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Age determination protocols for jack mackerels (*Trachurus* spp.) in New Zealand waters

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P.L. Horn,
C. Ó Maolagáin

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This report documents the age determination protocols for a group of important New Zealand middle-depth finfish species: jack mackerels (*Trachurus novaezelandiae*, *T. declivis*, and *T. murphyi*). It describes the most recent scientific methodologies used for otolith preparation and interpretation, ageing procedures, and the changes in these methodologies over time. Digital image examples of otolith preparations are presented and fully illustrate the zone interpretation used in determining fish age for the three jack mackerel species. Associated difficulties and idiosyncrasies related to ageing prepared otoliths are also described.

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 Fisheries New Zealand) held a fish ageing workshop in Wellington in May 2011 and produced a document "Guideline for the development of fish age determination protocols" based on the workshops results (Ministry of Fisheries Science Group 2011). From this, it was anticipated that age determination protocols would be developed for each species that was routinely aged under Fisheries New Zealand fisheries research projects.

This report describes the age determination protocol for a suite of important New Zealand middle-depth finfish species from the genus *Trachurus* (jack mackerels): *T. declivis*, *T. novaezelandiae*, and *T. murphyi* (Figure 1). The New Zealand jack mackerel fishery has produced landings of 21 000–50 000 t annually since 1987 (and more than 40 000 t annually since 2005). In most years, over 75% of the jack mackerel landings are taken from Fishstock JMA 7, comprising Fishery Management Areas (FMAs) 7, 8, and 9 off the west coasts of North Island and South Island. Stock assessments for jack mackerel are complicated by the reporting and management of three species under a single code. However, estimates of commercial catch-at-age are available, separately by species, for the JMA 7 catch annually since 2006–07. The purpose of the protocol reported here is to provide a practical guide for ageing, and to describe the methodologies and techniques used by National Institute of Water and Atmospheric Research (NIWA) otolith readers to prepare, interpret, and read otoliths, and to convert zone counts into estimates of fish age. The current best methods are documented to ensure that future consistency and accuracy for age estimates of jack mackerels are maintained over time. It will also serve as a valuable training tool for new otolith readers.

No attempt was made to describe protocols related to daily increments in jack mackerel otoliths (usually associated with ageing larval or juvenile fish), investigations into otolith ultrastructure, and chemical composition of otoliths, because these were outside the scope of the current project. It is known, however, that daily zones are discernible in the otoliths of *T. declivis* (Jordan 1994), *T. novaezelandiae* (Stewart et al. 1998), and *T. murphyi* (Cerna et al. 2017). Three otolith pairs are present in the otic capsule of bony fishes, i.e., asteriscii, lapilli, and sagittae, but only the sagitta is usually used in age estimation (Panfili et al. 2002) and is used to age jack mackerels. Therefore, throughout this report, the use of 'otolith' will be synonymous with the sagittal otolith (Figure 1). A glossary describing otolith terminologies and ageing definitions outlined in the "Guideline for the development of fish age determination protocols" is given in Appendix 1.

Figure 1: Jack mackerels (*Trachurus* species): whole fish and distal surfaces of sagittal otoliths. *T. declivis* (top row), *T. novaezelandiae* (middle row), *T. murphyi* (bottom row). The otoliths were from fish of fork lengths 42 cm, 39 cm, and 44 cm, respectively.

2. AGE DETERMINATION PROTOCOLS FOR JACK MACKERELS

2.1 Background

Jack mackerels (genus *Trachurus*) are widespread throughout the world's oceans and are a significant fishery resource in many areas with recent annual landings world-wide being about a million tonnes (FAO 2019). However, the various species exhibit widely differing growth rates, and maximum sizes and ages (Froese & Pauly 2019). *Trachurus declivis* and *T. novaezelandiae* have clearly been present in New Zealand waters for many years (Stephenson & Robertson 1977) in contrast to *T. murphyi* which was first reported there in the mid-1980s (Kawahara et al. 1988).

Horn (1993) produced the first comprehensive report on the growth and productivity of *T. declivis* and *T. novaezelandiae* in New Zealand waters; previous information had been limited to some observations by Robertson (1978) on maximum age and length. Several growth studies of *T. declivis* from Australian waters were available in the early 1990s (Webb & Grant 1979, Gasior & Kompowski 1982, Stevens & Hausfeld 1982, Stevens et al. 1984). The data presented by those authors needed to be treated with caution, however, because many of the readings were made on whole otoliths, a technique not recommended for *Trachurus* species because in older fish, otolith growth tends to thicken rather than lengthen the otolith, which can result in the underestimation of true ring number (Eltink & Kuiter 1989, Lyle et al. 2000).

Horn (1993) examined transverse cross-sections of otoliths from *T. declivis* and *T. novaezelandiae*. Otoliths were broken transversely through the nucleus, the broken surface polished with a fine file, and heated near a flame or in an oven (275 °C for 10–20 min dependent on otolith size) until amber coloured. The sections were mounted in plasticine and coated with paraffin oil. Examination under a binocular microscope (×8–40), with illumination by reflected light just above the plane of the prepared surface, revealed a pattern of alternating dark and light zones. The number of complete rings (i.e., dark hyaline zones with lighter opaque material on both sides) on the otolith was counted. Oven-baking and flame-heating produced similar banding patterns in otolith pairs where the two heating methods were compared. Horn (1993) concluded that baking generally produced a clearer pattern of alternating opaque and hyaline zones, but the clarity varied considerably between species and otoliths. Otoliths of *T. declivis* were generally less clear than those of *T. novaezelandiae*, whereas otoliths of both species from the Bay of Plenty were generally clearer than those from the west coast of North Island (Horn 1993).

Horn (1993) used the progression of modes in length-frequency distributions to validate the growth of *T. declivis* up to age 4, and the progression of weak and strong year classes through estimated age distributions to about age 13 provided further support for the ageing method. Bomb radiocarbon analyses also supported the ageing method (Kalish et al. 2001). Validation of the ageing method for *T. novaezelandiae* was similarly achieved using the progression of modes in length-frequency distributions up to age 3, and the progression of weak and strong year classes up to age 15 (Horn 1993).

Subsequently, Lyle et al. (2000) compared age estimates using sister otolith pairs from Australian *T. declivis* where one was baked and cross-sectioned while the other was sectioned transversely to produce a 0.3 mm slice that was mounted on a microscope slide. Age estimates from the sister otoliths were found to be similar, so both preparation methods can be used for ageing this species. They also showed that counts from whole otoliths started to deviate from section counts after about 7 years. An ageing manual for *T. declivis* was produced as appendix 5 in Lyle et al. (2000).

The ageing of *T. novaezelandiae* caught off New South Wales was investigated by Stewart et al. (1998, 1999) using transverse thin sections taken from untreated otoliths. The sections were examined against a black background using illumination by reflected light. The estimated von Bertalanffy L_∞ for Australian fish (28 cm) was considerably smaller than those estimated by Horn (1993) for New Zealand *T. novaezelandiae* (35–40 cm). The otolith interpretation was similar between the two studies, however, and because many larger and older fish were found in the New Zealand relative to the Australian samples it was concluded that either they lived longer and grew larger in New Zealand, or, more likely, that the inshore fishery off New South Wales did not exploit the oldest, largest fish (Stewart et al. 1998). Validation of the annual formation of opaque zones in otoliths of *T. novaezelandiae* was also reported by Stewart et al. (1999). Fish were captured, marked with oxytetracycline, kept in captivity for one year, and then periodically sampled. Opaque otolith zones were formed during the year in fish aged 0 to 7 years. These marks were formed in winter, but did not become visible until early summer in some fish, particularly the older and slower-growing individuals.

Both *T. declivis* and *T. novaezelandiae* have a protracted spawning season extending in some years from October to March (Jones 1990). An arbitrary birthday of 1 January was selected for New Zealand jack mackerels, although it is possible for fish from the same age class to have been spawned at least 6 months apart. Horn (1993) examined otolith margins on cross-sections and found that it was often difficult to determine whether the margins for older fish were opaque or translucent. He concluded, however, that the opaque zone generally begins to form (or become visible) about early October, and hence, fish are on average 9 months old at the completion of the first translucent zone.

Stewart et al. (1999) concluded a similar formation time for the opaque zone in *T. novaezelandiae* from Australian waters.

