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Tini a Tangaroa

Age estimation protocols for black oreo (*Allocyttus niger*) and smooth oreo (*Pseudocyttus maculatus*)

New Zealand Fisheries Assessment Report 2018/45

P.L. Horn
P. McMillan
C. Ó Maolagáin

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Ministry for Primary Industries
PO Box 2526
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TABLE OF CONTENTS

EXECUTIVE SUMMARY

Horn, P.L.; McMillan, P.J.; Ó Maolagáin, C. (2018). Age estimation protocols for black oreo (*Allocyttus niger*) and smooth oreo (*Pseudocyttus maculatus*).

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This report documents the age estimation protocol for two important New Zealand deepwater finfish species in the Family Oreosomatidae: black oreo (*Allocyttus niger*) and smooth oreo (*Pseudocyttus maculatus*). It describes the most recent scientific methodologies used for otolith preparation and interpretation. In addition, otolith reference collections of 100 preparations for *A. niger* and 100 preparations for *P. maculatus* were compiled and documented from previously prepared archived samples. Agreed readings and ages determined for the reference collections are stored in a reference table in the *age* database. The reference samples were taken from the Chatham Rise area and included comprehensive size and age ranges of both fish species.

Digital images of otolith preparations are presented to illustrate the zone interpretation methods used in estimating fish age for the two oreo species. 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 rate, 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.

This report describes the age determination protocol developed for two important New Zealand deepwater finfish species in the Family Oreosomatidae: black oreo (*Allocyttus niger*) and smooth oreo (*Pseudocyttus maculatus*). Oreos are managed as a species group, which also includes spiky oreo (*Neocyttus rhomboidalis*) (Ministry for Primary Industries 2015). The Chatham Rise is the main fishing area. The main fishstocks of this species group fall within Tier 1 of the National Fisheries Plan for Deepwater Fisheries, with service strategies that promote regular stock assessment, thus utilising catch-at-age information. The purpose of the protocol is to provide a practical guide for age determination, describing methods and techniques for otolith preparation, viewing and interpreting visible zones within otoliths, and converting annual zone counts into fish age. This protocol will ensure that the best methods are used in determining as accurate an estimate of fish age as possible, and that consistency in ageing is maintained over time. It will also serve as a valuable training tool for new otolith readers.

No attempt was made to document protocols related to daily increments in otoliths (usually associated with ageing larval or juvenile fish) or investigations into otolith ultrastructure or chemical composition. In addition, of the three otolith pairs present within the otic capsules of bony fishes (asteriscus, lapillus, sagitta), only the sagitta, generally the largest and most often used in age estimation (Panfili et al. 2002), was used to age black oreo and smooth oreo. Therefore, throughout this report, the use of 'otolith' will be synonymous with the sagittal otolith. A glossary describing otolith terminologies and ageing definitions relevant to this species is included in this report for reference purposes (Appendix A).

Stock assessments used to inform decisions about TACC levels depend upon estimates of biomass and on the species productivity, the latter often determined by age and growth analyses. While there is increased confidence in the current age interpretations of deepwater species, and proposed mechanisms for why deepwater fishes can attain a high longevity (Cailliet et al. 2001), most age estimates are based on growth zone counts from otoliths, and many remain unvalidated.

1.1 Previous oreo age determination studies

Age determination has proved difficult for both black oreo and smooth oreo, but there was a consensus that these species were slow growing and long-lived. An initial investigation of both species by Davies et al. (1988) concluded that growth zones visible on the surface and in sections of the otoliths were not useful for age determination. However, otolith thin section preparations were subsequently used in investigations of age in both New Zealand and Australia (transverse sections about 0.2 mm thick, through untreated otoliths). In New Zealand, maximum estimated otolith zone counts were 153 years (45.5 cm TL fish) for black oreo, and 86 years (51.3 cm TL fish) for smooth oreo (Doonan et al. 1995). Smith & Stewart (1994) estimated maximum ages of 100 (43.5 cm TL fish) and 78 (54.0 cm TL fish) years for black oreo and smooth oreo, respectively, from Australian waters. The age estimates reported by Doonan et al. (1995) were subsequently used to develop life history parameters for both oreo species (Doonan et al. 1997, McMillan et al. 1997), but no further age estimation work for stock assessment was completed for black oreo until 2010 under MPI project DEE2010-08 (Doonan et al. 2016), and for smooth oreo in 2007–08 under MPI project OEO2006-03 (Doonan et al. 2008).

Smith & Stewart (1994) and Doonan et al. (1995) reported that in black oreo otoliths the first 4–5 increments are generally broad and distinct, and these are followed by a rapid transition to (often less distinct) narrow increments. For smooth oreo, the first 6–7 increments are quite broad, after which they gradually narrow to become very fine regular increments at the otolith margin in older fish (Doonan et al. 2008).

Three other oreo species have been recorded in New Zealand waters: the ox-eye oreo (*Oreosoma atlanticum*), the spiky oreo (*Neocyttus rhomboidalis*), and the warty oreo (*Allocyttus verrucosus*). No age determination studies have been reported for the ox-eye oreo. Age determination of warty oreo using counts of zones in transversely sectioned otoliths produced a maximum age of 130 years (Stewart et al. 1995). Validation of that age determination method used lead-radium dating, where the natural decay of radium-226 (^{226}Ra) into lead-210 (^{210}Pb) acts as a natural clock and can provide an estimate of age that is independent of growth zone counting, and resulted in a maximum estimated age of 132 ± 15 years, similar to the zone count estimate. The use of whole otoliths in this radiometric validation method has known problems, however, as it is reliant on an assumed otolith mass growth rate model (Paul et al. 2002), an issue avoided in more recent studies of other species by using otolith cores (e.g., Andrews et al. 2009). Nevertheless, a high longevity was clearly indicated for warty oreo. Zone counts in the sectioned otoliths of spiky oreo were produced similarly to those for warty oreo (Smith & Stewart 1994), giving an estimated maximum age of 128 years. Sectioned spiky oreo otoliths were found to be the clearest of the four oreo species examined by Smith & Stewart (1994).

1.2 Black oreo and smooth oreo age validation studies

Fenton (1996) attempted an age validation of both black oreo and smooth oreo using lead-radium dating, as described above for warty oreo. While Fenton's work supported a high estimated longevity for each species (i.e., about 100 years for black oreo, and at least 45 years for smooth oreo), it also suffered from the problems resulting from pooling whole otoliths (described above for warty oreo).

Otolith core samples from young and old New Zealand black oreo and smooth oreo were analysed for ^{210}Pb and ^{226}Ra to determine the feasibility of applying a refined radiometric ageing technique (Andrews & Tracey 2003). Both species have very small otoliths, and there were few samples to select from. Ideally, juvenile otoliths would have been used, but few were available. This feasibility study showed that a full radiometric age validation study of black oreo, using otolith cores and the refined ^{210}Pb:^{226}Ra radionuclide method, would be challenging because of these two issues (otolith size, and few samples of suitably sized fish).

Morison et al. (1999) trialled the "bomb chronometer" method of radiocarbon ageing (Kalish 1993) to validate the age estimates derived from otoliths of black oreo and smooth oreo otolith zone counts. This method assumed that surface-dwelling juvenile oreos would pick up the pulse of radiocarbon (^{14}C) in their otolith cores that resulted from the atmospheric testing of thermonuclear bombs during the 1950s and 1960s, and that the level of radiocarbon in each core defines the approximate year of birth of that fish, and thus an age. These ages could then be compared with ages derived from growth zone counts in otolith thin sections. The attempted age validations by Morison et al. (1999) and Kalish (2002) were based on very broad trends, and the resolution of the application needed to be improved. Sparks (2000) concluded that the limited data presented by Morison et al. (1999) showed only a poor correlation between ^{14}C and zone count ages.

