Pacific Hake (*Merluccius productus*) Otolith Age Determination Manual

Shayne E. MacLellan, Joanne Groot and Judy McArthur

Fisheries and Oceans Canada
Science Branch, Pacific Region
Pacific Biological Station
3190 Hammond Bay Road
Nanaimo, BC
V9T 6N7

2021

Canadian Technical Report of Fisheries and Aquatic Sciences 3417

Fisheries and Oceans Canada Pêches et Océans Canada

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Canadian Technical Report of Fisheries and Aquatic Sciences

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Cat. No. Fs97-6/3417E-PDF ISBN 978-0-660-37341-6 ISSN 1488-5379

Correct citation for this publication:

MacLellan, S.E., Groot, J. and McArthur, J. 2021. Pacific Hake (*Merluccius productus*) Otolith Age Determination Manual. Can. Tech. Rep. Fish. Aquat. Sci. 3417: v + 55 p.

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ABSTRACT

MacLellan, S.E., Groot, J. and McArthur, J. 2021. Pacific Hake (*Merluccius productus*) Otolith Age Determination Guide. Can. Tech. Rep. Fish. Aquat. Sci. 3417: v + 55 p.

This manual describes protocols used by the Fisheries and Oceans Canada Sclerochronology Lab program at the Pacific Biological Station in Nanaimo, British Columbia to prepare otoliths to determine the age of Pacific hake (*Merluccius productus*). Production of best quality age data is dependent on producing high quality preparations as well as consistent and correct application of criteria to interpret annual growth patterns. The manual sets out procedures which have evolved and were developed to be the current operational standards for the DFO Pacific Region. A further purpose is to provide information to other agencies and individuals wishing to employ established otolith methods for Pacific hake age determination.

RÉSUMÉ

MacLellan, S.E., Groot, J. and McArthur, J. (2021). Pacific Hake (*Merluccius productus*) Otolith Age Determination Guide. Can. Tech. Rep. Fish. Aquat. Sci. 3417: v + 55 p.

Ce manuel décrit les protocoles utilisés par le programme du laboratoire de sclérochronologie de Pêches et Océans Canada à la Station biologique du Pacifique à Nanaimo, en Colombie-Britannique, pour préparer des otolithes afin de déterminer l'âge du merlu du Pacifique (*Merluccius productus*). La production de données d'âge de la meilleure qualité dépend de la production de préparations de haute qualité ainsi que de l'application cohérente et correcte des critères d'interprétation des schémas de croissance annuels. Le manuel présente des procédures qui ont évolué et ont été élaborées pour devenir les normes opérationnelles actuelles pour la région du Pacifique du MPO. Un autre objectif est de fournir des informations à d'autres organismes et individus souhaitant utiliser des méthodes otolithiques établies pour la détermination de l'âge du merlu du Pacifique.

1.0. INTRODUCTION

The Fisheries and Oceans Canada (DFO) Sclerochronology Lab (SCL) program was formed at the Pacific Biological Station in the fall of 1977. Its purpose, at the time, was to develop and standardize age determination methods for and to age commercially important groundfish species for DFO in the Pacific Region. It was intentionally established as a separate entity to ensure that age data production would not be affected by data users and investigators biases and expectations. The program's first motivation has been to create the best quality age data possible. The mandate of the program has since grown to include pelagic, shellfish and salmon species. The SCL currently ages around 120,000 fin/shellfish each year, about 12% being groundfish. Ageing Pacific hake (*Merluccius productus*) (Fig.1) from offshore and Strait of Georgia (SOG) stocks has been part of the program's workload on an annual and ongoing basis since inception.

Image credit: B. Guild Gillespie/www.chartingnature.com

Figure 1. Image of Pacific hake (*Merluccius productus*).

Historically, offshore stock Pacific hake (herein referred to as hake) have been an important commercial midwater fishery in the Pacific northwest (Bailey 1982). DFO's need for hake age data intensified when Canada's jurisdiction was extended to 200 miles in 1977. Over time, sample sources have included commercial domestic, foreign and joint venture fisheries as well as scientific surveys. SOG stock hake are one of the most abundant fish species in the Strait of Georgia supporting a small commercial fishery as well as being a major food source for seals and other fishes (McFarlane and Beamish 1985). Sagittal otolith pairs (Fig. 2) are collected for age determination. Age data has been utilized mainly for stock assessment purposes. The offshore stock age data is provided cooperatively to U.S. agencies for trans-boundary management with occasional sample exchanges to compare precision between the two countries. Precision results have been acceptable and exchanges are an ongoing endeavour through the auspices of the Committee of Age Reading Experts (CARE) sponsored by Pacific States Marine Fisheries Commission for the Technical Subcommittee of the Canada/U.S. Groundfish Committee. Since 1990 the SCL has aged 89,428 (2018 numbers) hake for an overall average of 3,200 per year. Annual numbers have reduced somewhat since 2007 to an average of 2,000. In recent years the majority of the age data has been generated from off shore samples with only a small component coming from the resident SOG stock.

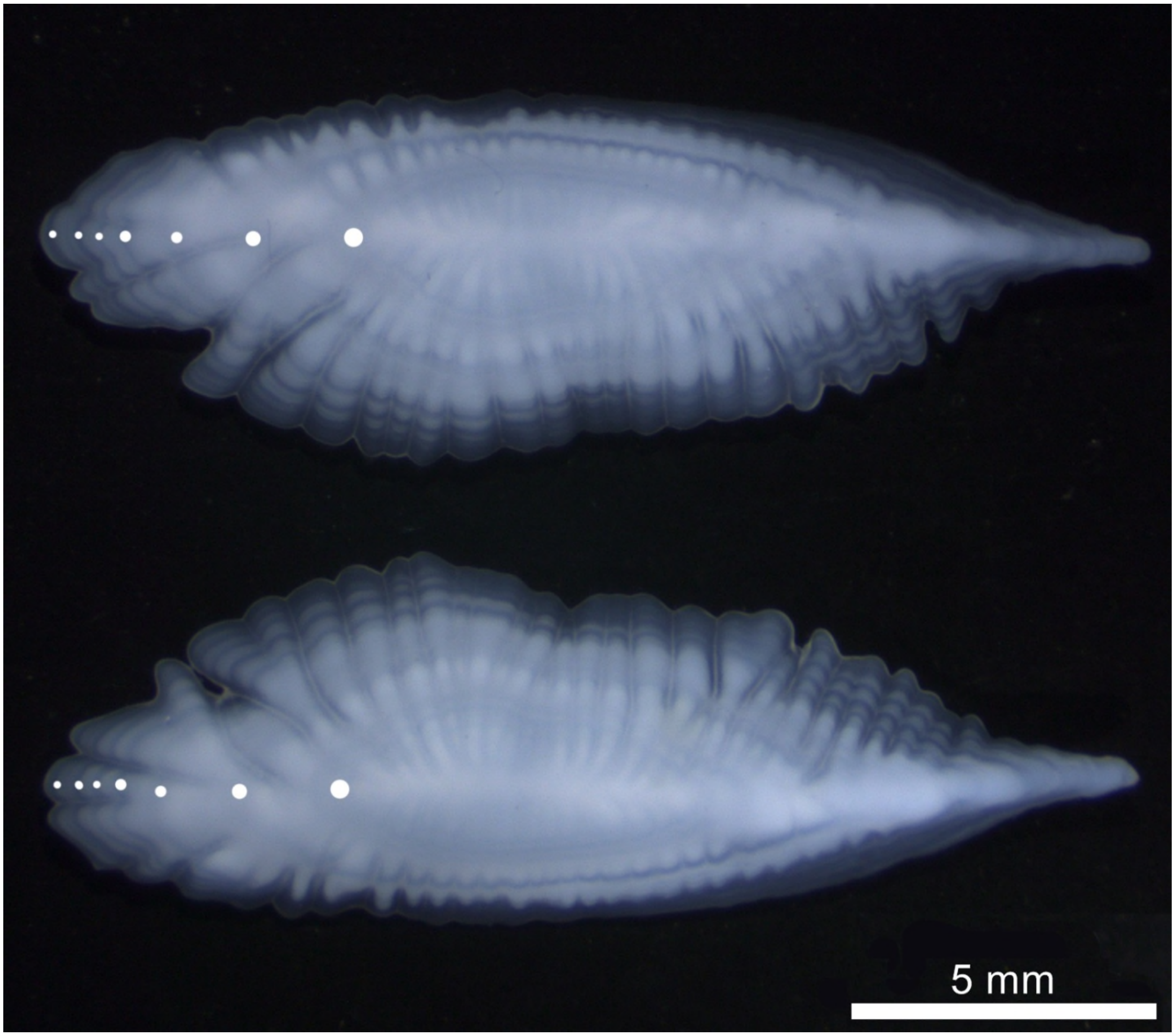


Figure 2. Whole surface or distal view of a pair of sagittal offshore stock hake otoliths lit by reflected light, age 7(7+). The narrow dark (translucent) growth zones marked by dots are counted to produce age.

1.1. Life history and age determination background

The SCL ages two hake stocks. The coastal stock, referred to as the offshore stock in this document, is migratory while the SOG stock remains resident in British Columbia (B.C.) waters (Bailey 1982). The SOG stock was established as a separate stock when Kabata and Whitaker (1981) published the absence of the parasite, *Kudoa paniformis,* that is found in the offshore stock. In spring the adult SOG hake spawn in the deeper waters of south central Strait of Georgia moving northward and dispersing in the fall

(McFarlane and Beamish 1985). Offshore hake spawn off southern California during the winter months. In spring the larger and older fish migrate north to forage off the west coast of B.C. during the summer (Helser and Alade 2012), then return to southern California in the fall . Fifty percent of hake are mature at age 3 and most by age 4 (Dark 1975). Offshore hake normally begin to show up in commercial B.C. catches at age 5-6 (Beamish et al 2004) and persist into their early teens when they begin to disappear from the fishery. Ages up to the late teens and early twenties have been generated by the SCL, but these are fairly rare, 22 being the oldest age to date. Large year classes have been identified over the years (Hallowed 1992) and accurately tracked through SCL age determinations.

Originally the SCL produced hake age estimates by examining growth patterns on the whole otolith (distal) surface only. In the late 1970’s the SCL changed to the otolith thin cross-section method (Beamish 1979). Beamish had demonstrated that the surface method was under-ageing adult hake and recommended preparing otolith thin cross-sections (Charles et al 2013) to improve accuracy. The SCL then shifted to a third method that involved breaking and burning otoliths (Chilton & Beamish 1982, MacLellan 1997) by the fall of 1981. Preparation time was quicker and pattern clarity was enhanced. Problematic ages from burnt sections, though, were secondarily checked using thin-sections until 1982. By sometime in 1983 all adult hake otoliths were aged using the burnt section method only. It should be noted that, to date, it has been the program’s general practise to age juvenile fish (0-3 years) using the surface otolith pattern only as this method is accurate for ageing juveniles (Dark 1975). This saves some time as no preparation is required.

Hake sagittal otoliths can be large attaining 2cm or more in length (Fig. 2). The growth patterns of the offshore stock are relatively easy to interpret, generally having a clear and consistent annual pattern. As a result, compared to most longer-lived species, this moderately-long lived stock is rated easier to age by SCL readers. Therefore, offshore hake is recommended as one of the first species to train novice fish agers with when learning the otolith burnt section method. On average, an experienced reader can age about 25 hake per hour (production rate). This fast rate reflects the relative ease of otolith preparation and ageing process. Production age rates for longer-lived (40 – 100 years) species, such as rockfish (*Sebastes* species) or sablefish (*Anoplopoma fimbria*), are about half that (Table 1).

Table 1. Production SCL reader rates (number of fish aged per hour) for hake versus two long-live species, sablefish and a rockfish, established in 2010.

Species	Reader rate (#fish aged/hr)
Hake	25
Sablefish	12
Pacific ocean perch	15

This manual outlines procedures for preparing and ageing hake otoliths for both the offshore and SOG stocks but mainly focuses on the offshore stock. There are no differences regarding preparation, but there are some with respect to growth pattern criteria. Offshore and SOG hake otoliths generally have similar annual growth pattern characteristics with some differences. The offshore stock fish are larger at age as are their otoliths (McFarlane and Beamish 1985). Offshore otoliths tend to have relatively larger annual zones, especially the first three, and generally have fairly clear patterns. SOG otoliths tend to be more difficult to age because their growth patterns are not as clear, having more compact annual zones and more prominent non-annual growth checks which can lead to over-ageing. Fish agers must know which stock they are ageing in order to produce best age estimates. There is no separate section regarding ageing SOG otoliths in this manual. Instead, any differences will be described with respect to offshore patterns, within individual method sections. Although SOG otoliths present a more difficult pattern to age the SCL has similar precision statistics for both stocks generally achieving 50% agreement and 80-100% within one year for most samples.

The size at age, shape and pattern clarity of offshore otoliths has undergone subtle changes over the decades (Fig. 3). Long time SCL hake readers have observed that offshore stock otoliths from fish hatched in the 1960’s and ‘70’s tended to be larger at age and had very clear surface patterns, even up to 8-10 years and older. In the 1980’s the stock had few successful year classes being dominated by a single large 1980 year class (MacLellan and Saunders 1995). This year class experienced reduced growth during the 1982/83 El Niño event (Smith et al 1990). This was also reflected in otolith growth patterns. Otoliths from offshore hake hatched in the1980’s tended to have patterns that were more difficult to interpret (unclear and compacted) than those from the 60’s and 70’s. The 1990’s and 2000’s saw a general improvement in otolith pattern clarity, but still somewhat less clear than seen in the 60’s and 70’s.

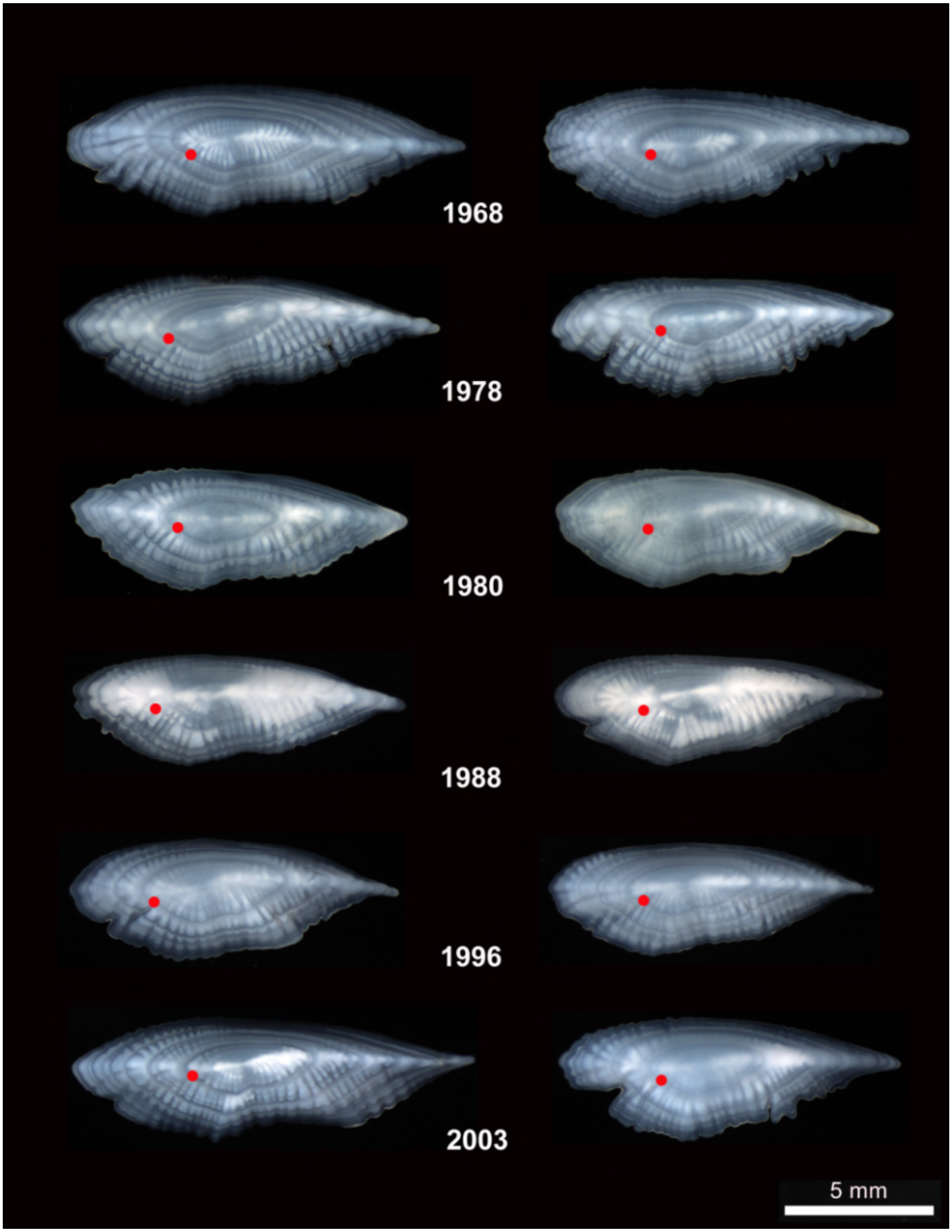


Figure 3. Examples of some typical looking offshore hake otoliths, whole surface view, taken from fish hatched in the late 1960's to early 2000's. Size at age and pattern clarity have been somewhat plastic over time. One otolith from 2 different fish for each birth year is provided for comparison. All otoliths were 8 years old and are at the same magnification. A dot indicates the first annulus.

2.0. METHODS: Quality Assurance and Quality Control Procedures

This manual is meant to provide instruction and act as a reference to those who wish to age Pacific hake otoliths from the offshore and SOG stocks captured in B.C. fisheries. It documents current quality assurance and quality control (QA/QC) procedures employed by the SCL program. This includes equipment, ergonomics, reference material, training and production ageing method standards. Readers are directed to consult the glossary at the back for terminology definitions. The manual will mostly focus on the burnt otolith section method for the offshore stock because it represents the bulk of annual hake age data requests to the SCL. Where appropriate, the otolith whole surface technique and SOG pattern criteria will be described.

2.1. Equipment and ergonomic standards

Standard, equivalent state-of-the-art equipment with specialized ergonomic parts and best quality optics and lighting are a part of the SCL's QA/QC system and are available to each reader (Fig. 4). This facilitates the production of consistent and accurate age data as more than one reader is involved in ageing each sample and multiple readers work as a team to complete sample requests.

Age determination involves long hours of repetitive work sitting or standing at a microscope which can lead to repetitive injuries. Ergonomic workstations ensure the physical health of readers so they are able to focus and work pain free over their careers. Readers possess unique and difficult-to-learn skills so the program does all it can to retain expertise in the long-term to promote age data continuity.

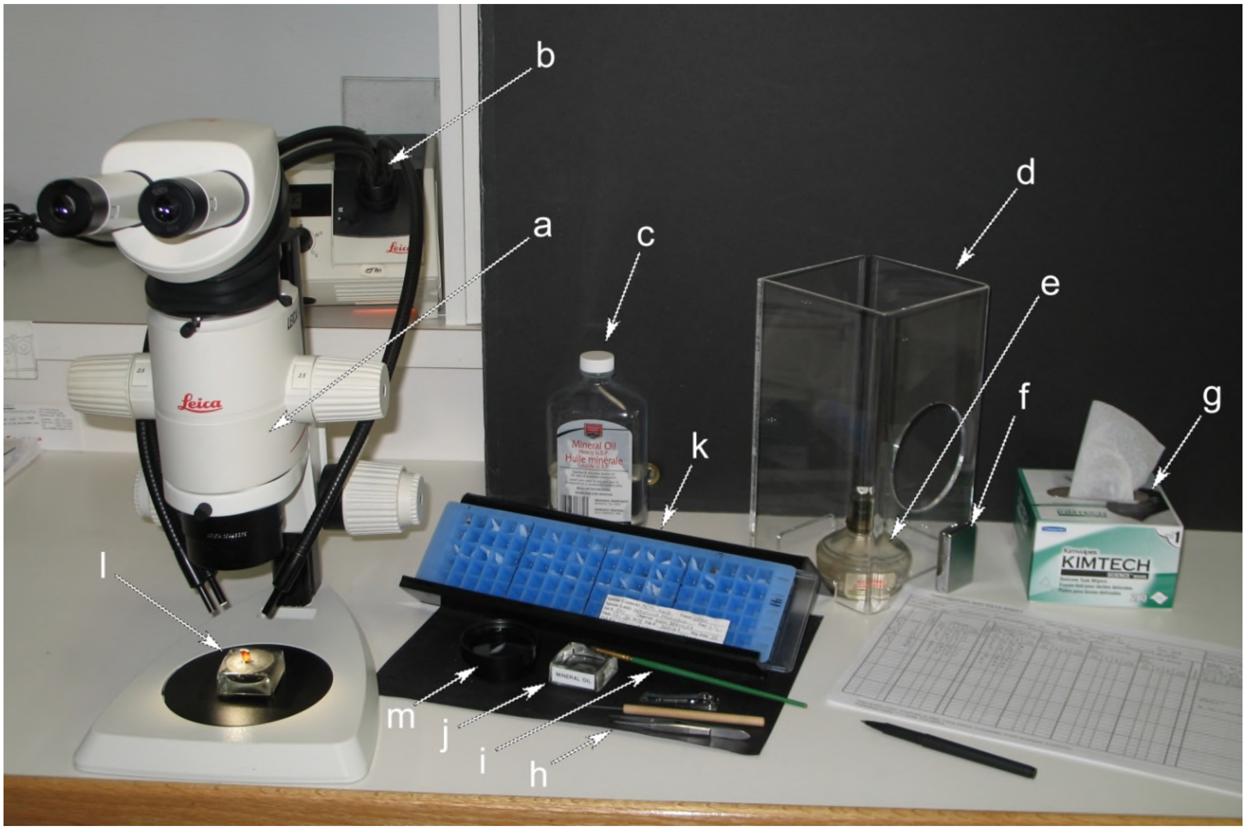


Figure 4. Equipment and materials the SCL uses to age hake otoliths. a=dissecting microscope, b=fibre optics with power source, c=mineral oil, d=protective shield, e=alcohol lamp, f=zippo lighter, g=tissues, h=clippers, probe, forceps, i=paint brush, j=mineral oil dish, k= tray sample holder, l=plasticine holder, m=black dish.