Trachurus declivis and *T. novaezelandiae* have moderate life-spans, with fish older than 20 years being relatively uncommon in New Zealand (Horn et al. 2019). The maximum recorded age was 28 years for both species (Horn 1993). Most of the commercial and research catch comprises fish aged from about 2 to 15 years (Horn et al. 2019).

Investigations into the age and growth of *T. murphyi* in the south-eastern Pacific were first reported by Kaiser (1973) and Carrera & Collantes (1978), although both these studies examined whole otoliths. Little subsequent work was formally published before the 1990s, although some 'grey literature' reports were produced. Horn (1993) examined some *T. murphyi* otoliths from New Zealand waters and saw the need to examine transverse sections rather than whole otoliths, but found it difficult to achieve distinct annuli using the break and burn technique. Kochkin (1994) prepared thin sections of *T. murphyi* otoliths and studied their inner structure using polarised light, estimating a maximum age of 10 years. Alegría et al. (1995) examined whole and sectioned otoliths, investigated daily zones, summarised available data, and presented data to a maximum age of 14 years. Based on their analysis of otolith margin classification and daily zones they concluded that opaque zones were formed annually in otoliths. Subsequent investigations of presumed annual and daily growth zones produced a collection of markedly different growth zones for *T. murphyi* sampled off the south-eastern Pacific coast (Dioses et al. 2013), leading to a recommendation for an integrated approach to tackle the uncertainties in their ageing. A subsequent workshop identified that there were substantial differences in the otolith reading criteria adopted by different laboratories, and resulted in a documented protocol for interpretation of the otoliths (Troncoso et al. 2016).

Trachurus murphyi otoliths sampled from western New Zealand waters have been aged, using otolith thin sections, from each year since 2006–07 (Horn et al. 2019). The ageing methodology used was unvalidated, but was based on the interpretation of *T. declivis* otoliths. However, as the time series of annual aged samples grew, it became apparent that there was a clear progression of a group of year classes in the New Zealand population (Horn et al. 2019), and hence, that the interpretation method was very likely to be correctly interpreting annual zones. The maximum recorded age was 34 years and most of the commercial catch comprises fish aged from about 8 to 22 years (Horn et al. 2019).

2.2 Methods

Sagittal otoliths are the primary structure used for determining the age of jack mackerels. All scientific methodologies described in the following sections will be associated with age determination using transverse thin sections from untreated sagittal otoliths, currently the best practice preparation method. Although initial New Zealand investigations into ageing *Trachurus* species used the bake and cross-section method, and this method was found to produce satisfactory results, it was subsequently concluded that thin sections across untreated otoliths produced slightly better preparations in little extra time (author's observations). The methodology aimed to provide robust, permanent, ordered, and compact sets of aged otolith preparations. This method is used, occasionally with minor modifications, to prepare otoliths from a variety of deepwater, middle-depth, and inshore species. The following report sections present additional information pertinent to jack mackerel age determination.

2.3 Otolith preparation and examination

Post extraction, jack mackerel otoliths are cleaned of adhering tissue and blood, and stored in paper envelopes labelled with sample details, including trip code, station number (or landing number for market samples), fish number, date, and fish length and sex. Age determination studies should record sex as a mandatory requirement for each fish selected for analysis even though jack mackerels show no statistically significant differences in growth between the sexes (Horn 1993). The envelopes are stored in labelled box files relating to the year of collection and source of the otoliths (i.e., research surveys, commercial catch samples at sea, or on-shore samples of commercial landings), and are archived at NIWA, Wellington.

Whole otoliths selected for preparation are marked with a pencil line dorsoventrally across the nucleus to denote the required cross-section position (Figure 2). Sets of four otoliths are embedded in blocks using a Bisphenol A-based clear epoxy resin with an isophorone diamine-based hardener. Once cured at 50 °C, a transverse section ~380 µm thick is cut from each block through the primordia using a high-speed precision saw. The thin section is washed, dried, and embedded under a cover slip on a glass microscope slide, using the same epoxy resin above (Figure 3). Details identifying the species, sample year, and otolith number in the sample are written on each slide. Thin sections are read with a bright field microscope at up to ×100 magnification, with illumination by transmitted light. A pattern of bright (translucent) and darker (opaque) zones was apparent. If the zonation pattern is unclear, improvements can sometimes be achieved by slightly altering the plane of focus or by introducing contrast by differential interference techniques. Zone counts are based on the number of complete opaque zones (i.e., opaque zones with translucent material outside them), which are counted to provide data for age estimates. Subsequently in this document, 'zone' refers to the paired structure of one opaque zone outside one translucent zone. The number of opaque zones is counted. Fish length and sex are unknown to the otolith reader.

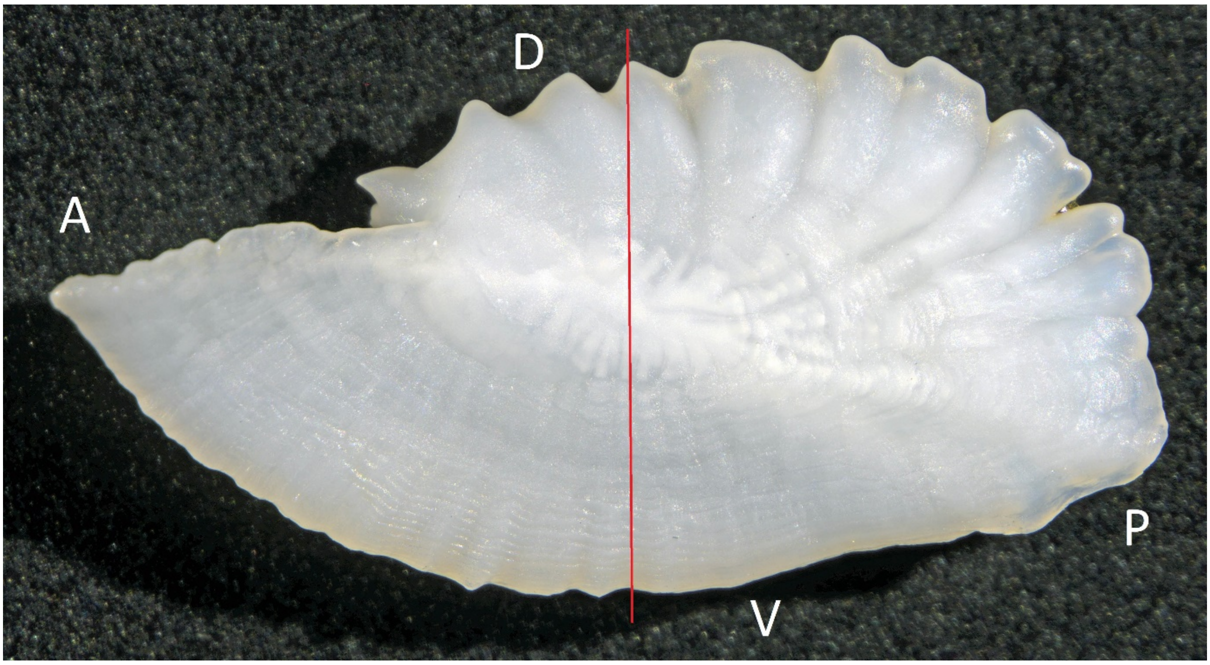

Figure 2: Untreated sagittal otolith (distal surface), with red line indicating the position of the cross-section. A, anterior; P, posterior; D, dorsal; V, ventral.

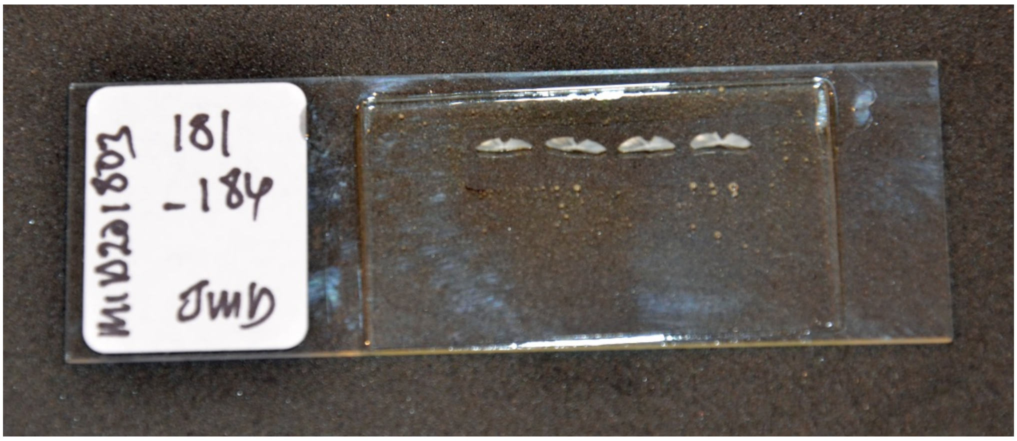

Figure 3: Microscope slide with a mounted resin block slice holding four *T. declivis* otolith thin sections.