A study by Neil et al. (2008) measured ^{14}C levels in core micro-samples from large, previously-aged (by zone-count) black oreo and smooth oreo otoliths. The ^{14}C measurement for each fish was compared with the value on the reference curve of Kalish (1993) for the calculated birth year of the fish. The data supported the age estimation technique using growth zone counts for black oreo, and this species was also shown (using stable isotope analyses) to have a near-surface juvenile phase. Smooth oreo were shown to not have a near-surface pelagic early life history phase, but comparison of their ^{14}C measurements with an alternative deep water ^{14}C curve suggested that the age estimation technique using growth zone counts was appropriate. The analysis of Neil et al. (2008) on black oreo and smooth oreo was subsequently refined and published formally (Horn et al. 2018).

1.3 Development of an age determination protocol

Age determination of black oreo was recommenced in 2015 as part of a Ministry for Primary Industries project routinely ageing deepwater species for stock assessment purposes (Project MID2015-01). A sample of 600 black oreo otolith preparations from Fishstock OEO 3A (southwest Chatham Rise) was read and analysed to produce an estimate of the age distribution of the commercial fishery catch (Doonan et al. 2017). Objective 2 of that project required the preparation of this manual describing the methods used to prepare, and interpret the growth zones, in the otoliths of both black oreo and smooth oreo.

Small subsets of otolith sections where two readers agreed on the interpretation and final zone counts were previously compiled for both black oreo and smooth oreo. The first protocol set of 21 black oreo sections was developed in 1995 (Doonan et al. 1995) and a second protocol set of 46 otoliths was developed in 2010 using sections selected from the original 1995 age study (Doonan et al. 2016). A protocol set of 21 smooth oreo sections was developed in 1995 (Doonan et al. 1995). New, larger reference otolith collections were developed for both species for this study; see Section 6 below.

2. OTOLITH PREPARATION

The otolith preparation method is the same for both black oreo and smooth oreo. One of each pair of otoliths is chosen for sectioning, with neither the left or right otolith consistently chosen. The main selection criterion is a clean and complete otolith that was not ‘crystalline’ (i.e., comprised partially or completely of vaterite). Each otolith was marked with a dorso-ventral line through the primordium along the section (reading) axis (Figure 1). Groups of three otoliths were oriented in a mould with all the reading axes lined up, embedded in resin (Araldite K142), cured at about 50°C for a minimum of 4 hours and left to harden overnight. After curing, a thin section 250 ± 20 µm wide was taken through the three otoliths along the marked axis line using a high precision Struers Secotom sectioning saw. The sections were cleaned in detergent and water to remove any residual particulates, then air dried before being mounted on a glass microscope slide in epoxy resin under a glass cover slip.

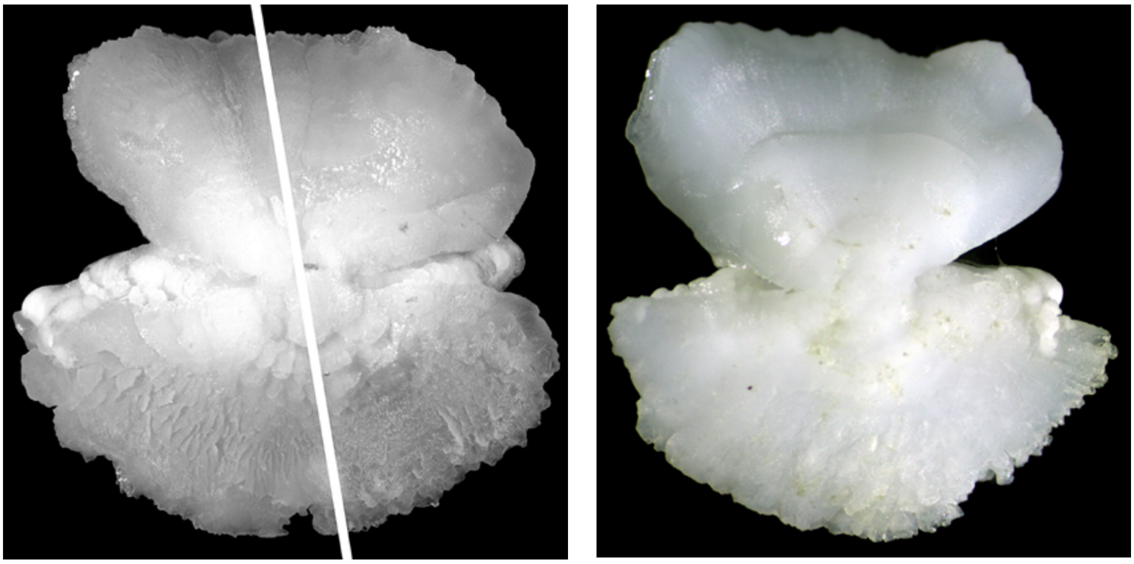

Figure 1: Proximal surfaces of whole black oreo and smooth oreo otoliths. Left panel – black oreo, white line indicates the approximate axis of the sectioning plane. Right panel – smooth oreo. Dorsal, top; ventral, bottom.

3. OTOLITH INTERPRETATION

Otolith sections for both black oreo and smooth oreo were examined using the same overall approach, as follows:

- View sections under transmitted light.
- The preferred sector for counting zones is on the ventral (thicker) lobe of the otolith section (e.g., Figure 2 for black oreo, Figure 8 for smooth oreo). The dorsal lobe, partially shown on the right hand side in Figures 2 and 8, is much more difficult to interpret, because most sections lack clear outer (fine) zones although the thicker inner zones can be observed.
- Counts can be made from the inner (convex, proximal) side to the outer (concave, distal) side of the otolith or vice versa (e.g., Figures 2 and 8).
- Counts of zones from the outer side, which has the narrowest zones, are important because that region can produce the majority of the total zone count and is the principal region for determining the readability of the otolith.
- It is useful to identify a path with the best readable zones from one side of the section to the other before starting a complete zone count for the section.
- Count dark (opaque) growth zones.
- Start by viewing each section under ×100 magnification.
- Switch to higher magnification, i.e., up to ×200 magnification, for fine zones in the outer region of large otoliths.
- Before starting to read otoliths from a new research sample (i.e., after a break of some weeks/months since any previous readings), read a random sample of about 30 otoliths from the protocol set, compare the results with the agreed counts, and then resolve any interpretation issues. This process should 'recalibrate' the reader before any new counts are produced.

3.1 Scales of readability for black oreo and smooth oreo

For each otolith section that is read, the reader will assign readability categories on a 5-stage scale as defined below.

1. Otolith structure is exceptionally clear with unambiguous zones. This is very rare for both black oreo and smooth oreo for sections prepared with the techniques described above. It is unknown if better readability can be achieved with different preparation methods. This readability scale is potentially possible for very young fish where there are fewer than 6 inner zones.
2. A complete zone count from primordium to edge can be made relatively easily. Repeat counts of the very best preparations gave differences of fewer than 5 zones for large/old fish.
3. A complete zone count can be made with some difficulty. Parts of the section are ambiguous, e.g., there may be split zones or the zones at the edge may be faint. There may be small (up to about 10 zone) differences between repeat counts for large/old fish.
4. A zone count is possible with extreme difficulty and the count is probably unreliable.
5. The section is unreadable due to failed preparation or unreadable structure, e.g., no readable zones visible in outer region, or the section clearly missed the primordium.