2.1.1. Equipment and materials

- Dissecting zoom microscope (e.g. Leica MZ7.5) with 10X eyepieces
- Light source (e.g. Leica model KL2500 LCD) with 2 or 3 “goose-neck” fibre optics
- Burning shield
- Forceps, probe, alcohol burner, zippo lighter, black dish, small paint brush, dish with green/blue/black plasticine
- Thumb nails or nail clippers
- 95% ethanol, tissues, heavy U.S.P. mineral oil, lens cleaner

Microscope: A dissecting microscope with high quality light gathering optics, especially with a zoom objective lens, is desirable so that growth zones can be viewed at various magnifications. A zoom lens provides a smooth transition between magnification changes helping readers to keep their eyes on track when following a counting axis or on a specific point or feature when counting. The SCL ages hake with scopes outfitted with 10X eyepieces resulting in maximum magnification of 50X. Higher power magnification is not usually necessary as hake otoliths are relatively large and cross-section growth zones can adequately be seen at 50X or less. The SCL does not advocate set microscope magnifications when determining age. However, it is recommended to start off assessing age at the lowest or a lower magnification and increase upwards as necessary. Starting small provides a first overall perspective of the annual pattern and age. It also discourages over-ageing as high magnifications can emphasize the prominence of growth checks, especially those found in the juvenile zones. Highest magnifications need only be employed to interpret the small growth zones on the margins of older hake otoliths ≥ 8-10 years.

Lights: Multiple goose-necked fibre optics can be adjusted to shine reflective light onto otoliths at the best angles for clear viewing of growth patterns. Fibre optics provide light that is cool, direct and intense. This enhances the reader's ability to clearly differentiate and count microscopic and closely spaced annual growth zones on hake otolith burnt sections.

Other materials: The burning shield protects the reader from flame and hot pieces that may explode off the otolith on occasion. Forceps and probes are needed to hold and manipulate hot and cooled otoliths. The zippo lighter lights the alcohol burner filled with ethanol. A water filled black dish can be used to view the whole otolith or to clean off dirt, membranes and storage medium if a tissue is not adequate. The paint brush is used to apply mineral oil to the burnt otolith surface and the plasticine provides support for the burnt otolith when viewing under the scope. Blue/green/black plasticine has been found to best contrast with and show up the burnt pattern.

2.1.2. Ergonomics

Proper ergonomic workstations in the SCL have been a good investment for staff retention. The program strives to ensure that all equipment and workstations can safely be used by all staff without incurring repetitive injuries. Specialized equipment, ergonomic parts and setup have improved the comfort and health of age determination work. If workstations are not adjustable injuries to the legs, back, neck, wrists and hands may occur over the long-term. This can compromise age data quality and lead to lost time and expertise impacting program capacity.

SCL workstation ergonomics:

1. Adjustable eyepieces for focus and inter-pupillary distance to avoid eye strain.
2. Tilt-able scope heads to ensure straight ahead vision to prevent neck strain.
3. Scope height controls to adjust to different heights and promote good posture (sitting with a straight back and neck).
4. Coarse and fine or powered focus knobs to avoid thumb and wrist problems.
5. Focus controls close to counter to avoid elbow pressure points and allow wrists to be kept as straight as possible.
6. Adjustable bench/desk heights so that readers can sit or stand to work.
7. Rounded counter edges to avoid pressure ridges on arms.
8. High backed chairs with adjustable height, seat and back tilt and adjustable arms to provide good support to back, shoulders and arms while promoting good posture.
9. Document holders to avoid neck strain.

2.2. Reference material

Documentation is a regular part of SCL activities and all staff are expected to participate. The SCL uses written materials, photographs and specific physical samples as references and as part of their QA/QC process. This facilitates training, ensures age data quality, enhances demonstration, contributes to publications and helps avoid reader drift. Reader drift usually occurs when readers unknowingly change how method criteria are applied and may result in random or systematic over-/under-ageing. This can happen over a long period of time, within one reader or many, or quite suddenly when new readers come on board. Reader drift can have a profound impact on stock assessment analysis.

2.2.1. Written documentation

Over time age determination methods and criteria used by the SCL have gone through purposeful updating or changes. These needed to be explained and discussed with data users and had to be thoroughly documented in writing. Written documentation is a strong tool that helps avoid reader drift. When hake samples are aged, SCL readers will add any new information to a cumulative record for each stock in a living Word document that exists on a shared network drive (see example below).

"2000, Aug 11 -Set 6, near Flamingo Inlet, QCI #1-65: The majority of the sample were 3 yr olds but the ages ranged from 2 to 16 yrs. The amount of plus growth varied as did the size of the 1st year. Checks were common in the first three years. The otolith surface was opaque making it difficult to verify whether there was a check in the 2nd and/or 3rd years or if they were vague annuli. The 2 yr olds tended to have a larger 1st yr than the 3 yr olds. (00/10/19 JG)"

2.2.2. Image documentation

Photographic documentation is a very effective means to ensure that methods and criteria remain consistent over time. Age determination is a visual pattern recognition skill where a picture truly is worth a thousand words. The SCL has standard protocols for capturing and annotating digital images. All readers are expected to contribute to the photographic archives. Readers identify hake otoliths to be photographed as samples are aged. Digital images are stored on the shared network drive for all staff to access. An Excel image log documents photos for each species (Fig. 5). In some cases images may be linked to the Word document that describes samples and ageing issues.

Species: Hake

file name .extensi	SAMPLE#	OOK/TF	ID#	AC	CAUGHT YY/MM/	AREA/ LOCAT	STC	STRUCTU	IMAGE DESCRIPTION	IMAGE CAPTURE NOTES	COMMENTS	IMAGE CREDIT/D/	ARCHIVE CD#
hake1.tif	1-9		1	0	10/09/09	4B	H143	otolith	whole surface, oil	LeicaMZ8, DFC322, 0.63x2.5	young of year	JG 10/10/28	
hake2.tif	1-9		1, 9	0, 0	10/09/09	4B/3C	H143	otolith	whole surface, oil	LeicaMZ8, DFC322, 0.63x2.0	comparison young of year SoG/WCVI, in oil	JG 10/10/28	
hake2a.jpg	1-9		1, 9	0, 0	10/09/09	4B/3C	H143	otolith	whole surface, oil	LeicaMZ8, DFC322, 0.63x2.0	annotated - comparison young of year SoG/WCVI, in oil	JG 10/10/28	
hake3.tif	1-9		6	1	10/09/09	4B	H143	otolith	whole surface, oil	LeicaMZ8, DFC322, 0.63x1.25	good juv	JG 10/10/28	
hake3a.jpg	1-9		6	1	10/09/09	4B	H143	otolith	whole surface, oil	LeicaMZ8, DFC322, 0.63x1.25	annotated - good juv	JG 10/10/28	
hake4.tif	1-9		1, 6	0, 1	10/09/09	4B	H143	otolith	whole surface, oil	LeicaMZ8, DFC322, 0.63x1.25	comparison 0 vs 1yr	JG 10/10/28	
hake4a.jpg	1-9		1, 6	0, 1	10/09/09	4B	H143	otolith	whole surface, oil	LeicaMZ8, DFC322, 0.63x1.25	annotated - comparison 0 vs 1yr	JG 10/10/28	
hake5.tif	201002530001-52		2		10/05/27	3C	H141	otolith	whole surface, oil	LeicaMZ8, DFC322, 0.63x1.25x(	clear sfc pattern, May edge (manual)	JG 10/11/16	
hake6.tif	201002530001-52		6	6	10/05/27	3C	H141	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x1.6	young, dorsal clear, ventral 5th vague	JG 10/11/16	
hake7.tif	201002530001-52		6	6	10/05/27	3C	H141	otolith	B&B XS, dorsal close-u	LeicaMZ8, DFC322, 0.63x2.5	young, dorsal clear pattern	JG 10/11/16	
hake8.tif	201001930001-49		6	11	10/05/08	3D	H141	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x1.6	older, clear ventral	JM 10/11/16	
hake9.tif	201001930001-49		6	11	10/05/08	3D	H141	otolith	B&B XS, ventral close-	LeicaMZ8, DFC322, 0.63x3.2	older, clear ventral	JM 10/11/16	
hake10.tif	201002530001-52		6	6	10/05/27	3C	H141	otolith	B&B, XS, ventral close-	LeicaMZ8, DFC322, 0.63x2.5	young, ventral 5th vague	JG 10/11/16	
hake11.tif	201002530001-52		17	11	10/05/27	3C	H141	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x1.25	teen, good pattern, thick dark edge =12yrs?	JG 10/11/16	
hake12.tif	201002530001-52		17	11	10/05/27	3C	H141	otolith	B&B, XS, dorsal close-	LeicaMZ8, DFC322, 0.63x2.5	teen, thick dark edge - +1 yr?	JG 10/11/16	
hake13.tif	201002530001-52		17	11	10/05/27	3C	H141	otolith	B&B, XS, ventral close-	LeicaMZ8, DFC322, 0.63x2.5	teen, good pattern, edge not questionable	JG 10/11/16	
hake14.tif	201002820061-113		65	3	10/06/05	3D	H142	otolith	whole surface, oil	LeicaMZ8, DFC322, 0.63x1.25x(	juv, prom. nucleus, check in 3rd, small + growth	JG 10/11/16	
hake15.tif	201002820061-113		65	3	10/06/05	3D	H142	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x1.25	juv, prom. nucleus, check in 3rd, small + growth	JG 10/11/16	
hake16.tif	201002820061-113		65	3	10/06/05	3D	H142	otolith	B&B, XS, dorsal close-	LeicaMZ8, DFC322, 0.63x2.5	juv, prom. nucleus, check in 3rd, small + growth	JG 10/11/16	
hake17.tif	201002820061-113		65	3	10/06/05	3D	H142	otolith	B&B, XS, ventral close-	LeicaMZ8, DFC322, 0.63x2.5	juv, prom. nucleus, check in 3rd, small + growth	JG 10/11/16	
hake18.tif	201002820061-113		93	11	10/06/05	3D	H142	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x1.25	teen - 1st,2nd and 3rd double ann.	JG 10/11/16	
hake19.tif	201002820061-113		93	11	10/06/05	3D	H142	otolith	B&B, XS, closer	LeicaMZ8, DFC322, 0.63x1.6	teen - 1st,2nd and 3rd double ann.	JG 10/11/16	
hake20.tif	201001930001-49		7	14	10/05/08	3D	H141	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x2.8	teen - vague 1st , clear pattern	JM 10/11/19	
hake21.tif	201001930001-49		7	14	10/05/08	3D	H141	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x5.0	teen - dorsal, vague 1st , clear pattern	JM 10/11/19	
hake22.tif	201003050001-38		5	6	10/06/14	3D	H142	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x4.0	juv, prom. nucleus, check with 2nd, small 1st - 4th	JM 10/11/19	
hake23.tif	201003050001-38		5	6	10/06/14	3D	H142	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x5.0	juv, dorsal, prom. nucleus, check with 2nd, small 1st - 4	JM 10/11/19	
hake24.tif	201003050001-38		10	8	10/06/14	3D	H142	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x3.2	darker burn, clear pattern	JM 10/11/19	
hake25.tif	201003050001-38		10	8	10/06/14	3D	H142	otolith	B&B, XS	LeicaMZ8, DFC322, 0.63x5.0	darker burn, dorsal, clear pattern	JM 10/11/19	
hake26.tif	201004070201-259		253	6	10/08/09	3C	H145	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.2!	normal 1st (larger) checks within	JM 10/12/21	
hake27.tif	201004070201-259		253	6	10/08/09	3C	H145	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x2.5	dorsal, normal 1st (larger) checks within	JM 10/12/21	
hake28.tif	2251254-1353		1259	3	10/09/04	3D	H143	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.7!	very small 1st? clear ventral and dorsal	JM 10/12/21	
hake29.tif	2251254-1353		1259	3	10/09/04	3D	H143	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x2.5	dorsal, very small 1st? clear ventral and dorsal	JM 10/12/21	
hake30.tif	2251154-1253		1204	3	10/09/04	3D	H142	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.7!	vague 1st	JM 10/12/21	
hake31.tif	2251154-1253		1204	3	10/09/04	3D	H142	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.7!	dorsal, vague 1st	JM 10/12/21	
hake32.tif	2251154-1253		1221	9	10/09/04	3D	H142	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.4	8th and 9th check	JM 10/12/21	
hake33.tif	2251154-1253		1221	9	10/09/04	3D	H142	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.4	dorsal, 8th and 9th check	JM 10/12/21	
hake34.tif	2251154-1253		1222	5	10/09/04	3D	H142	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.7!	1st and 2nd check	JM 10/12/21	
hake35.tif	2251154-1253		1222	5	10/09/04	3D	H142	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.4	dorsal, 1st and 2nd check	JM 10/12/21	
hake36.tif	2251154-1253		1229	12	10/09/04	3D	H142	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.3!	dark burn, small 8th, 8th check, larger 11th and 12th, 12	JM 10/12/21	
hake37.tif	2251154-1253		1229	12	10/09/04	3D	H142	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x2.5!	dorsal, dark burn, small 8th, 8th check, larger 11th and	JM 10/12/21	
hake38.tif	201005200001-53		1	4	10/09/18	5E	H146	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.6	clear pattern, small first with prom. Nucleus, 2nd double	JM 10/12/22	
hake39.tif	201005200001-53		1	4	10/09/18	5E	H146	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x2.5	dorsal, clear pattern, small first with prom. Nucleus, 2nd	JM 10/12/22	
hake40.tif	201005200001-53		6	5	10/09/18	5E	H146	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.5	clear pattern, check with 1st	JM 10/12/22	
hake41.tif	201005200001-53		6	5	10/09/18	5E	H146	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x2.5	dorsal, clear pattern, check with 1st	JM 10/12/22	
hake42.tif	201003450001-64		63	13	10/07/01	5B	H142	otolith	B&B, XS	LeicaWildM8, DFC322, 0.63x1.2!	off centre break, older fish with unclear pattern	JM 10/12/22	

Sheet1 Sheet2 Sheet3

Figure. 5 Example of Excel file that SCL employs as a Photo Log. The blue file names link to photos.

2.2.3. Reference samples

To avoid reader drift, some fisheries agencies prefer to assemble and maintain permanent or semi-permanent sample reference collections for training and testing. These may or may not be known-age. Reference collections have their place, but they work best for labs with one to a few readers and/or when numbers of species/stocks aged are small. The SCL employs 9-10 readers and production ages large numbers of groundfish species (>40), 2 shellfish species, several herring stocks and 4 salmon species consisting of 100's of stocks on an annual basis. Although the program assembled some reference collections years ago, it took an enormous amount of effort to do so and the labour to maintain them was significant, impacting production. The SCL no longer keeps specific reference collection samples. Instead, the program relies more on published and unpublished documentation (manuals and reports with photos) and established QA/QC standards and procedures, including use of known-age hake and documented previously-aged samples as references to help ward off reader drift. Specific demonstrative past/recently aged samples are used for training and testing. All hake otolith samples are maintained and permanently stored for future reference in a large library that goes back to the 1970's.

Known-age samples are a QC tool utilized by the SCL to validate ageing methods and data whenever possible. These opportunities are generally rare for most species. In the case of hake some known-age samples do exist. The large 1980 year class of the offshore stock was documented for reference when a natural tag was formed within the annual growth pattern on their otoliths during the 1982/83 El Niño (MacLellan and Saunders 1995). The natural tag was tracked on the otoliths of the 1980 year class until the fish disappeared in the late 1990's as older teenagers extending validation of the ageing method from 4 to 21 years of age.

2.3. Training system

The training and development policy of the SCL is intended to expand and preserve its expertise against loss through retirement and unforeseen events and to address regional stock assessment/research project needs. The work culture of the program is unique, complex and dynamic. It takes several (5-10) years for a newcomer to absorb and participate in it all. SCL readers eventually learn how to age all species using all methods employed by the program. To continue development, readers must demonstrate that they can maintain the designated level of expertise required to age the species they are already trained in over the long term. Depending on need and other duties, it generally takes only one season for a novice SCL reader to learn to age hake otoliths.

Any skilled and experienced hake reader in the SCL may be asked to train others. They are chosen according to demonstrated abilities, experience and in regards to availability within the program's work schedule. Good coaching skills are important. The trainer is responsible for developing a written training plan with input from the student and is subject to the supervisor's approval. The trainer's task is to follow through with the process according to set policies and procedures and to objectively assess the student's progress and skill levels and report on progress. A training plan (see end of this section) provides a step by step reference with clear goals and expectations for both the trainer and student. If progress does not go as expected it is up to the trainer to investigate and work with the student to overcome problems. It is equally important for the student to indicate when they need help to ensure that they achieve the expected goals. It should be noted that the SCL may abbreviate the plan below if an experienced reader, rather than a novice, is being trained.

The SCL assesses two key technical competencies, quality and quantity, to measure a student's age determination skills. The first is imperative while the second is necessary. Both are measured against standard target values. Quality is assessed by measuring precision and accuracy. Productivity is measured against established productivity target rates (e.g. #fish aged/hour). Both competencies are important to develop and assess in stages by the trainer.

Comparative tests against self and experienced readers or known-age samples are used to assess a student's precision and accuracy to identify biases so that adjustments can be made with further coaching. Students learning to age hake otoliths are expected to meet agreement (precision) targets of 80% ± 1 year for both difficult, as well as easy, to age samples to achieve expert status. Progress and results are collated in an Excel training form consisting of samples (Fig. 6). Students must also learn to eventually meet productivity targets. The SCL is responsible for ageing 1,500-2,000 hake each year during a short turnaround time of around two months along with other high volume priority age requests such as salmon. Students must learn to age swiftly with certainty. In order to become an expert reader a novice must develop good judgment and decisiveness so as not to spend too much time agonizing over the age assessment of a single fish. They need to know and understand the limits of the methods and criteria they are employing. Generally, if an age cannot be determined within 5 minutes it is best to assign a best estimate, record low confidence in age quality, and move on to the next fish. By and large, and especially for hake, this would not represent a high percentage of most samples.

Training requires a considerable effort. While teaching, the trainer's participation in production ageing and other regular duties are impacted, lowering SCL capacity. Training, especially novices, must be very carefully planned into the annual work schedule in order to ensure that all the region's priority age requests can be met on time. There is a limit to how much time and effort can be invested in training any one person. Not all people have the disposition and the skill-set suited for this meticulous yet fast-paced unique work. If a novice cannot establish technical competencies within the framework of the training plan then they are deemed not to possess the aptitude required to work successfully in the program.

2.3.1. Example Hake Training Sample Form

Form preamble: *Student X has aged numerous rockfish species but hake training will start at a novice level as the breaking and burning of their otoliths and growth patterns are different than rockfish. Dependent on precision test results fish numbers in some/all stages may be reduced.*

Date caught	Sample Tray #	Set #	Total #	Expert Reader % Agreement	Date Aged & Trainee initials	% Agreement (X vs Expert)	Precision (n)	X Self-precision % agreement	Comments
Stage 1 - some samples will be used in Stage 2 as precision and self-precision samples.									
	Jar samples				15/09/16 X				
2014 May 20	201202190001-60	6	30	50/90/100	15/09/17 X	Read w/ ages			
2014 June 28	201403130001-60	5	60	50/100	15/09/18 X	Read w/ ages			juveniles
2014 June 09	201402560001-100	11	30	90/100	15/09/17 X	62/93/100	30		teens
2014 June 28	201403120051-100	7	50	50/100	15/09/18 X	65/100	50		
2014 July 12	201403320181-236	30	56	50/100	15/09/21 X	25/85/95	56		juveniles 1-5 yr olds
2014 July 27	225001-50	900	50	50/100	15/09/21 X	72/100	50	30/75/95	young - mid teens
2014 Sep 28	D 40701-762	900	62	100	15/09/24 X	45/95/100	62		edge growth
			338						
Stage 2									
2014 Oct 08	201406130151-200	34	50	80/100	15/09/30 X	30/75/90	50		
2014 Oct 09	201406130201-250	38	50	80/100	15/09/30 X	40/80/90	50		
2014 June 02	225001-50	900	50	80/100	15/09/30 X	70/100	50		juveniles included
2014 July 05	D 43001-60	900	60	60/90/100	15/09/30 X	50/85/100	60	58/80/93	teens
2014 Aug 27	2250501-600	14	50	100	15/10/05 X	90/100	10		juveniles included
2014 June 09	201402560001-100	11	30	90/100	15/10/06 X	47/90/98	30	31/73/94	young - mid teens
			290						
Stage 3									
2014 May 25	201402340101-161	8	30	50/70/100	15/10/15 X	47/93/97	30		older fish
2014 Jan 31	201308220001-50	3	30	30/100	15/10/28 X	47/83/90	30	60/90/100	mix of young and old
2014 May 25	201401980121-180	25	30	60/83/97	15/10/26 X	43/87/100	30	50/90/100	
2014 July 8	40341-40382	900	40	80/100	15/10/28 X	90/100	40		teens
2014 Aug 28	4001-4025	900	25	60/96/100	15/10/28 X	60/88/96	25		teens
			155						
Stage 4 - Strait of Georgia (Area 4B); growth patterns differ from Offshore patterns									
2011 Mar 24	201-299	14,16	99	80/100	15/11/03 X	70/100	50	80/90/100	
2013 July 29	201304200001-49	1	49	100	15/11/13 X	90/100	49	80/100	juveniles (0-4 yrs)
2014 June 03	225001-50	900	50	50/100	15/11/19 X	50/80/100	50		mix of juv. & teens
2014 Aug 04	225001-50	900	50	80/100	15/11/25 X	60/100	50		
			248						

Fig. 6 Example of Excel form used to record training goals and progress of a reader learning to age Pacific hake.