2.4 General otolith interpretation

Otolith cross-sections show that initial growth occurs in width (dorsal-ventral) and depth (proximal-distal). At around 6–8 years of age for *T. declivis* and *T. murphyi*, and 5–6 years of age for *T. novaezelandiae*, the growth in width slows markedly, although growth in depth continues (Figure 4). This truncation of growth in width is why counts of zones made on whole otoliths will often underestimate the age of older fish. Little growth occurs on the distal side of the otolith at any age, so the primordium is always quite close to the distal surface.

The zonation pattern is generally clearest on the ventral side of the otolith cross-section (Figure 4), and most counts (and all measurements) are made in this area. The dorsal part of the cross-section is frequently examined, however, primarily as a check on the ventral count but sometimes to produce the actual count if the ventral section is unclear. Counts can be made in various locations of the ventral side: along the apex, adjacent to the sulcus, or in the central part of the section (Figure 4). Counts on the dorsal side are generally made relatively close to the sulcus.

In most otolith cross-sections of all three species a relatively large opaque area is apparent surrounding the primordium (Figure 4). The size of this area can vary markedly, probably owing to whether the fish was spawned early or late in the protracted spawning periods (Jones 1990), but also to how near the primordium the cross-section was made. [When four otoliths are set in a block for sectioning it is relatively easy for one to be out of alignment, resulting in a cut to one side of the primordium.] Outside that central opaque area, alternating translucent and opaque zones are visible. The first annual zone is usually separated from the central opaque area by an area that is translucent, or at least appears markedly lighter than the central area. The distance between successive opaque zones generally decreases, although this can become less apparent for zones laid down after about 10–15 years of age.

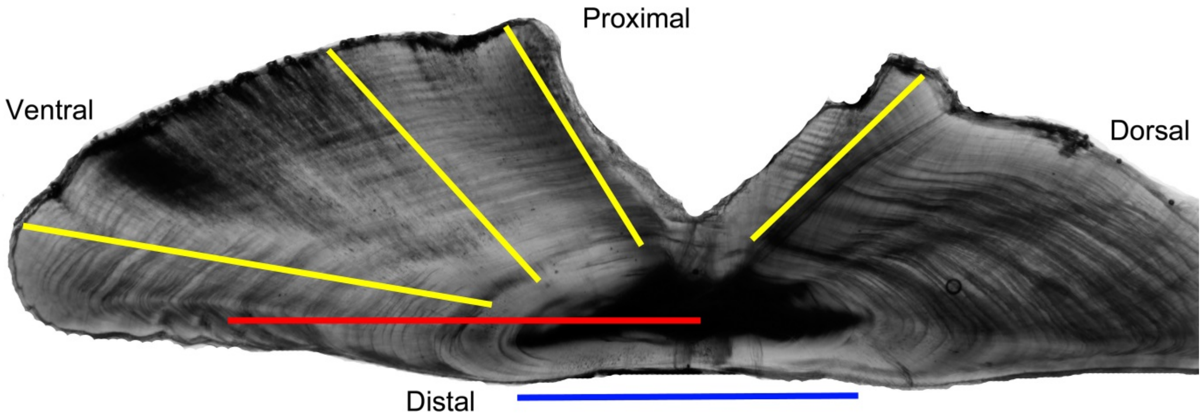

Figure 4: Jack mackerel (*Trachurus declivis*) otolith image (B&W) of a transverse section illuminated using transmitted light, illustrating otolith terminology. Radial measurements are made along the horizontal red line, and subsequent zone counts are usually made in the vicinity of one or more of the yellow lines. The blue lines indicate the approximate extent of a relatively large opaque zone that is usually apparent within the first annual zone.

The relative width of the otolith margin (i.e., the distance outside the last opaque zone as a proportion of the width of the last complete zone) is classified using a 3-stage scale: narrow, medium, or wide. Because of the often diffuse nature of the opaque zones and the very narrow width of zones in older fish, it is often difficult to confidently classify the margins. This information is, however, used to allocate an age to the fish: where the margin classification is narrow or medium, the age is taken to be the count of opaque zones; where the margin is wide, the age is the opaque zone count plus one. Some readers also provide an overall readability for an otolith section on a 5-stage scale where 1 is a certain count and 5 is unreadable.

Jack mackerel catches in New Zealand frequently comprise more than one species, and it is apparent that species identifications of some fish are incorrect (based on plotted age-length data where age estimates for individuals identified as *T. declivis* fall on the growth curve for *T. novaezelandiae*, or vice versa). It is likely that about 5% of jack mackerels in Observer samples are mis-identified to species (e.g., see Horn et al. 2019). Outwardly the otoliths from the three mackerel species appear quite similar, although those from *T. murphyi* are often more slender with a relatively longer rostrum than those of the other two species (see Figure 1). Transverse sections do, however, generally exhibit some differences between *T. declivis* and *T. novaezelandiae*. In *T. novaezelandiae* the ventral wall of the sulcal groove is steeply sloped, whereas the dorsal wall has a much flatter slope; in *T. declivis* the slopes of the walls are generally similar producing a more even ‘V’ shape (Figure 5). In addition, material is generally laid down in the sulcal groove of *T. novaezelandiae*, whereas the *T. declivis* groove is often unfilled (Figure 5). These characteristics can be used to help confirm the mis-identification of any fish with apparently incongruous age estimates.

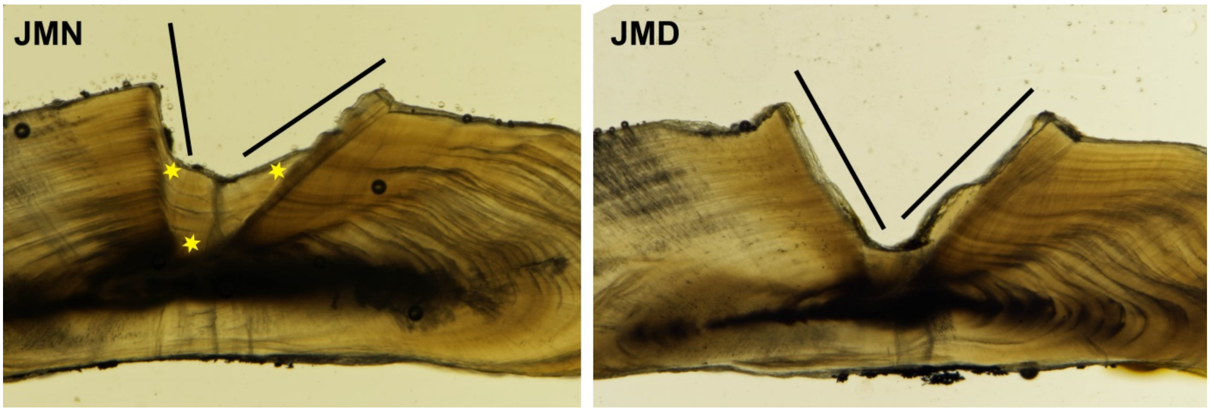

Figure 5: Images showing the general shape of the cross-sections through the sulcal groove in otoliths from *T. novaezelandiae* (JMN) and *T. declivis* (JMD). The three yellow stars on the JMN section define a triangular area of the sulcal groove that is usually filled in otoliths of *T. novaezelandiae* but relatively empty in otoliths of *T. declivis*. Ventral side of the otolith is to the left in both sections.

