhibit squat growth in the dorso-ventral plane and others are much wider across this plane.

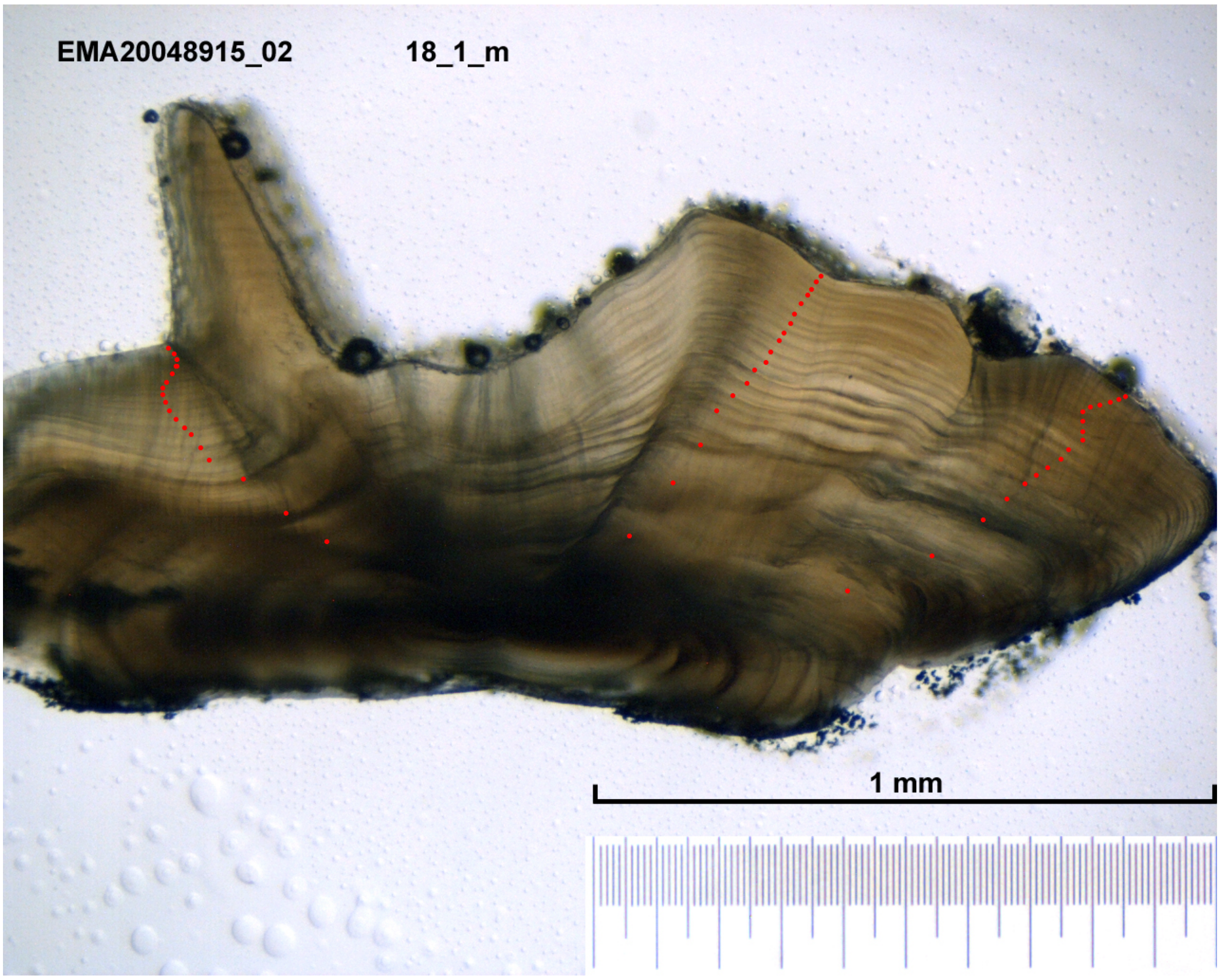


Figure B1: Example of an old, clear to read otolith: red dots mark the observed annual zones on various axes. The primary axis for reading is the dorsal side of the sulcus (in this case the middle chain of dots). This individual shows 18 fully formed annual zones, was given a readability of 2, and exhibited a marginal zone of medium thickness.