3.2 Black oreo (*Allocyttus niger*)

Counts were made from the primordium to the section outer edge (Figure 2). A sectioned black oreo otolith exhibited three regions each with different zone types. The descriptions of these regions, and tasks pertinent to their interpretation, are listed below.

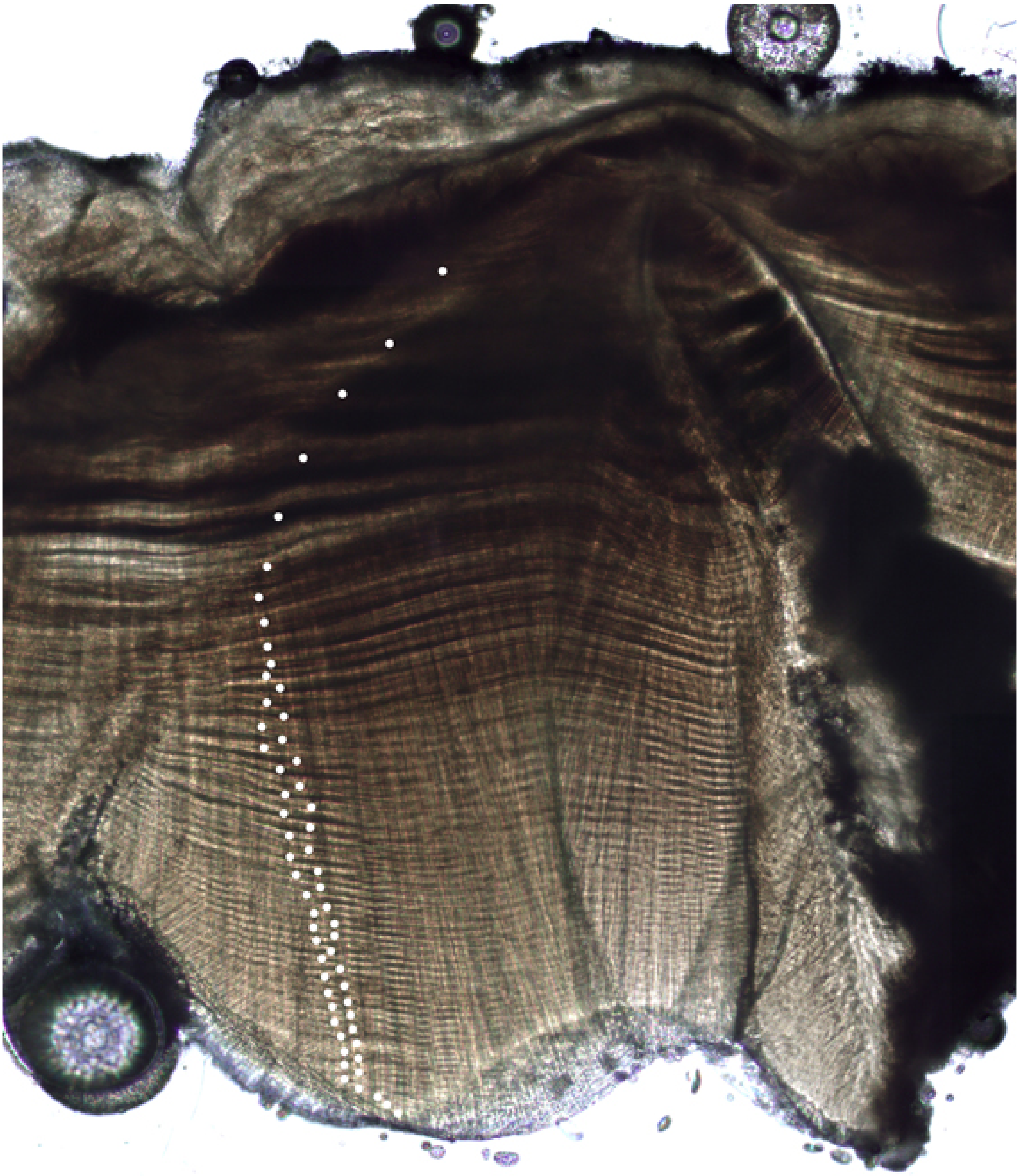

Figure 2: Black oreo otolith section showing the preferred reading sector with zone counts shown as white dots, (TAN0213, station 17, fish #11, slide #113_3, 31.1 cm TL male), estimated age 59 years, readability 2.

3.2.1 Inner region

The inner region is usually comprised of 4–5 broad inner zones of similar width. The first zone is sometimes difficult to determine and there may be split zones (e.g. Figure 2), so it is best to look along the zone to aid interpretation (i.e. side to side across the section) and also to examine this region under low magnification and to ignore finer split zones. This region is assumed to represent relatively fast growth in a near-surface environment where temperatures are higher and food is high-energy. Individuals less than about 4 years old were not caught by bottom trawl sampling. About 20 intact very small black oreo were taken from the stomachs of other fish (e.g., smooth oreo) between 1980 and 2016.

These juveniles had enlarged abdomens (with a very large liver), and cryptic colouration (suggesting an environment with ambient light), and some had stomach contents dominated by copepods. Otoliths are available from only three of these.

In order to correctly interpret this zone, it is necessary to:

- Identify the primordium and determine the positions of the first 4–5 zones.

3.2.2 Intermediate region

The intermediate region consists of about 10 zones, narrower than the broad inner zones, which are sometimes split (paired) and which gradually decrease in width towards the otolith margin. This region is assumed to represent the settlement of the individual to a near-bottom mode of life with consequent slowed growth at lower temperatures.

In order to correctly interpret this zone, it is necessary to:

- Determine where the inner broad zones finish, and thus, the position of the first intermediate region zone. This is easier for larger otoliths but can be difficult for small otoliths where there is not much width outside the last broad inner zone, i.e., otoliths with only 5–15 zones in total.
- Note that the initial zones of this region are relatively broad, but the width of subsequent zones decreases rapidly, sometimes making this a difficult part of the section to interpret. There may also be double or split zones present, but these can often be resolved by moving in and out of focus (resulting in split zones merging to produce a single diffuse band, while true zones are still clearly differentiated). It is also helpful to scan along an individual zone (across the section) to see if it is consistent in width and spacing with the zones above or below it.

3.2.3 Outer region

The outer region is a region of narrowing zones which can be numerous in large otoliths and require higher magnification (up to ×200) to achieve reliable counts. No obvious or consistent transition zone is observed in this region, i.e., a point where the fish possibly reaches sexual maturity and spawns. The estimated (mean) age at first maturity for black oreo, based on an analysis of gonad stage data, was 27 years (Ministry for Primary Industries 2015).

In order to correctly interpret this zone, it is necessary to:

- Examine the edge of the section to determine where the clearest zones are located and decide on the best path for a complete count (primordium to edge). It may be necessary to move across the section (laterally) at a convenient point to enable the count to continue, i.e., there may be a particularly dark zone or some distinctive mark or structure on the section to use as a marker to enable the count to continue.
- Understand that finding readable zones on the otolith edge may require close study, and adjustments of focus, and increased magnification.
- Note that zone resolution for the outer edge often determines the overall readability of the section. There may be a substantial area where zones are not clearly visible even after exploring the section and adjusting focus and magnification, resulting in an unreadable section (readability score 5). A tentative count may be possible after considerable effort to interpret unclear parts of the section (where readability score 4 should be assigned). Better readability scores (3 or less) should be assigned for repeatable and similar counts.
- Changing to a higher magnification during reading is possible if a distinct mark on the section is located to enable the zone count to continue. If no obvious marks are visible it is best to read the whole section using the higher magnification.

Examples of sections with a range of readability are shown in Figures 3–7.