2.5 Otolith interpretation: *Trachurus declivis*

Descriptions of the general interpretation of *T. declivis* otoliths are given in section 2.4 above. An example of the relatively large opaque area surrounding the primordium, but inside the first annual zone, is shown in Figure 6. The position of the first annual increment is often difficult to determine as the opaque zone marking it can be much lighter in colour than subsequent annual zones and the primordial opaque area (Figure 6). A further complicating factor is that the size of the otolith when the first annual zone is formed can vary substantially because of the relatively long spawning season and the accuracy of the otolith sectioning. However, some *T. declivis* otolith sections exhibit distinct indentations of the distal face, which help identify the locations of the initial zones (Figures 6 and 7). These indentations are not apparent on every otolith, and although they occur occasionally out past the fifth zone, they are seldom distinct beyond the second zone. Presumed split or sub-annual zones are frequently observed out to age 3 years (Figures 6–9) but can often be clearly observed in older zones (e.g., see zones 4 and 5 on the ventral side of the section in Figure 7, and between zones 6 and 7 on Figure 9). Examples of the annual zonation pattern in otolith sections from relatively old *T. declivis* are shown in Figures 10 and 11. Zones representing ages 8 to 23 in Figure 11 show no discernible reduction in thickness as the fish age increases.

The relatively long spawning season results in a relatively broad size range of radial distances to the first few annual zones. The mean distances from the primordium to the outer edges of the first three annual dark (opaque) zones on the ventral sides of otoliths sections were determined (Table 1). These measurements can be used to help define the likely positions of annual zones when multiple sub-annual, or indistinct annual zones, are present.

Table 1: Measurements (minimum, maximum, and mean) from the primordium to the outer margin of the first three opaque zones on the ventral side of *T. declivis* otolith sections, with estimated 95% confidence intervals (CI).

	1st zone	2nd zone	3rd zone
Minimum (mm)	1.18	1.50	1.78
Maximum (mm)	1.60	1.90	2.15
Mean (mm)	1.33	1.71	1.96
CI (mm)	±0.27	±0.28	±0.27
N	35	35	33

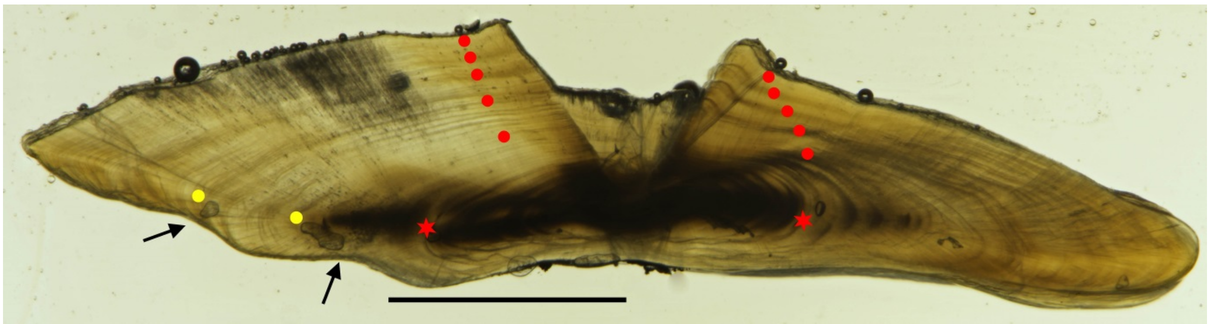

Figure 6: ***T. declivis*** **otolith section showing all annual zones (red dots), approximate extent of the dark opaque region within the first year's growth (area between red stars), and the indentations on the ventral side of the distal face (black arrows) that often occur in association with the first few annual zones (the first two here indicated by yellow dots). Estimated age is 5 years. Scale bar is 1 mm.**

Figure 7: ***T. declivis*** **otolith section showing all annual zones (red dots) and the indentations on the ventral side of the distal face (black arrows) that often occur in association with the first few annual zones. Estimated age is 6 years. Scale bar is 1 mm.**

Figure 8: ***T. declivis*** **otolith section showing all annual zones in two locations on the ventral side (red and yellow dots). On this section, the zonation pattern is not clear on either side of the sulcus. Estimated age is 9 years. Scale bar is 1 mm.**

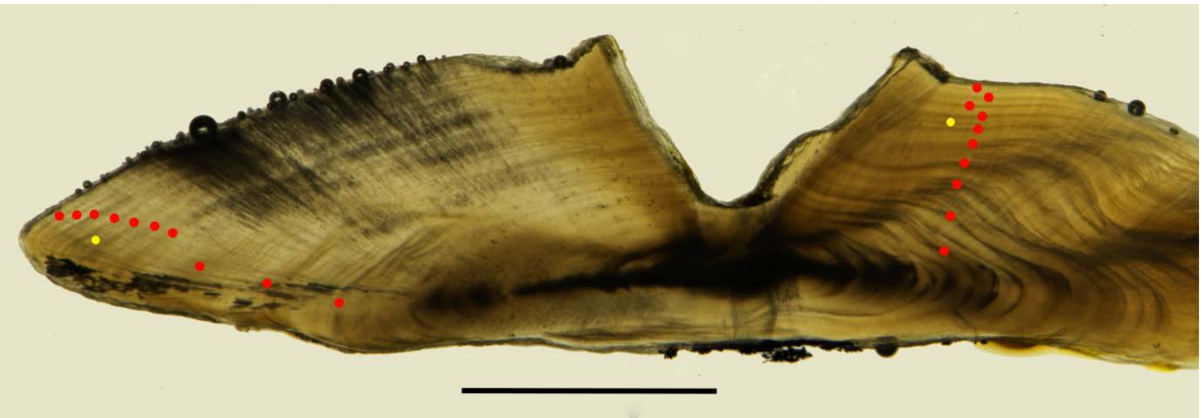

Figure 9: ***T. declivis*** **otolith section showing all annual zones (red dots). A presumed sub-annual 'split zone' visible on both the ventral and dorsal parts of the section is indicated by yellow dots. Estimated age is 10 years. Scale bar is 1 mm.**

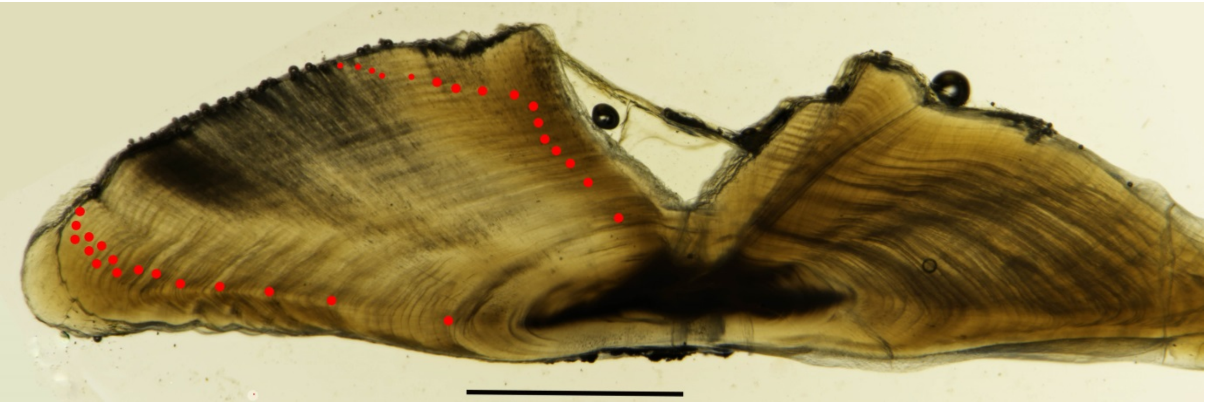

Figure 10: ***T. declivis*** **otolith section showing all annual zones (red dots). The zonation pattern is relatively clear in several locations on the section. The decrease in otolith growth in the dorsal-ventral axis after about age 7 is very apparent in this example. Estimated age is 16 years. Scale bar is 1 mm.**

