Fisheries New Zealand

Tini a Tangaroa

Age determination protocol for ling (*Genypterus blacodes*)

New Zealand Fisheries Assessment Report 2021/31

P.L. Horn

ISSN 1179-5352 (online)
ISBN 978-1-99-100929-6 (online)

June 2021

New Zealand Government

Requests for further copies should be directed to:

Publications Logistics Officer
Ministry for Primary Industries
PO Box 2526
WELLINGTON 6140

Email: brand@mpi.govt.nz
Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries websites at:
http://www.mpi.govt.nz/news-and-resources/publications
http://fs.fish.govt.nz go to Document library/Research reports

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Horn, P.L. (2021). Age determination protocol for ling (*Genypterus blacodes*).

***New Zealand Fisheries Assessment Report 2021/31.* 19 p.**

This report documents the age determination protocol for an important New Zealand middle-depth finfish species: ling (*Genypterus blacodes*). It describes the most recent scientific methodologies used for otolith preparation and interpretation, ageing procedures, and the estimation of ageing precision. In addition, an otolith reference collection of 480 preparations has been compiled and documented. Agreed readings and ages determined for the reference set are stored in a reference table in the *age* database. The reference set sample was generally a random selection from fish stocks and seasons to account for spatio-temporal variations in otolith readability; however, the selection process also ensured a comprehensive range of fish size and age was included.

Digital image examples of otolith reference set preparations are presented and fully illustrate the zone interpretation used in determining fish age for ling. Difficulties and idiosyncrasies related to ageing prepared otoliths are also documented. There is a wide variation in length-at-age for ling in New Zealand waters related to sex and location.

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 a part of the Ministry for Primary Industries (MPI)) held a fish ageing workshop in Wellington (May 2011), producing a document “Guideline for the development of fish age determination protocols” based on the workshops results. From this, it was anticipated that age determination protocols would be developed for every species that was routinely aged through the Fisheries New Zealand fisheries research projects.

This report describes the age determination protocol for an important New Zealand middle-depth finfish species: ling (*Genypterus blacodes*) (Figure 1). Significant fishstocks (LIN 1–8) for this species fall within Tier 1 of the National Fisheries Plan for Deepwater and Middle-depth Fisheries, with service strategies that promote regular stock assessment, thus utilising routinely collected catch-at-age information. The purpose of the protocol is to provide a practical guide for ageing, describing the methodologies and techniques used by otolith readers in how otoliths (‘ear bones’) should be prepared, how the visible zones within the otoliths are best examined and interpreted, and how the annual zone counts are best converted into estimates of fish age. This protocol will ensure 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 has been made to document protocols related to daily increments in ling otoliths (usually associated with ageing larval or juvenile fish) or investigations into otolith ultrastructure or chemical composition, because these are seen to be outside the context of the current project. In addition, of the three otolith pairs present in the otic capsule in heads of bony fishes (asteriscae, lapillae, sagittae), the largest is most often used in age estimation (Panfili et al. 2002), i.e., the sagittae, and has been used to age ling. Therefore, throughout this report, the use of ‘otolith’ will be synonymous with sagittal otolith (Figure 1). A glossary describing otolith terminologies and ageing definitions outlined in the “Guideline for the development of fish age determination protocols” has also been included in this report for reference purposes (Appendix 1).

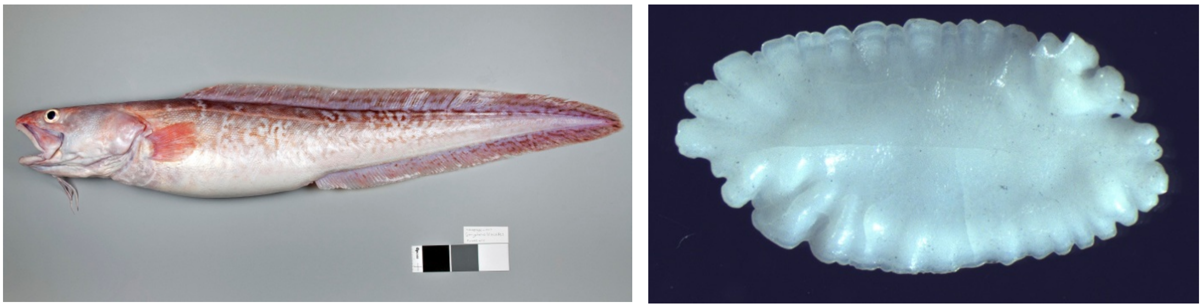

Figure 1: Ling (*Genypterus blacodes*): whole fish and sagittal otolith. (Fish photo by Peter Marriott, NIWA.)

2. AGE DETERMINATION PROTOCOL FOR LING

2.1 Background

Attempts to age ling (*Genypterus blacodes*) were first reported by Wrzesiński (1984) using specimens off Argentina and Withell & Wankowski (1989) using specimens from off south-eastern Australia. Both studies reported unvalidated methodologies. Wrzesiński (1984) counted opaque zones on broken and burned otoliths, with the broken surface coated with glycerine. Withell & Wankowski (1989) counted complete annuli (i.e., an annulus of an inner opaque band and an outer hyaline band) on dorso-ventral sections of otoliths 0.5–0.6 mm thick, after first embedding whole otoliths in a clear polyester resin. Withell & Wankowski (1989) initially examined whole untreated otoliths immersed in water and then trialled other techniques (staining, breaking and burning, heating, sectioning, and immersion in other clearing agents) to see if the readability of otoliths could be improved. They concluded that only immersion in water and the sectioning technique described previously resulted in significantly improved clarity.

Subsequently, partially validated studies were produced for Chilean (Chong & Aguayo 1990) and New Zealand (Horn 1993) *G. blacodes* based primarily on the changing state of otolith margins over one year. A partially validated age study of *Genypterus capensis* (the kingclip off South Africa) was also produced around the same time; this used a similar validation method (Japp 1990).

The New Zealand partial validation was based on classifications of otolith margins throughout the year, using marginal increment measurements for fish aged 3 to 7, and marginal state (i.e., either translucent or opaque) for fish older than 7 years, and on counts of otolith zones in fish from four adjacent juvenile length-frequency modes (Horn 1993). Before reading, all otoliths were broken transversely through the nucleus, the broken surface polished with fine sandpaper, and heated in an oven until amber-coloured. The sections were mounted in plasticine and coated with paraffin oil. Examination under a binocular microscope (×30), with illumination by reflected light, revealed a pattern of alternating dark and light zones. Complete rings (i.e., dark translucent zones with lighter opaque material on both sides) on the otolith were counted. Increment width (i.e., the width of opaque material outside the last completed translucent zone) of fish with 3–7 otolith bands was highest in autumn to early spring. For fish with more than 7 bands, translucent margins were least prevalent in July and most common in October. These data implied that in general for ling the translucent zone forms around winter, and that most fish are laying down opaque material again by spring. Horn (1993) noted, however, that it was often difficult to precisely define opaque-translucent boundaries owing to the diffuse structure of many zones and the narrowness of older zones. The number of zones visible in otoliths from each of four juvenile modes (within a length range from 20 to 59 cm total length, with zone counts ranging from 1 to 5) generally increased by one for each consecutive mode. These lines of evidence suggested that for ling aged 1–7 years, and probably for older age classes as well, that pairs of opaque and translucent zones are formed annually.