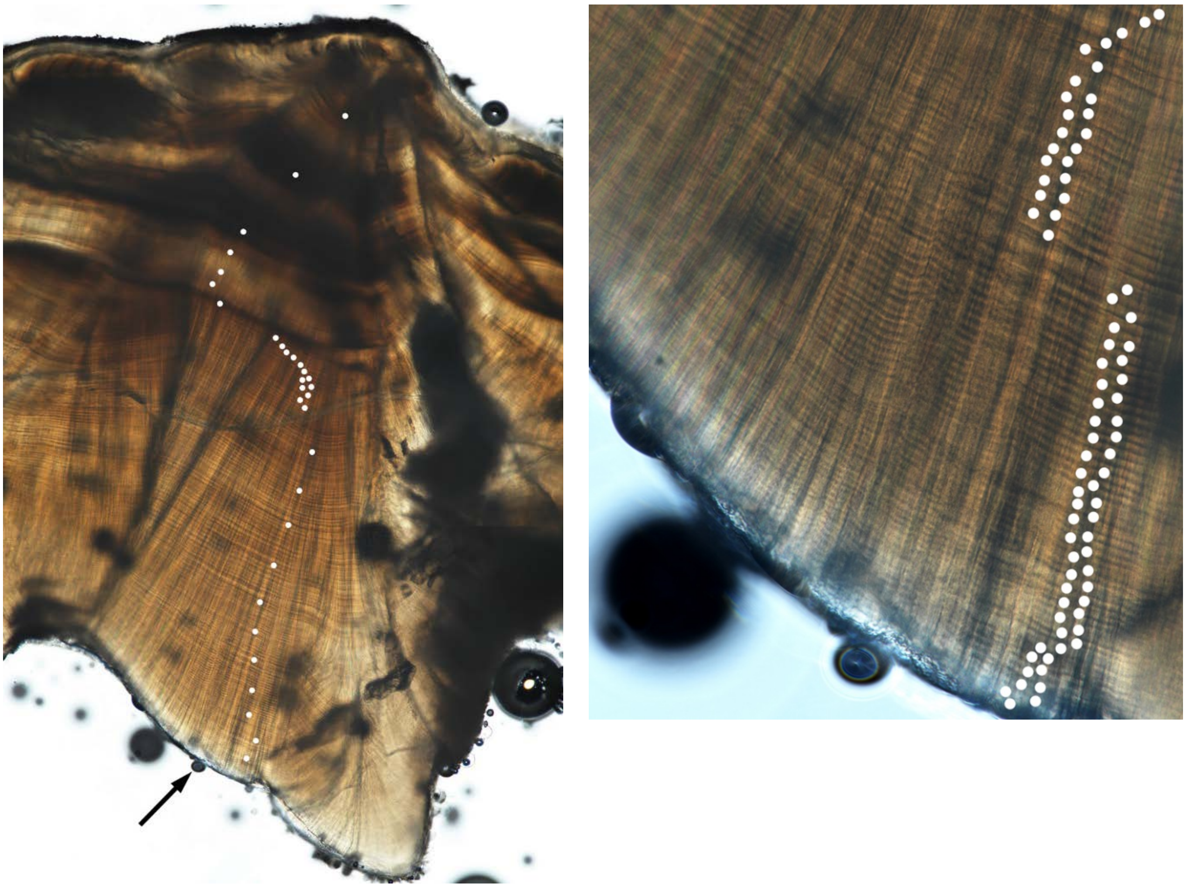

Figure 3: Black oreo otolith (SWA1402, station 22, fish #9, slide #67_2, 33.8 cm TL male), estimated age 138 years, readability 3. Left image of whole cross section showing the first 20 zones (white dots) followed by each 10th count. Right image enlarged showing a count of 76 zones out to the section edge. Black arrow on left image shows bubble seen in right image.

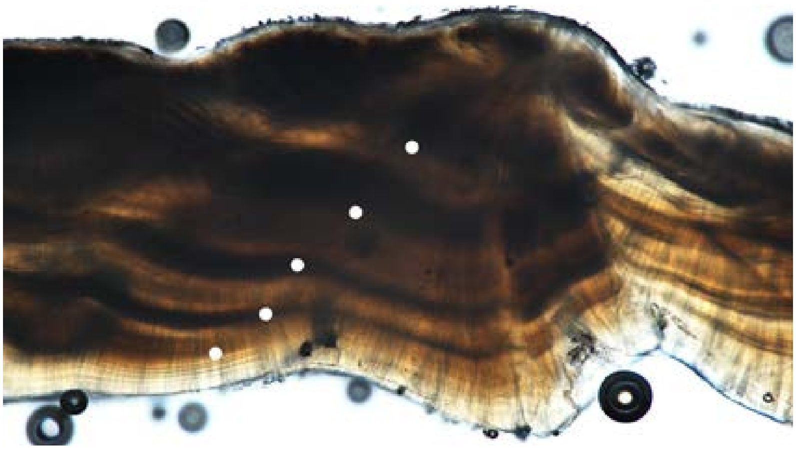

Figure 4: Black oreo otolith (SWA1402, station 15, fish #34, slide #139_3, 23.9 cm TL female), estimated age 5 years, readability 2.

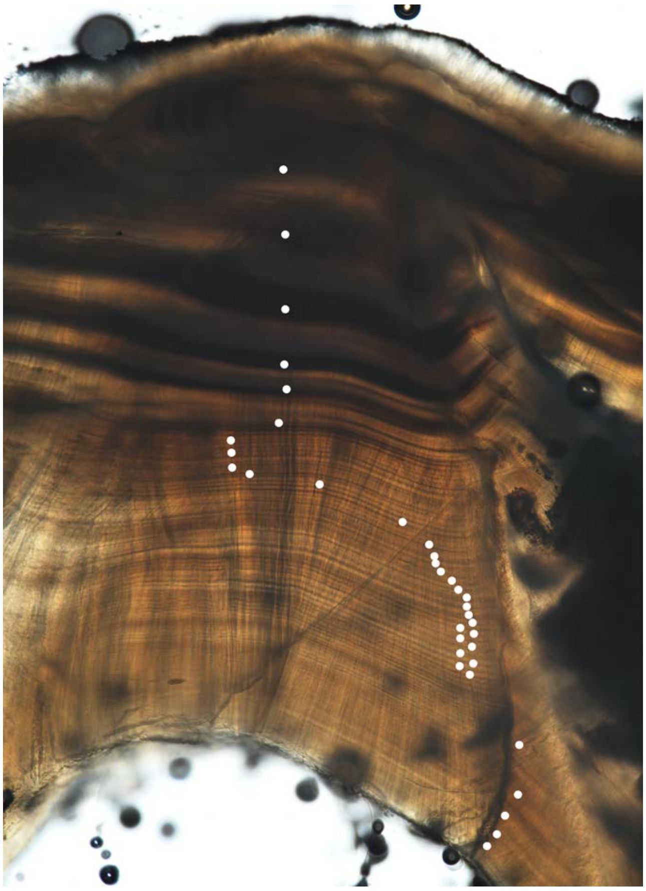

Figure 5: Black oreo otolith (SWA1402, station 22, fish #25, slide #70_2, 33.4 cm TL female), estimated age 87 years, readability 3, showing the first 30 zones followed by each tenth count.