The zones to the transition, approximately zone 8, tend to exhibit much splitting and false checks though they are usually more regular than the juvenile zones. The contrast between the dark and light bands in these zones is usually quite clear.

The zones from the transition to the otolith margin edge are often the easiest to resolve. They generally show good contrast between the dark and light bands. The zones are usually closely and regularly spaced, getting closer as you progress towards the edge. Clear examples have discrete obvious zones, but they can also exhibit many split zones and false checks.

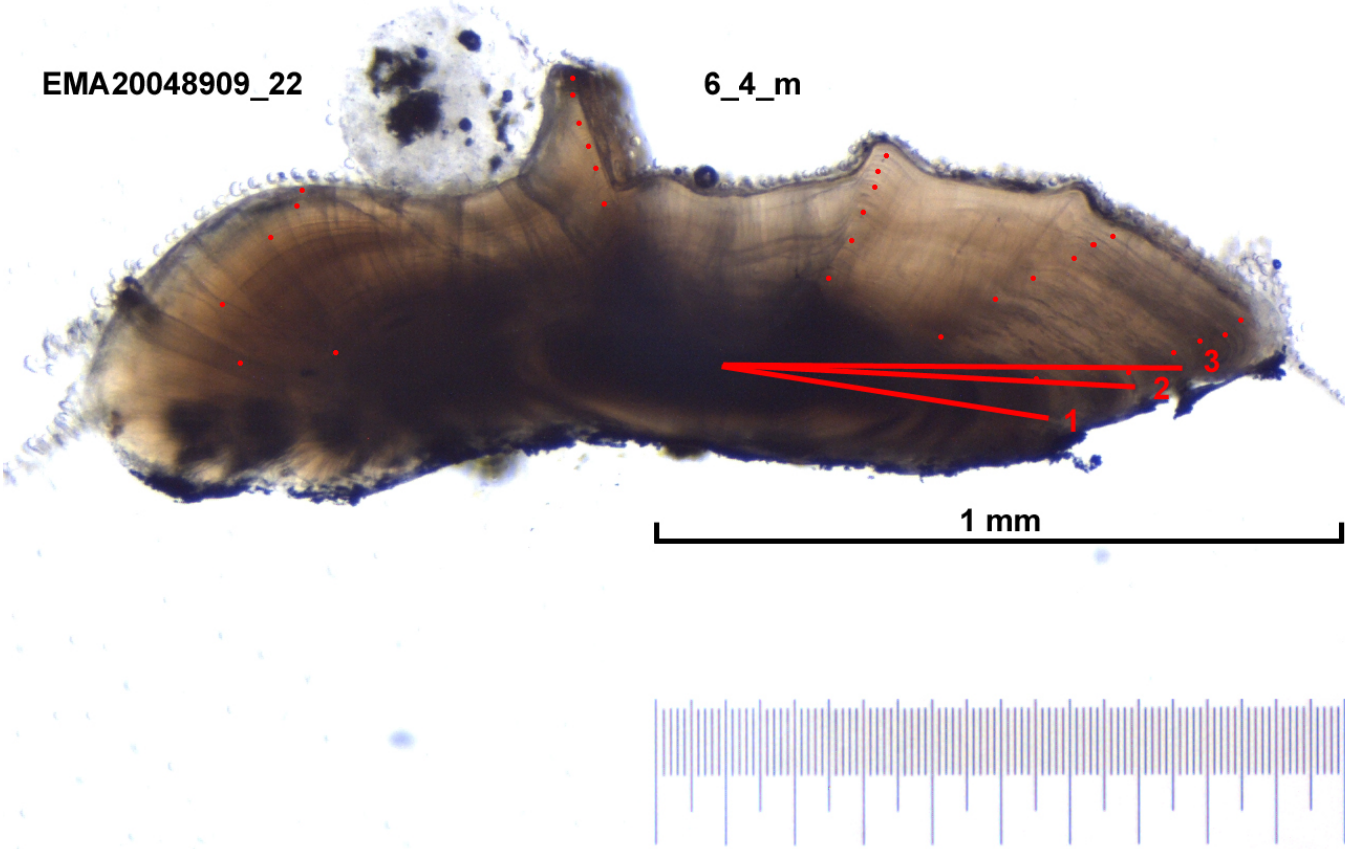


Figure B2: Measuring the first three zones. On the section surfaces the radii were measured from the primordium to the greatest extent of the zone along the dorsal aspect of the otolith.