2.3.2. Novice hake reader training plan:

Goal: *To participate in production ageing of offshore and SOG hake otolith samples meeting both: 1. Quality (precision of 50% agreement and an overall 80% ± 1 year agreement) and 2. Productivity targets (age an average 25 fish/hr) with consistency over the long term.*

Expectations:
By end of training the student must consistently demonstrate they can meet a minimum precision target of 50% agreement (no differences) and attain a total agreement of 80% ± 1 year with themselves and/or expert readers to begin full participation in routine

production ageing of offshore and SOG hake. This will require reviewing/ageing otoliths from about 800-1200 hake. Productivity targets of determining the age of 16-29 fish/hr, or an average of 25 fish/hr, are expected to be achieved within the training period. Expert status is achieved when quality and quantity targets are met and maintained with difficult as well as easy to age samples. The SCL reader is expected to participate in both first reading and precision testing of samples and to maintain, broaden and deepen expertise with both offshore and SOG hake stocks over the long term as required.

Literature and Sample references: Age Determination Methods of Fishes Studied by the Groundfish Program at PBS (Chilton and Beamish 1982, MacLellan 1997, Hake Ageing Manual, MacLellan et al 2019), SCL hake photo binders, training reference samples and jar samples.

Stage 1:

- *Review otoliths from ~200 already aged offshore hake to become familiar with the scope of growth pattern characteristics and their variations.*
- *The goal is to produce ages similar to those of an experienced reader and be consistent with self, most of the time, while looking at previously aged samples and having access to age data. Similar means generating the same age 60-70% of the time.*
- *Once confident with recognising and interpreting these patterns, the student will age ~200 additional, previously aged hake otoliths without the benefit of age data.*
- *Again, the goal is to produce ages similar to experienced readers most of the time. Differences are to be reviewed and discussed with the trainer. If consistency with self and others is 60-70% proceed to stage 2.*

Stage 2:

- *Independently age (no access to original ages) an additional ~200-300 easy to age offshore hake samples.*
- *Conduct two precision tests; one self and one against an expert.*
- *Differences are to be reviewed and resolved with the trainer.*
- *Once agreement is being met most of the time (~70-80% ± 1) the student can begin to participate in a limited role in production ageing, as the first reader. These samples, initially, will all be second read by an experienced reader.*
- *When the student consistently meets/exceeds the precision targets within the training period set for stage 2 they graduate to stage 3.*

Stage 3:

- *Independently age ~200 fish with more difficult patterns.*
- *Conduct precisions against trainer and achieve 80% ± 1 agreement with some consistency.*
- *At this point the student may participate in production ageing of offshore hake as the first reader.*
- *First few samples will be 2nd read by trainer.*
- *Once meeting precision targets consistently the student may also participate in testing as well as doing a first read.*

- *During this stage the student will work incrementally towards and meet the expected average production rate of ageing an average of 25 fish/hr while meeting precision target.*
- *Both quality and quantity targets are expected to be maintained over time.*

Stage 4:

- *This stage expands the depth and breadth of a student's hake expertise, learning to age otoliths from the SOG stock.*
- *The student will be provided instruction and samples (~100 fish) from the SOG stock that are more challenging to interpret.*
- *Stages 1-3 will be repeated and depending on the facility of the student may be moderated and/or reduced somewhat.*

2.4. Production ageing system

The SCL's production ageing system is complex, designed to manage workload and includes a series of procedures with checks and balances to produce best quality age data efficiently to meet stock assessment deadlines. A monthly sample tracking Excel file (Fig. 7) is kept to monitor sample progress and the roles of various staff are identified to ensure best quality age data through a series of QC steps and application of standards.

					#	% Agreement			Agers
Species	Sample numbers			Set #	Fish	+/- 0	+/- 1	+/-2	Initials
Hake	21120395	1	50	7	7				
	21120395	51	104	12	7				
	21120395	111	162	19	7				
	21120395	171	225	32	7				
	21120395	231	280	41	7				
	21120395	281	334	45	7				
	21120395	341	395	56	7				
	21120395	401	454	60	7				
	21120395	461	515	72	7				
	21120395	521	576	30	7	45	100		MH>JG
Hake	20120398	1	62	2	8				
	20120398	71	133	15	8				
	20120408	71	120	7	7				
	20120428	1	60	9	8				
	20120450	1	62	5	8				
	20120450	63	126	10	8				JM>
Hake	20120468	1	50	6	7				
	20120468	51	107	11	8				
	20120468	111	166	18	7				
	20120468	171	225	33	7				
	20120468	231	287	37	8				
	20120468	291	343	46	7				
	20120468	351	404	57	7				
	20120468	411	465	60	7				
	20120468	471	527	67	8				JG>
Hake	20120491	1	50	8	7				
	20120491	101	150	11	7				
	20120493	1	63	9	8				
	20120493	71	133	17	8				
	20120493	141	203	25	8				
	20120498	1	53	5	7				
	20120525	1	67	1	9				
	20120526	1	50	3	7				
	20120537	1	63	8	8				KC>

TOTAL GROUNDFISH RESOLVED IN NOVEMBER = 695
TOTAL GROUNDFISH UNRESOLVED IN NOVEMBER = 814

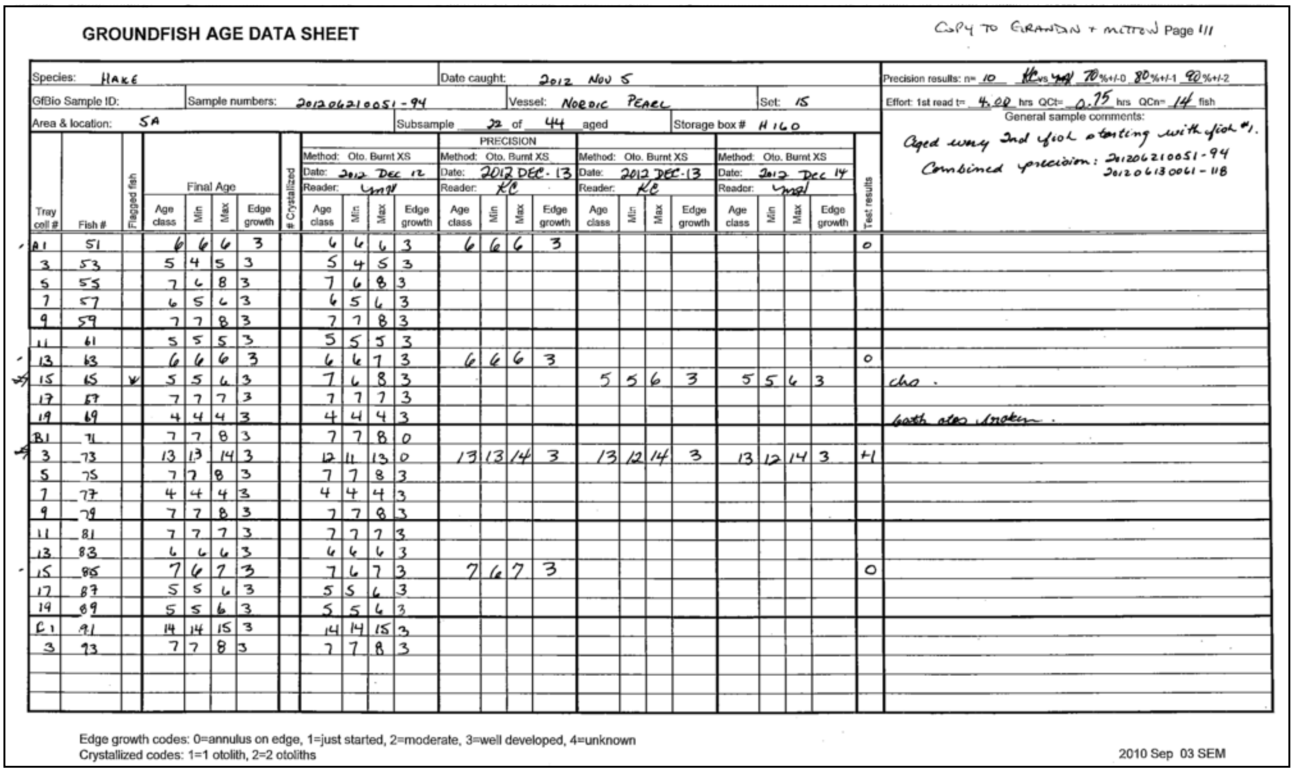
Fig. 7 Example of Excel file used by the SCL to track the progress of sample processing and QA/QC results.

For the most part, two SCL readers are involved with processing a sample. Team members pair up to coordinate and complete samples keeping due dates in mind. They are designated as the first (primary) or the second reader (tester). The first reader ages the whole sample while the tester ages a randomized independent subsample. Post precision, the tester will also re-age fish flagged by the first reader for review. Sometimes a third is called in to help out when agreement between the first two is elusive. Independent (blind) measurement of quality is carried out through precision tests. Non-independent (access to first readings) data is generated during the resolution process after precision testing is concluded. This is a matter of expediency as continuing the independent processes beyond the test phase would significantly increase the time and effort required to complete samples and negatively impacts SCL capacity.

2.4.1. Age data sheet standards

The following protocols and standards were developed by the SCL to ensure that consistent high quality data is created and recorded and to make certain that data users understand the scientific defensibility of age data they use. Some historical protocols will be described to provide reference and timelines for changes over time. This is specifically important for data users when using historically generated age data that may have been created with somewhat different protocols as compared to the present.

The most recent hake age data sheet and recording conventions were developed by S. MacLellan and R. Stanley, tested on a difficult to age species (sablefish) and adopted in 2010 for all groundfish species (Fig. 8). The new form was accompanied by changes to the groundfish database (GfBio) allowing almost all data, but the comments section, of the new ageing sheet to be captured for analysis. The header contains spaces for sample metadata (species, sample numbers, catch date, location, vessel, set etc.). It also captures reading effort, precision results, reader ID, date aged and method(s) used to determine age. Each row records the age determination process for each fish through working columns with multiple readings in the middle (minimum, maximum, age class and edge growth) to the final age on the left. The age class column contains the preferred age that the reader puts forward as their best estimate and falls on/within the limits of the minimum and maximum ages. The standard January 1st birthdate (INPFC 1957) is used to generate and record age class.



GROUNDFISH AGE DATA SHEET

COPY TO GRANDIN + MCTROW Page 1/1

Species: HAKE | Date caught: 2012 NOV 5
GfBio Sample ID: | Sample numbers: 20120621 0051 - 94 | Vessel: NORDIC PEARL | Set: 15
Area & location: 5A | Subsample 22 of 44 aged | Storage box # H160

Precision results: n= 10 KC vs YMW 70 %+/-0 80 %+/-1 90 %+/-2
Effort: 1st read t= 4.00 hrs QCt= 0.75 hrs QCn= 14 fish
General sample comments: Aged using 2nd fish starting with fish #1. Combined precision: 20120621 0051-94, 20120613 0061-118

Tray cell #	Fish #	Flagged fish	Final Age: Age class	Min	Max	Edge growth	# Crystallized	Method: Oto. Burnt XS; Date: 2012 Dec 12; Reader: YMW — Age class	Min	Max	Edge growth	PRECISION — Method: Oto. Burnt XS; Date: 2012 DEC. 13; Reader: KC — Age class	Min	Max	Edge growth	Method: Oto. Burnt XS; Date: 2012 DEC. 13; Reader: KC — Age class	Min	Max	Edge growth	Method: Oto. Burnt XS; Date: 2012 Dec 14; Reader: YMW — Age class	Min	Max	Edge growth	Test results	Comments
A1	51		6	6	6	3		6	6	6	3	6	6	6	3									0	
3	53		5	4	5	3		5	4	5	3														
5	55		7	6	8	3		7	6	8	3														
7	57		6	5	6	3		6	5	6	3														
9	59		7	7	8	3		7	7	8	3														
11	61		5	5	5	3		5	5	5	3														
13	63		6	6	6	3		6	6	7	3	6	6	6	3									0	
15	65	✓	5	5	6	3		7	6	8	3					5	5	6	3	5	5	6	3		cho.
17	67		7	7	7	3		7	7	7	3														
19	69		4	4	4	3		4	4	4	3														both otos broken.
B1	71		7	7	8	3		7	7	8	0														
3	73		13	13	14	3		12	11	13	0	13	13	14	3	13	12	14	3	13	12	14	3	+1	
5	75		7	7	8	3		7	7	8	3														
7	77		4	4	4	3		4	4	4	3														
9	79		7	7	8	3		7	7	8	3														
11	81		7	7	7	3		7	7	7	3														
13	83		6	6	6	3		6	6	6	3														
15	85		7	6	7	3		7	6	7	3	7	6	7	3									0	
17	87		5	5	6	3		5	5	6	3														
19	89		5	5	6	3		5	5	6	3														
C1	91		14	14	15	3		14	14	15	3														
3	93		7	7	8	3		7	7	8	3														

Edge growth codes: 0=annulus on edge, 1=just started, 2=moderate, 3=well developed, 4=unknown
Crystallized codes: 1=1 otolith, 2=2 otoliths

2010 Sep 03 SEM

Figure 8. Image of the new SCL groundfish age data sheet demonstrating how age assessments are recorded for hake otoliths. Note there are columns for a "preferred", minimum and maximum age class as well as edge growth for each reading by a reader and in the Final Age columns.

Note: From 1977-2010 the SCL had recorded age using a designation method developed by the lab founder, Dr. Richard Beamish (Beamish and Chilton 1982, MacLellan 1997). It was referred to as the bracket system and is described in Fig 9. Basically, for ages such as 2(2+) or 2(3), the age class precedes the first bracket. Within brackets the reader records what they actually see on the ageing structure. A plus sign indicates the presence of opaque growth, that light will not shine through, on the otolith margin. The absence of a + indicates that a translucent zone, that light can shine through, is present on the margin. The age class is interpreted based on the type and amount of growth visible on the otolith margin with the catch date and January 1st birthdate factored in. The new age data sheet developed for groundfish in 2010 did not support recording this age designation method. However, the bracket system has not been abandoned. The principals are still applied when calculating age class. It is just not recorded in the same format on the new sheet. Readers now indicate the type and amount of plus growth in the Edge growth column to explain age class interpretation. Readers may still record the bracket system in the comments section of the age data sheet if they wish, but not in the age data columns for data capture. Figures in this manual will use the bracket system to express age.

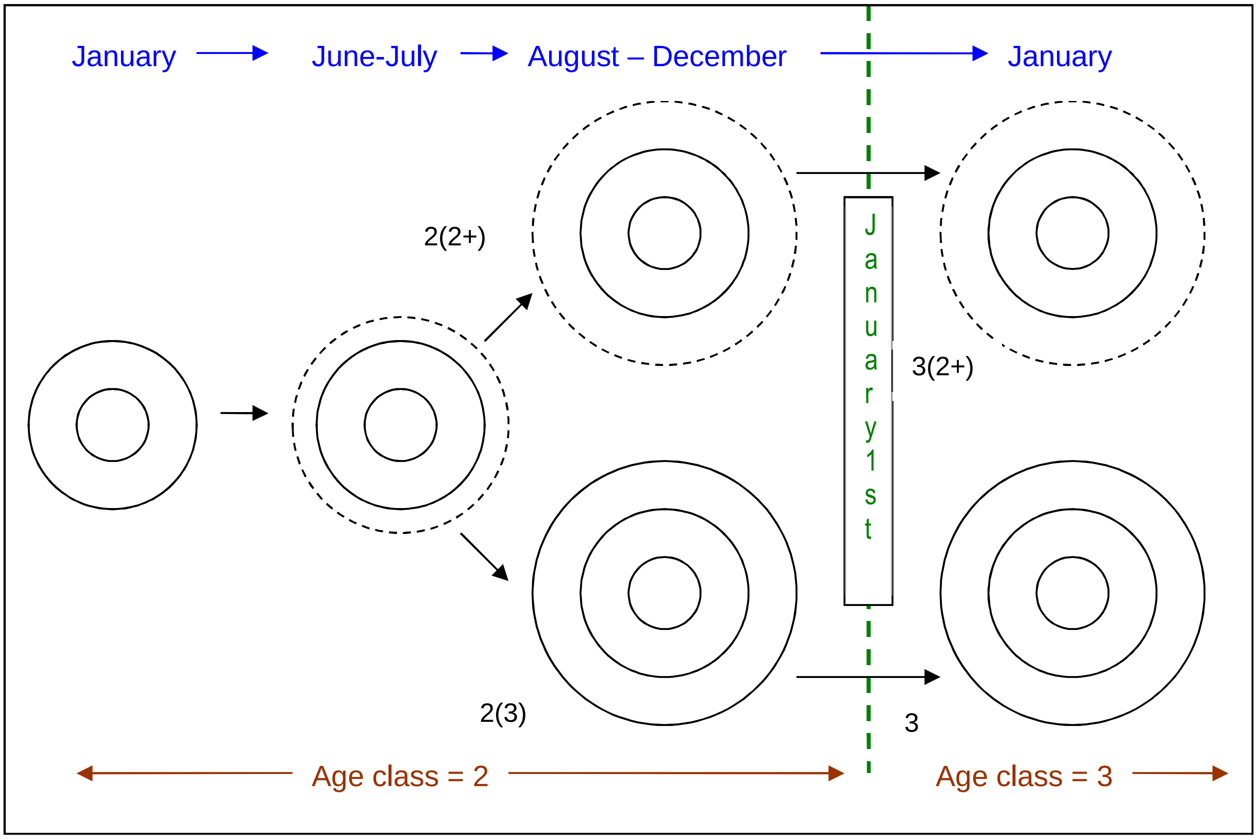


Figure 9. Drawing that explains how the January 1st birthdate is applied to assign age class throughout the calendar year (left to right) according to catch date and amount and type of marginal growth. Solid lines represent annuli (translucent growth) and the white area between lines is opaque summer growth. New incomplete opaque growth (delimited by dashed circle) generally begins to appear on most Pacific northwest groundfish species' otoliths around June and continues to be added through the summer and perhaps into fall. Depending on a number of factors, including age and species, an annulus may begin to form on otoliths as early as late August - September. This incomplete annulus would not be counted until the sample catch date crosses into January 1st of the next calendar year (bottom right corner). Alternatively, if a large amount of opaque growth (top right corner) is present on the otolith margin early in the year (Jan. ~ Jun.) it would be considered to have formed the previous calendar year and is counted as another year. The latter example may be seen on otoliths from the oldest individuals where annual zones have become quite small and it is difficult to discern if an annulus is present on the otolith margin early in the year. The bracket system age designations (black text) are added for reference. A 3(3) designation was always shortened to just a 3.

2.4.1.1 Age designation standards

Initially in the 1970's, the SCL developed and recorded a confidence index (Table 2) on age data sheets to describe the relative quality of age assessments for groundfish species. With a few evolutions over time the index came to include quantitative and qualitative factors and ages coded as; good (G), fairly good (FG), fair (F), fairly poor (FP) or poor (P). The quantitative component indicated within or self repeatability and qualitative pattern clarity. The index was a rough tool to identify age determination issues for the SCL and also provided some guidance to data users if they wanted to make decisions on which age data to use for analysis.

Although the confidence index was found to be a satisfactory estimate of pattern clarity and age repeatability for many years, it was found that multiple expert readers over time expressed differing opinions on assessment resulting in index inconsistencies that could be seen when reviewing multiple readings for the same fish. This was due to a number of factors, e.g. time fading and affecting clarity of the burnt otolith patterns, different readers re/processing the same/opposite otolith half or pair, slightly different microscope or lighting set up or just having different opinions regarding interpretation of the pattern. This index was eliminated from the SCL's groundfish age data sheets in 2010 when a new keypunch friendly sheet was adopted to provide the reader with a more objective way of expressing uncertainty that could be quantified by the data users. However, readers may still use and record the index in the comments column as needed. Including it in this manual provides a reference when looking back at historical age data sheets.

The degree of uncertainty, or reader confidence, for all groundfish age data is now recorded in newly added minimum and maximum age columns on the age data sheets (Fig. 8). The reader must provide three ages per fish; a preferred, a minimum and a maximum, all interpreted to an age class. The preferred age is used for analysis. The min-max ages also provide the reader with the opportunity to express an age range that is not normally distributed around the preferred age. A greater departure from the preferred age articulates increased uncertainty and therefore a poorer quality age estimate. A data user wanting to include only high confidence age data can make decisions by reviewing the min-max differences surrounding the reader's preferred age. For instance, if the preferred age is 25 and the min-max ages are also 25, then the reader is very confident with their preferred age estimate. Alternatively, if the min-max ages were 15 and 29 the reader is expressing much less confidence.

Table 2. Former confidence index used by the SCL. The index was both qualitative and quantitative. The descriptive aspect addresses pattern clarity and the quantitative aspect expresses within repeatability. The index also takes into consideration longevity when assessing the quantitative aspect. For example, a variation in age estimates of five years is considered to be a FAIR estimate for a fish aged 50 years or older, but would be POOR if the fish was aged <20 years.