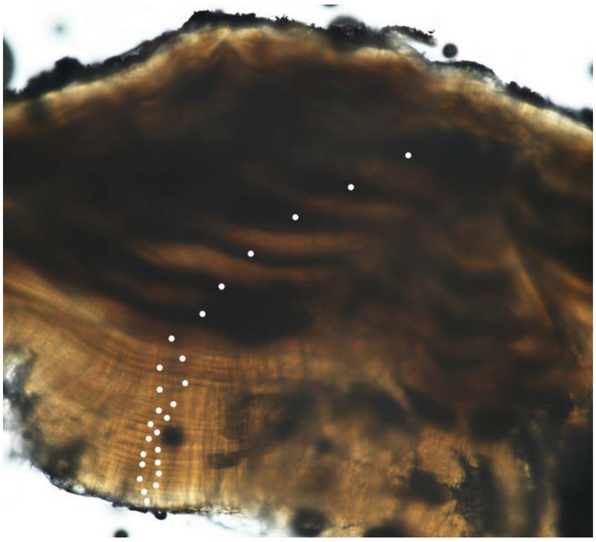

Figure 6: Black oreo otolith (SWA1402, station 11, fish #31, slide #38_3, 34.1 cm TL female), estimated age 26 years, readability 4.

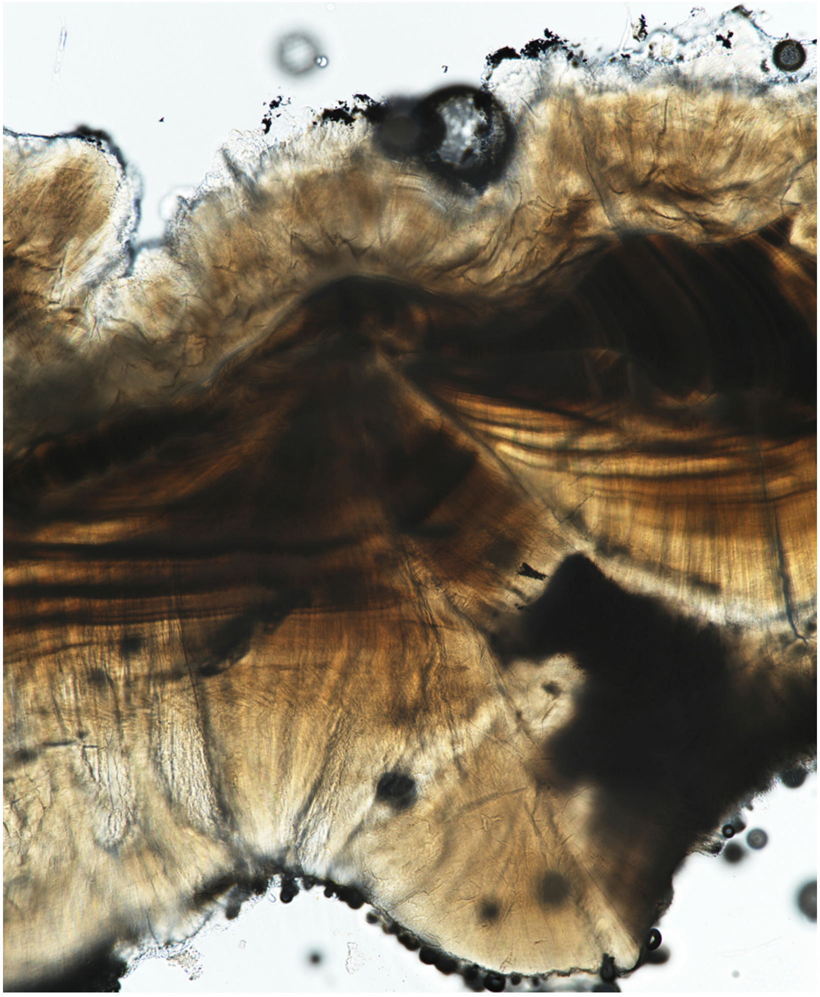

Figure 7: Black oreo otolith (SWA1402, station 11, fish #31, slide #43_1, 34.1 cm TL female). Readability 5, i.e., no count deemed possible.

3.3 Smooth oreo (*Pseudocyttus maculatus*)

Counts were made from the primordium to the section outer edge (see Figure 8). A sectioned smooth oreo otolith exhibited three regions each with different zone types. The descriptions of these regions, and tasks pertinent to their interpretation, are listed below.

3.3.1 Inner region

The inner region is usually comprised of 6–7 broad inner zones of similar width. This region is assumed to represent relatively fast growth. It is unknown where fish of this size live but individuals less than about 6 years old were rarely caught by bottom trawl sampling, i.e., there are fewer than 10 records of very small individuals caught from about 1980 to 2016.

In order to correctly interpret this zone, it is necessary to:

- Identify the primordium and determine the positions of the first 6–7 zones. The inner region zones for smooth oreo are often not as dark or distinctive as those from black oreo otoliths.

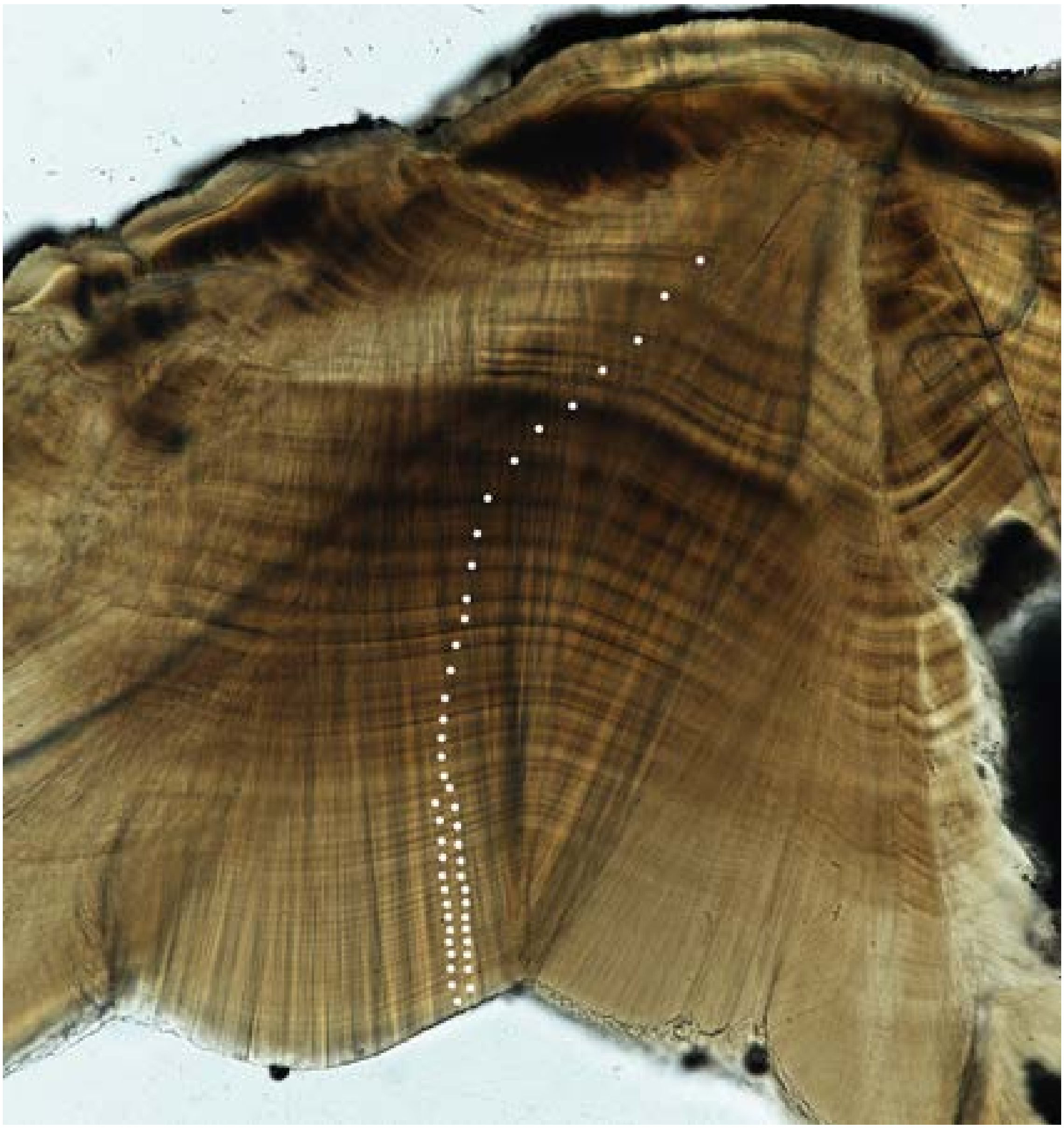

Figure 8: Smooth oreo otolith section showing the preferred reading sector, zone counts shown as white dots (TAN9812, station 33, fish #7, slide #37_5, 37.7 cm, male), estimated age 47 years, readability 2.

