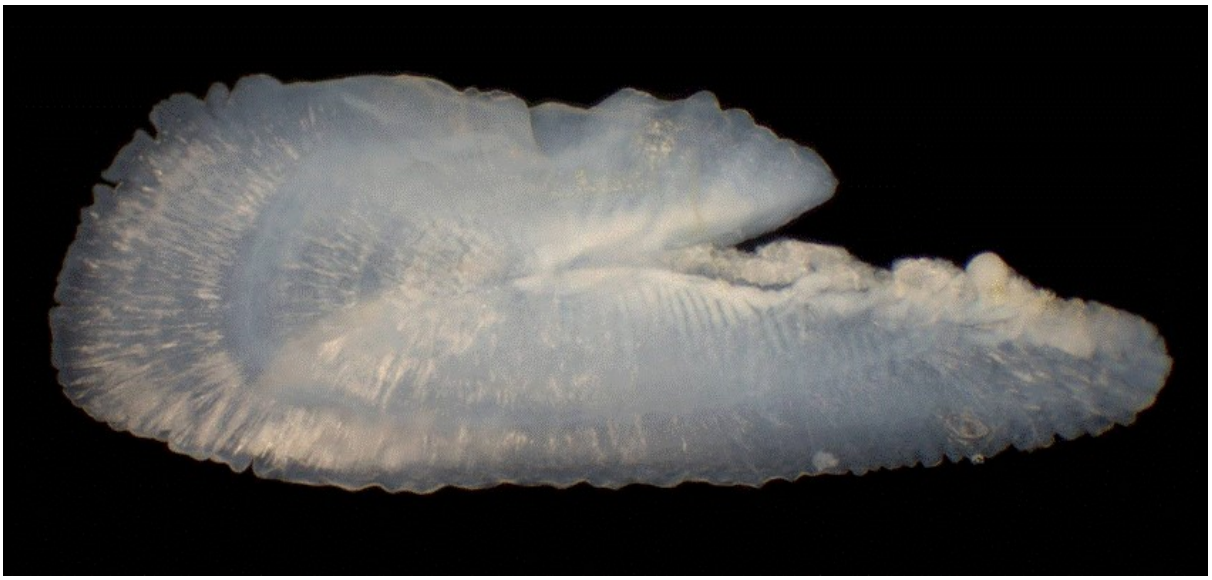
Ageing Protocol

Grey Mackerel (*Scomberomorus semifasciatus*)



Queensland Government

This publication has been compiled by Fishery Monitoring within Fisheries Queensland, Department of Agriculture and Fisheries. This protocol has been contributed to by many staff, in particular, Michelle Stewart, Wayne Hagedoorn, Sue Helmke, Olivia Whybird and Stuart Hyland. James Cook University (on behalf of Welch et al, 2009 and Cameron and Begg, 2002) supplied otoliths and corresponding data for our original reference collection.

The Department of Agriculture and Fisheries proudly acknowledges all First Nations peoples (Aboriginal peoples and Torres Strait Islanders) and the Traditional Owners and Custodians of the country on which we live and work. We acknowledge their continuing connection to land, waters and culture and commit to ongoing reconciliation. We pay our respect to their Elders past, present and emerging.

Cover; Whole grey mackerel (*Scomberomorus semifasciatus*) otolith viewed with stereomicroscope using reflected light.

Contents

Acronyms

FM	Fishery Monitoring, a work unit of Fisheries Queensland within the Queensland Department Agriculture and Fisheries
DAF	Queensland Department of Agriculture and Fisheries
IAPE	Index of average percent error
LAS	Leica Application Suite

Purpose

Fishery Monitoring, part of the Department of Agriculture and Fisheries, follows a standardised approach for routinely estimating fish age using otoliths (Fisheries Queensland, 2018). This document contains species-specific information to assist:

- the interpretation of the incremental macrostructure of east coast grey mackerel otoliths (*Scomberomorus semifasciatus*),
- the criteria for achieving competency for reading of grey mackerel otoliths,
- the estimation of grey mackerel age using otolith-derived data.

.

Preparation

The procedure of otolith removal is described in the sampling protocol for grey mackerel (Fisheries Queensland 2020). Otoliths are cleaned and dried with facial tissues and stored in labelled plastic vials. Annual age estimation occurs after the otoliths are sufficiently dry, either 4 weeks or longer after removal, or after being placed in an oven at 40°C for 4 to 5 days).