Confidence Index	**Abbreviation**	**Qualitative meaning** (Pattern clarity)	**Quantitative meaning** (Repeatability)	**Age examples**
Good	G	Pattern is very clear with no interpretation problems	Reader always gets the same age	8^{G},25^{G},63^{G}
Fairly good	FG	Pattern is clear with a few easy interpretation problems	Reader gets the same or close to the same age most of the time	8^{FG},$25^{\pm 1FG}$,$63^{\pm 2FG}$
Fair	F	Pattern is fairly clear with some areas presenting easy & moderate interpretation problems	Reader gets close to the same age most of the time	$8^{\pm 1F}$,$25^{\pm 2F}$,$63^{\pm 6F}$
Fairly poor	FP	Pattern is fairly unclear presenting a number of difficult interpretation problems	Reader more likely to produce ages with fairly significant differences most of the time	$8^{\pm 2FP}$,$25^{\pm 3FP}$,$63^{\pm 8FP}$
Poor	P	Pattern is very unclear presenting significant interpretation problems	Reader has little confidence in repeatability of age – significant differences	$8^{\pm 3P}$,$25^{\pm 5P}$,$63^{\pm 15P}$

2.4.1.2. Other information recorded

Besides age data, the reader is also expected to record observations regarding the condition of the otolith (e.g. crystallized, broken, single otolith only, poor burn, off-centre break, etc.) and record growth pattern issues or problems (e.g. small/large years of growth, presence of checks, problematic years etc.). These remarks help to explain age data quality issues or how the age was derived. Otolith condition information can provide good feedback to samplers and identify problems experienced when ageing a particular fish. Comments may also help to identify synchronous or atypical growth patterns in a population which may be useful to both the reader and the data user.

2.4.2. Productivity standards

The SCL has established productivity targets and capacity by documenting the time for two activities in the age determination process; the first reading and the quality control (QC) effort of the tester. The first reading rate (productivity) is the speed (fish/hr) with which the first reader can prepare and age hake otoliths. The rate for hake is based on 8 years of data, includes results from 9 readers and was calculated from 23,282 fish comprising 499 samples aged between 1999-2006. The average first read rate is 25 fish/hour. The acceptable range (25th and 75th quartile) for readers to achieve during production ageing is 16 – 29 fish/hour. Readers are expected to maintain productivity over time. Capacity, or effort expended to complete a sample by a team, is calculated by weighting sample numbers and combining the effort of first reading and testing. This includes the time it takes to first read and for the second reader to precision test and resolve differences. It also takes into account the number of readings required to do this. The SCL's capacity to age hake samples is, on average, 15 fish/hour and is lower than the first reading rates because it takes into account most (but not all) of the additional effort it takes both readers to resolve and finalize ages for each sample. It should be noted that not every step in the production ageing process was captured in our capacity calculation, meaning the above numbers will under-estimate overall effort to age samples of hake to some degree.

2.4.3. Data quality standards

2.4.3.1. Precision

The SCL generally applies the same QA\QC measures when production ageing hake otoliths as for other groundfish species. The readers have access to sample information such as species, date caught and area/location. **They do not have access to biological data such as fish length, weight or sex which are considered to be biasing.** This does not preclude the use of biological data after the fact to reassess suspect ages or to make adjustment to criteria if needed, especially since only a small portion of all samples receive a second reading.

The first reader ages a sample completely and then produces a precision test for the second reader. Once finished a sample, the first reader rolls a die to randomly decide the first fish to be tested and then identifies every Xth fish thereafter to re-age a minimum 15% of the total sample independently. The second reader (tester) ages the test fish without having access to the original ages. Precision targets for hake have been set at 50% agreement and overall 80% +/- 1 year. Once the test results are calculated by the tester they will attempt to resolve any differences as well as re-age any fish flagged by the first reader needing a second look. This step is done with access to all previous age data and is not independent. If precision targets are not met, biases are identified and additional otoliths are reviewed. Both readers will work together to resolve any disagreements to ensure best quality age data is produced. Any significant differences in application of standard method criteria would be discussed and resolved between readers. In some cases, documentation may be required for future reference.

2.4.3.2. Accuracy

Beamish and McFarlane (1983) demonstrated the need to validate the accuracy of fisheries age determination methods. The SCL's methods to age hake otoliths have been validated through various methods. Length-frequency data validated criteria for the first three years of growth (Dark 1975). The SCL used a natural tag to validate the ages of mature offshore stock hake (MacLellan and Saunders 1995). The 1982-83 El Niño Southern Oscillation (ENSO) event produced a natural tag on otoliths of the strong 1980 year class of the offshore stock in the form of a relatively small fourth annual growth zone. The tag established known-age fish and validated the burnt otolith section method for up to 12 years of age. The SCL subsequently extended the validation to 21 years. Figure 10 illustrates how annual zones were deposited on otolith sections in the years post natural tag formation.

Of interest, when the 1980 year class of offshore hake first showed up in B.C. waters in the summer of 1984 the SCL was faced with a significant pattern interpretation issue regarding the criteria identifying the fourth year on otolith surfaces versus cross-sections. Readers were ageing otolith surfaces at 4 years but cross-section ages at 3 years old. Protocol prioritized the section age. After submitting age data to and consulting with the data users it was revealed that the large year class was generally being under-aged by one year. The data users knew to expect a large number of 4 rather than 3 year olds. The conundrum was resolved with readers and data users working together and by processing samples over the next two catch-years (1985 and 1986). The SCL was able to identify that an anomalous small fourth year of growth had formed and was difficult to differentiate from a commonly seen check in the third year of otolith cross-sections on four year old otoliths. Once one to two more years of growth formed on the otoliths in subsequent years it was easier to identify the small 4^{th} year. This was documented for future reference in the SCL's internal system and published (MacLellan and Saunders 1995). The tag became a unique tool to accurately track the 1980 year class. This demonstrates the importance of age readers and data users working together to work out issues and continuously assess and update age determination methods to ensure best quality age data.

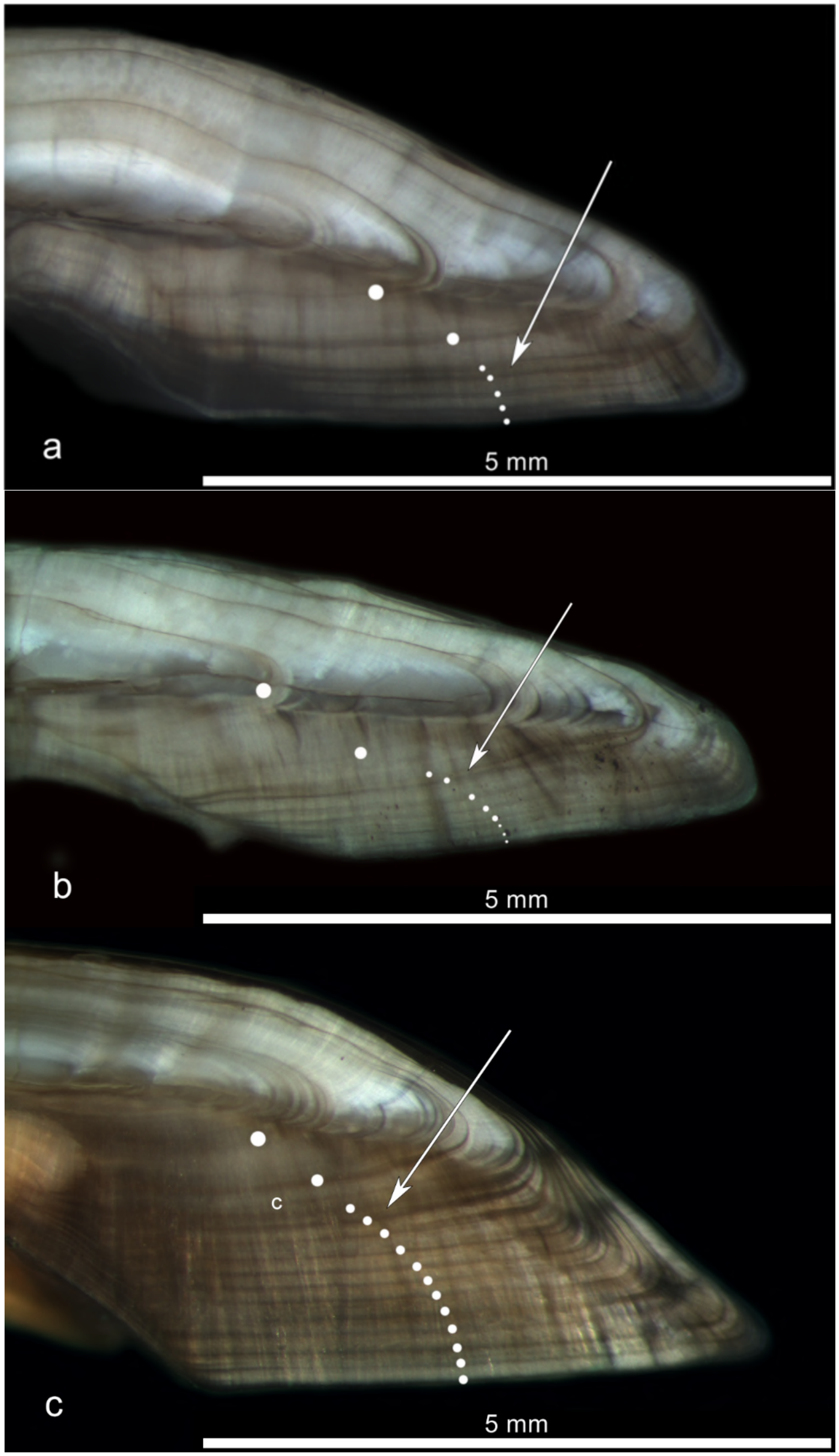


Figure 10. Photos of hake otolith burnt sections lit with reflected light illustrating the 1982-83 El Niño natural tag in progressively older offshore stock hake caught from 1988 to 1994. a) Age 7 yrs (1988), b) Age 10 yrs (1990) and c) Age 14 yrs (1994). Note that an annual zone has formed after the tag for each additional year the fish was caught beyond the tag year of 1983 (arrow). Dots=annuli and c=checks.

2.5. Otolith burnt section age determination method

Many age determination protocols and standards are commonly employed by the SCL when ageing otoliths for all groundfish species, such as terminology and generating age data. There are, however, differences when it comes to species specific criteria, such as counting axes and the interpretation of otolith growth and pattern characteristics.

2.5.1. Terminology standards

Communicating with standard terminology ensures that readers are seeing and talking about the same thing and promotes the generation of consistent age data. The glossary at the back of this manual defines terminology adopted by the SCL and used in this manual.

A reader must learn to identify three basic growth zones in order to age Pacific hake otoliths and clearly understand their definitions.

1. Summer zones (plus growth) appear opaque; correspond to a time of fast growth and form generally from spring through to fall.
2. Annuli (winter zones) appear translucent; correspond to a slowing of growth and form only once a year generally from the fall through to spring.
3. Checks have similar characteristics to annuli. These zones also appear translucent and correspond to a slowing of growth due to some kind of stress. However, unlike annuli, they form during the normally fast-growing season, within the summer zone, and may form more than once a year. Usually, but not always, they are less prominent than annuli.

An annual growth zone is comprised of one summer zone followed by one annulus.

Checks that look very similar to annuli are sometimes incorrectly counted, resulting in over-ageing. Essentially an experienced reader must learn to differentiate checks from annuli in order to accurately assess age. This presupposes that the method used includes criteria powerful enough to identify checks and annuli with a fairly high rate of precision (consistency) and accuracy.

2.5.2. Age data generation standards

Age class is assigned to otoliths based on the amount and type of growth (winter or summer) visible on the otolith margin in relation to both the January 1st birthdate and the date caught (Fig. 9). These factors are important to consider as there are certain times when the catch-year's summer or winter growth may not be visible at the time of capture or is incomplete. The amount or extent of edge growth is evaluated relative to the size of the previous year's annual zone, albeit expecting a reduction in annual growth zone size over time.

2.5.2.1. Date of capture

The SCL will not age a sample unless the date of capture is provided. To assess age accurately, the date of capture is necessary and must be available to the reader. The year is not important, but the month is essential. The day is also very useful in terms of placement within a month; early, mid or late. This can be critical to the age class decision-making process, especially in spring and fall when otolith growth zone deposition is changing from fast or slow growth conditions. The month and day provide expectations for the type and size of the last growth zone (summer/winter) on the otolith margin (Fig. 9). The date requirement ensures that the last annual growth zone visible on the otolith edge, complete or not, is assigned to the correct calendar year of formation, either the previous or the catch year. Without specific catch date information a fish can be miss-aged by one year.

2.5.2.2. Jan 1st birthdate

The SCL uses a standard January 1st birthdate (INPFC 1958) to assign age or age class to almost all fin/shellfishes they age, irrespective of a species' actual biological birthdate. There is one exception, English sole (*Parophrys vetulus*) (MacLellan and Fargo 1995). The convention ensures that northern hemisphere fish born within the same calendar year are combined into the same cohort for analytical purposes, such as stock assessment, regardless of their biological birthdate.

2.5.2.3. Location/stock

The SCL needs to know the location hake samples were collected from to know if they are ageing offshore or SOG stock fish. Minor-nuanced age criteria exist for each stock based on the size of annual zones at age (i.e. the first 3 years) and presence of prominent checks throughout the pattern. SOG otoliths are smaller at age than offshore otoliths (McFarlane and Beamish 1985) and therefore have relatively smaller annual zones, in particular the first. The first annual zone of a SOG otolith is very similar to the size of the nucleus check on offshore otoliths. Not knowing which stock samples are from could lead to over-ageing of offshore stocks and under-ageing of SOG stocks.

2.5.3. Otolith preparation procedures

The production method used by the SCL to age hake is the otolith burnt section method (Christensen 1964, MacLellan 1997), also known as the "break and burn". It takes a fair amount of experience to produce a good quality broken and burnt otolith. If the preparation is not done properly it can impact age data quality. Done correctly, burning will enhance the clarity of an otolith's growth pattern and facilitate identification of annual zones and counting. Hake otoliths have a different appearance from most other fish species' otoliths aged by the SCL. They are fairly flat with little to no concavity and are relatively brittle, having a "smooth-glassy" consistency and appearance.

2.5.3.1. Cleaning the otolith

The SCL ages otoliths stored in a 50:50 solution of glycerine and water that contains a small amount of thymol. It is important to remove all fluids and tissues (e.g. blood, otic membrane) from the otolith that might be displaced onto the cross-section surface during the breaking process. Paper tissues can be used to wipe the otolith of any remaining organic tissues or liquid storage medium and ensure it is dry before breaking and burning. If desired, the otolith can be swept through water to help remove the storage medium. Particular care must be taken to clean out the sulcus groove. Left behind, tissues will burn dark and stick to the otolith's cross-section surface obscuring the growth pattern (MacLellan 1997). NOTE: The SCL is currently in the process of changing their storage protocol for otoliths from a liquid medium to dry for long term storage. They still receive hake in the liquid medium as they are aged soon after arriving. These otoliths will be washed and stored dry once aged.

2.5.3.2. Breaking the otolith

Hake otoliths have a relatively large first annual growth zone, easily seen by eye. If desired, the otolith can be immersed in water in a black dish to view the overall surface distal pattern and to establish approximate age, especially juvenile annual growth. The SCL standard is to break hake otoliths in a dorso-ventral or transverse cross-section plane where the first year's growth is widest rather than directly through the nucleus centre (Fig. 11). This ensures a standard preferred plane that provides a consistent looking pattern for interpretation. Readers need to develop the skill to reliably make clean, even breaks to aid pattern interpretation so that quality age data is produced. An even break is described as having a relatively flat cross-section plane. Off-center or irregularly broken otoliths can be aged. An experienced reader can recognize when a non-standard break plane occurs by the shift in size and shape of the first 3-4 annual growth zones in cross-section.

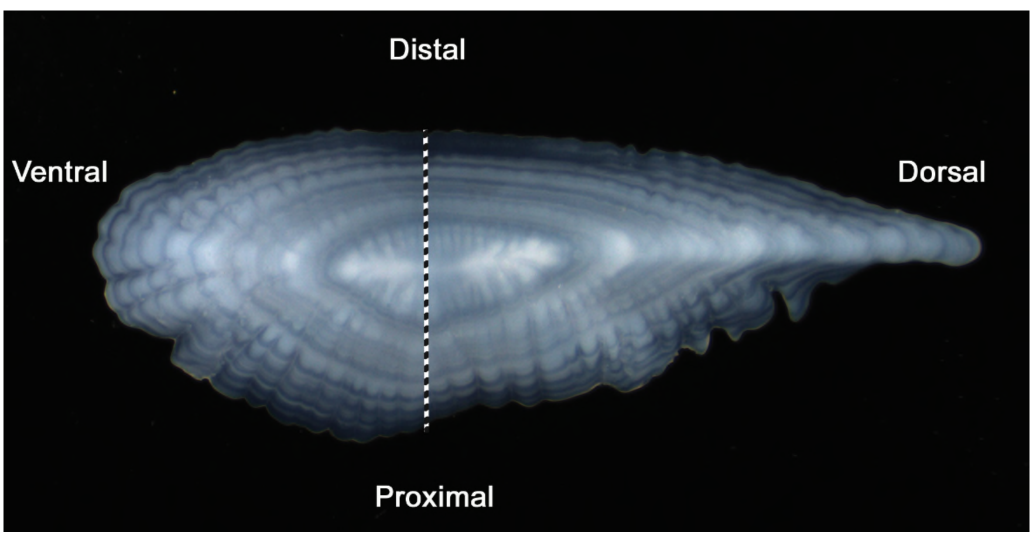


Figure 11. Distal surface of an offshore hake otolith identifying topography and the location of the standard break line (dashed line).

Otoliths can be broken using various methods. SCL readers most commonly use their index finger and thumb nails (Fig. 12) or nail clippers (Fig. 13) to bisect hake otoliths in preparation for burning. When using hands to break avoid touching the broken surface with fingers as skin oils will be transferred and may form a dark deposit when burnt obscuring the growth pattern. The slightly "rough" broken surface is preferred to the partially polished plane produced by saw blades. Once burnt, a polished surface can result in a glassy or hazy looking pattern that SCL readers find more difficult to age.

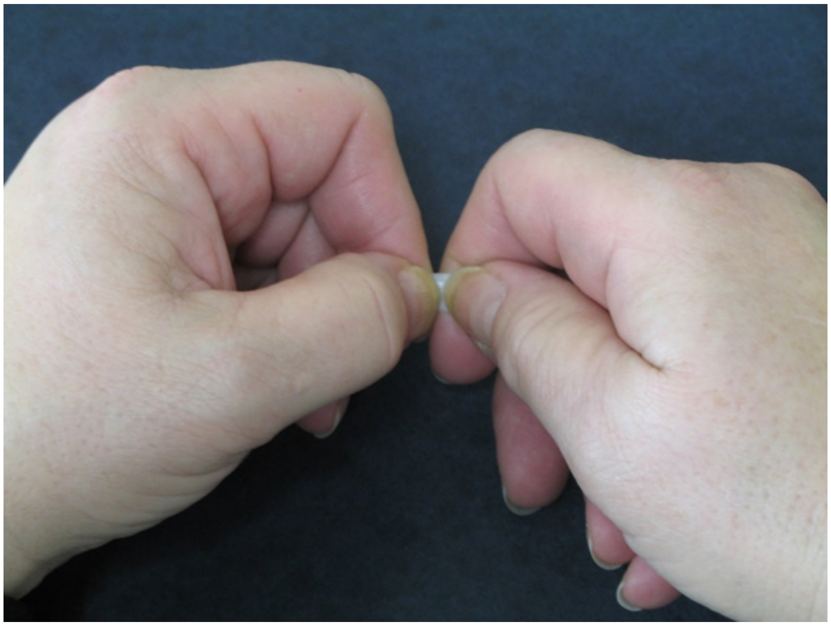


Figure 12. Otoliths can be broken by hand by holding the otolith with the distal surface facing the reader. The thumbnails are lined up along the cross-section plane as above. They are pushed into the otolith with a snapping motion to break the otolith in two.

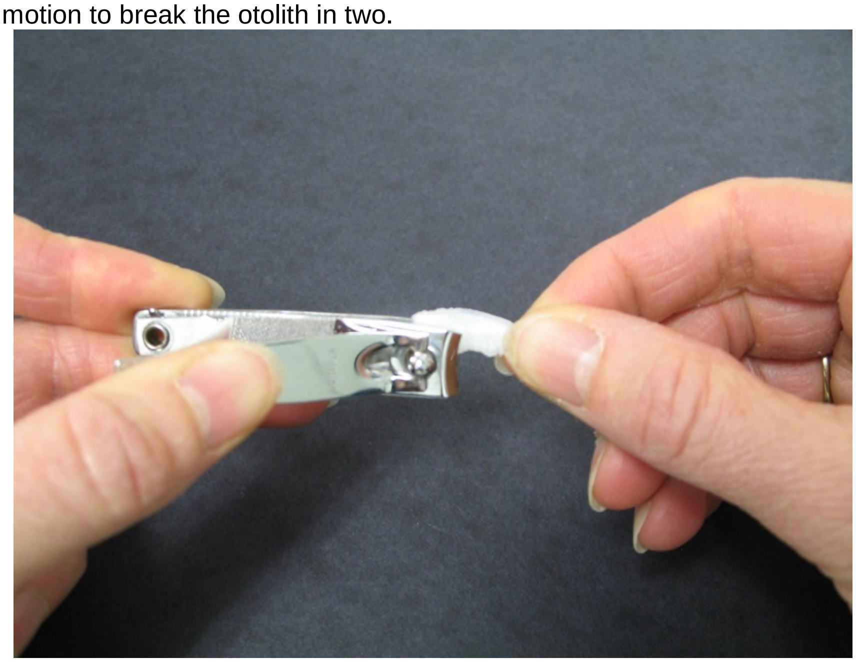


Figure 13. Nail clippers can be used to cut otoliths in half by lining up along the break plane and slightly to the dorsal side of the dorso-ventral break plane.