3.3.2 Intermediate region

The intermediate region is a region of about 10–15 zones, narrower than the broad inner zones, which are sometimes split (paired) and which gradually decrease in width towards the otolith margin. This region is assumed to represent the settlement of the individual to a near-bottom mode of life with consequent slowed growth at lower temperatures.

In order to correctly interpret this zone, it is necessary to:

- Determine where the inner broad zones finish, and thus, the position of the first intermediate region zone. This is easier for larger otoliths, but can be difficult for small otoliths where there is not much width outside the last broad inner zone, i.e., otoliths with only 6–15 zones in total.
- Note that the initial zones of this region are relatively broad, but the width of subsequent zones decrease rapidly, sometimes making this a difficult part of the section to interpret. There may also be double or split zones present, but these can often be resolved by moving in and out of focus (resulting in split zones merging to produce a single diffuse band, while true zones are

still clearly differentiated). It is also helpful to scan along an individual zone (across the section) to see if it is consistent in width and spacing with the zones above or below it.

3.3.3 Outer region

The outer region is a region of narrowing zones which can be numerous in large otoliths and require higher magnification (up to ×200) to achieve reliable counts. No obvious transition zone is observed, i.e., where the fish reaches sexual maturity. The estimated mean age at first maturity for smooth oreo, based on an analysis of gonad stage data, was 31 years (Ministry for Primary Industries 2015).

In order to correctly interpret this zone, it is necessary to:

- Examine the edge of the section to determine where the clearest zones are located and decide on the best path for a complete count (primordium to edge). It may be necessary to move across the section (laterally) at a convenient point to enable the count to continue, i.e., there may be a particularly dark zone or some distinctive mark or structure on the section to use as a marker to enable the count to continue.
- Understand that finding readable zones on the otolith edge may require close study, and adjustments of focus, and increased magnification.
- Note that zone resolution for the outer edge often determines the overall readability of the section. There may be a substantial area where zones are not clearly visible even after exploring the section and adjusting focus and magnification, resulting in an unreadable section (readability score 5). A tentative count may be possible after considerable effort to interpret unclear parts of the section (where readability score 4 should be assigned). Better readability scores (3 or less) should be assigned for repeatable and similar counts.
- Changing to a higher magnification during reading is possible if a distinct mark on the section is located to enable the zone count to continue. If no obvious marks are visible it is best to read the whole section using the higher magnification.

Examples of sections with a range of readability are shown in Figures 8–13.

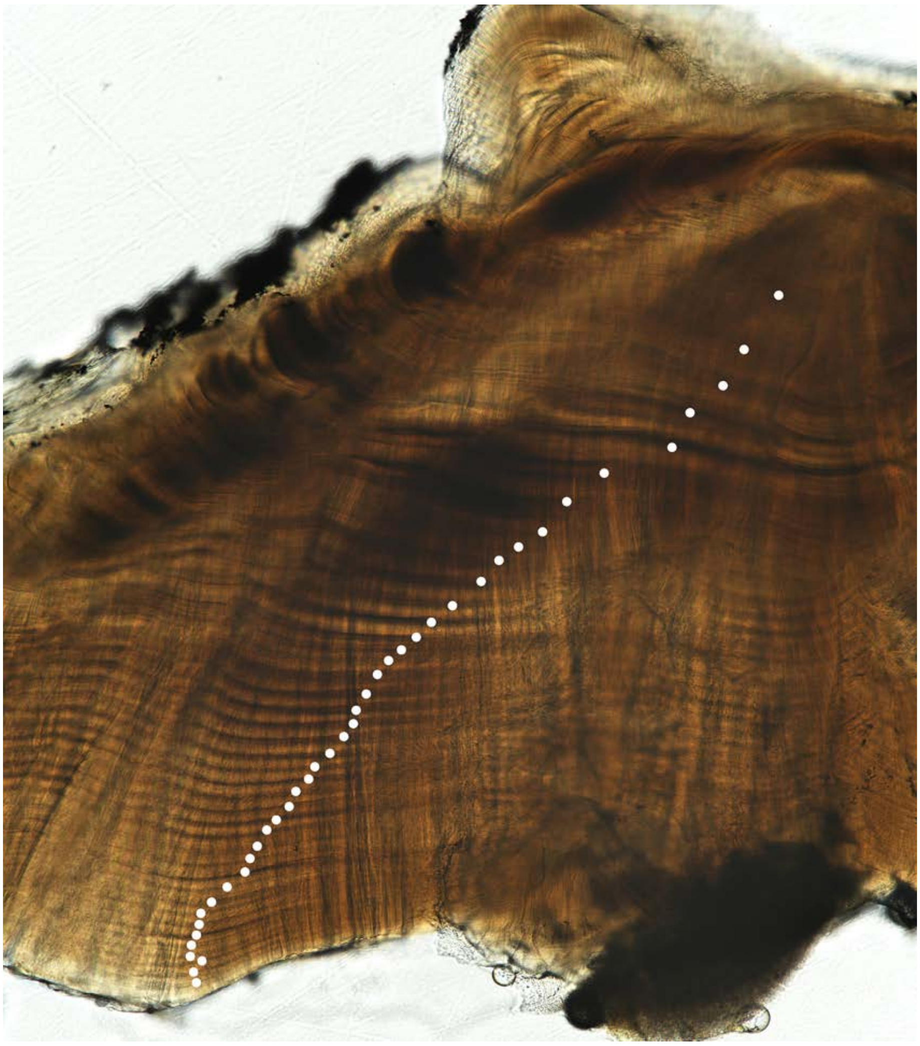

Figure 9: Smooth oreo otolith (SWA0501, station 32, fish #12, slide #29_4, 37.3cm, male), estimated age 41 years, readability 2.

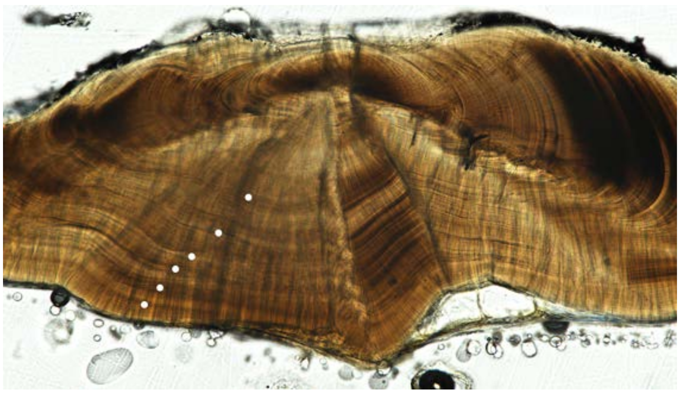

Figure 10: Smooth oreo otolith (SWA0501, station 69, fish #10, slide #78_4, 17.5 cm, female), estimated age 6 years, readability 2.