Confirmation of the annual formation of pairs of opaque and translucent zones in ling otoliths was provided from a study using the bomb radiocarbon chronometer method (Kalish et al. 2002). The tested otoliths were sampled from the western Bass Strait, Australia. Measurements of $\Delta^{14}C$ from ling otolith cores plotted against birth date determined from counts of zones in otolith sections were shown to fall on or close to the curve reflecting the increase in ^{14}C in the south-west Pacific Ocean between the late 1950s and early 1970s (Kalish 1993).

Ling has a moderate to long life-span, with fish aged over 25 years being relatively common in New Zealand samples. The oldest recorded age determined for a ling in the New Zealand Exclusive Economic Zone was 46 years, for a female (170 cm) and a male (112 cm) captured on the Chatham Rise in 2004 and 2017, respectively. However, only about 0.4% of ling aged from New Zealand waters were older than 30 years and most of the commercial and research catch comprised fish aged from about 5 to 20 years (Horn & Sutton 2019).

New Zealand ling spawn during winter-spring on Chatham Rise and off the west coast South Island (WCSI) and during spring-summer on the Campbell Plateau and Bounty Plateau (Horn 2005), so a 'birthday' was set arbitrarily at 1 October. At about this time, the translucent zone is just being completed and opaque material is visible on most otolith margins. Hence, because of the relatively long spawning season, the nuclei in otoliths can be either translucent or opaque dependent on whether the fish were spawned early or late. Consequently, ranges of length-at-age can be relatively broad for juvenile fish.

2.2 Otolith preparation and examination

Sagittal otoliths are acknowledged as the primary structure for determining the age of ling (Chong & Aguayo 1990, Horn 1993). The method used to prepare ling otoliths for ageing is the bake and embed method which was developed in the early 1990s (Hanchet & Uozumi 1996), with the aim to provide robust, permanent, ordered, and compact sets of aged otoliths. The method has subsequently been used, occasionally with minor modifications, to prepare otoliths from a variety of deepwater, middle-depth, and inshore species. The following sections present additional information pertinent to ling age determination.

Post extraction, ling 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. Because ling show differential growth between the sexes, where females attain a larger average size than males at a given age (Horn 2005), age determination studies should record sex as a mandatory requirement for each fish selected for analysis. 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), and then baked in an oven until amber-coloured (270 °C for about 4 min). The baked otoliths are then embedded in blocks of clear epoxy resin in flexible silicone moulds. Sixty otoliths are embedded in each block; there are five rows of 12 otoliths, each row being four wide and three high (Figure 3). In each row, the pencil lines (which are still clearly visible after baking) are aligned with marks on the moulds so that a single saw-cut will bifurcate all 12 otoliths along the marked lines. The cross-sectioning is carried out using a rotary diamond-edged saw (0.3 mm thick) with water lubrication to produce a series of short resin sub-blocks that fit easily on a microscope stage (Figure 3). Details identifying the block and row number are written on each block section.

For examination and zone counting, the prepared sub-blocks (each with 12 otolith cross-sections) is coated in paraffin oil, illuminated by reflected light with an incident angle of about 45°, and examined under a binocular microscope (×25). A pattern of dark-brown (translucent) and light-brown (opaque) zones is apparent. If the zonation pattern is unclear, improvements can sometimes be achieved by altering the angle of incidence of the illumination and/or by slightly altering the plane of focus. Subsequently in this document, 'zone' refers to the paired structure of one opaque band inside one translucent band. The number of translucent zones is counted. Fish length and sex are unknown to the otolith reader.

Figure 2: Untreated sagittal otolith (distal surface), with pencil line indicating the position of the cross-section.

Figure 3: Resin block (block #8 of the ling reference set), comprising six sub-blocks, holding 60 cross-sectioned baked otoliths in five groups of 12.

2.3 Otolith interpretation

Clearest banding generally occurred beside the sulcus (either dorsally or ventrally) or along either of the long axes (although more frequently along the ventral axis) of the prepared otolith surface (Figure 4). Older growth zones are also often clear along much of the proximal side between the sulcus and otolith tip (Figure 4). Banding was generally indistinct on the distal surface and zone counts are never made in this region. Most counts are made on the ventral side of the otolith cross-section. The dorsal part of the cross-section is sometimes used, however, if the ventral part is unclear, or as a check on the ventral count if the dorsal part is obviously clear.

Counts are made of complete translucent zones (i.e., translucent zones with opaque material on both sides of them). Translucent zones appear dark when illuminated by reflected light.

In general, otoliths from male ling had a clearer banding pattern than those from females. From 19 552 readings (8785 males and 10 767 females) where otolith clarity grades on a 5-stage scale had been recorded (where 1 = unambiguous count, to 5 = unreadable), 14.4% of males were graded 2 or better, and 4.2% of females were similarly graded.

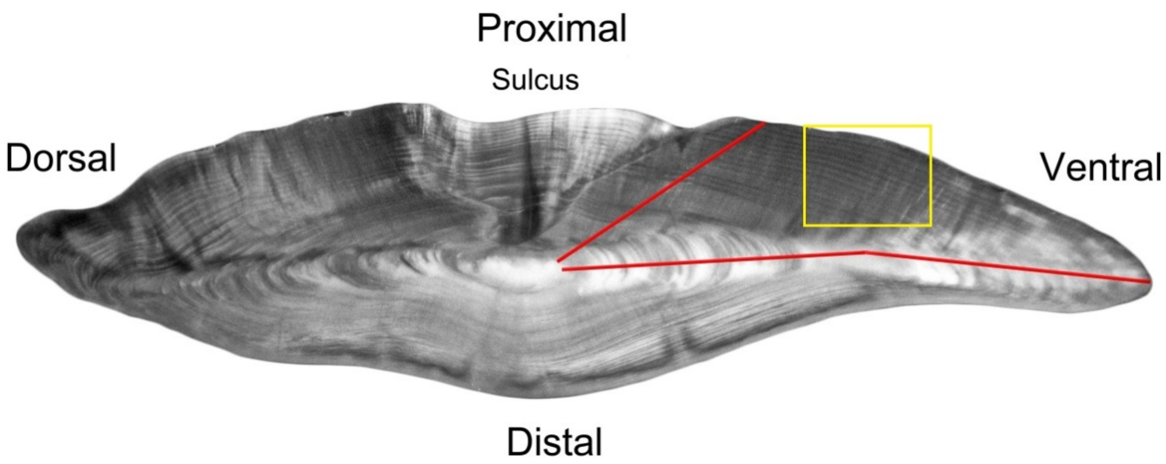

Figure 4: Ling otolith image (B&W) of a baked transverse cross-section under reflected light, illustrating otolith terminology. The two red lines indicate the areas where zone counts are often made. Older growth zones are also often very clear in the area outlined in yellow. This is reference set otolith #013 and is illustrated with zone interpretations later in this document. All otolith section images presented below are shown in the same orientation as this example.