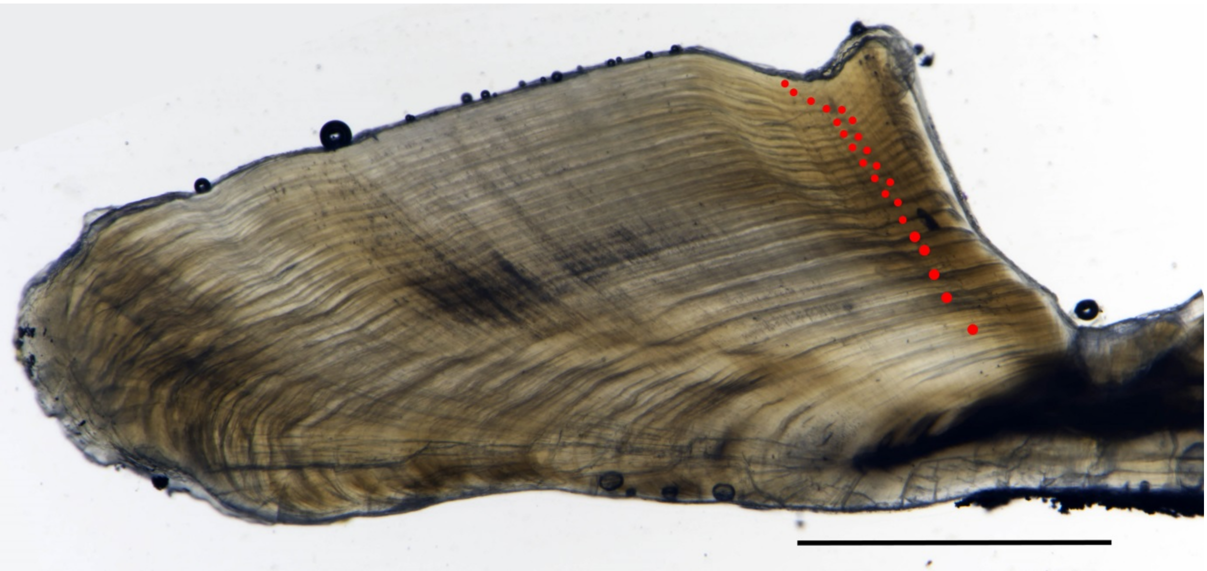

Figure 11: ***T. declivis*** **otolith section showing all annual zones (red dots) on the ventral area. This is an example of one of the oldest specimens of this species. Estimated age is 23 years. Scale bar is 1 mm.**

2.6 Otolith interpretation: *Trachurus novaezelandiae*

Descriptions of the general interpretation of *T. novaezelandiae* otoliths are given in section 2.4 above. An example of the relatively large opaque area surrounding the primordium, but inside the first annual zone, is shown in Figure 12. The position of the first annual increment is often difficult to determine because the opaque zone marking it can be much lighter in colour than subsequent annual zones and the primordial opaque area (Figure 13). A further complicating factor is that the size of the otolith when the first annual zone is formed can vary substantially because of the relatively long spawning season and the accuracy of the otolith sectioning. Presumed split or sub-annual zones are frequently observed out to age 3 years (Figures 12–15) and can often be clearly observed in older zones (e.g., see between zones 3 and 4 on Figure 14). Examples of the annual zonation pattern in otolith sections from relatively old *T. novaezelandiae* are shown in Figures 16 and 17. Zones representing ages 10 to 23 in Figure 17 show no discernible reduction in thickness as the fish age increases, and there was virtually no increase in otolith width on the ventral side after age 5.

The relatively long spawning season results in a relatively broad size range of radial distances to the first few annual zones. The mean distances from the primordium to the outer edges of the first three annual dark (opaque) zones on the ventral sides of otoliths sections were determined (Table 2). These measurements can be used to help define the likely positions of annual zones when multiple sub-annual, or indistinct annual zones, are present. Figure 16 shows an example where there are two possible interpretations for the position of the first annual zone. The radial measurements to the two posited first zones are close to the maximum and minimum values in Table 2. In this example, the reader chose the early spawned scenario (age 23 years) rather than the late spawned scenario (age 24 years).

Table 2: Measurements (minimum, maximum, and mean) from the primordium to the outer margin of the first three opaque zones on the ventral side of *T. novaezelandiae* otolith sections, with estimated 95% confidence intervals (CI).

	1st zone	2nd zone	3rd zone
Minimum (mm)	1.22	1.53	1.68
Maximum (mm)	1.58	1.90	2.10
Mean (mm)	1.36	1.70	1.89
CI (mm)	±0.26	±0.26	±0.30
N	36	36	36

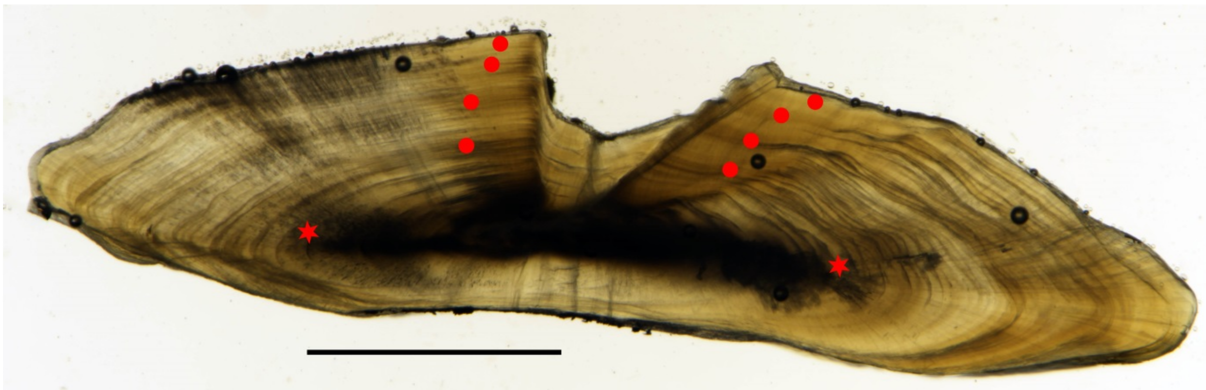

Figure 12: *T. novaezelandiae* otolith section showing all annual zones (red dots) and the approximate extent of the dark opaque region within the first year's growth (area between red stars). Estimated age is 4 years. Scale bar is 1 mm.

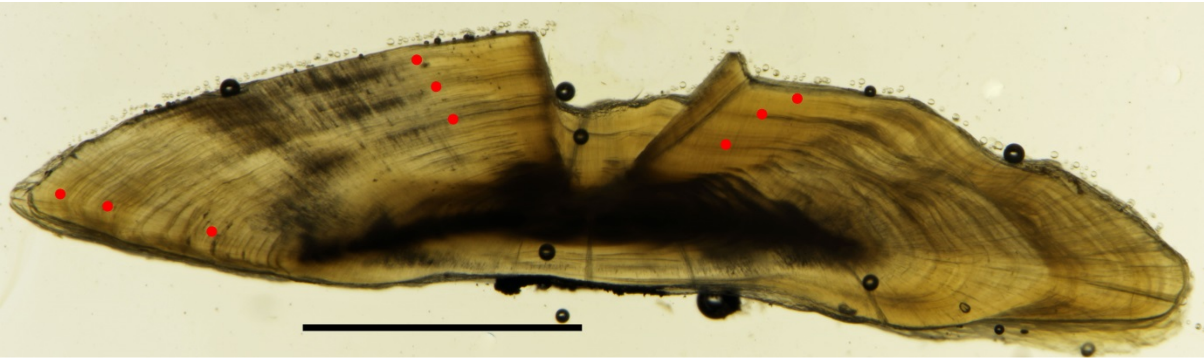

Figure 13: ***T. novaezelandiae*** **otolith section showing all annual zones (red dots). The zonation pattern is clearly visible at various locations on the section. Estimated age is 3 years. Scale bar is 1 mm.**

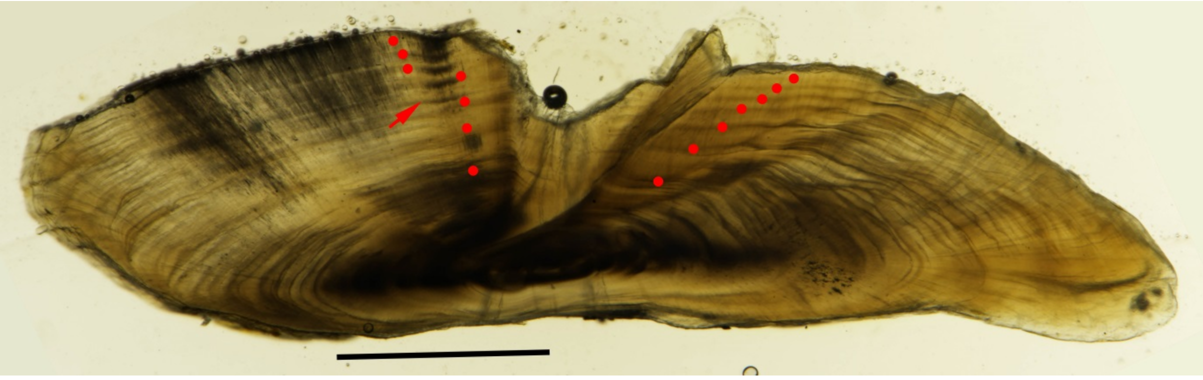

Figure 14: ***T. novaezelandiae*** **otolith section showing all annual zones (red dots). A split third annual zone is apparent on the ventral (broader) part of the section; the sub-annual zone is indicated by the red arrow. Estimated age is 7 years. Scale bar is 1 mm.**