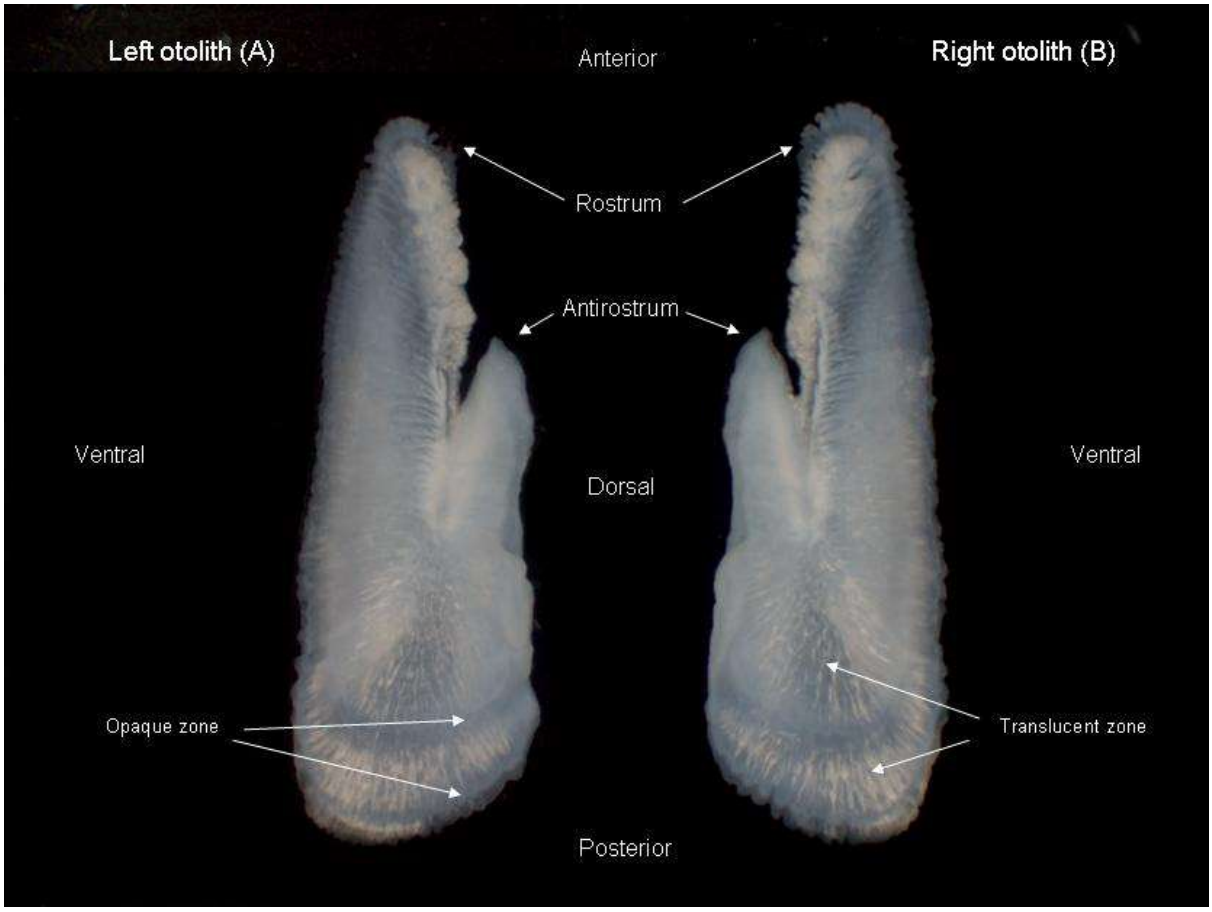


Figure 1 Distal view of left and right whole grey mackerel otoliths.

Observation

Grey mackerel otoliths are placed in a petri dish and immersed in baby oil for viewing using a microscope. Water is placed between the stage plate and bottom of the petri dish to improve the contrast between the background and otolith. After the opaque bands of the otolith are interpreted, it is removed, blotted on tissue paper and rinsed in fresh 100% ethanol. It is then dried thoroughly with tissue paper and returned to the vial. The 100% ethanol must be changed daily or refreshed when it appears cloudy.

Otolith orientation can be determined using Figure 1. The left otolith is selected and viewed distal (concave) side up with reflected light and a black background (Figure 2). The right otolith is viewed if the left otolith is not available or is difficult to read. Otoliths are viewed stereomicroscope with magnification of 12.5X (objective 1.25x & eyepiece 10x).