The main assumptions made when interpreting zones in transversely sectioned ling otoliths are:

1. The translucent zone (dark in baked cross-section preparations) first becomes visible in winter to early spring.
2. The theoretical 'birthday' for all ling is 1 October (although mid spawning season timing does vary across geographical areas; see section 2.1).
3. Translucent zones are counted. For otoliths aged from winter samples (i.e., primarily the WCSI and Cook Strait otoliths sampled concurrently with the hoki spawning season fisheries), the margin can be very wide opaque, translucent, or vary narrow opaque. Where the margin is very wide opaque, 1 is added to the translucent zone count; similarly a translucent margin is also counted as if it is complete (i.e., has opaque material outside it). These adjustments are made so that all fish from the spawning fisheries are aged as though they have all just had their birthday (even though they have been captured before 1 October).

Zone width tends to decline steadily up to about ages 7–10, and then becomes relatively uniform and often quite clear. Sometimes, however, more than one reading from more than one region is required to attain a final zone count. Zone deposition on different parts of the otolith section may not always appear to be equal and, if discrepancies occur between counts, the default is to use the higher estimate.

The conversion of a zone count to an age estimate involves considering the relationship between the date of the increment formation, the date of capture, and the nominal birthdate (Panfili et al. 2002). This is relatively straightforward for ling because the counted translucent zone begins to appear in winter to early spring just before the chosen birthdate (1 October). The zone count thus corresponds with the age of the fish in whole years (with the exception of otoliths with wide opaque margins in June–September, as noted in assumption 3 above). Three categories of ling age samples are produced routinely (Horn & Sutton 2019). 1) Trawl fishery samples from off the west coast South Island in winter (characterised generally by otoliths with translucent dark margins), and from the Chatham Rise and Campbell Plateau from late spring to early autumn (characterised by otoliths with opaque light margins of varying widths). 2) Line fishery samples extend over various months (i.e., Chatham Rise, June–October; Puysegur spawning, October–December; Campbell Plateau non-spawning, February–July) and can have otoliths with opaque or translucent margins depending on sampling time. 3) Trawl survey samples are generally collected during the summer (Chatham Rise and Campbell Plateau (Sub-Antarctic survey), characterised by medium width opaque margins), but occasionally during the winter (WCSI, translucent margins).

Otolith readers know the source of the sample they are reading (i.e., the fishery or survey they are derived from), and they know that otoliths are prepared roughly in temporal order (i.e., early season first, and late season last). The timing of the deposition of the newly formed zones may vary slightly between individual fish, stocks, and years, but, by knowing the sample source, readers are able to anticipate the expected otolith margin in comparison to what is actually seen. This is primarily an issue for otoliths collected during early winter when a very wide opaque margin may be apparent (i.e., no translucent material is yet apparent, so the reader must add 1 to the zone count).

A 5-stage readability scale is currently used when ageing ling otoliths. Each section was scored subjectively for readability as follows; 1, unambiguous count; 2, count ±1; 3, some increments not clear, or some uncertainty in distinguishing 'true' increments; 4, many increments not clear and alternative counts possible; 5, unreadable.

An example of otolith section interpretation (Figure 5) shows how the count was made initially along the long axis and then moves to the more central part of the dorsal side where the older zones are clear. The same count in similar areas can be derived on the ventral side of the section.

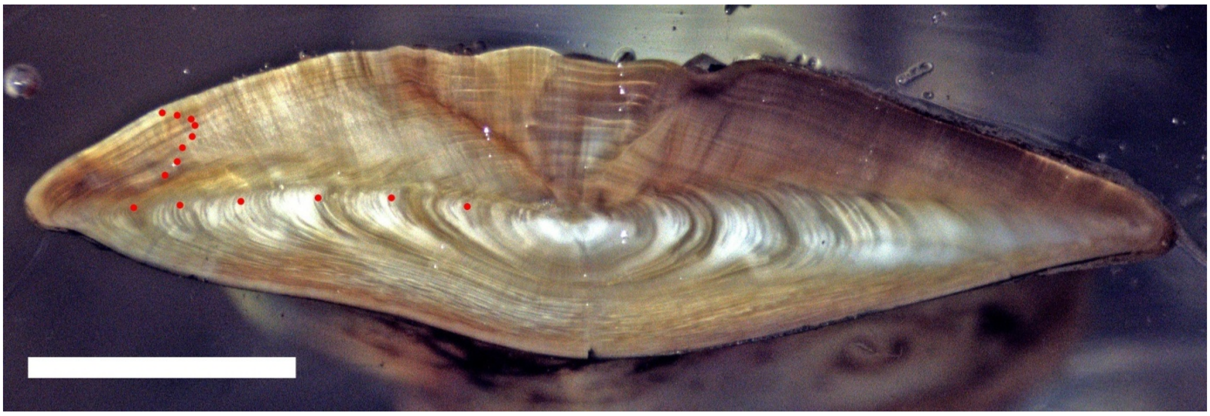

Figure 5: Otolith section image for an 86 cm male ling (Sub-Antarctic, December) estimated to be 14 years old, with a readability classification of 2 (reference set otolith #001). The counted dark zones are indicated by red dots. This fish was sampled during summer and exhibits a medium width margin. White bar = 2 mm.

2.4 Characteristics of sections

A good ling otolith section is one where the first three to six annual zones are clear and unambiguous (even though some sub-annual zonation might still be apparent) and where subsequent annual zones are clear and distinct and exhibit a declining width as the margin is approached along the long axis, although will be relatively evenly spaced in the direction of the proximal surface.

The following images (Figures 6–15) illustrate the interpretation of otolith sections from a range of fish sizes and a range of otolith readabilities. Some characteristics of zonation patterns in ling otolith sections are described.

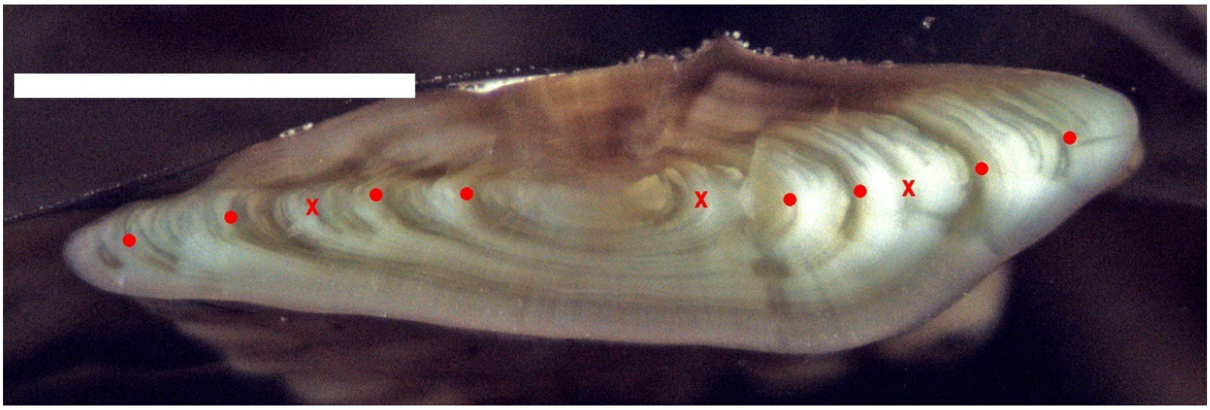

Figure 6: Otolith section image for a 51 cm male ling (Sub-Antarctic, December) estimated to be 4 years old, with a readability classification of 3 (reference set otolith #098). The counted dark zones considered to be annual are indicated by red dots, with false checks marked with red crosses. The assumed annual zones are relatively clear (although diffuse) adjacent to the sulcus. White bar = 2 mm.