Table B1. Mean measurements of the radius of the first three zones, from the primordium to the greatest extent of the dorsal aspect.

Zone 1 (mm)	Zone 2 (mm)	Zone 3 (mm)
0.49	0.63	0.74

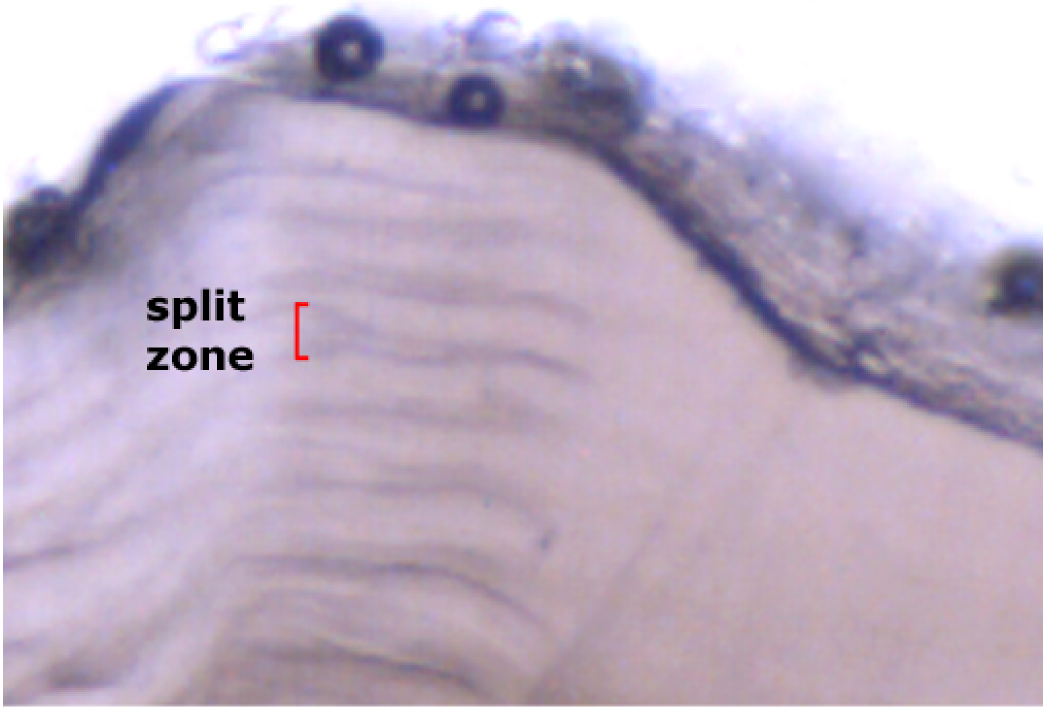


Figure B3: Split zone. Bracket shows a split zone that reconverges into a single zone.