2.5.3.3. Burning the otolith

An experienced reader can burn an otolith half properly in approximately 10-30 seconds. To burn, the reader must clamp the otolith in forceps and hold it over the flame of an alcohol lamp (Fig.14).

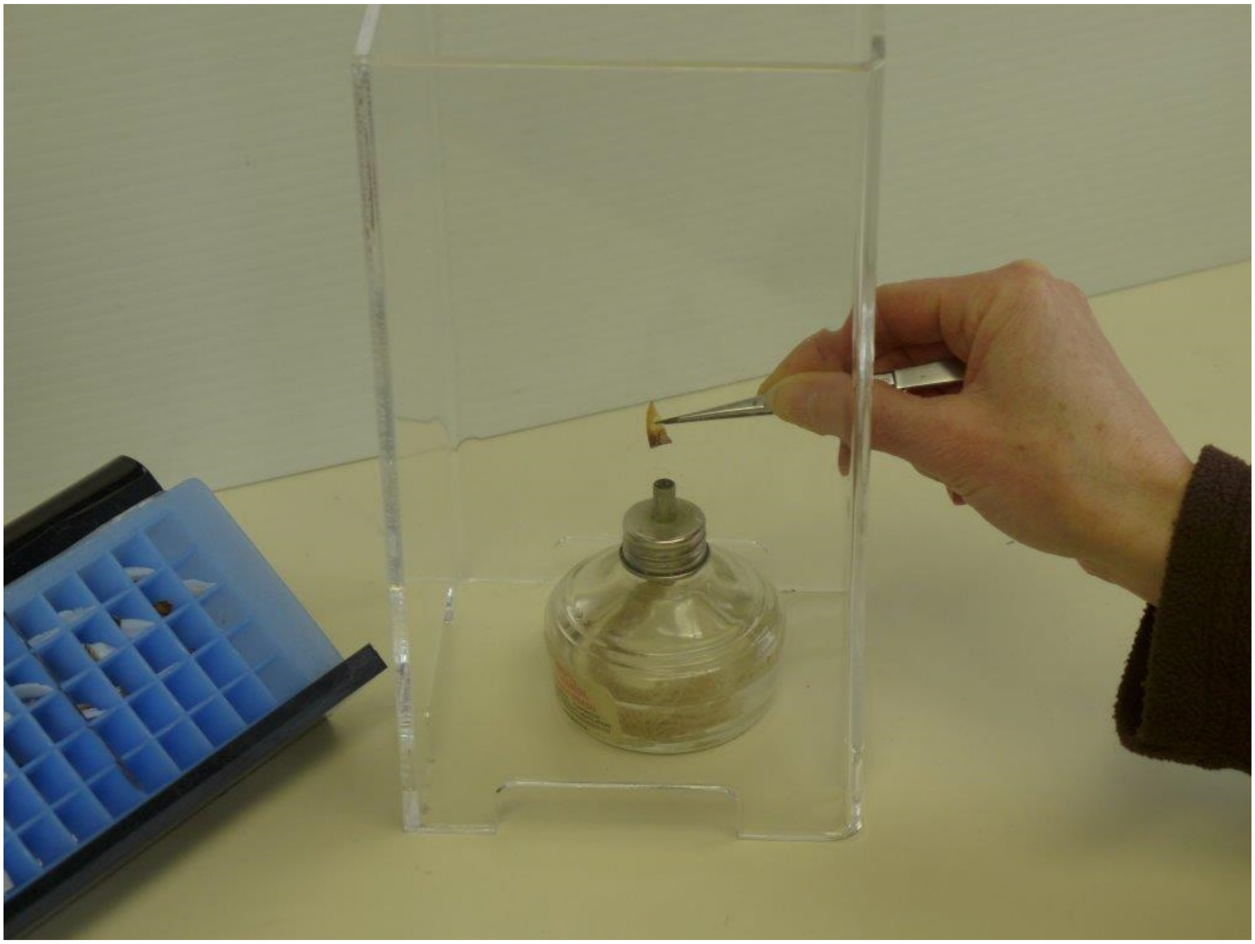


Fig. 14 A broken hake otolith is held over an alcohol burner flame to turn the annuli brown for age determination. Burning takes place inside a shield to keep drafts away from the flame and to protect the reader from flames and hot pieces of otolith that may pop off during the process.

> **Health:** The SCL keeps lit alcohol lamps inside a plastic shield (Fig. 14) to shelter the flame from drafts and to protect eyes, skin and clothing from flames and hot otolith fragments that may pop off. Burnt otoliths should never be touched until they have cooled. Always use forceps. Hot otoliths can singe skin and clothes. The best practice is to douse a burnt otolith in mineral oil to help quench the heat before placing it into plasticine for viewing. In the past, concerns were expressed that breathing the "smoke" from burning otoliths may impact a reader's health. In 2005 Health Canada conducted air testing in the SCL and it was determined that burning otoliths is not a hazard to health (Clay and MacVicor 2005).

The annual growth on unprepared hake otoliths consists of one opaque white summer zone followed by one translucent winter zone or annulus. Under reflected light, against a dark background, the translucent zones of an unburnt otolith appear dark and the opaque zone appears light. Burning causes translucent zones to turn brown while the opaque summer zones remain a whitish colour. It is important to produce "evenly" burnt otoliths (Fig.15) so that the reader is presented with a clear growth pattern to generate quality age estimates. This takes some practice and expertise to accomplish with consistency. The reader must watch and check the otolith as burning progresses to ensure that all areas of the cross-section plane are burnt to the same intensity of colour. This process requires good eye-hand control and focus to coordinate subtle movements and to hold the otolith at the appropriate distance above the flame.

Figure 15. Photos of burnt offshore hake otolith sections. a) A 9 year old section that has been evenly burnt. Note that the annuli can be seen clearly in all areas of the section. b) An 8 year old section with over-burnt tips that have lost material due to too much heat. Also, annuli are not clear in the preferred areas for ageing (arrows).

When burning, otolith sections advance through colour changes; white to yellow-orange to reddish-brown to brown to black to grey followed by white. If in doubt, progress can be checked by assessing pattern clarity under the scope. The pattern will not appear as discrete as when mineral oil is applied but clarity changes can be seen. The goal is to produce uniformly distinct dark brown annuli across the whole section plane and to especially avoid over-burning the otolith section's thinner dorsal and ventral tips. This is important as the section is aged on both the ventral and dorsal sides. The reader maintains control over the burning rate and quality with slight movements over and above the flame. The further the otolith is held above the flame, the more control in burning speed. If portions of the otolith appear to be burning too quickly the otolith can be lifted, tilted or flipped to present the portions needing more burning closer to the flame.

Hake otoliths, perhaps because of their "glassy" consistency, can be more difficult to burn well. The contrast between the summer and winter zones is not as discrete when burnt, appearing more diffuse on the ventral side (Fig. 16a) and can lack the clarity and contrast of other groundfish species' otoliths, e.g. rockfish (Fig. 16b). Generally, it is best to burn small juvenile hake otoliths a lighter red-brown (Fig. 17a). Larger - thicker otoliths, from older hake, should be burnt longer to produce more discrete darker annuli (Fig. 17b) especially on the dorsal side of the section where growth is more compact. The reader has four otolith halves to work with if there is a need to make adjustments to burning. An experienced reader usually needs to break just one of the otoliths to produce an age. They may or may not burn both halves.

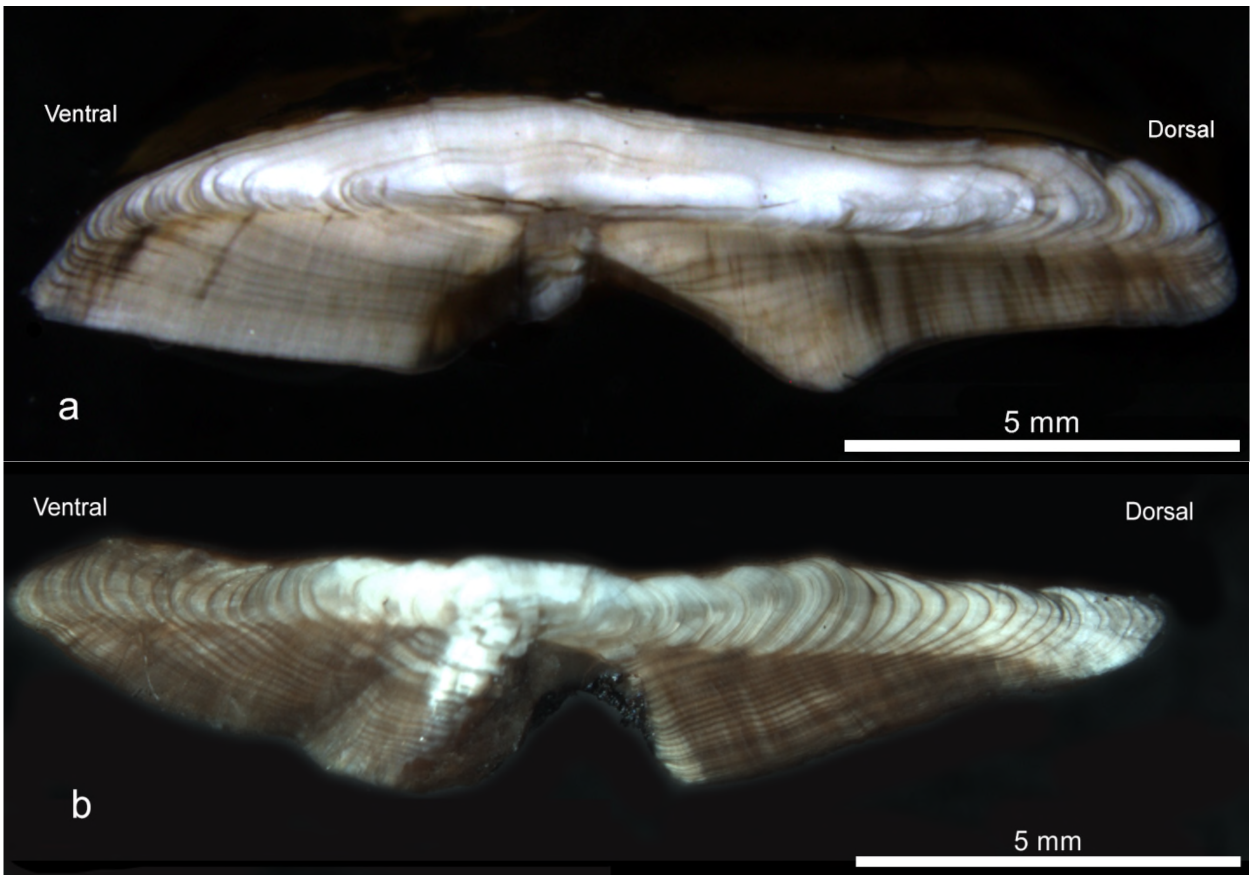


Figure 16. Photos of properly burnt offshore hake and rockfish otolith sections with ventral and dorsal sides identified. Hake otoliths do not burn with the same contrast and discrete clarity as rockfish otoliths, especially on the ventral side of the section. a) Burnt offshore stock hake otolith section. b) Burnt yelloweye rockfish (*Sebastes ruberrimus)* otolith section.

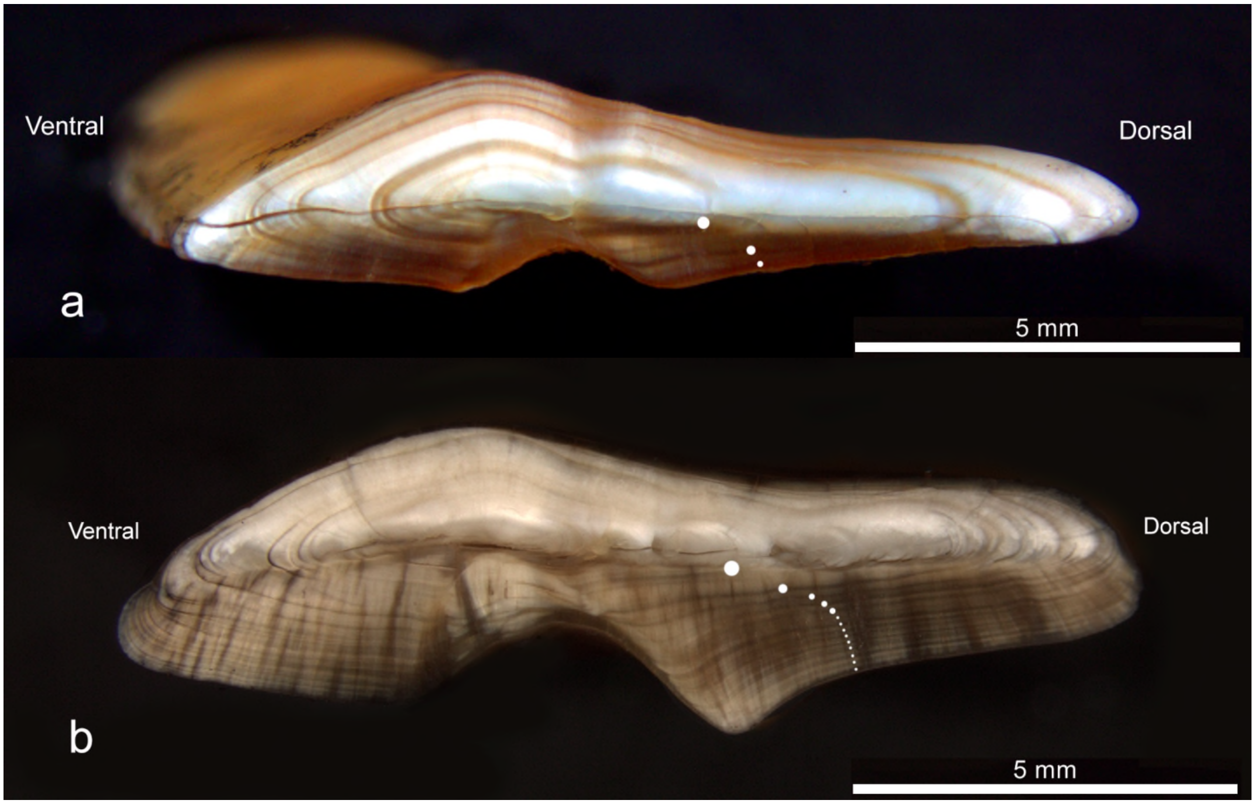


Figure 17. Burnt otolith section from a) 3 year old and b) 14 year old offshore hake. Annuli are identified by dots. Note that the section from the younger otolith is burnt less, being a light red-brown colour. The older section was burned longer to produce darker more discrete annuli.

2.5.3.4. Viewing the otolith

The burnt otolith section must be placed in plasticine at appropriate viewing angles with respect to the microscope objective lens and fibre optic lights (Fig. 18). Normally the otolith is positioned fairly upright (section plane facing up). The readers are encouraged to adjust the position of burnt otoliths and the angles that reflected light hits the burnt section plane to improve pattern clarity as needed. Mineral oil is applied to the section plane to enhance pattern clarity, acting like a coverslip. A paint brush is used to apply oil onto the burnt surface or the otolith may be dipped directly into the oil before placing it in the plasticine. Heavy mineral oil is used because it evaporates slower, requiring fewer applications.

> **Health:** Mineral oil is an inert substance which does not react with the hot otolith to produce toxic fumes and is therefore safe to work with.

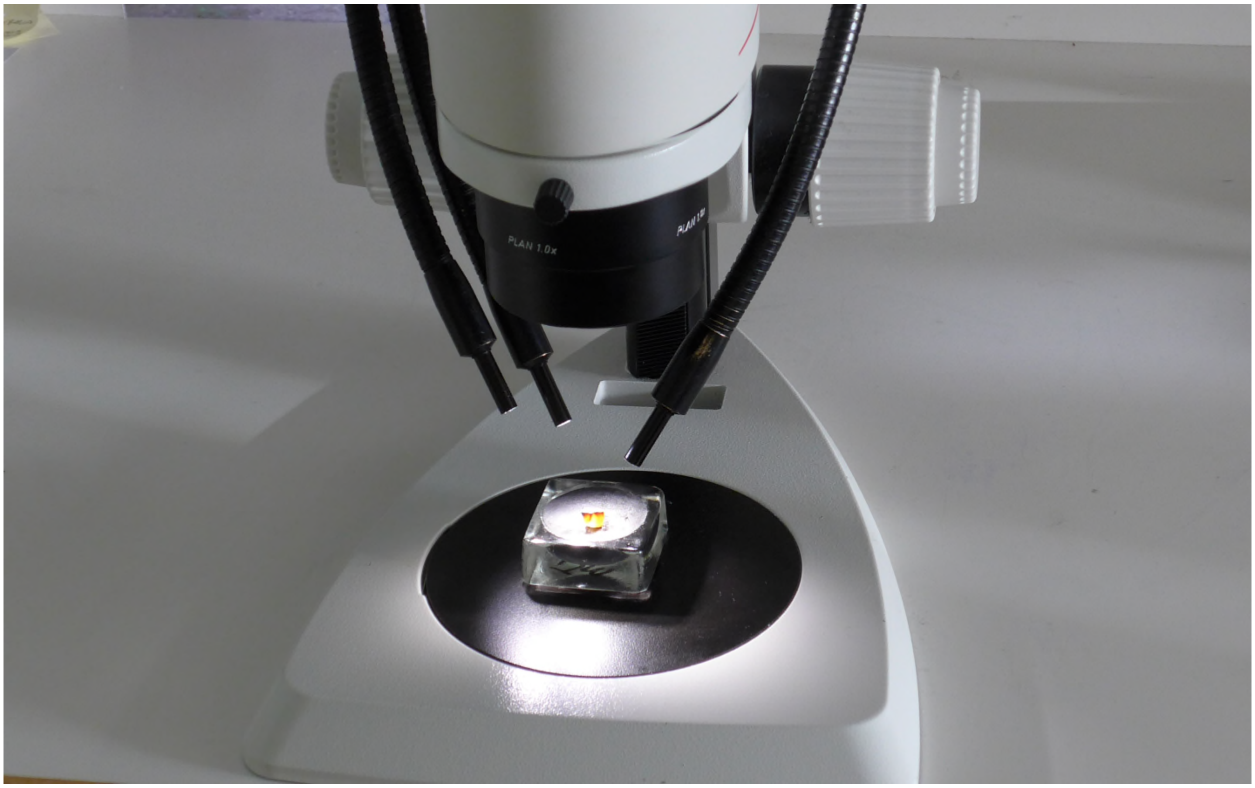


Figure 18. After orienting the burnt otolith in the plasticine, the gooseneck fibre optic lights are bent into position and angled to best illuminate the growth pattern for counting.

2.5.4. Ageing criteria

Criteria are standardized rules that the SCL has developed through research and established as standards for readers to employ when interpreting growth patterns on otoliths. Consistent and correct application of criteria should result in good quality age data and avoid reader drift.

2.5.4.1. Preferred counting axes

A whole or cross-sectioned otolith presents a number of growth axes for counting annuli (Fig 19). Some axes are more reliable than others because they present a clearer well defined pattern with fewer noticeable checks and/or contain all annuli. Use of standard preferred axes promotes production of consistent and accurate age data. The reader should become accustomed to counting in either direction along an axis, i.e. from the otolith margin “inwards” to the first annulus and vice versa. The same age should be attained either way.

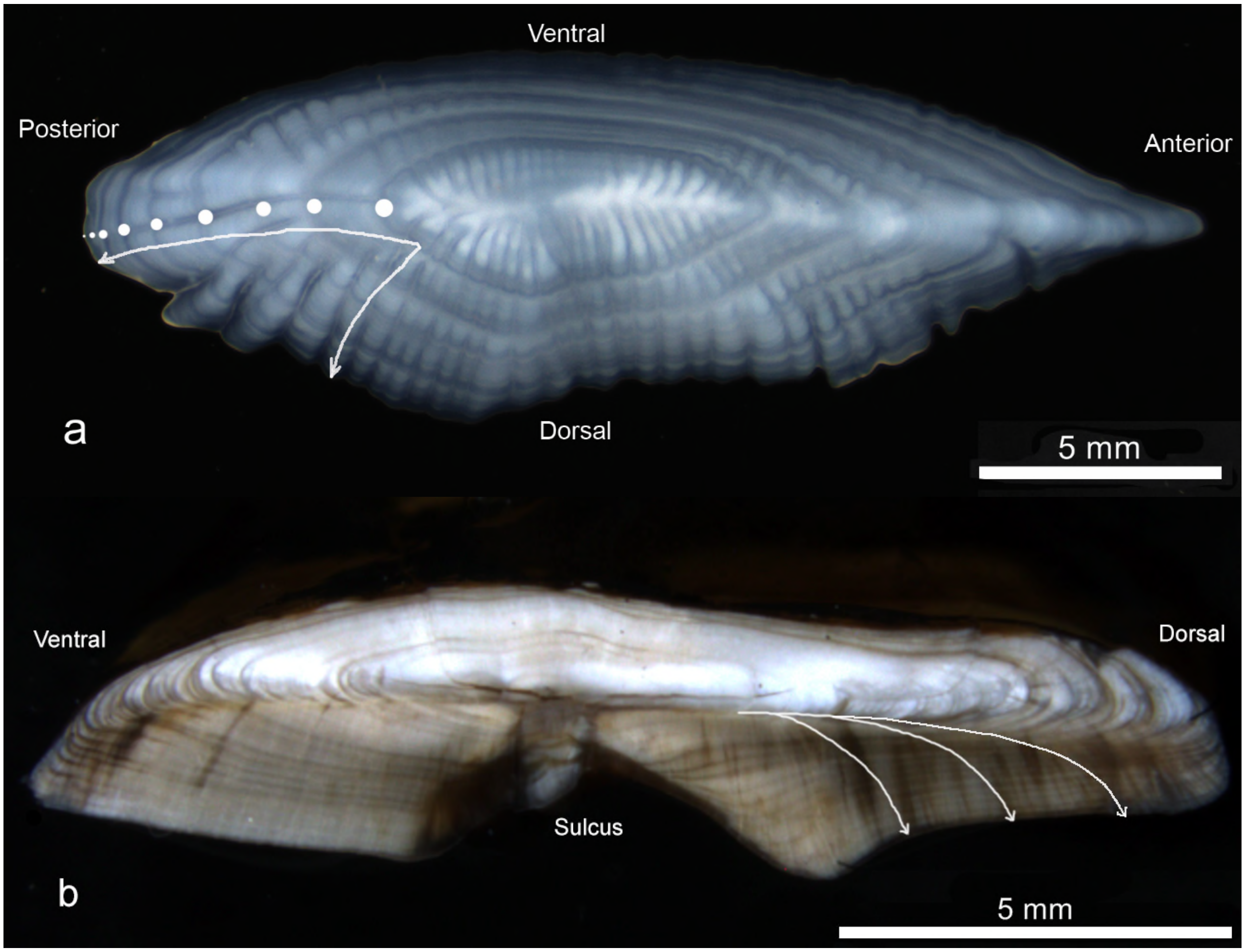


Figure 19. Photos showing the preferred counting axes (arrows) on offshore hake otoliths a) Distal surface. b) Otolith burnt section. Both the dorsal and ventral side of the section can be aged but the dorsal is more preferred. The anterior, posterior, dorsal and ventral areas are pointed out as well as the sulcus on the cross-section.