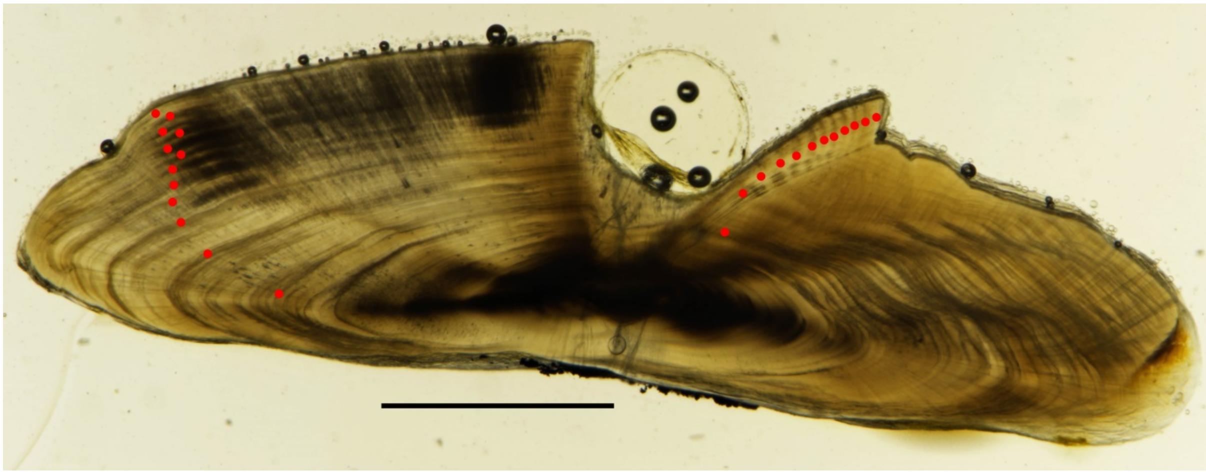

Figure 15: ***T. novaezelandiae*** **otolith section showing all annual zones (red dots). The decrease in otolith growth in the dorsal-ventral axis after about age 5 is very apparent in this example. Estimated age is 12 years. Scale bar is 1 mm.**

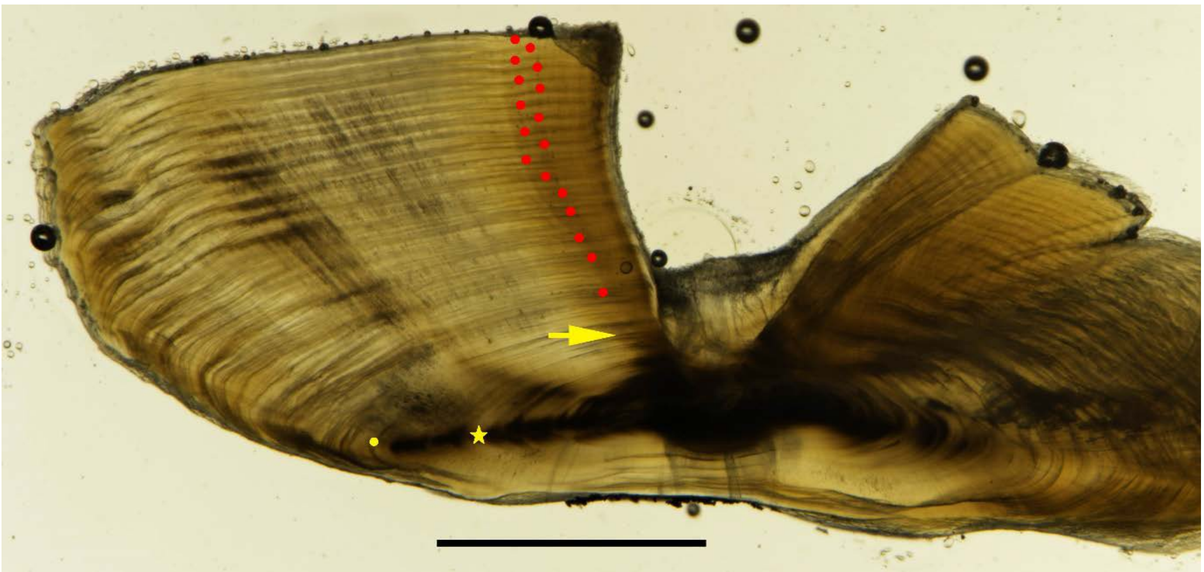

Figure 16: ***T. novaezelandiae*** **otolith section showing all annual zones (red dots). The decrease in otolith growth in the dorsal-ventral axis after about age 5 is very apparent in this example. Estimated age is 17 years. It is possible, however, that this fish was spawned very late in the season and that the first annual zone is at the position indicated by the yellow arrow and yellow dot (not the first red dot) and the edge of the central dark opaque zone is indicated by the yellow star (rather than the yellow dot). Scale bar is 1 mm.**

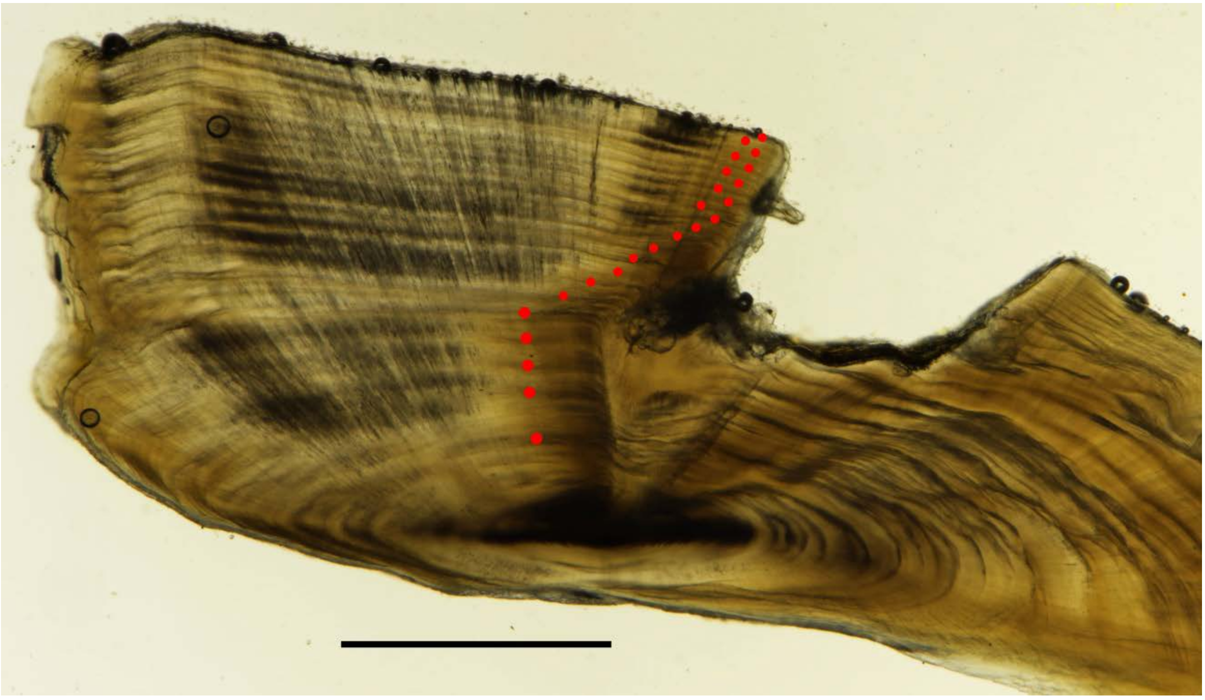

Figure 17: ***T. novaezelandiae*** **otolith section showing all annual zones (red dots) on the ventral area. The decrease in otolith growth in the dorsal-ventral axis after about age 5 is very apparent in this example. This is an example of one of the oldest specimens of this species. Estimated age is 23 years. Scale bar is 1 mm.**

2.7 Otolith interpretation: *Trachurus murphyi*

Of the three species of jack mackerel in New Zealand waters, *T. murphyi* is the most problematic for ageing. Descriptions of the general interpretation of *T. murphyi* otoliths are given in section 2.4 above. An example of the relatively large opaque area surrounding the primordium, but inside the first annual zone, is shown in Figure 18. The position of the first annual increment can be difficult to determine because the opaque zone marking it can be much lighter in colour than subsequent annual zones and the primordial opaque area (Figures 18–20); however, there is often an area of inflection on the distal surface that is associated with the end of the first annual zone (Figures 18 and 19). Presumed split or sub-annual zones are frequently observed out to age 3 years and can often be judged present in older zones also. Examples of the annual zonation pattern in otolith sections from relatively old *T. murphyi* are shown in Figures 18–20. Zones representing ages greater than about 10 years show no discernible reduction in thickness as the fish age increases.