From 2020, the microscope has been connected to an image analysis system (Leica Application Suite, LAS) via a high resolution video camera. When imaging via LAS, particular attention is paid to the clarity of the 1st increment and the closely spaced increments towards the margin of older fish. More than 1 image is taken if necessary. Optimal images are achieved by:

- adjusting the focus for enhanced definition of important features
- adjusting the light source for enhanced definition of opaque and translucent zones;
- rotation of the otolith to obtain a different perspective of the reading area.

Otolith interpretation is evaluated on the screen. A high resolution image is saved of each otolith for records and quality control testing.

Interpretation

Otolith Growth

Grey mackerel otoliths are elongate and laterally compressed. During the first two years of life grey mackerel exhibit rapid growth rates. However, growth in following years appears to slow dramatically. Otolith size is no indication of age (Figure 2).

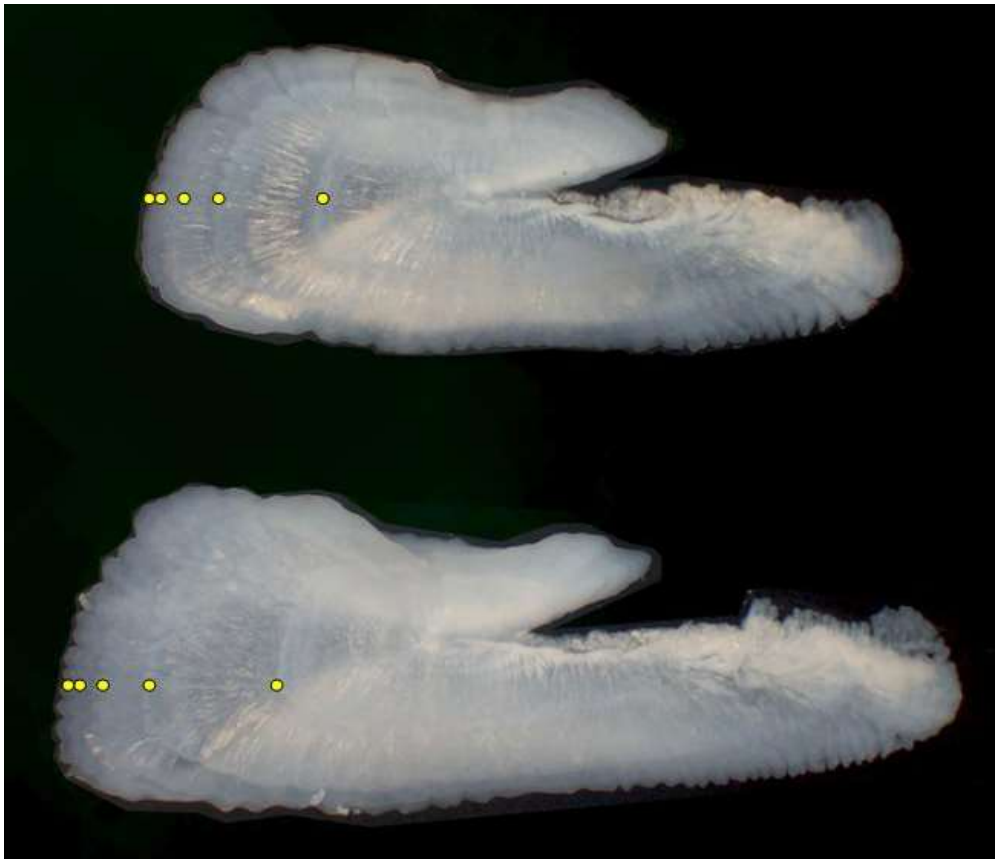


Figure 2 Distal view showing difference in size grey mackerel otoliths from two different fish, both with 5 increments. Yellow dots indicate annual increments.

Reading

Grey mackerel otoliths exhibit annual increments (herein referred to as increments) which consist of a translucent zone and an opaque zone. Grey mackerel increments are read within a defined area of the otolith called the Primary Reading Area between the primordium and the posterior margin (Figure 3). The Primary Reading Area has been chosen as it exhibits a greater distinction between the translucent and opaque zones.