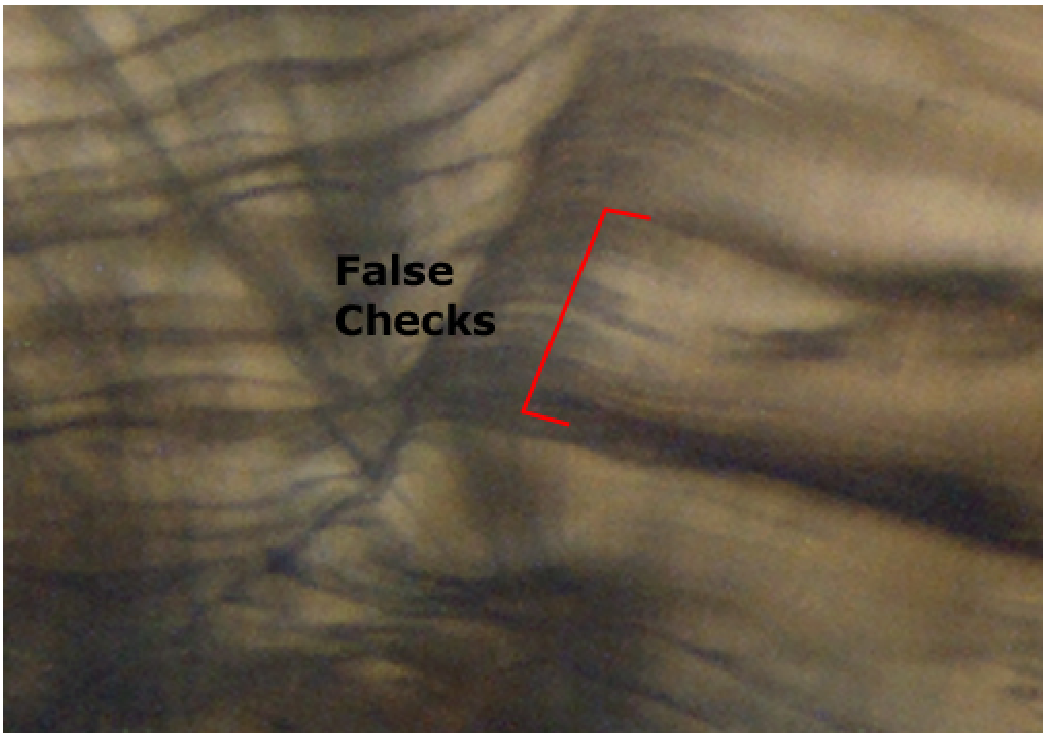


Figure B4: False checks. Bracket shows a zone that contains many false checks.

The primary axis should be read more than once to ensure a consistent interpretation of the zones observed. Attempt to read secondary axes, or at least portions of them, to gain supporting evidence for the counts achieved on the primary axis. This also gives weight to our confidence estimate of the zones counted (i.e., readability scale, see below)

If you cannot count discrete zones along the entire length of a growth axis you can count partway out along an axis of growth then shift out to an adjacent axis and continue counting where the zone structure is clearer. But you have to be very sure when shifting sideways that you are still following the same zone around the otolith.

Once you have agreed on a zone count for an otolith you then give the reading a confidence estimate. This is an arbitrary scale that estimates what you think the reliability or accuracy of your zone count is for each individual otolith.

Readability scale

1 = zones very clear (the reader had a high level of confidence in their zone count);
2 = zones relatively clear (the reader may be up to 1 zone out);
3 = zones average in clarity (the reader may be up to 2 zones out);
4 = zones relatively unclear (the reader was not confident, possibly more than 2 zones out);
5 = zones unreadable.

Marginal state

Finally, for each otolith the marginal state is classified. This is an estimate of the relative width of the marginal zone. The distance from the last visible opaque zone to the otolith edge is classified as either narrow (N), medium (M), or wide (W), based on the relative distance between the two outer most opaque zones. This observation is used in the conversion of zone counts to estimated ages.

Summary information

Additional information that should be recorded is the reader’s name and date of reading. Table B2 below shows an excerpt from some blue mackerel age data. It is important that all age estimates are made ‘blind’, that is, there was no prior knowledge of the fishes sex, length, weight, or other readers’ zone count estimates.

Table B2. Sample of age data generated for blue mackerel otoliths.

block_no	origin	year	trip_code	sample_no	area	fish_no	lgth	sex	Age	Reada	Edge	Reader	Date read
1	SMP	2004	20046551	1	CEE	1	51	1	19	3	w	Marriott	21/10/2005
2	SMP	2004	20046801	1	AKE	1	48	2	14	3	n	Marriott	21/10/2005
	SMP	2004	20046801	1	AKE	2	45	1	14	3	n	Marriott	21/10/2005
	SMP	2004	20046801	1	AKE	3	50	1	13	2	w	Marriott	21/10/2005
	SMP	2004	20046801	1	AKE	4	44	2	12	3	n	Marriott	21/10/2005
	SMP	2004	20046801	1	AKE	5	46	1	13	2	w	Marriott	21/10/2005
3	SMP	2004	20046801	1	AKE	6	49	1	12	3	m	Marriott	21/10/2005
	SMP	2004	20046801	1	AKE	7	44	2	13	3	n	Marriott	21/10/2005
	SMP	2004	20046801	1	AKE	8	49	2	12	3	m	Marriott	21/10/2005
	SMP	2004	20046801	1	AKE	9	46	1	9	3	w	Marriott	21/10/2005