2.5.4.1.1. Whole otolith axes

The most consistent counting axes on the whole distal surface are in the posterior-dorsal and posterior areas towards the rounded (post- rostrum) end of the otolith (Fig 19a). Once past 8-10 years annuli usually become so crowded on the otolith distal surface margin they can't be differentiated or they may not be visible at all. This is due to a change in growth morphology as the fish ages where the otolith begins to get thicker, forming annual zones only on the sulcus side of the otolith (Beamish 1979).

2.5.4.1.2. Burnt sectioned otolith axes

Both the dorsal and ventral sides of hake otolith burnt sections can be used to assess age reliably (Fig 19b). When the thin-section method was adopted in the 1970's the ventral was the only preferred counting side. This protocol was continued when the SCL changed over to the burnt section method in 1983. However, over the next decade, with a change in pattern clarity (more checks), the SCL shifted to prefer counting along the dorsal side of the section where growth is more compacted and checks are less obvious. Readers usually confirm the dorsal count by ageing the ventral side as well.

2.5.4.1.2.1. Juvenile axes

The compact distal counting axis is preferred to first identify the 3 juvenile growth zones on the burnt otolith section. They are normally fully deposited there as overburden. Overburden refers to sequential overlapping of annual growth on the distal side of the section. Normally the 4th annual zone (1st adult year for most hake) does not extend entirely around to the section's distal surface forming only a small amount of overburden at the dorsal and ventral tip areas (arrows Fig. 20). Juvenile annuli are usually continuous throughout all regions (distal, ventral, proximal and dorsal) of the burnt otolith section. They can also usually be identified near and to either side of the sulcus on the proximal side of the section. Once found on the distal axis they can then be traced towards the dorsal and ventral tips of the section and into the sulcus area on the proximal side of the section (Fig. 20). Sometimes it is difficult to trace juvenile annuli on the ventral side of the section into the sulcus (Fig. 21). Therefore, it is especially important to confirm age with a dorsal side count.

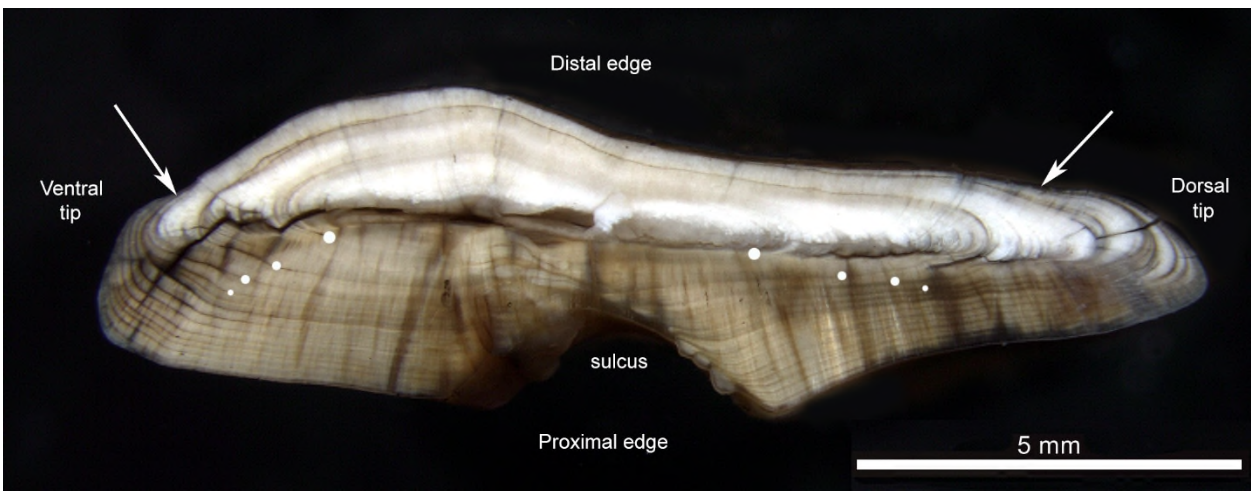


Figure 20. Photo of a 14 year old offshore hake otolith burnt section indicating the dorsal, ventral, proximal, distal and sulcul areas. The first 3 annuli (dots) are visible and can be traced continuously in/on all areas/axes. Arrows point out where 4th year overburden forms partially on the distal side of the dorsal and ventral tips but does not extend all the way over the distal surface.

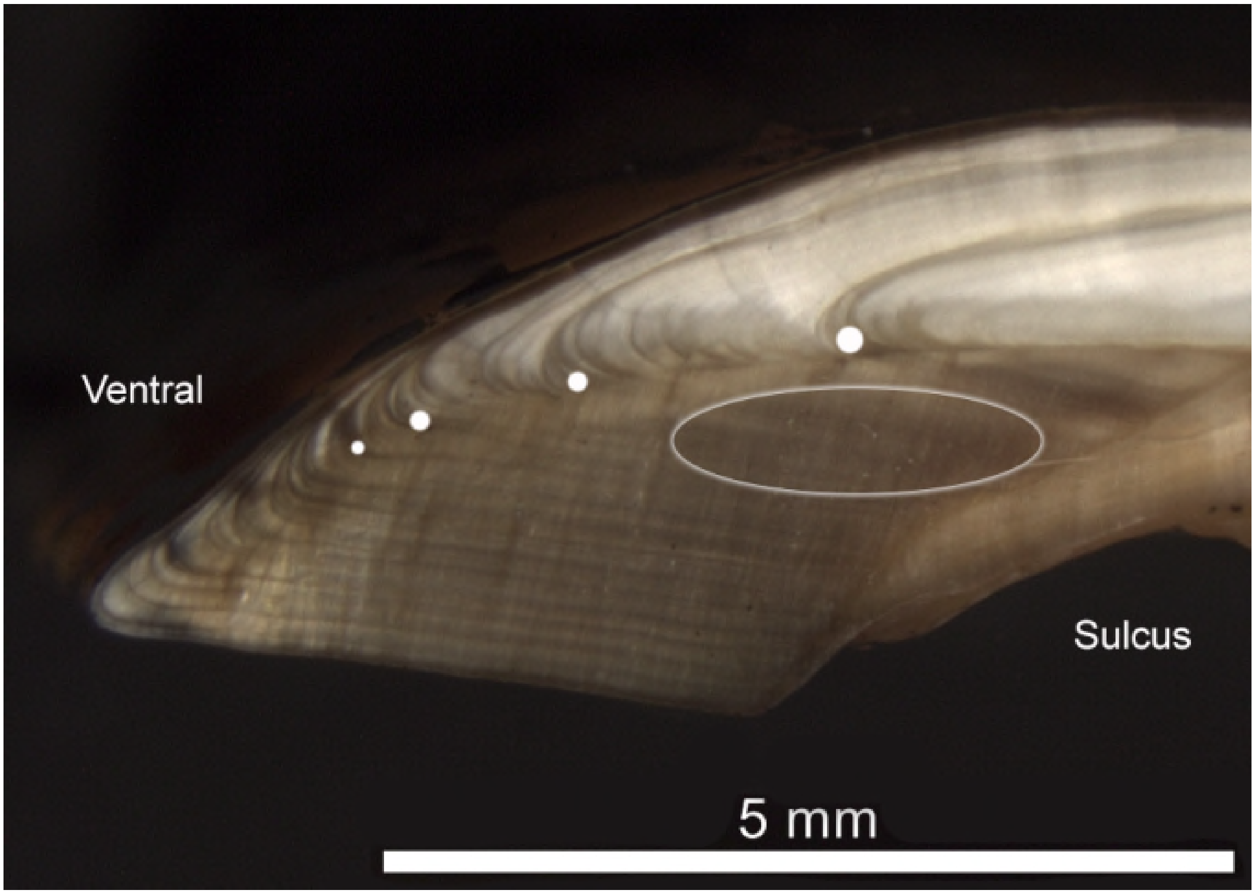


Figure 21. Photo of 14 year old offshore hake burnt otolith section that illustrates how difficult it can be to trace juvenile annuli from the ventral tip to sulcus area. Dots mark the first 4 annuli and the circle captures where the 2^{nd} and 3^{rd} become vague and hard to trace.

2.5.4.1.2.2. Mature axes

The dorsal side of the burnt section is the preferred side for counting mature annuli. Normally, all prominent dark zones visible on both the dorsal and ventral sides of the burnt section following the 4th year are counted as annuli. The 4^{th} annulus should be tracked from the dorsal or ventral tips towards the sulcus area to count subsequent annuli on the section. Counts should be confirmed by ageing both the dorsal and ventral sides of the otolith section.

2.5.4.1.2.3. Plus growth axes

Plus growth (new year's) on hake otoliths can be observed on some counting axes sooner than others and does not show up in equal amounts simultaneously on all growth axes. The preferred location to see new plus growth on the distal whole surface, early in the year, is the post rostrum of the posterior region (Fig 22). It becomes visible on the rostrum and antirostrum of the dorsal end of the whole otolith later in the growing season for juvenile fish. On the burnt section, plus growth will be visible on the dorsal tip before the ventral tip. It is important to trace the last visible annulus on the section out to the dorsal tip to properly assess the extent of plus growth. Plus growth shows up sooner and in greater amounts at the dorsal tip area than in the sulcus region (Fig 23).

Figure 22. Photo of a 5 year old offshore hake otolith, surface view, caught in June. New plus/opaque growth is visible on the otolith margin only on the post-rostrum (arrow) at this time of the year.

Figure 23. Photos of offshore hake otolith burnt sections showing the amount and location of edge growth visible on the margin in a) June age 5 and b) September age 5. Note that plus growth is only visible on the dorsal side tip of the sections in a) and both sides later in the year b).

2.5.4.2. Pattern characteristics

The typical Pacific hake otolith has three relatively large first years of growth. This is followed by an intermediate sized fourth year (age of maturity) and subsequent annual zones that are much reduced in size that generally become progressively smaller each year. This is observed both in cross-section and the whole distal surface (Fig. 24).

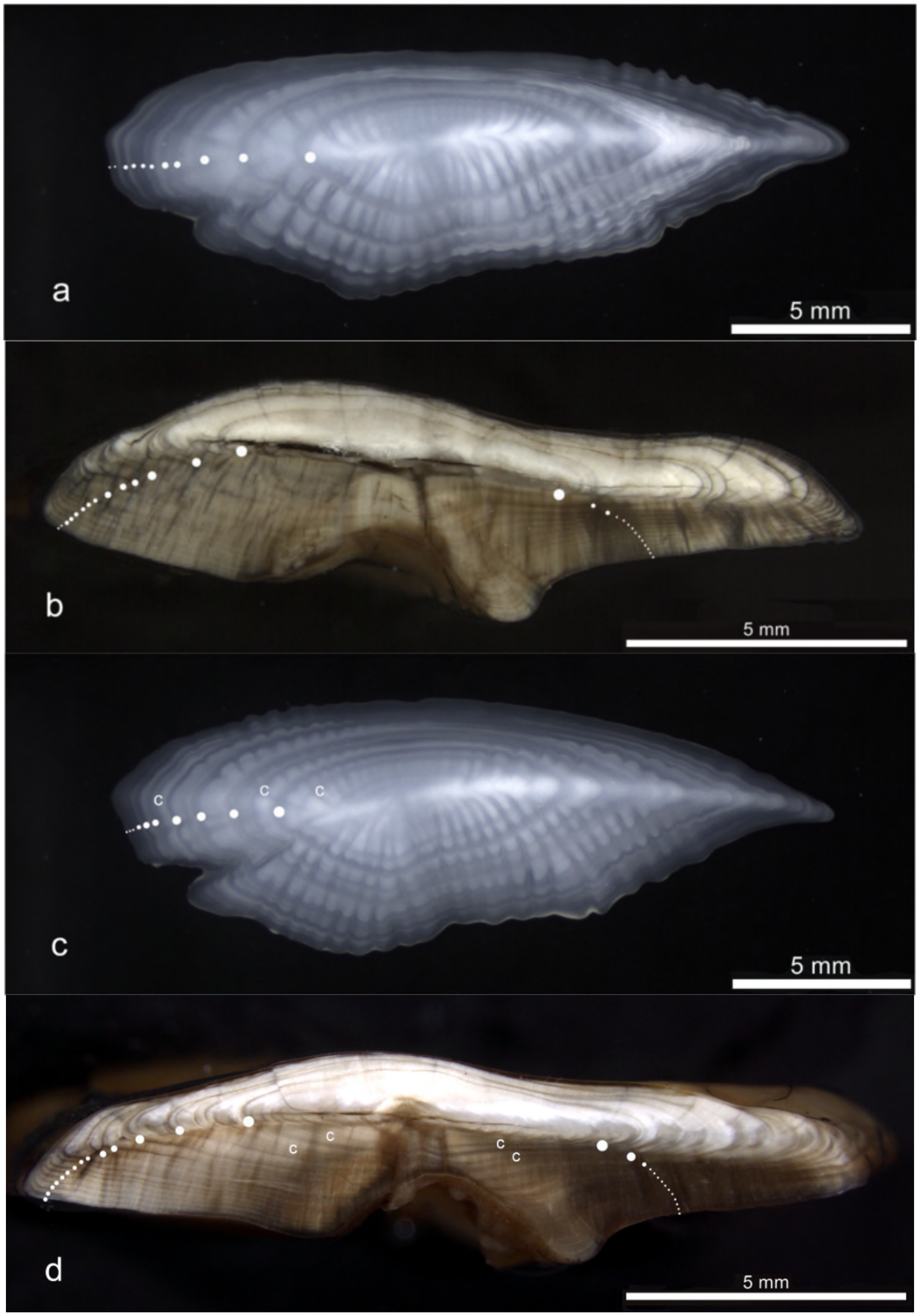


Figure 24. Photos of the otolith distal surfaces and subsequent burnt sections from two different 14 year old offshore hake (a/b and c/d) showing typical growth patterns. Annuli (dots) and checks (c) are indicated. Note how it becomes difficult to discern annuli past age 8-10 on the distal surfaces (a and c). The older annuli (>8) are easily seen in cross-section on the dorsal and ventral sides (b and d).

2.5.4.2.1. Juvenile annual zone criteria (years 1-3)

2.5.S4.2.1.1. Shape and size

Juvenile growth zones on Pacific hake otolith sections are distinctive in shape (Fig. 25a), particularly on the ventral side. Overall, the first annual zone is typically shaped like a compressed oval. The second and third annuli usually form a subtle "hook" at the ventral tip. Sometimes the first annulus, particularly if somewhat large, will also form a bit of a hook as well. Hake otoliths have only three large juvenile years of growth. If there appears to be less/more and/or they are atypical in shape the reader should check to see if the otolith was broken through the preferred plane. Otolith breaks somewhat to the side of the preferred break line result in the first years appearing small and mis-shaped (Fig. 25b). If the break is way off line the first year could be missed altogether and then the first year would appear extra-large as it is actually the second annulus.

Even though the first year on an offshore hake otolith is quite large, under-ageing can occur if a break is too far off-plane. The resulting smaller first year could be mistaken for the nucleus check and go uncounted. As SOG otoliths tend to have a smaller first year, an off-plane break has even more impact. The reader needs to develop a strong sense of the size of the first three annual zones for each stock. To learn juvenile growth pattern characteristics it is helpful to review both the surface and section growth patterns of many 0-4 year old hake otoliths from both stocks.

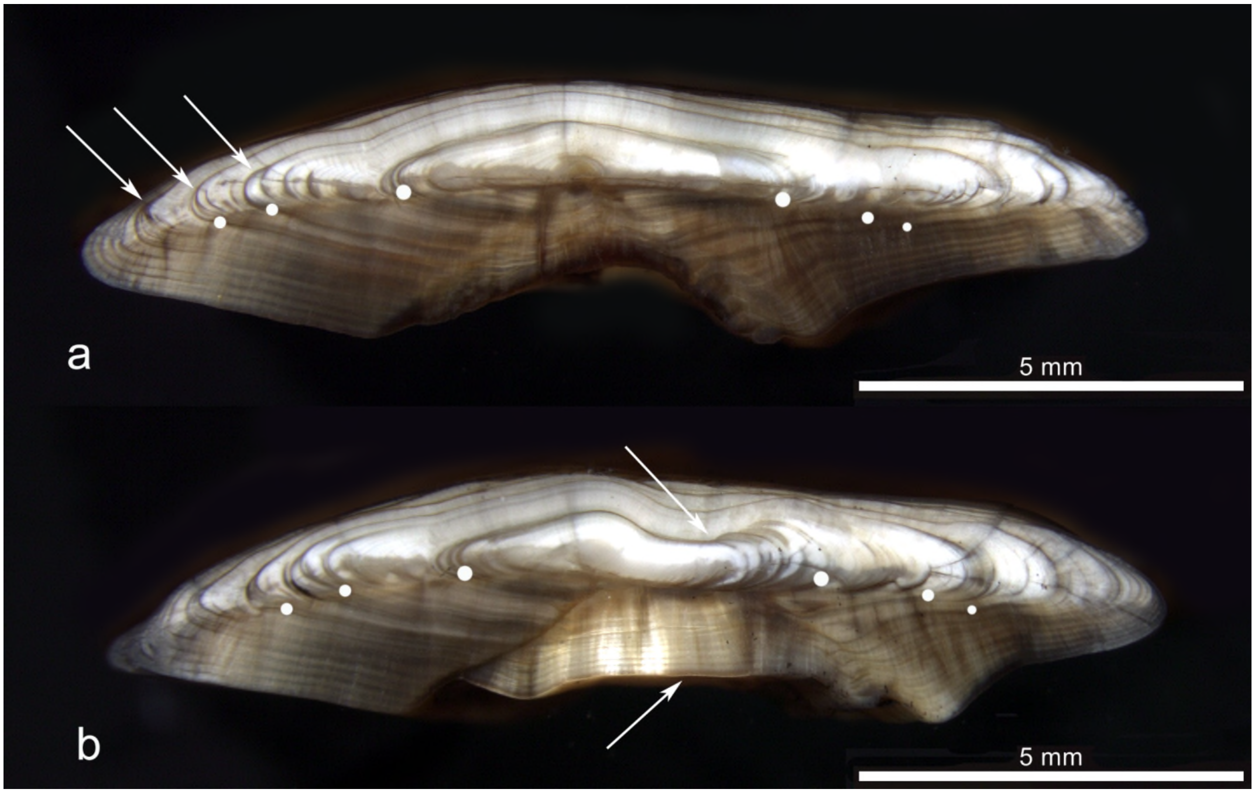


Figure 25. Photos of burnt sections from the same 10 year old offshore hake otolith. Note how size and shape of annual zones, especially the 1st 3 juvenile years (dots) change from a to b when the section plane is cut to one side of the preferred break plane. a) Typical section pattern when broken through the correct plane. The first annual zone is shaped like a compressed oval. Note the slightly hooked tip formed by the 2nd and 3rd annuli on the ventral side (arrows). b) Same otolith broken further away from the preferred plane. There are distinctive changes in the shape and size of the 1st year of growth and the sulcus area (arrows) of the cross-section.

The growth pattern on the surface is a good tool to help age the burnt section, especially to identify juvenile years. Aside from keeping juvenile otoliths on hand for reference, marked probes or the serrations on forceps tips can be used as crude measuring tools when estimating the size of the first year of growth on burnt sections or surfaces (Fig. 26) under the microscope. These tools can be used at any magnification.