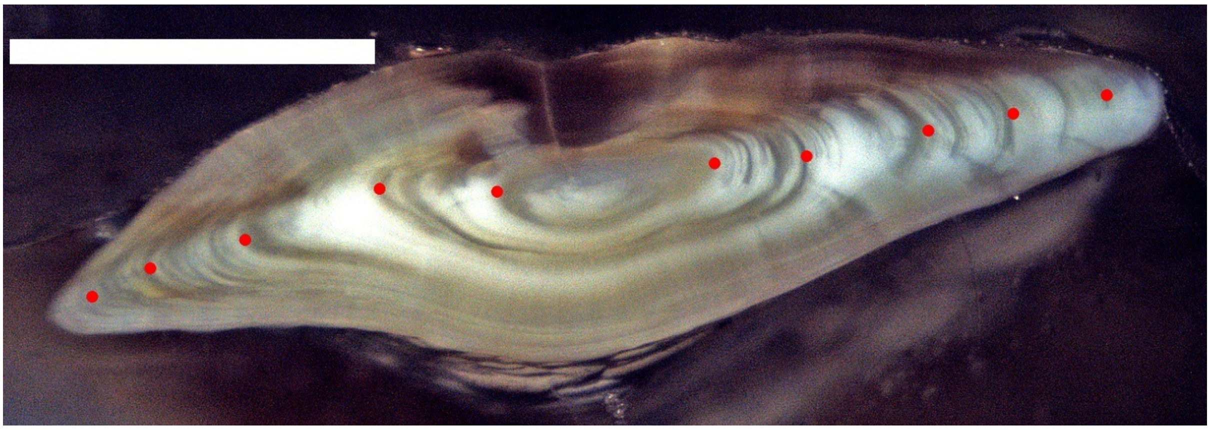

Figure 7: Otolith section image for a 58 cm male ling (Chatham Rise, January) estimated to be 5 years old, with a readability classification of 2 (reference set otolith #086). The counted dark zones considered to be annual are indicated by red dots. This section illustrates a common situation where the distance between the first and second zones is less than between the second and third zones. White bar = 2 mm.

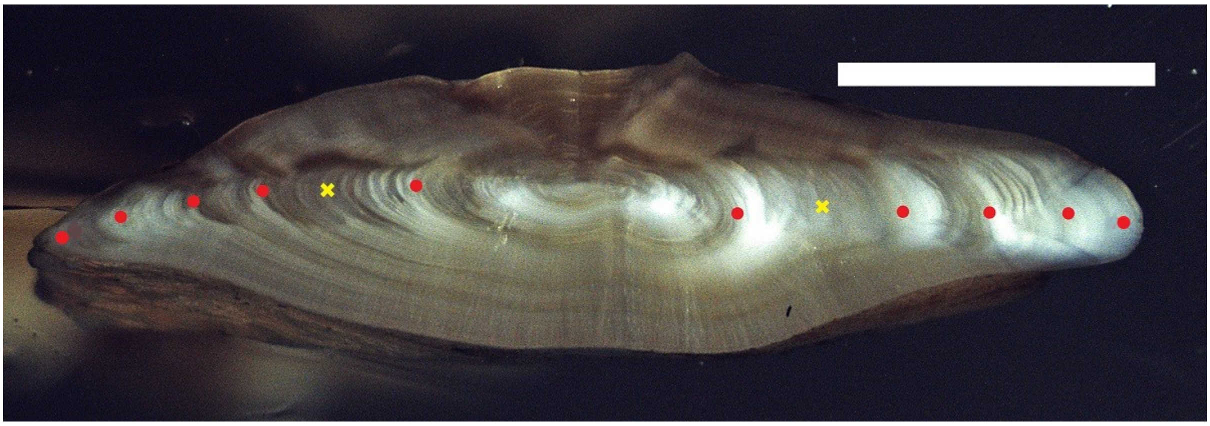

Figure 8: Otolith section image for a 70 cm female ling (WCSI, August) estimated to be 5 years old, with a readability classification of 3 and a narrow margin (reference set otolith #056). The counted dark zones considered to be annual are indicated by red dots. This fish may be a year older if the second annual zone is weak and actually at the location indicated by the yellow crosses. White bar = 2 mm.

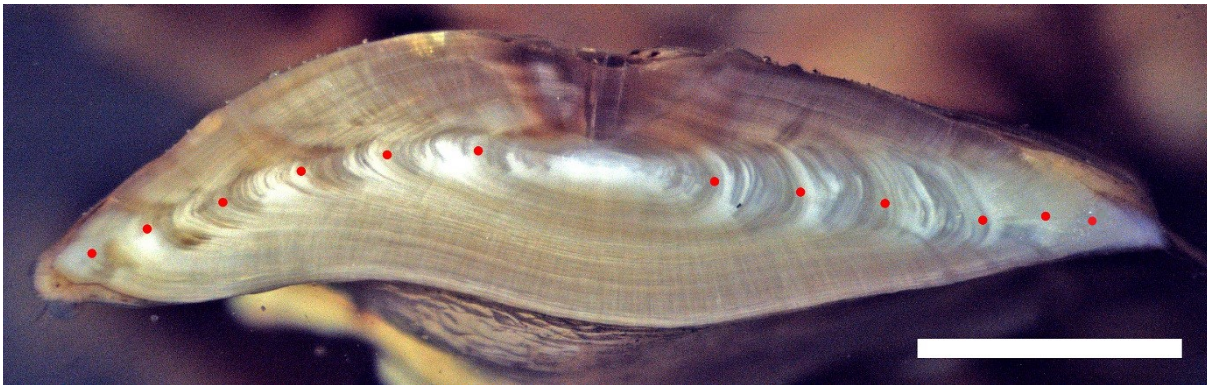

Figure 9: Otolith section image for a 78 cm male ling (WCSI, August) estimated to have 6 translucent zones, with a readability classification of 2 (reference set otolith #029). The counted dark zones are indicated by red dots. This fish was sampled in winter and has a wide margin, so would be classified as being 7 years old. White bar = 2 mm.