Given the relative difficulty of determining the age of *T. murphyi* it is recommended that readers re-age and re-train on up to 25% of the previously aged sample, to maintain as consistent an interpretative approach between sampling years as possible. A sub-set of good preparations (i.e., those with readability scores of 1 or 2) should also be used as a preliminary reference tool. Determining early growth zone patterns from years 1 to 3 can be especially difficult and using distance cues can be helpful, either by eyepiece micrometer or interactive digital measurement. Marginal increment interpretation is further complicated in older fish by the appearance of multiple well-defined finer rings, often with splits, which may or may not be annual zones. Thus, the width classification of the margin may of necessity need to be apportioned using date of capture (i.e., the 'forced margin' technique – see Appendix 1). As there is no formal age validation study for *T. murphyi* from New Zealand, interpretative errors can only be subjectively minimised by re-ageing and re-training on prior aged sets.

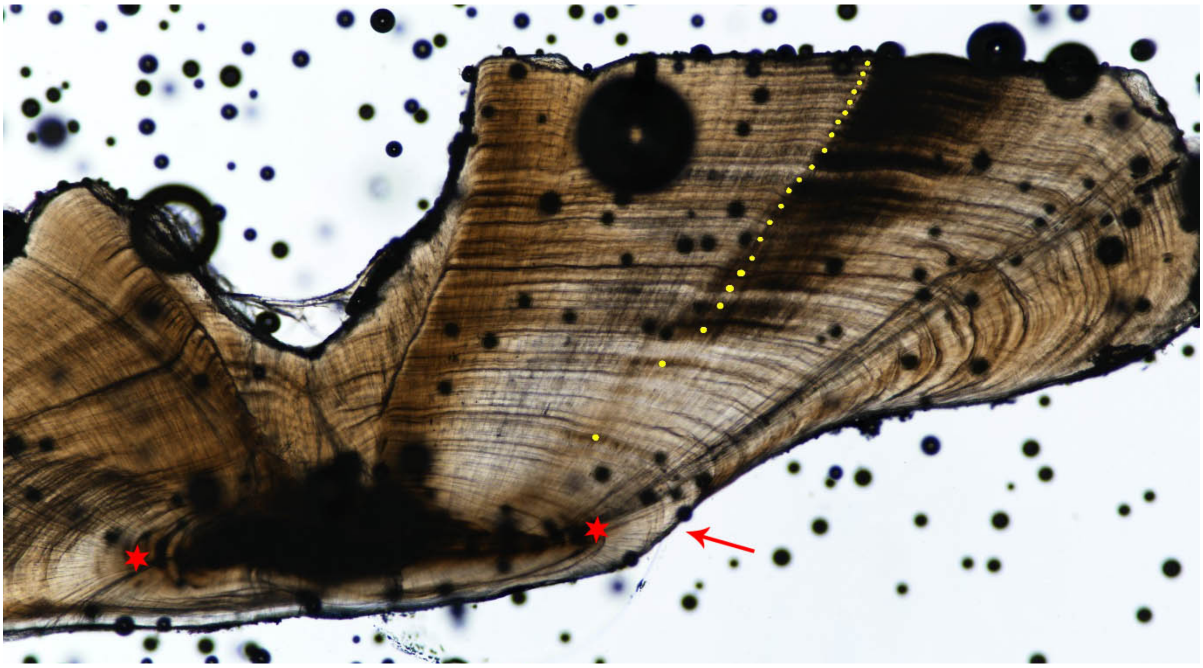

Figure 18: *T. murphyi* otolith section showing all presumed annual zones (yellow dots) on the ventral area and the approximate extent of the dark opaque region within the first year's growth (area between red stars). Sub-annual zones are abundant in the first 3–4 years of growth. The red arrow indicates an area of inflection on the distal surface that is often associated with the end of the first annual zone. Estimated age is 23 years (22 annual zones plus a wide margin).

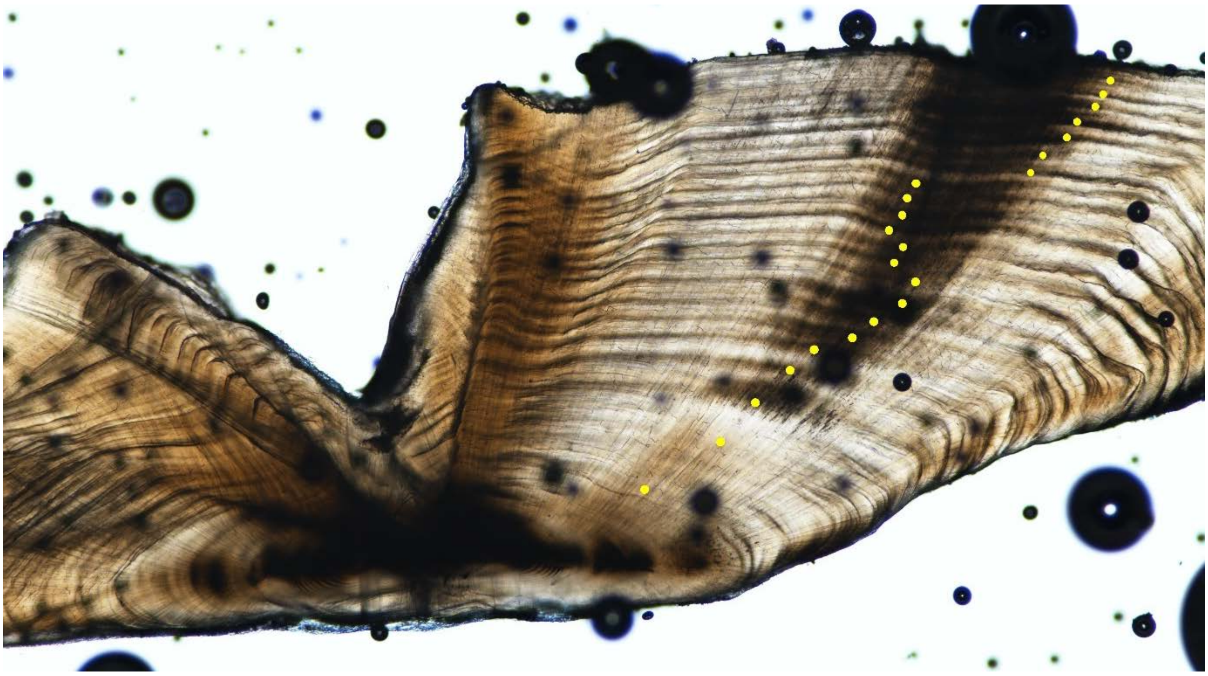

Figure 19: ***T. murphyi*** **otolith section showing all presumed annual zones (yellow dots) on the ventral area. This otolith has relatively clear zonation, with a low frequency of sub-annual zones. Inflection of the distal surface at the end of year 1 is apparent (as on Figure 18). Estimated age is 23 years (22 annual zones plus a wide margin).**