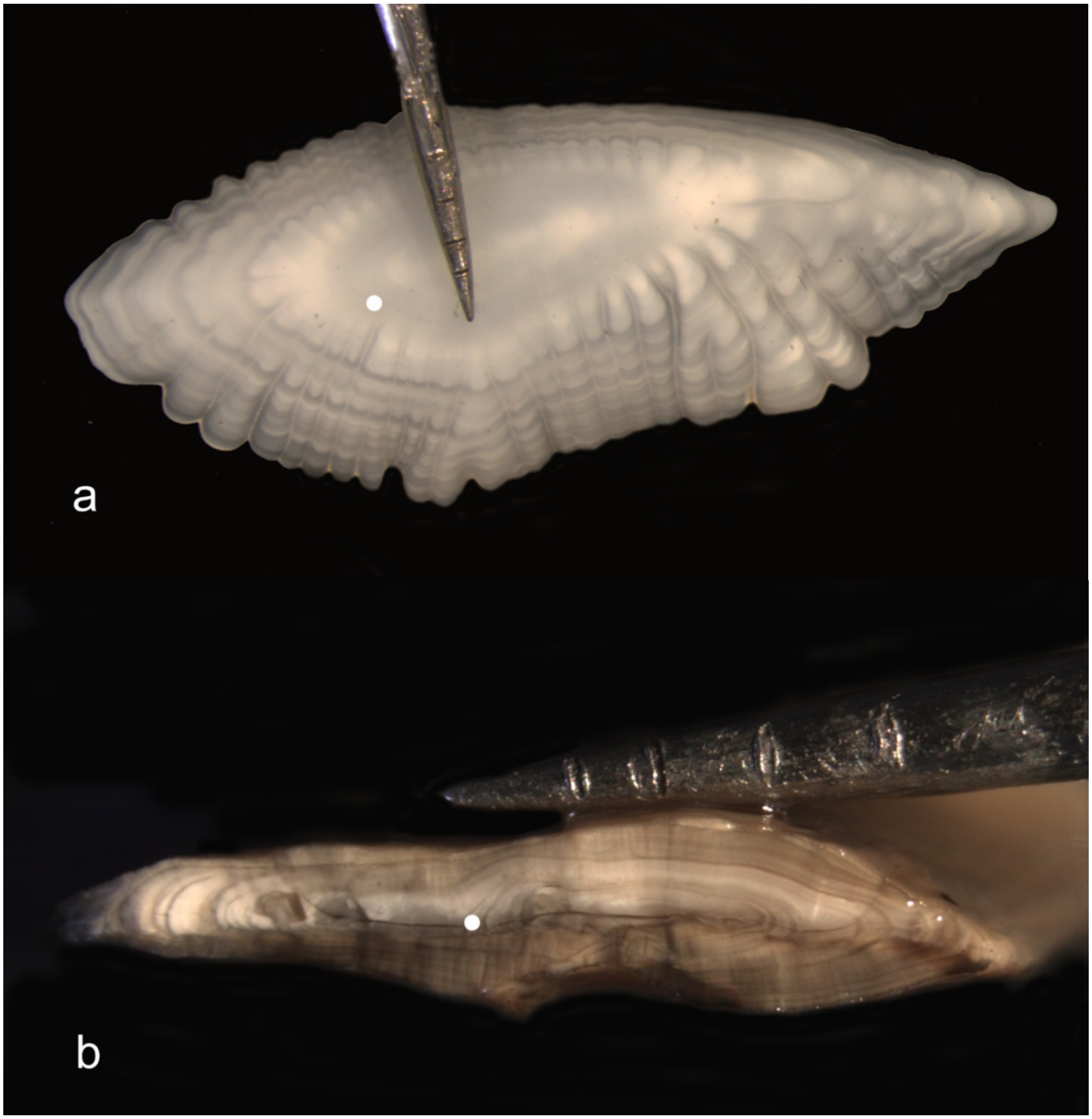


Figure 26. Photo of the same a) a whole and b) a broken and burnt offshore hake otolith showing how to use a marked probe to estimate size/width of annuli. The width of the sectioned first annual zone should measure approximately the same on both views. Magnification does not factor into this process.

The size of the first year of growth on most SOG hake otoliths is visibly smaller compared to that of an offshore otolith (Fig. 27). Historically the SCL used samples of juvenile hake (ages 1 to 3), along with fish lengths, to determine pattern shape and annual growth size criteria for both offshore and SOG stock otoliths. If the stock origin is not known the first annulus of a SOG otoliths can be mistaken for a prominent nucleus seen on most offshore otoliths and may be discounted causing under-ageing. This underlines the importance of knowing which stock is being aged in order to provide the most accurate age data possible.

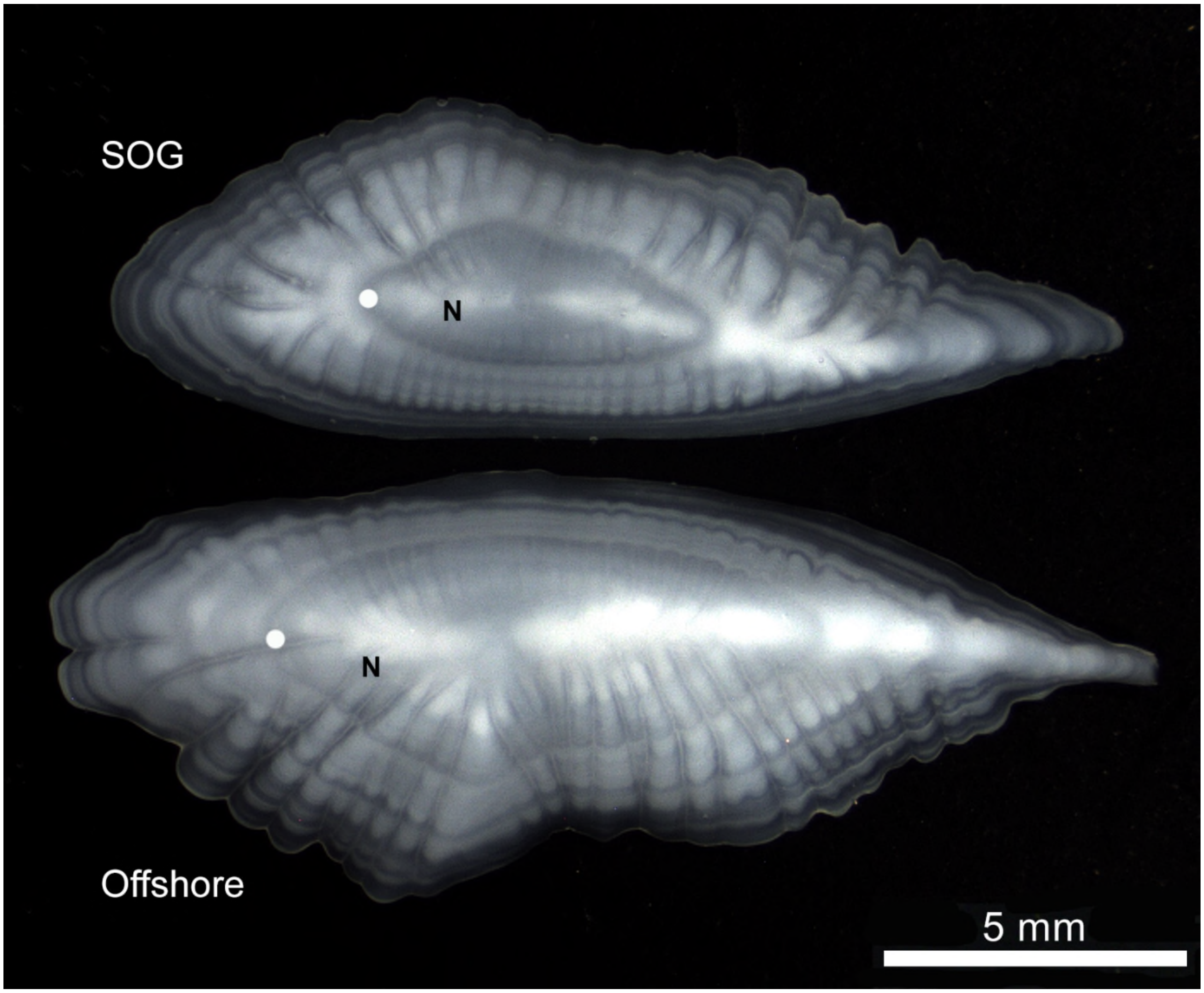


Figure 27. Photo of otoliths from a 6 year old SOG (top) and a 6 year old offshore (bottom) hake. Offshore otoliths tend to be larger at age and therefore have a relatively larger first annual growth zone. (dot). Note the nucleus (N) on each and how the size of the first annulus on the SOG otolith is similar to the size of the offshore otolith's nucleus.

Sometimes juvenile annuli may appear vague or indistinct (Fig. 28) and be missed, resulting in under-ageing. It is important to use the shape and size criteria of the first three years to determine if this is the case. A vague first annulus will cause the first annual zone to appear extra-large (e.g. size of the 2nd year) and the pattern will look to have only 2, rather than 3, large first years. Also, the apparent first annulus will have the significant ventral tip hook of the second annulus. In some cases the first annulus may be easier to identify on the otolith surface. The reader can check size with the probe or forceps on the surface to decide if a 1st annulus may have gone uncounted (Fig. 26).

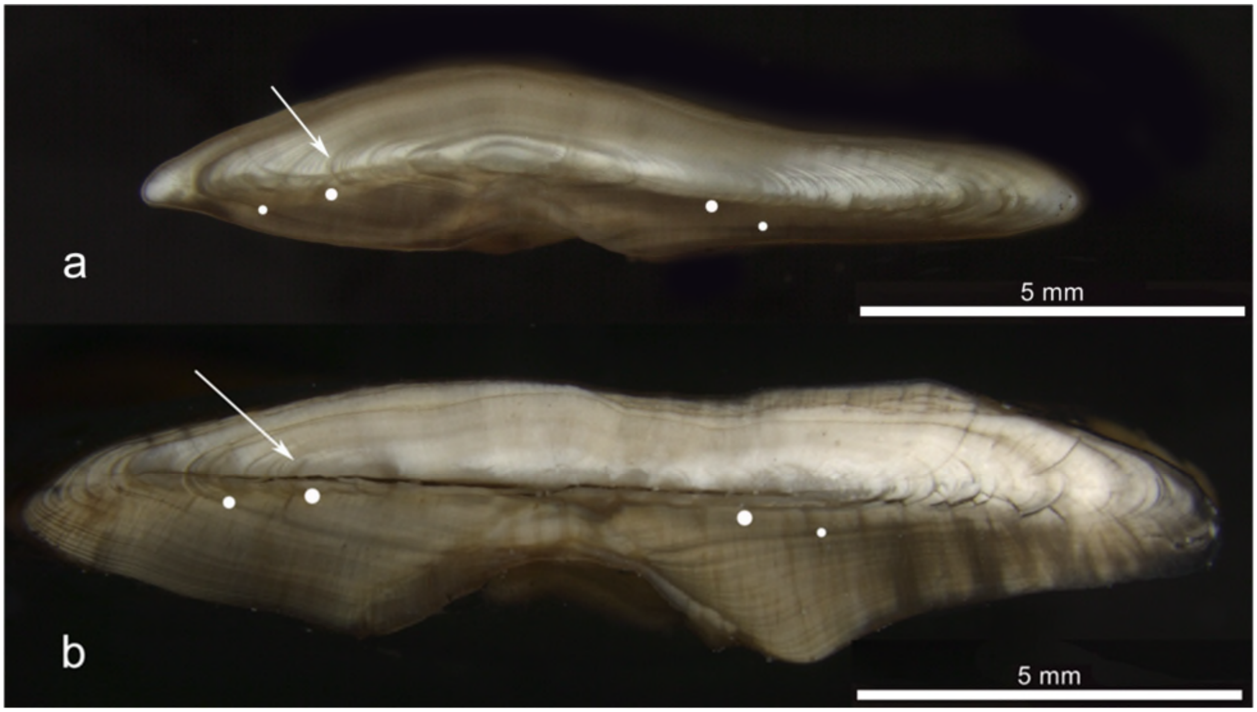


Figure 28. Photos of a young a) and an older b) offshore hake otolith burnt section taken at the same magnification with vague 1st (arrow) and clear 2nd annuli (dots). Note that the first prominent appearing annulus (actually the 2nd) appears too large for the 1st and/or has a hook on the ventral tip.

2.5.4.2.1.2. Checks

In general, checks are: 1) less prominent than annuli, 2) merge with annuli and/or 3) are not continuous along preferred counting axes (Fig. 29). Checks are difficult to differentiate when interpreting juvenile growth because they appear more conspicuous than when formed in mature years. They tend to be most noticeable along the longer, less compressed, growth axes and the ventral side of the burnt otolith section.

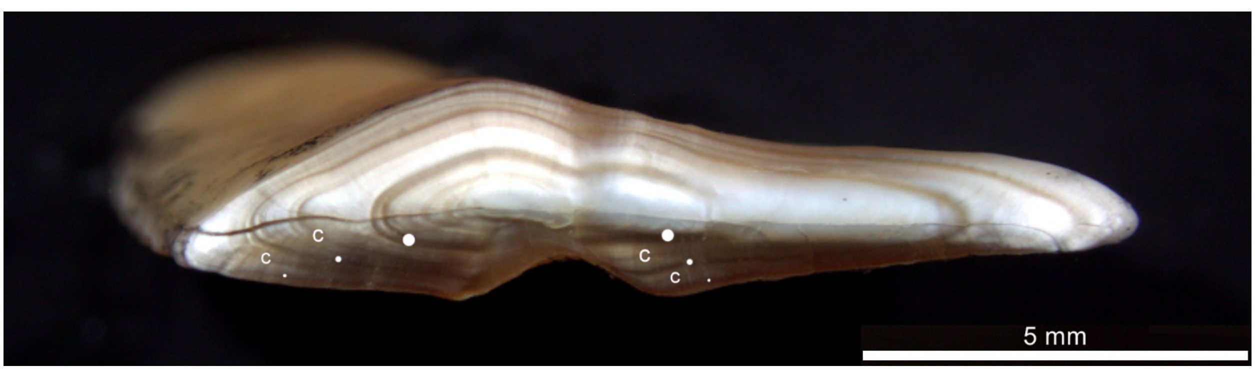


Figure 29. Examples of prominent checks on a young offshore hake otolith burnt section. They generally are less prominent than annuli and may merge with them or become discontinuous. Annuli (dots) and checks (c) are indicated.

A check, referred to as the nucleus, may be visible inside the first annulus (Fig. 30). It can be as prominent as the first annulus. In cross-section it also is often shaped like a compressed oval, like the first annulus, but is normally about two thirds or less the size. Confirm size with a marked probe and verify if the otolith was broken on the preferred plane to identify this check. Also, if it appears as though there are 4, rather than 3, large first years of growth a prominent nucleus may be present. Be sure to use the surface pattern to help make this decision.

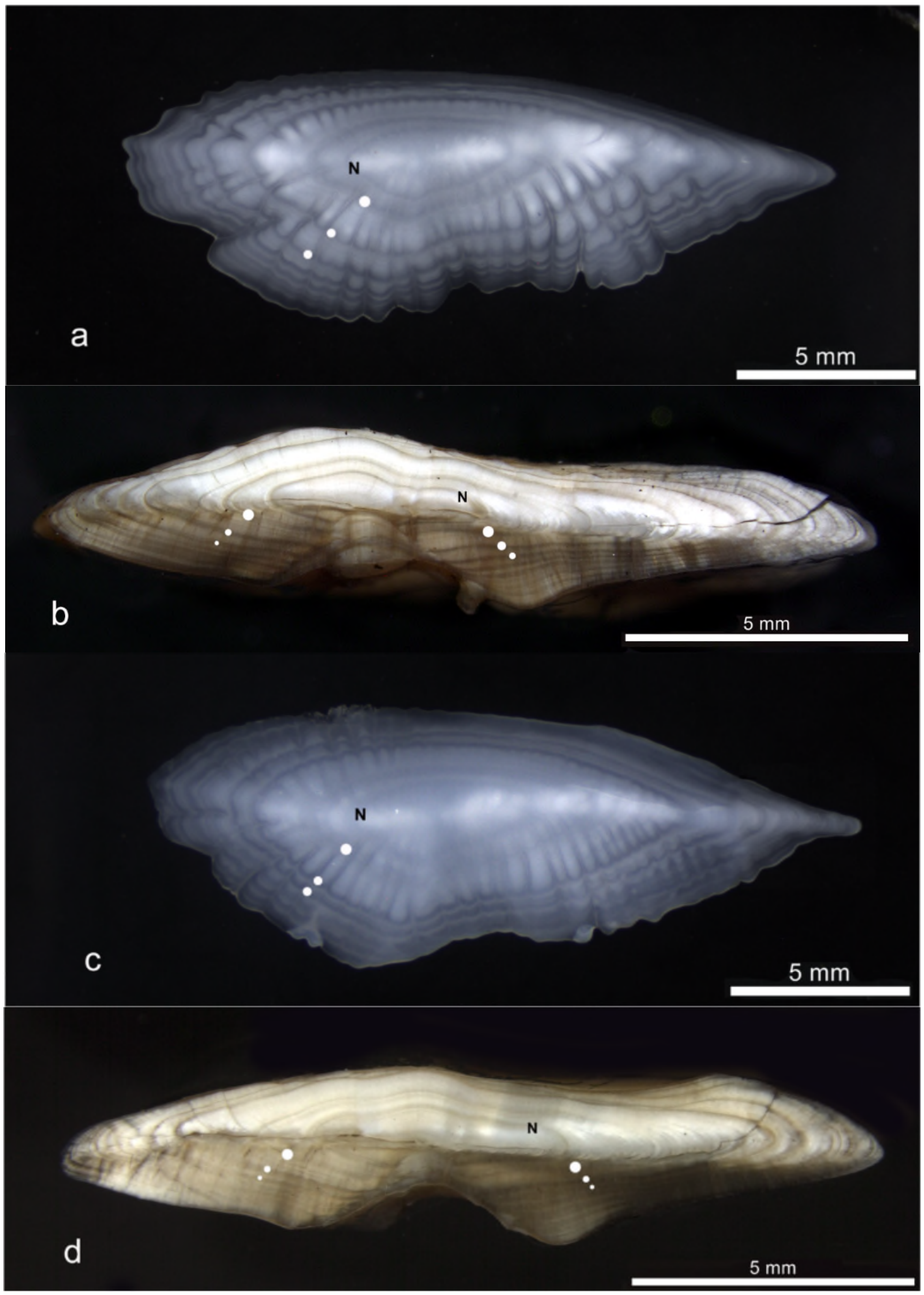


Figure 30. Photos of paired (a/b and c/d) surface and burnt section offshore otoliths from 2 different hake. The first 3 annuli are marked with dots. Both examples show a prominent nucleus check (N) visible on the distal surface (a, c) as well as the burnt section (b, d).

2.5.4.2.2. Mature annual zone criteria

2.5.4.2.2.1. Shape and size

Most hake mature during their 4th year of life which produces an intermediate sized annual zone on their otoliths that is sandwiched between the large 3rd and the much reduced 5th years. In cross-section, the fourth annual zone generally does not form much overburden on the distal surface of the otolith. Though, there is usually some small amount visible on the ventral and dorsal tips of the section (Fig. 31). Beyond the 4th year, annual growth zones form little to no overburden at either the ventral or dorsal tips of the section. Exceptions do exist (Fig. 31b, c, d). Mature annual zones generally become progressively smaller and narrower being visible only on the proximal portions of the section. Normally SCL readers count all prominent dark zones (annuli) seen on burnt sections past age 4.

2.5.4.2.2.2. Checks

After age four checks forming in mature annual growth zones tend to be less obvious because of reduced size and compaction in later years. If present they are usually found along longer faster-growth axes. Checks tend to be more of an issue for the ventral side of the section which has larger growth zones relative to the dorsal side. They are usually less prominent and are discontinuous or merge with annuli when traced from the section tips into the sulcus area (Fig. 32).

2.5.4.2.2.3. Colour

Sometimes older annual zones are hard to distinguish on the proximal sides of burnt otolith sections because the small compacted summer and winter zones may burn to an overall brown colour, especially if not burnt enough. However, annual zones can be distinguished by paying close attention to slight variations or shades of brown which demark each annual zone (Fig. 33). On close inspection, annuli will always be slightly darker. The otolith section can be further burnt to a grey-brown colour to help differentiate the summer and winter zones better, especially on older otoliths. The annuli will look more discrete. The ventral side of the otolith section is sometimes a better axis to count older mature zones (teens) as the annual growth is less compacted (wider) than the dorsal side making it easier to see.