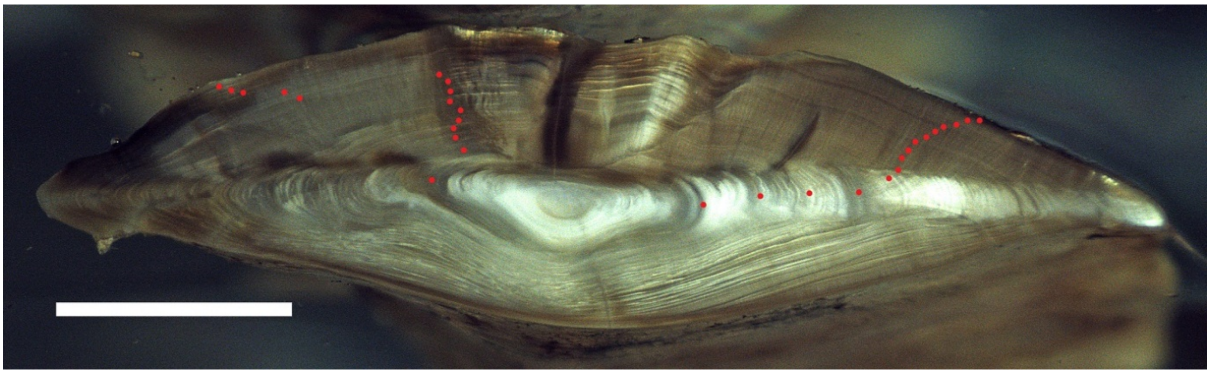

Figure 10: Otolith section image for a 102 cm female ling (Sub-Antarctic, December) estimated to be 15 years old, with a readability classification of 4 (reference set otolith #017). This otolith had relatively indistinct zones in most areas. By connecting some areas with moderately clear zones and comparing counts on both the ventral and dorsal sections, it was possible to derive the zonation pattern indicated by the red dots. White bar = 2 mm.

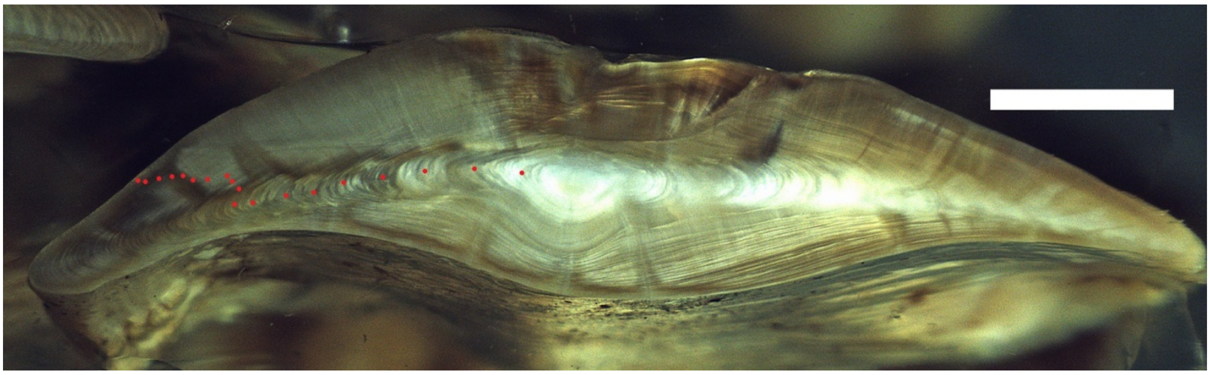

Figure 11: Otolith section image for a 132 cm female (Chatham Rise, January) estimated to be 19 years old, with a readability classification of 4 (reference set otolith #024). The counted dark zones are indicated by red dots. The dorsal section was very unclear. The ventral section was confusing with numerous zones considered to be sub-annual in the first 8–10 'true' annual zones. A perceived annual zonation pattern adjacent to the ventral side of the sulcus was used to help interpret 'true' zones closer to the ventral tip. White bar = 2 mm.

Figure 12: Otolith section image for a 127 cm female (Chatham Rise, January) estimated to be 20 years old, with a readability classification of 4 (reference set otolith #055). The counted dark zones are indicated by red dots. The youngest six zones and oldest six zones are clear, but the intermediate zones lack clarity and appear to exhibit multiple sub-annual zones. White bar = 2 mm.

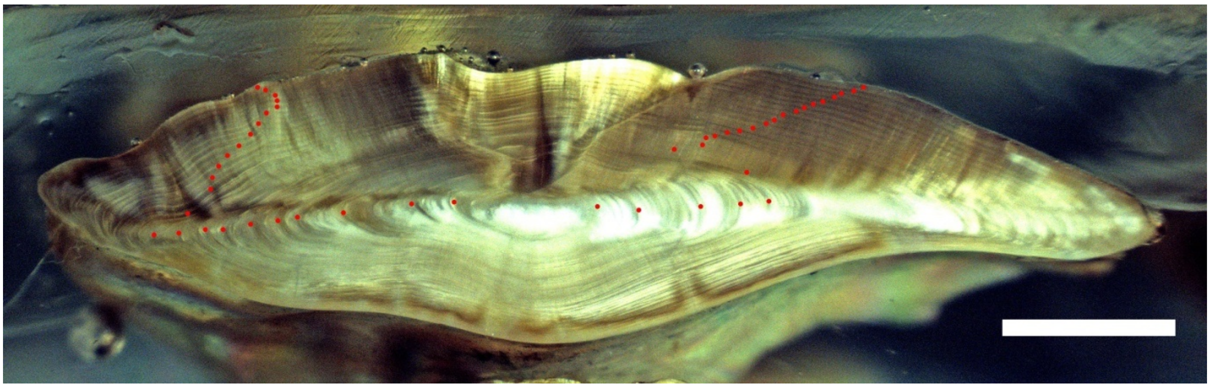

Figure 13: Otolith section image for a 151 cm male (WCSI, August) estimated to be 24 years old, with a readability classification of 3 (reference set otolith #013). The counted dark zones are indicated by red dots. On both the ventral and dorsal sections the 14 most recently formed zones and the six juvenile zones are relatively clear, but the intermediate zonation pattern is open to some interpretation. White bar = 2 mm.

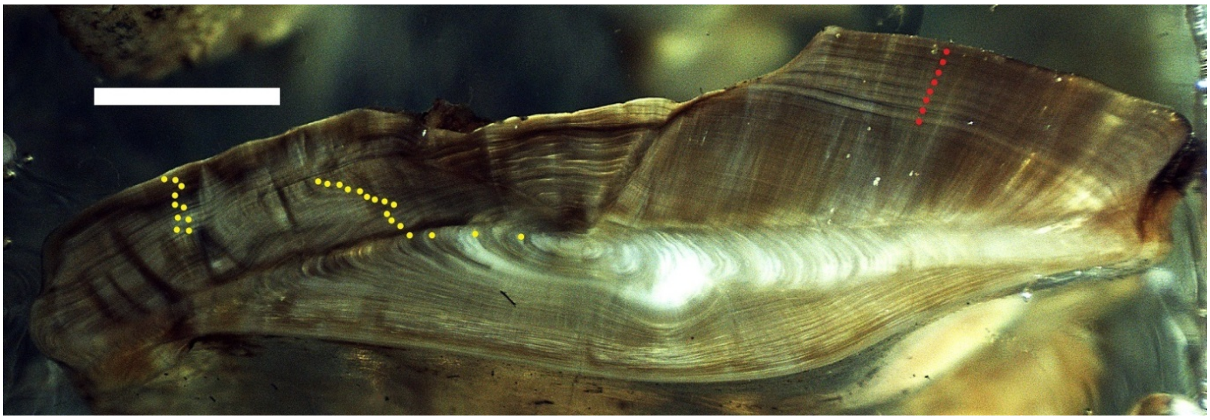

Figure 14: Otolith section image for a 145 cm female (WCSI, August) estimated to have 26 translucent zones, with a readability classification of 4 (reference set otolith #012). The counted dark zones are indicated by yellow dots. A marked change in the otolith growth pattern appears to have occurred about 8 years before death (see red dots). The most recently formed 10 zones and the first four juvenile zones are clear, but the intermediate zonation pattern is difficult to interpret. This fish was sampled in winter and has a wide margin, so would be classified as being 27 years old. White bar = 2 mm.