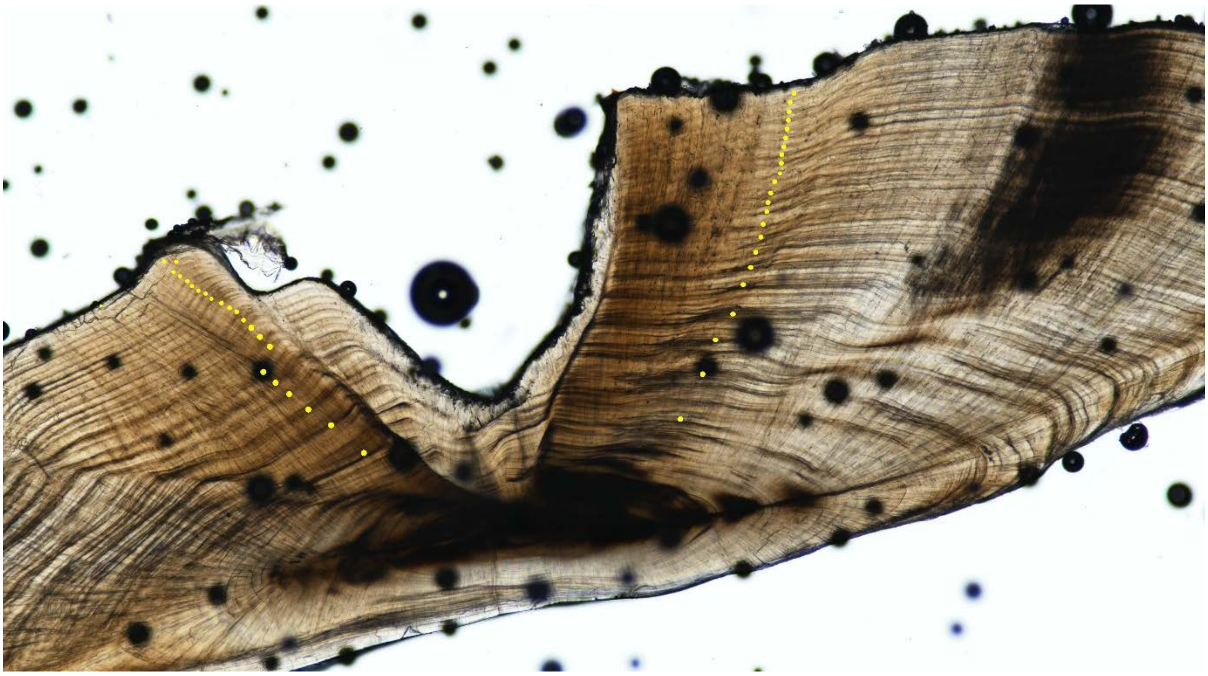

Figure 20: ***T. murphyi*** **otolith section showing all annual zones (yellow dots). This otolith has relatively clear zonation adjacent to the sulcal groove on the dorsal part of the section, with a low frequency of sub-annual zones. Sub-annual zones are abundant in the first 3–4 years on the ventral part of the section. Curvature of the distal surface at the end of year 1 is not apparent on this otolith. Estimated age is 24 years (23 annual zones plus a wide margin).**

2.8 Reference collection

The primary role of a 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). No formal reference collection has been created for any of the three *Trachurus* species in New Zealand waters. However, hundreds of aged sections of each of these species are available in the otolith archive at NIWA (Wellington). Sections aged by readers 10 or 77 (see 'reader' in the age database, section 2.9) can be used as reference material. Because jack mackerels have a moderate life-span (i.e., relatively few sampled fish are older than 20 years), a reference collection of about 500 otolith preparations per species is believed to be more than adequate for quality control monitoring purposes.

2.9 Format for data submission to *age* database

NIWA (Wellington) currently undertake the role of Data Manager and Custodian for fisheries research data owned by Fisheries New Zealand. This includes storing physical age data (i.e., otolith, spine, and vertebral samples) and the management of electronic data in the *age* database. A document guide for users and administrators of the *age* database exists (Mackay & George 1993). This database contains several tables, outlined in an Entity Relationship Diagram which physically shows how all tables relate to each other, and to other databases.

When research has been completed, NIWA receives the final age data (usually in an Excel spreadsheet format) from the research provider and performs data audit and validation checks prior to loading these data to the *age* database (Table 3).

Table 3: An example of jack mackerel age data submitted for loading to the *age* database. Data from samples of each of the three species are included. Block number (when used) refers to slide number; result1 is zone count; result2 is the margin classification (Wide, Medium, Narrow); error1 (when used) is a readability classification on a 1–5 stage scale.

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	error1	age	proj_code
SOP	2018	5119	85	-1	CHA	JMD	2	1		1	2019-04-16	1	30	10	17	W		18	MID201803
SOP	2018	5119	85	-1	CHA	JMD	3	2		1	2019-04-16	1	30	10	16	M		16	MID201803
SOP	2018	5119	85	-1	CHA	JMD	4	3		1	2019-04-16	1	30	10	24	M		24	MID201803
SOP	2018	5119	85	-1	CHA	JMD	13	4		1	2019-04-16	1	30	10	18	N		18	MID201803
SOP	2018	5119	95	-1	AKW	JMD	5	5		1	2019-04-16	1	30	10	4	N		4	MID201803
SOP	2018	5119	95	-1	AKW	JMD	7	6		1	2019-04-16	1	30	10	18	N		18	MID201803
SOP	2018	5119	103	-1	CHA	JMD	9	7		1	2019-04-16	1	30	10	3	N		3	MID201803
SOP	2018	5137	60	-1	AKW	JMN	10	55		1	2019-05-02	1	30	124	14	M		14	MID201803
SOP	2018	5137	61	-1	AKW	JMN	6	56		1	2019-05-02	1	30	124	10	W		11	MID201803
SOP	2018	5137	61	-1	AKW	JMN	8	57		1	2019-05-02	1	30	124	15	M		15	MID201803
SOP	2018	5137	61	-1	AKW	JMN	19	58		1	2019-05-02	1	30	124	3	N		3	MID201803
SOP	2018	5137	62	-1	AKW	JMN	4	59		1	2019-05-02	1	30	124	7	M		7	MID201803
SOP	2018	5137	62	-1	AKW	JMN	11	60		1	2019-05-02	1	30	124	5	M		5	MID201803
SOP	2018	5137	62	-1	AKW	JMN	14	61		1	2019-05-02	1	30	124	10	N		10	MID201803
SOP	2018	5119	106	-1	CHA	JMM	1	1	1-1	1	2019-04-29	1	30	77	20	W	3	21	MID201803
SOP	2018	5119	114	-1	CHA	JMM	1	2	1-2	1	2019-04-29	1	30	77	21	W	3	22	MID201803
SOP	2018	5119	114	-1	CHA	JMM	2	3	1-3	1	2019-04-29	1	30	77	17	W	4	18	MID201803
SOP	2018	5119	114	-1	CHA	JMM	4	4	1-4	1	2019-04-29	1	30	77	20	W	4	21	MID201803
SOP	2018	5119	114	-1	CHA	JMM	5	5	2-1	1	2019-04-29	1	30	77	19	W	3	20	MID201803
SOP	2018	5119	114	-1	CHA	JMM	6	6	2-2	1	2019-04-29	1	30	77	19	W	4	20	MID201803
SOP	2018	5119	114	-1	CHA	JMM	44	7	2-3	1	2019-04-29	1	30	77	22	W	4	23	MID201803

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APPENDIX 1: Glossary of otolith terminology and ageing definitions.

Based on Kalish et al. (1995) "Glossary for otolith studies", but with some added items including definitions for "fishing year age-class" and "forced margin" to describe New Zealand practice.

Accuracy – the closeness of a measured or computed value to its true value.
Age estimation, age determination – these terms are preferred when discussing the process of assigning ages to fish. The term 'ageing' should not be used because 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 neither 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.
Asteriscus (pl. Asteriscii) – one of three otolith pairs found in the membranous labyrinth of osteichthyan fishes.
Bias – The systematic over or under estimation 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 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 micro-increment that appears dark when viewed with transmitted light and appears as a depressed region when acid-etched and viewed with a scanning electron microscope. This component of a micro-increment contains a greater amount of organic matrix and a lesser amount of calcium carbonate than the L-zone. Referred to as a 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 the 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., sub-daily, 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 micro-increment 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 micro-increment 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.

Micro-increment – increments that are typically less than 50 um in width; the prefix "micro" serves 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 sub-daily increments. See Increment.

Microstructural growth interruption – a discontinuity in crystallite growth marked by the deposition of an organic zone. It may be localised 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 fibrillary material surrounding one or more optically dense nuclei from 0.5 um to 1.0 um in diameter. In the early stages of otolith growth, if several primordia are present, they generally fuse to form the otolith core.

Rostrum – anterior and ventral projection of the sagitta. Generally longer than the antirostrum.

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 age estimation 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. A thickened portion of the otolith 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.