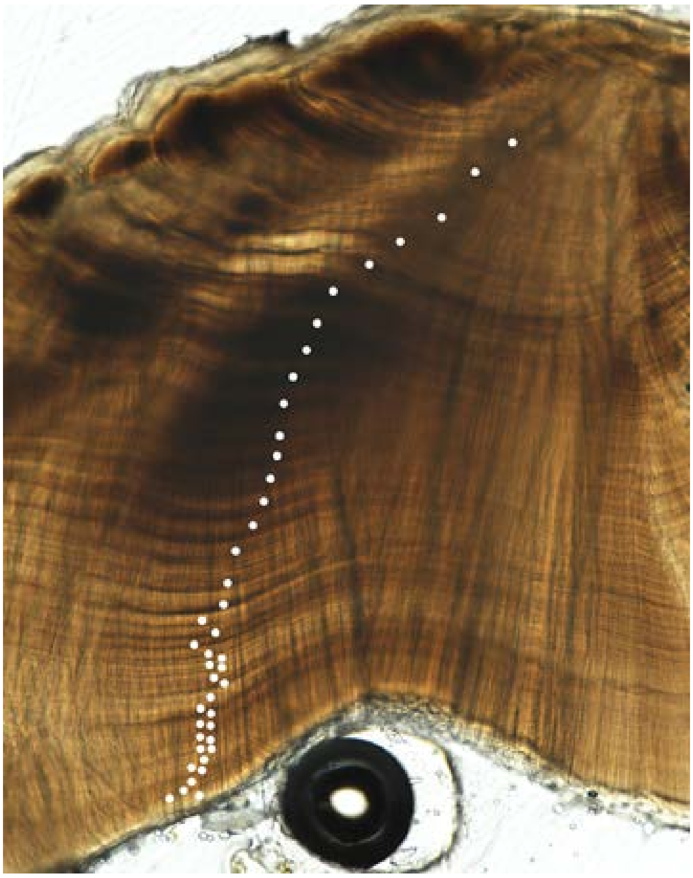

Figure 11: Smooth oreo otolith (TAN9812, station 55, fish #51, slide #72_3, 44.9 cm, female), estimated age 43 years, readability 3.

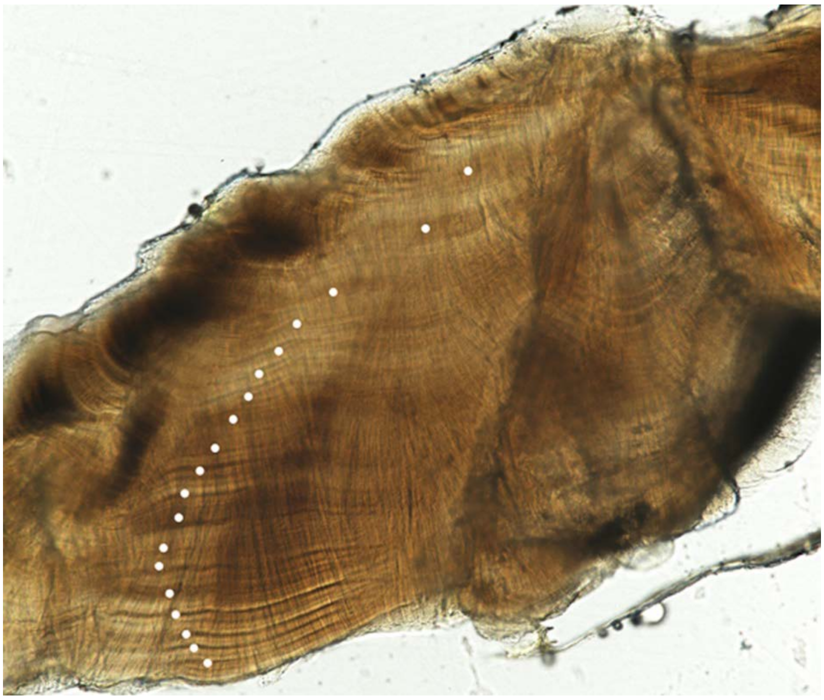

Figure 12: Smooth oreo otolith (SWA0501, station 34, fish #9, slide #34_1, 30.5 cm, female), estimated age 19 years, readability 4.

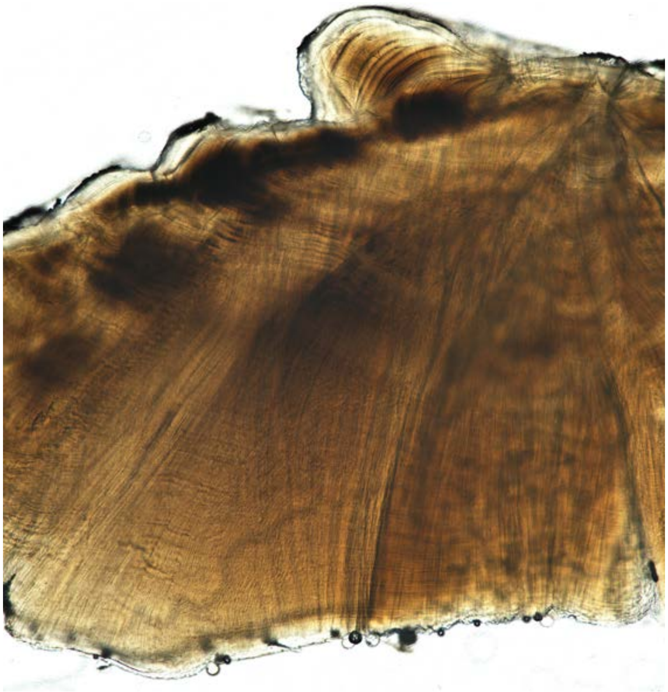

Figure 13: Smooth oreo otolith (SWA0501, station 28, fish #11, slide #23_4, 39 cm, female), readability 5, i.e., no count deemed possible.

3.4 Use of digital images in age estimation

Using digital images for ageing both of these oreo species might be difficult because relatively few preparations provide a clear zone count from primordium to edge. Most sections require manipulation of position and focus to track zones and resolve ambiguous parts of the section. Two or more digital images would be required for large/old fish where the inner zones are counted at ×100 and the outer zones at ×200. Annotated images of a reference (protocol) set of otoliths would be beneficial, however, for re-calibration for experienced readers and initial calibration (i.e., training) of new readers.

<u>Advantages of direct microscopic examination</u>

- Greater ability to perceive variations in intensity, density and colour of otolith zones.
- Greater ability to interpret three-dimensional structure by altering the focal plane and magnification.
- Greater resolution and ability to alter light intensity and contrast (e.g., with condenser).
- Faster for routine age estimates required for fisheries stock assessment where black oreo and smooth oreo sample sizes of about 500 per species are required. Sections with relatively large numbers of zones take more time to count. Experienced and careful readers can expect to read 30–50 sections per day on average. Extra time would be required if all images were captured digitally.

<u>Advantages of digital images</u>

- Ability to mark, count and display growth zones on the image for later re-examination, discussion, and dissemination.
- Reduced chance of counting same zone twice, or missing zones (if zones are marked on-screen while counting).

4. PRECISION and ACCURACY

In age estimation studies, 'accuracy' refers to the closeness of an age estimate to the true age whereas 'precision' is a measure of the variability between individual readings, either within or between readers (Campana 2001).