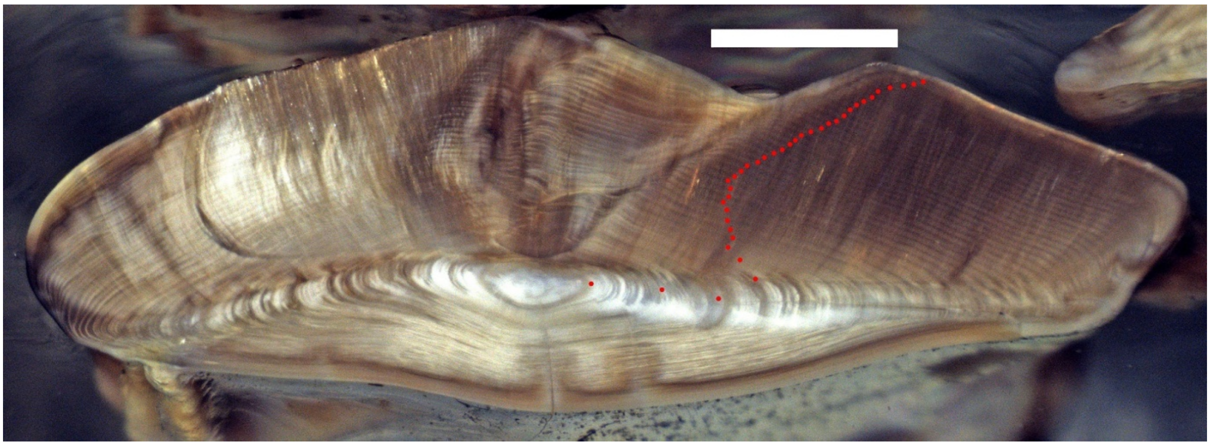

Figure 15: Otolith section image for a 130 cm male (Chatham Rise, January) that can be relatively clearly aged as 38 years, with a readability classification of 3 (reference set otolith #245). The counted dark zones are indicated by red dots. Sub-annual zones are apparent in the first four years of growth and the second zone is relatively diffuse and indistinct. This section provides a clear example of how, for old fish, otoliths continue to increase steadily in thickness but little in width. White bar = 2 mm.

2.5 Geographical differences in growth

Horn (2005) showed that New Zealand ling exhibited significant differences in mean length-at-age across different geographical areas, as well as between sexes within areas. Studies of ling growth off Chile and Australia have also demonstrated between-sex differences (Chong & Aguayo 1990, Tuck 2013). For example, in New Zealand waters, records of ling aged 15 years old range in length from 74 to 155 cm total length. Consequently, it was considered desirable to present growth curves for several area-sex combinations because these curves may be useful to help determine whether 'outlier' age-length data points are truly erroneous or not. Using age-length data produced by the current author up to January 2020, von Bertalanffy equations (by sex) were estimated for five different areas in New Zealand waters, postulated to hold distinct ling stocks (Horn 2005). Growth curves were fitted using the nonlinear least squares procedure in the FSA R statistical package (Ogle et al. 2020). In some areas the t_0 parameter was poorly defined primarily owing to the lack of very young and small fish in the samples. Consequently, von Bertalanffy parameters presented here were all produced after fixing the t_0 value at -0.1 years, a value that forced the fish to have a small positive length at time of hatching, essentially estimating the growth trajectory of young fish and thus producing a more biologically realistic growth model (Table 1). The growth curves from the five areas (Figure 16) clearly demonstrate the very broad range in the length-at-age of ling in New Zealand waters, related to both sex and location.

Table 1: Estimated von Bertalanffy parameters (with 95% confidence intervals, 95% CI), by area and by sex, where in all cases the t_0 parameter has been fixed at -0.1 years. *N*, sample size.

Area	Sex	*N*	Age range	L_∞	95% CI	*K*	95% CI
Cook Strait	male	3 122	2–42	142.6	141.1–144.0	0.122	0.119–0.125
	female	2 899	3–32	159.6	157.5–161.7	0.112	0.109–0.115
WCSI	male	6 132	2–36	134.4	133.4–135.4	0.116	0.114–0.118
	female	6 729	2–38	157.7	156.5–158.8	0.0978	0.0963–0.0992
Chatham Rise	male	11 855	1–46	111.0	110.6–111.4	0.150	0.149–0.151
	female	12 352	1–46	148.7	147.9–149.5	0.101	0.0997–0.102
Bounty Plateau	male	492	7–41	121.9	120.1–123.7	0.133	0.126–0.140
	female	678	5–40	143.1	140.4–145.7	0.107	0.102–0.112
Sub-Antarctic	male	9 307	1–36	92.6	92.3–92.8	0.215	0.213–0.217
	female	13 910	1–37	109.9	109.6–110.3	0.163	0.161–0.165