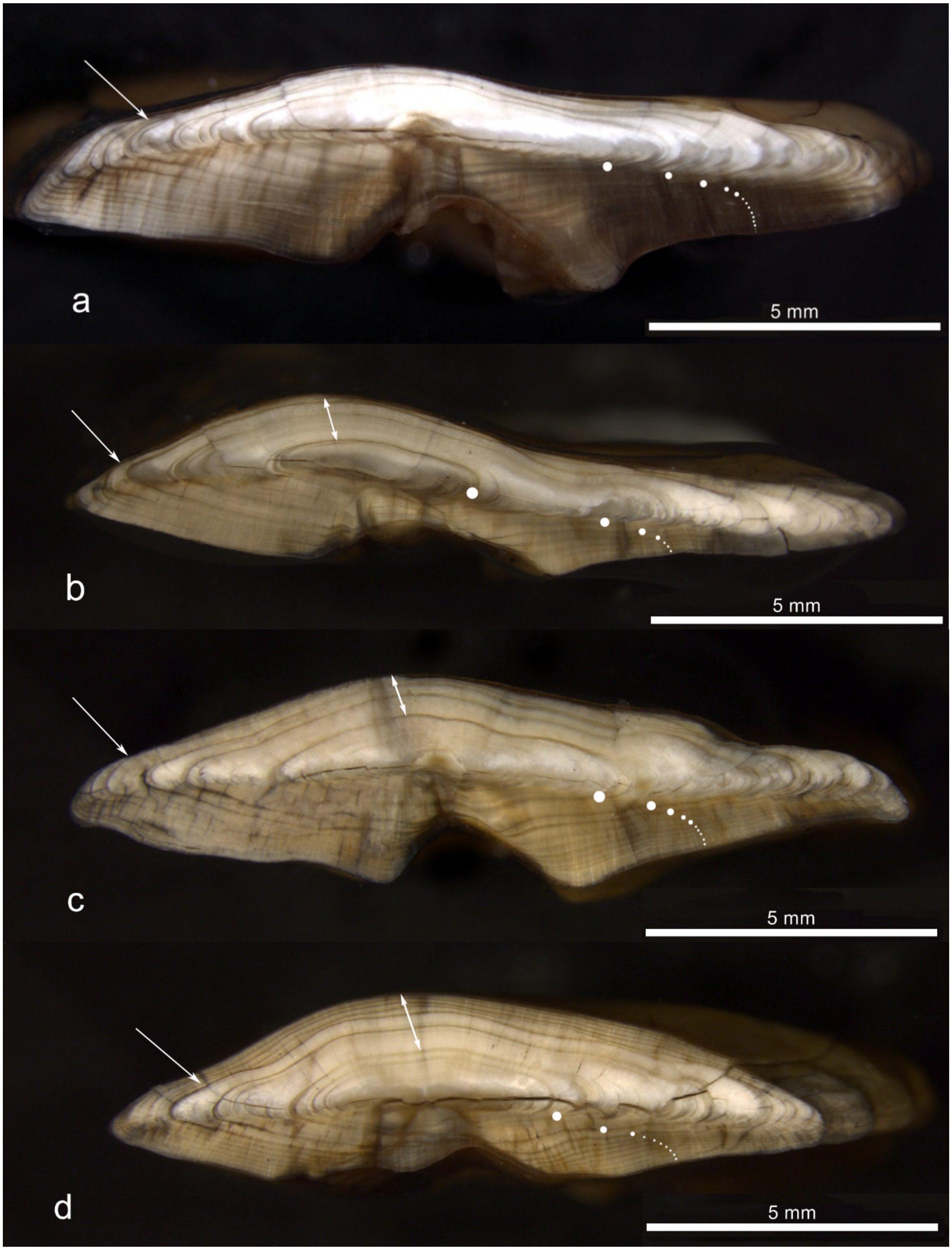


Figure 31. Photos of burnt offshore otolith sections from 4 different mature hake with differing amounts of overburden deposited in the 4th (arrows) and subsequent years on the ventral side. Annuli (dots) are plotted along the preferred counting axes. The typical growth pattern is illustrated in a) showing overburden much reduced in the 4th year on the ventral side of the section with only 3 years visible on the distal axis. The last three images show atypical overburden (double headed arrow) in the distal area of the otolith section with more than 3 years visible. a) Age 14 b) Age 7 c) Age 10 d) Age 10.

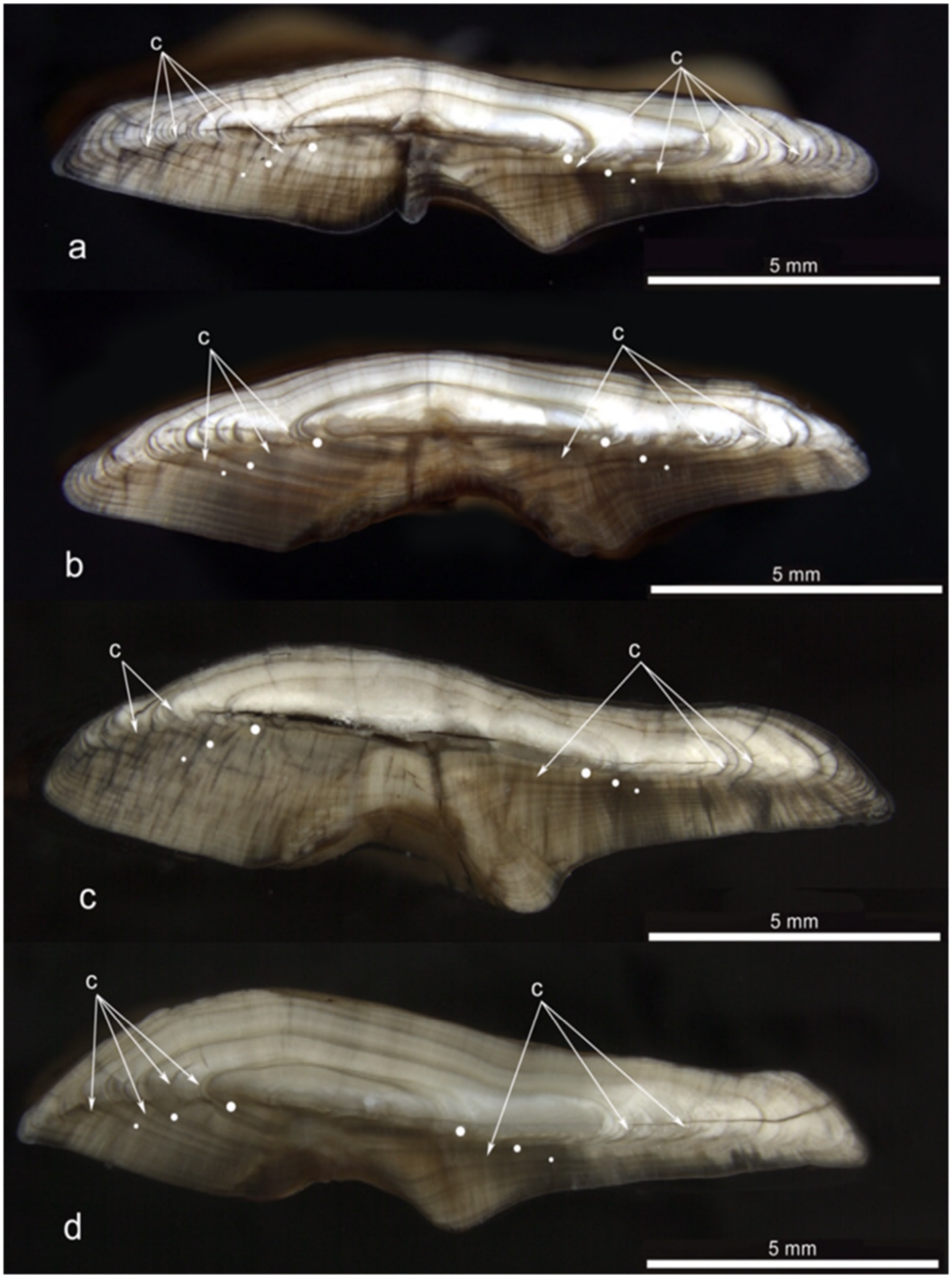


Figure 32. Photos of older burnt section offshore otoliths showing checks (c) that merge with annuli (dots) or are discontinuous. a) Age 10 b) Age 10 c) Age 14 d) Age 7.

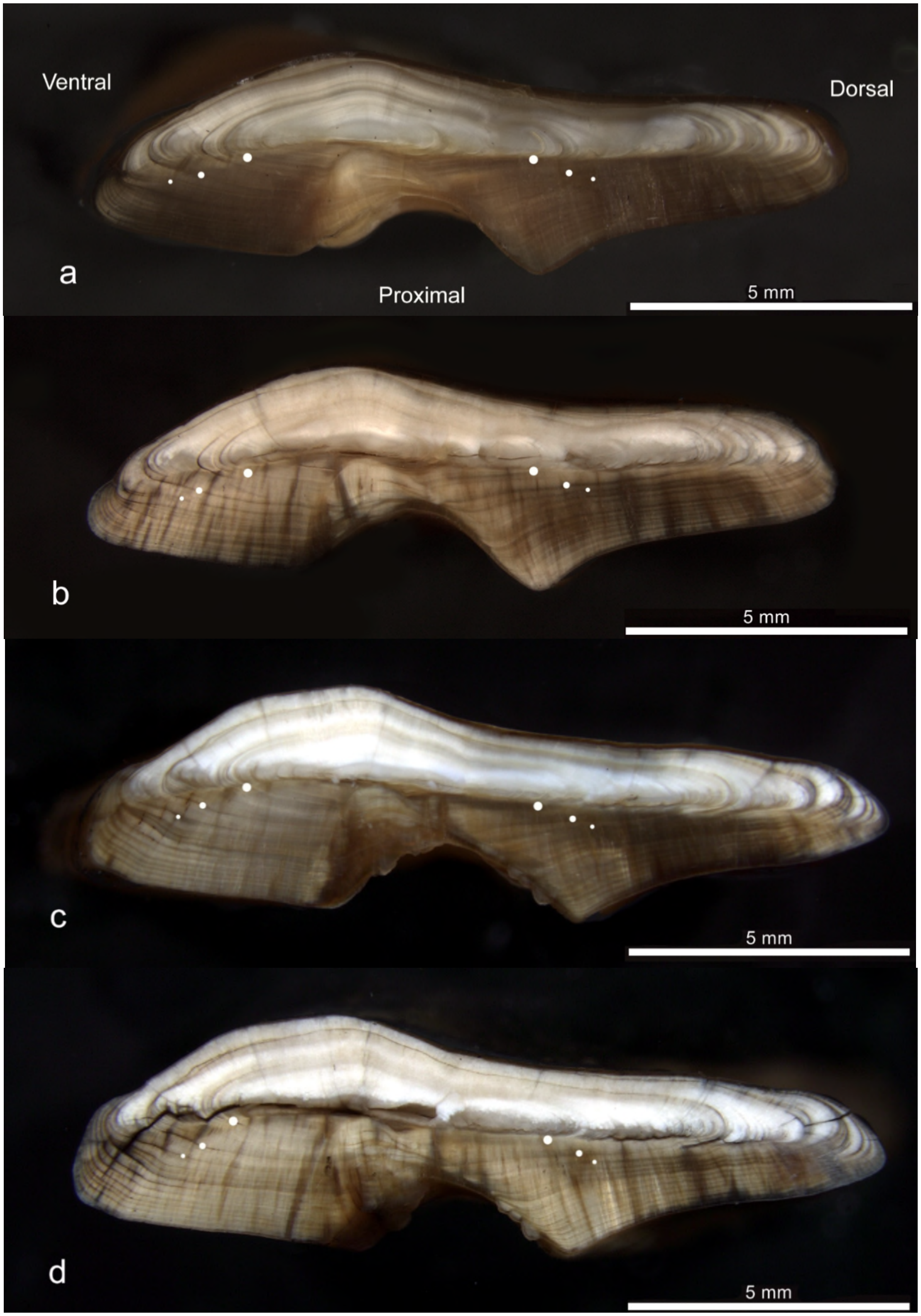


Figure 33. Photos of burnt otolith sections from two different offshore hake. Each pair (a/b and c/d) shows how further burning of sections to an ash-brown colour helps to differentiate the mature annual zones, especially in the more compact proximal growth zones. a) and c) have been burnt lightly to shades of brown while b) and d) were burnt longer to ash-brown.

2.5.4.2.3. Edge/plus growth criteria

Edge or plus growth are terms used to indicate incomplete annual growth present on the margin or edge of an otolith surface or section. Normally, SCL readers are referring to opaque summer growth with no visible annulus. But, late in the year this may include an incomplete annulus forming on the margin. The size of the opaque zone on B.C. caught hake otoliths increases throughout the growing season (May/June to August) to be followed by the formation of a translucent zone (annulus) during the slower growing months, September through May/June (Fig. 23). Age class should be assigned based on the greatest extent and type of growth that can be identified as the last annual zone forming in the catch year. It is important to note that otoliths from juvenile hake generally tend to start and finish forming opaque growth on their otoliths sooner and later in the year than most adult fish as they are not channelling energy into reproduction.

It is a challenge to correctly place the last growth zone on an otolith's margin into the correct calendar year. The question is; does it belong to the year the fish was caught or the previous year? Catch date is very important in making this decision. Interpretation is based on identifying the type (summer, winter, check) and amount of growth and then relating that to the time of year the fish was caught with respect to a Jan. 1st birthdate. Fairly complete looking amounts of opaque plus growth on the burnt section margin of spring caught fish should be attributed to the previous calendar year. When the same is observed in the late summer or fall it should be attributed to the catch year. In the latter case, even though annual growth looks fairly complete, the fish is not considered to be a year older until it has crossed the next January 1st birthdate (Fig 9). SCL readers often identify an annulus forming on hake otolith burnt sections as early as September, but discount the annulus as incomplete. If it were counted, over-ageing by one year would occur.

Late June-early July is a difficult time to assign age class for older mature hake otoliths as even small amounts of plus growth may appear close to the size of the previous year's growth. If edge growth is not interpreted correctly older fish may be under/over-aged by one year. July caught fish are particularly difficult to assign an age to as it seems as though the new year's growth appears all at once during the month of July. In these cases it becomes very important to pay close attention to the exact catch day to determine which calendar year plus growth should be attributed. Sometimes it helps to have reference samples caught before and after July to establish the progression of plus growth on mature hake otoliths.

In summary, the SCL finds the ageing of Pacific hake otoliths to be fairly straight forward. The criteria described above have generally been found to produce good quality age data that has allowed data users to follow large year classes successfully over the last 45 years. It should be noted, though, that slight variations in otolith annual growth pattern (e.g. size at age, pattern clarity) have been observed over time connected to climate and ocean environment (Beamish et al 2004). It is therefore important that the SCL be diligent with ongoing monitoring, assessment, documentation and updating of criteria used to age this species.

ACKNOWLEDGEMENTS

We would like to thank Dr. R.J. Beamish for reviewing this document and providing valuable comments and recommendations.

GLOSSARY

Accuracy – measures closeness to true age

Age class – estimated age

Annulus/Annuli – translucent “winter” zone(s) that forms on otoliths once a year during the slower growing months (fall through to early spring in Northern Hemisphere)

Annual growth zone – consists of a summer zone followed by a winter zone

Anterior – towards the nose

Antirostrum – small projection on otolith anterior-dorsal

Biological birthdate – actual time of birth

Break and burn technique – method used to prepare otoliths for age determination where the otoliths are broken in half and the growth zones of the cross-section surface are burnt to turn the annuli dark (brown)

Capacity – amount of fish that can be aged by the program

Check(s) – similar growth zone to an annulus, also translucent but may form more than once a year and forms within the fast-growing summer zone

Confidence index – indicators of certainty associated with individual age estimates

Counting axis/axes – location and direction to count annuli on otoliths

Cohort – fish born in the same calendar year

Cross-sectioned otolith – otolith that has been broken or cut through its transverse axis

Crystallized otolith – vaterite otolith where the calcium carbonate has formed differently than normal

Dissecting microscope – a microscope that generally uses reflected light to view 3-dimensional objects at lower magnifications (1-50X)

Distal surface/axis – the non-sulcus side of an otolith (opposite to proximal)

Dorsal side – side of otolith that is oriented towards the dorsal (top) fin of the fish (opposite to ventral side)

Dorso-ventral – transverse line or axis along which an otolith is broken in cross-section through the first year of growth

Dorsal tip – most dorsal end of the dorsal side of a cross-sectioned otolith

Edge growth – see plus growth

Even break – produced when an otolith is broken in half resulting in a plane that is fairly smooth

Eyepiece – ocular lens on a microscope closest to the eye

Expert level reader – reader that consistently ages fish with a high level of precision

First read rate – number of fish per hour aged by the first reader of a sample

First reader – person that reads otolith sample first

First reading – age produced by the first reader

Groundfish – finfish that live at or near the sea bottom

Growth axes – direction that growth formed on an otolith over time

January 1st birthdate – internationally accepted birthdate for fish created to group fish born in the same calendar year and age class regardless of their biological birthdate

Juvenile annuli/growth zone(s) – early growth zones formed on otoliths during the years of a fish's life when it was not reproductive

Margin – outer edges of an otolith where growth is added

Mature annuli/growth zone(s) - later growth zones formed on otoliths during the years of a fish's life when it was reproductive

Moderate to long-lived species – species that lives up to 20 or more years

Natural tag – a distinctive and specific annual zone that can be identified that was formed through natural sources (e.g. lack of food)

New growth – see edge/plus growth

Nucleus – translucent zone (check) that forms within the first year of opaque growth on an otolith

Objective – lens on a microscope closest to the stage

Offshore stock – Pacific hake stock that spawns off of southern California and migrates north to feed off of B.C. in the summer

Otolith(s) – non-skeletal calcium carbonate bone that forms in a boney fish's middle ear

Opaque zone(s) – white growth zone on an otolith that does not allow the passage of light

Overburden – sequential layers of overlapping annual growth deposited on the distal side of an otolith

Otic tissue – middle ear membrane that envelops the otolith

Plus growth – incomplete growth forming on the margin/edge of an otolith when the fish was caught

Post rostrum – rounded posterior end of an otolith closest to the posterior (tail) of the fish

Posterior – towards the tail

Precision – measures the repeatability of age assessments, i.e. consistency

Precision test – independent reading of an otolith sample where the reader does not have access to previously generated age estimates

Preferred axes – directions to count annuli that will provide the most consistent age estimates

Production method – age determination method used to mass produce age data for species on a regular basis (i.e. annual, biennial)

Production rate – number of fish aged per hour by a reader using a production method

Productivity – rate at which a reader produces age estimates

Proximal – sulcus side of an otolith (opposite to distal)

Quality assurance – program of practices (standards, procedures, policies) to ensure that age data is of best quality

Quality control – specific procedures to ensure age data quality

Reader drift – when a fish ager(s) purposely/unintentionally applies ageing criteria differently over time resulting in age data quality and continuity issues

Rostrum – pointed anterior end of an otolith closest to the anterior (nose) of the fish

Sagittal otolith(s) – largest pair of otoliths for most fish

Sclerochronology – the study of how time is recorded in the hard tissues of aquatic organisms

Strait of Georgia stock – stock of Pacific hake that are resident to the St. of Georgia in B.C.

Sulcus – auditory nerve groove on the proximal side of otoliths

Summer zone(s) – see opaque zone

Translucent zone – see annulus

Transverse – plane at right angles to the anterior-posterior axis of an otolith

Thin cross-section(s) - slivers of otoliths that are cut from an otolith and fastened to glass slides to view the growth pattern

Vague annuli – annuli that are not prominent and are difficult to discern

Validation – study that proves that an age determination method is accurate for all age classes of a species or stock

Ventral side – side of otolith that is oriented towards the ventral (bottom) side of the fish (opposite to dorsal side)

Ventral tip - most ventral end of the ventral side of a cross-sectioned otolith

Whole otolith surface – distal side of the otolith that does not have a sulcus

Winter zone(s) – see annulus

Year class – fish that are born in the same calendar year

Zoom objective lens – microscope lens closest to the specimen that has varied rather than fixed focal lengths

REFERENCES

Beamish, R.J. 1979. Differences in the age of Pacific hake (Merluccius productus) using whole otoliths and sections of otoliths. Journal of the Fisheries Research Board of Canada. 36:141-151.

Beamish. R.J., A.J. Benson, R.M. Sweeting and C.M. Neville. 2004. Regimes and the history of the major fisheries off Canada's west coast. Prog. in Ocean. 60:355-385.

Beamish, R.J and G.A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112:735-743.

Bailey, K.M., R.C. Francis and P.R. Stevens. 1982. The life history and fishery of Pacific whiting, *Merluccius productus*. NWAFC Processed Rep. 82-03:74p.

Charles, K.D., MacLellan, S.E., and Little, D. 2013. A guide to sectioning otoliths for age determination. Can. Tech. Rep. Fish. Aquat. Sci. 3037: iv + 35 p.

Chilton, D.E and R.J. Beamish. 1982. Age determination methods for fishes studied by the Groundfish Program at the Pacific Biological Station. Can. Spec. Pub. Fish. Aquat. Sci. 60: 102 p.

Christensen, J.M. 1964. Burning of otoliths, a technique for age determination of soles and other fish. J. Cons. Int/ l'Explor. de la Mer. 29: 73-81.

Clay, S. and D. MacVicar. 2005. Indoor Air Quality at the Fish Ageing Lab Pacific Biological Station Fisheries and Oceans Nanaimo, BC. 23 p.

Dark, T.A. 1975. Age and growth of Pacific hake, *Merluccius productus*. Fisher bulletin, U.S. 73:336-355.

Hallowed, A.B. 1992. Spatial and temporal distributions of Pacific hake, *Merluccious productus*, larvae and estimates of survival during early life stages. CalCOFI Rep. 33: 100-123.

Helser, T.E. and L. Alade. 2012. A retrospective of the hake stocks off the Atlantic and Pacific coasts of the United States: Uncertainties and challenges facing assessment and management in a complex environment. Fish. Res. 114:2-18.

International North Pacific Fisheries Commission. 1958. Proceedings of the annual meeting 1957. p. 73.

Kabata, Z. and D.J. Whitaker. 1981. Two species of Kudoa (Myxosporea: Multivalvulida) parasitic in the flesh of *Merluccius productus* (Ayres 1855) (Pisces: Teleostei) in the Canadian Pacific. Can. J. Zool. 59: 2085-2091.

MacLellan, S. E. 1997. How to age rockfish (*Sebastes*) using *S. alutus* as an example - the otolith burnt section technique. 1997. Can. Tech. Rep. Fish. Aquat. Sci.2146: 39p.

MacLellan, S.E. and J. Fargo. 1995. Validation of age and growth for English sole (*Parophrys vetulus)* in Hecate Strait, British Columbia. In Recent Developments in Fish Otolith Research. Eds D.H Secor, J.M. Dean and S.E. Campana. University of South Carolina Press. P341-360.

MacLellan, S.E. and M.W. Saunders. 1995. A natural tag on the otoliths of Pacific hake (*Merluccius productus*) with implications for age validation and migration. In Recent Developments in Fish Otolith Research. Eds D.H Secor, J.M. Dean and S.E. Campana. University of South Carolina Press. p567-580.

McFarlane, G.A and R.J. Beamish. 1985. Biology and fishery of Pacific whiting, Merluccius productus, in the Strait of Georgia. Mar. Fish. Rev. 47(2):23-34.

Smith, D.B., G.A. McFarlane, and M.W. Saunders. 1990. Variation in Pacific hake (*Merluccius productus*) summer length-at-age near southern Vancouver Island and its relationship to fishing and oceanography. Can. J. Fish Aquat. Sci 49:1-14.