Accuracy

Direct age validation of both black oreo and smooth oreos has proven problematic, so testing for accuracy is difficult. Horn et al. (2018) used the bomb radiocarbon ageing technique to provide independent age estimates from sagittal otolith cores. For black oreo, the ^{14}C values from the cores corroborated well with the ^{14}C surface water reference curve, indicating that the otolith zone count interpretation method described above does produce relatively accurate estimates of age. Unlike black oreo, smooth oreo do not appear to have a near-surface juvenile phase, so the ^{14}C values from the otolith cores did not correlate with the surface water reference curve. However, the values did correlate, to some extent, with a deepwater reference curve developed for bluenose (*Hyperoglyphe antarctica*) (Horn et al. 2010), indicating that the otolith interpretation method described above probably provides relatively accurate estimates of age.

Precision

Estimates of precision are a necessary tool for comparing within- and between-reader variability or error. They can indicate bias, provide an indication of "uncertainty" surrounding the age estimates, and indicate whether age estimates from a particular species are reliable for stock assessment purposes.

Estimates of between-reader and within-reader error were made for black oreo and smooth oreo age estimates by Doonan et al. (1995). Two otolith readers read all the smooth oreo and the black oreo otolith sets. Findings are summarised in Table 1. The variability of the first and final otolith readings made by two readers was higher for black oreo than for smooth oreo, reflecting anecdotal observation that the otoliths were "harder to read" than those of smooth oreo (Doonan et al. 1995).

Table 1: Within- and between-reader average percent errors for smooth oreo (SSO) and black oreo (BOE) otolith readings from Doonan et al. (1995).

	Between-reader	Within-reader	
		Reader 1	Reader 2
SSO	6.1	6.5	5.3
BOE	8.4	6.0	7.0

Further estimates for between- and within-error reading were made for smooth oreo by Doonan et al. (2008). They reported estimates of within-reader CV of 7.3 and 7.6% for readers 1 and 2 respectively. They estimated that the CV for the mean age for readings made by both readers was 6.6% but the estimate was 5.3% if there was no between-reader component. This indicates that the between-reader source CV was 4% and that the total error CV, including within and between-reader error, for any one otolith reading was 8.5%.

A further analysis of the precision of age estimates for black oreo was carried out by Doonan et al (2016). They concluded that the ageing of black oreo otoliths for that study was problematical and that there was a small between-reader bias and the overall precision of the age estimates had a CV of 15%.

5. 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 (ERD) which physically shows how all tables relate to each other, and to other databases.

When research has been completed, NIWA receives the documented age data (usually in an Excel spreadsheet format) from the research provider and performs data audit and validation checks prior to loading these data to the *age* database (Table 2).

Table 2: An example of black oreo age data from a research survey submitted for loading onto the *age* database. Data recorded for each otolith are: origin, source of sample, e.g., SWA = *San Waitaki* (vessel used for survey); yr, year of sample collection; trip_code, survey where sample was collected; sample_no, tow or station number; sub_sample_no, default is -1, i.e., no subsample; fish_no, fish number within the sample at each station; prep_no, is the unique number for each otolith (fish); slide_no, each slide is numbered but there may be 1–5 otolith sections on each slide; result1, total zone count; result2, section readability using a 5-stage scale; age, estimated age in years; project_code, age estimation project.

origin	yr	trip_code	sample_no	sub_sample_no	area	species	fish_no	prep_no	slide_no	reading_no	reading_date	reader	lgth	sex	result1	result2	age	proj_code
SWA	2014	swa1402	4	-1	SWCR	BOE	3	52	18-1	1	28-Jan-16	111	30.0	2	17	3	17	MID2015-01
SWA	2014	swa1402	4	-1	SWCR	BOE	6	53	18-2	1	28-Jan-16	111	31.3	1	24	3	24	MID2015-01
SWA	2014	swa1402	4	-1	SWCR	BOE	25	54	18-3	1	28-Jan-16	111	32.8	2	20	2	20	MID2015-01
SWA	2014	swa1402	4	-1	SWCR	BOE	38	55	19-1	1	28-Jan-16	111	38.0	2	52	4	52	MID2015-01
SWA	2014	swa1402	5	-1	SWCR	BOE	1	56	19-2	1	28-Jan-16	111	31.8	1	21	2	21	MID2015-01
SWA	2014	swa1402	5	-1	SWCR	BOE	10	57	19-3	1	28-Jan-16	111	30.9	1	49	4	49	MID2015-01

6. REFERENCE OTOLITH COLLECTIONS

The protocol or reference collections for both black oreo and smooth oreo included 100 sections. Previously prepared and read otolith sections were selected to represent a range of readability scales, and young to old age estimates. A new or revising reader can randomly select a subset of about 30 otoliths sections (work for about one day) from the reference set, and should also select a different subset of sections for subsequent training sessions. The black oreo reference collection included a subset of 46 slides previously selected as a reference subset plus an additional 54 sections chosen from otoliths read in 2016 for project MID201501 that had readability scales of 2–4 and zone counts (numbers of sections in parentheses) of 5–20 (14), 21–40 (20) and >41 (20). The smooth oreo reference collection includes 13 slides previously selected plus an additional 87 sections read in 2007 for project OEO200603 that had readability scales of 2–4 and zone counts (numbers of sections in parentheses) of 5–15 (18), 16–30 (50) and >31 (19). The objective is to replace the older reference otolith subsets for both species with more recent (better) preparations, i.e., embedded in resin under a coverslip, as they become available. Both collections were established and are held by NIWA. The agreed ages for these otoliths were taken as the readings by Reader 1. They were stored in a table (*t_reference*) in the *age* database (administered by NIWA for Fisheries New Zealand). The reference otoliths can be used for training new readers as well as monitoring their progress as they gain experience in ageing. Any new readings of the reference otoliths are also stored in the *age* database (in table *t_ref_reading*). New readings of the reference otoliths are stored in this separate table to distinguish each calibration or training reading from those used to estimate catch-at-age distributions or growth parameters.

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APPENDIX A: Glossary of otolith terminology and ageing definitions

Based on Kalish et al. (1995) "Glossary for otolith studies" but with some added items to describe New Zealand practice.

Accuracy – the closeness of a measured or computed value to its true value.
Age estimation, age determination – these terms are preferred when discussing the process of assigning ages to fish. The term ageing should not be used as it refers to time-related processes and the alteration of an organism's composition, structure, and function over time. The term age estimation is preferred.
Age frequency – the frequency distribution of fish by age group in a particular sample.
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.
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 (see Figure 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 primordia 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.
Drift – Shift with time in the interpretation of otolith macrostructure for the purposes of age determination.
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.
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.
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.
Postrostrum – one of the two posterior extensions of the otolith. The preferred sectioning plane is from the primordium to the postrostrum.
Precision – the closeness of repeated measurements of the same quantity. For a measurement technique that is free of bias, precision implies accuracy.
Primordial granule – the primary or initial components of the primordium. There may be one or more primordial granules in each primordium. In sagittae the granules may be composed of vaterite, whereas the rest of the primordium is typically aragonite.
Primordium (pl. Primordia) – the initial complex structure of an otolith, it consists of granular or fibrillar material surrounding one or more optically dense nuclei from 0.5 um to 1.0 um in diameter. In the early stages of otolith growth, if several primordia are present, they generally fuse to form the otolith core.
Rostrum – anterior projection of the sagitta (see Figure 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.
Sulcus acusticus (commonly shortened to ‘sulcus’) – a groove along the medial surface of the sagitta. 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 can be formed in otoliths 1) during metamorphosis from larval to juvenile stages, 2) at the onset of sexual maturity, 3) when growth rate changes markedly, or 4) 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.
Vaterite – a polymorph of calcium carbonate that is glassy in appearance and does not show opaque and translucent zones. 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.