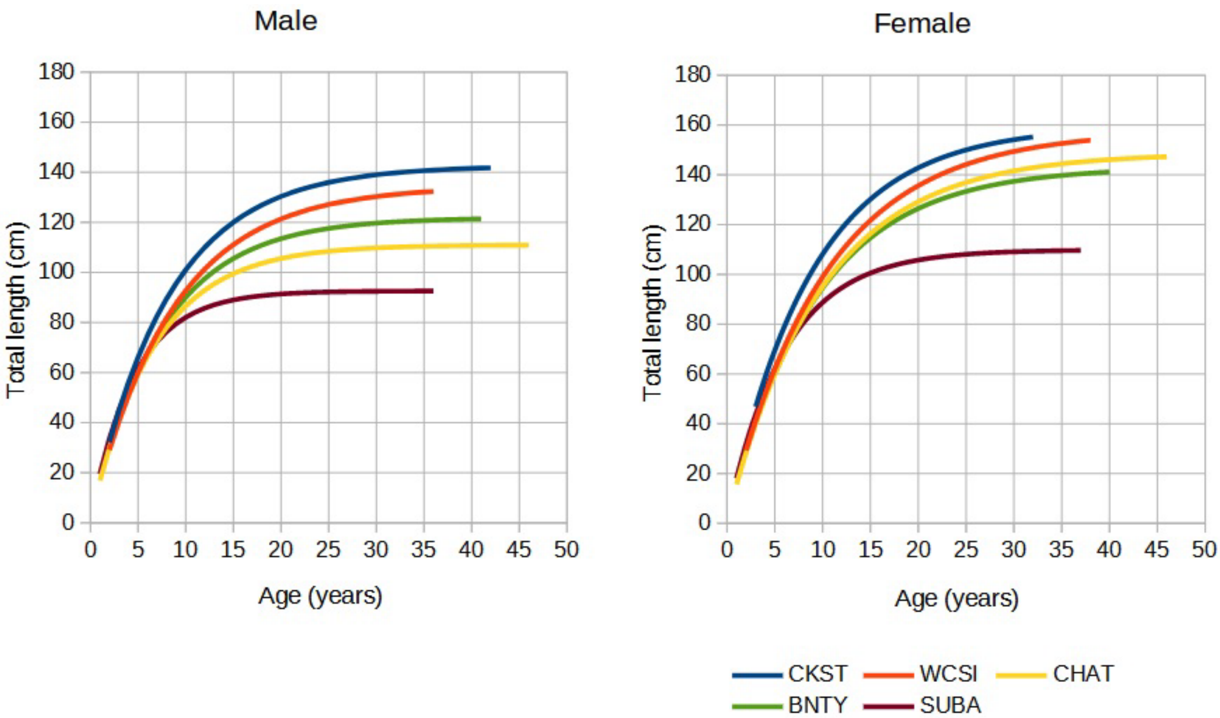

Figure 16: von Bertalanffy growth curves, by sex and area, all estimated with t_0 fixed at -0.1 years. The curves are plotted over the data ranges for which data were available. Areas: CKST, Cook Strait; WCSI, west coast South Island; CHAT, Chatham Rise; BNTY, Bounty Plateau; SUBA, Sub-Antarctic.

2.6 Reference collection

A collection of 480 ling otoliths has been selected for the reference collection, i.e., eight blocks each containing 60 preparations. This is expected to be sufficient for quality control monitoring in assessing reader performance, but it may be added to over time. The primary role of the reference set is to monitor ageing consistency (and accuracy) over both the short and long term, particularly for testing long-term drift, as well as consistency among age readers (Campana 2001). The ling reference collection was assembled from otoliths (archived at NIWA Wellington) collected from the main hoki fishery areas (west coast South Island, Chatham Rise, Campbell Plateau) during research trawl surveys from June 2012 to February 2013. The selection process of the reference set aimed to ensure that a seasonal distribution of otolith samples is represented, and that the full length range is covered, while not being dominated by any particular age class. Also, because growth variation between stocks (areas) and sexes has been reported for ling (Horn 2005), the otolith selection process also ensured approximately even sampling of these two categories. Examples of the otolith preparations for a range of fish sizes and ages are presented in Section 2.5 (Figures 6–15). Ling has a moderate to long life-span (i.e., few sampled fish are older than 25 years), so a reference collection of 480 otolith preparations is considered adequate for quality control purposes.

The estimated ('agreed') ages for otoliths selected for the reference set already exist on the *age* database (administered by NIWA for Fisheries New Zealand), and the data have been stored in a table (t_reference) created within this database. These preparations had already been aged two or three times in the past by the current author to produce a preferred age, and so can probably be treated with a reasonable level of confidence. The reference set may also be used for training new readers as well as monitoring their progress as they gain experience in ageing. Any new readings of the reference set collection (e.g., created before embarking on reading a new otolith collection) are stored on a second new table (table t_ref_age) to distinguish each calibration or training reading from those used to estimate catch-at-age distributions or growth parameters.

2.7 Format for data submission to *age* database

NIWA (Wellington) currently undertake the role of Data Manager and Custodian for fisheries research data owned by 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). Additional information that should be recorded include the date of reading, preparation method, and a description of how the agreed ages were derived from zone counts (e.g., from a single, or multiple readings by one or more readers).

Table 2: A example of ling age data submitted for loading onto the *age* database, where the sample contains otoliths originating from the observer programme (SOP) and a trawl survey (origin denoted by the vessel code TAN). Result1, translucent zone count; result2, margin classification; error1, readability category.

origin	yr	trip_code	sample _no	sub_sample _no	area	species	fish_no	prep_no	block_no	reading_no	material	reader	result1	result2	error1	age	project_code	length	sex
SOP	2012	3516	3	-1	SUB	LIN	5	160	3D	1	1	10	11	m	3	11	MID2010-01C	89	2
SOP	2012	3516	4	-1	SUB	LIN	1	161	3D	1	1	10	21	m	3	21	MID2010-01C	108	2
SOP	2012	3516	4	-1	SUB	LIN	2	162	3D	1	1	10	9	m	3	9	MID2010-01C	84	2
SOP	2012	3516	4	-1	SUB	LIN	3	163	3D	1	1	10	19	m	3	19	MID2010-01C	90	1
SOP	2012	3516	4	-1	SUB	LIN	5	164	3D	1	1	10	6	m	3	6	MID2010-01C	75	1
SOP	2012	3516	4	-1	SUB	LIN	7	165	3D	1	1	10	15	m	3	15	MID2010-01C	106	2
SOP	2012	3516	5	-1	SUB	LIN	1	166	3D	1	1	10	5	m	2	5	MID2010-01C	68	2
SOP	2012	3516	5	-1	SUB	LIN	2	167	3D	1	1	10	15	m	4	15	MID2010-01C	97	2
SOP	2012	3516	5	-1	SUB	LIN	3	168	3D	1	1	10	12	m	3	12	MID2010-01C	80	1
TAN	2006	tan0617	16	-1	PUKR	LIN	3007	112	2E	1	1	10	5		3	5	MID2006-01	63.4	2
TAN	2006	tan0617	17	-1	CAMP	LIN	3002	113	2E	1	1	10	10		3	10	MID2006-01	80.2	1
TAN	2006	tan0617	18	-1	STEW	LIN	3014	114	2E	1	1	10	4		3	4	MID2006-01	61.2	1
TAN	2006	tan0617	18	-1	STEW	LIN	3016	115	2E	1	1	10	7		3	7	MID2006-01	82.9	1
TAN	2006	tan0617	18	-1	STEW	LIN	3017	116	2E	1	1	10	5		3	5	MID2006-01	63.5	1
TAN	2006	tan0617	18	-1	STEW	LIN	3018	117	2E	1	1	10	3		3	3	MID2006-01	42.6	2
TAN	2006	tan0617	20	-1	STEW	LIN	3001	118	2E	1	1	10	11		3	11	MID2006-01	96.7	2
TAN	2006	tan0617	20	-1	STEW	LIN	3002	119	2E	1	1	10	12		2	12	MID2006-01	85.9	2
TAN	2006	tan0617	20	-1	STEW	LIN	3003	120	2E	1	1	10	16		4	16	MID2006-01	107.1	2

3. ACKNOWLEDGMENTS

This protocols report was funded by the author and NIWA and builds on decades of deepwater ageing projects funded by Fisheries New Zealand. I acknowledge the inclusion of a glossary of otolith terminology and additional figures and images that have been reproduced within this report from a number of fish ageing publications, particularly Kalish et al. (1995) and Marriott & Manning (2011). I thank Colin Sutton for otolith preparation, Alan Hart for assistance with otolith images, and Richard Saunders and Alan Hart for reviewing this document.

4. REFERENCES

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

Chong, J.; Aguayo, M. (1990). Determinación de edad y estimatión de los párametros de crecimiento del congrio dorado, *Genypterus blacodes* (Schneider, 1801) (Osteichthyes, Ophidiidae) en el Pacifico Sur-oriental. *Biología Pesquera 19*: 55–67.

Hanchet, S.M.; Uozumi, Y. (1996). Age validation and growth of southern blue whiting, *Micromesistius australis* Norman, in New Zealand. *New Zealand Journal of Marine and Freshwater Research 30*: 57–67.

Horn, P.L. (1993). Growth, age structure, and productivity of ling, *Genypterus blacodes* (Ophidiidae), in New Zealand waters. *New Zealand Journal of Marine and Freshwater Research 27*: 385–397.

Horn, P.L. (2005). A review of the stock structure of ling (*Genypterus blacodes*) in New Zealand waters. *New Zealand Fisheries Assessment Report 2005/59*. 41 p.

Horn, P.L.; Sutton, C.P. (2019). Catch-at-age for hake (*Merluccius australis*) and ling (*Genypterus blacodes*) in the 2017–18 fishing year and from research trawl surveys in 2018, with a summary of all available data sets from the New Zealand EEZ. *New Zealand Fisheries Assessment Report 2019/63*. 81 p.

Japp, D.W. (1990). A new study on age and growth of kingklip *Genypterus capensis* off the south and west coasts of South Africa, with comments on its use for stock identification. *South African Journal of Marine Science 9*: 223–237.

Kalish, J.M. (1993). Pre- and post-bomb radiocarbon in fish otoliths. *Earth and Planetary Science Letters 114*: 549–554.

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

Kalish, J.; Johnston, J.; Smith, D.; Morison, S.; Robertson, S. (2002). Validation of pink ling (*Genypterus blacodes*) age based on otolith radiocarbon. *In*: Kalish, J.M. Use of the bomb radiocarbon chronometer to validate fish age. *Final Report FRDC Project 93/109*. Pp. 130–138.

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

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

Ogle, D.H.; Wheeler, P.; Dinno, A. (2020). FSA: Fisheries Stock Analysis. R package version 0.8.30.9000, https://github.com/droglenc/FSA.

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

Tuck, G.N. (ed.) (2013). *Stock assessment for the southern and eastern scalefish and shark fishery 2012*. Part 1. Australian Fisheries Management Authority and CSIRO Marine and Atmospheric Research, Hobart. 199 p.

Withell, A.F.; Wankowski, J.W.J. (1989). Age and growth estimates for pink ling, *Genypterus blacodes* (Schneider), and gemfish, *Rexea solandri* (Cuvier), from eastern Bass Strait, Australia. *Australian Journal of Marine and Freshwater Research 40*: 215–226.

Wrzesiński, O. (1984). Some features of the *Genypterus* sp. populations off the coasts of Southwest Africa and Argentina. *Reports of the Sea Fisheries Institute, Gdynia 19*: 43–60.

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 not be continuous nor obviously concentric. The optical appearance of these marks depends on the otolith structure and the species and should be defined in terms of specific characteristics on the structure. This term has traditionally been used to designate year marks even though the term is derived from the Latin ‘anus’ meaning ring, not from ‘annus’ which means year. The variations in microstructure that make an annulus a distinctive region of an otolith are not well understood.
Antirostrum – anterior and dorsal projection of the sagitta. Generally shorter than the rostrum.
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 both age-group, year-class, and day-class.
Core – the area or areas surrounding one or more primordia and bounded by the first prominent D-zone. Some fishes (e.g., salmonids) possess multiple primordial and multiple cores.
Corroboration – a measure of the consistency or repeatability of an age determination method. For example, if two different readers agree on the number of zones present in a hard part, or if two different age estimation structures are interpreted as having the same number of zones, corroboration (but not validation) has been accomplished. The term verification has been used in a similar sense; however, the term corroboration is preferred because verification implies that the age estimates were confirmed as true.
D-zone – that portion of a microincrement that appears dark when viewed with transmitted light and appears as a depressed region when acid-etched and viewed with a scanning electron microscope. This component of a microincrement contains a greater amount of organic matrix and a lesser amount of calcium carbonate than the L-zone. Referred to as discontinuous zone in earlier works on daily increments; D-zone is the preferred term. See L-zone.
Daily increment – an increment formed over a 24-hour period. In its general form, a daily increment consists of a D-zone and an L-zone. The term is synonymous with ‘daily growth increment’ and ‘daily ring’. The term daily ring is misleading and inaccurate and should not be used. The term daily increment is preferred. See increment.
Drift – Shift with time in the interpretation of otolith macrostructure for the purposes of age determination.
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.
Forced Margin or Fixed Margin – Otolith margin description (Line, Narrow, Medium, Wide) is determined according to the margin type anticipated *a priori* for the season/month in which the fish was sampled. The otolith is then interpreted and age determined based on the forced margin. The forced

margin method is usually used in situations where fish are sampled throughout the year and otolith readers have difficulty correctly interpreting otolith margins.
Hatch date – the date a fish hatched; typically ascertained by counting daily increments from a presumed hatching check (see check) to the otolith edge.
Hyaline zone – a zone that allows the passage of greater quantities of light than an opaque zone. The term hyaline zone should be avoided; the preferred term is translucent zone.
Increment – a reference to the region between similar zones on a structure used for age estimation. The term refers to a structure, but it may be qualified to refer to portions of the otolith formed over a specified time interval (e.g., subdaily, daily, annual). Depending on the portion of the otolith considered, the dimensions, chemistry, and period of formation can vary widely. A daily increment consists of a D-zone and an L-zone, whereas an annual increment comprises an opaque zone and a translucent zone. Both daily and annual increments can be complex structures, comprising multiple D-zones and L-zones or opaque and translucent zones, respectively.
L-zone – that portion of a microincrement that appears <u>light</u> when viewed with transmitted light and appears and as <u>elevated</u> region when acid etched and viewed with a scanning electron microscope. The component of a microincrement that contains a lesser amount of organic matrix and a greater amount of calcium carbonate than the D-zone. Referred to as an incremental zone in earlier works on daily increments; L-zone is the preferred term. See D-zone.
Lapillus (pl. Lapilli) – one of three otolith pairs found in the membranous labyrinth of osteichthyan fishes. The most dorsal of the otoliths, it lies within the utriculus ("little pouch") of the pars superior. In most fishes, this otolith is shaped like an oblate sphere and it is smaller than the sagitta.
Margin/Marginal increment – the region beyond the last identifiable mark at the margin of a structure used for age estimation. Quantitatively, this increment is usually expressed in relative terms, that is, as a fraction or proportion of the last complete annual or daily increment.
Microincrement – increments that are typically less than 50 um in width; and 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 subdaily 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 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 primordial 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 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 otolithic membrane lies within the sulcus acusticus. The sulcus acusticus is frequently referred to in otolith studies because of the clarity of increments near the sulcus in transverse sections of sagittae.
Transition zone – a region of change in otolith structure between two similar or dissimilar regions. In some cases, a transition zone is recognised due to its lack of structure or increments, or it may be recognised as a region of abrupt change in the form (e.g., width or contrast) of the increments. Transition zones are often formed in otoliths during metamorphosis from larval to juvenile stages or during significant habitat changes such as the movement from a pelagic to a demersal habitat or a marine to freshwater habitat. If the term 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 because 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.