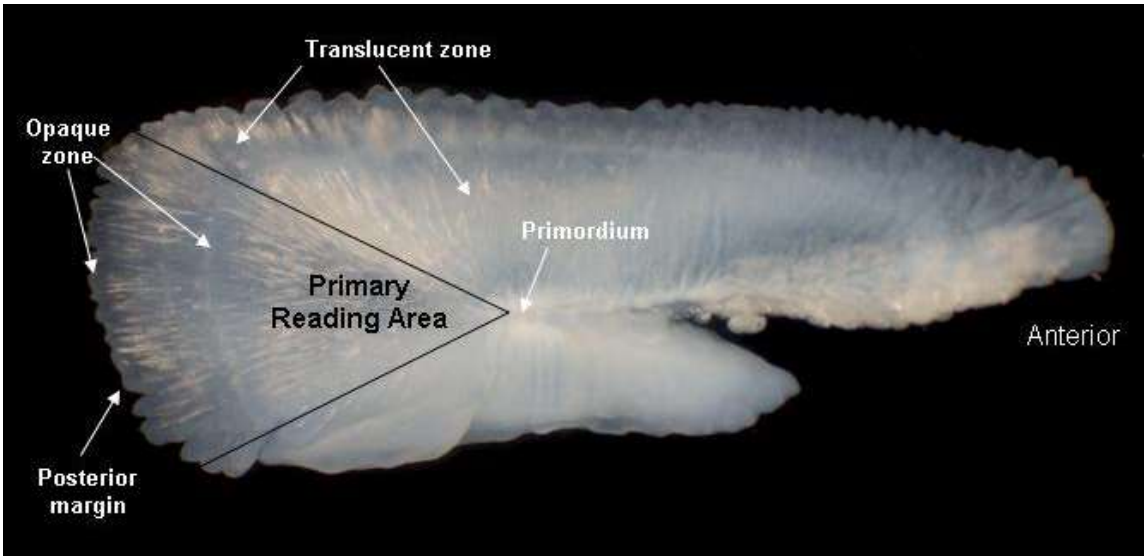


Figure 3 Distal view of whole grey mackerel otolith illustrating the Primary Reading Area.

The final decision on increment count and edge type must be made within the Primary Reading Area, however all areas of the otolith can be used to support information seen inside the Primary Reading Area.
Occasionally a reader may need to refer to a Secondary Reading Area of the otolith to help confirm the position of the first opaque zone (Figure 4). The Secondary Reading Area is located between the primordium and the first formed opaque zone.

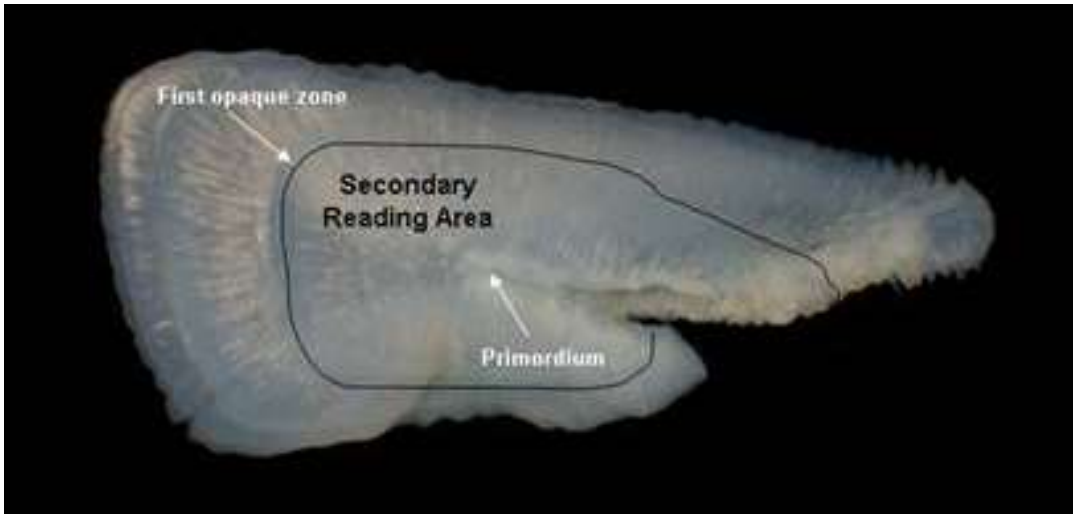


Figure 4 Distal view of whole grey mackerel otolith illustrating the Secondary Reading Area.

Within the reading area otoliths are assigned:
a) an increment count which is equal to the number of opaque zones,
b) an edge classification, and
c) a readability rating (see Fisheries Queensland 2018).

Identification of the first annual increment

An area of translucent material must exist between the primordium and an opaque zone before the opaque zone can be confidently recognised as the first increment. It is the presence of radial striae in the translucent zone between the primordium and the first opaque zone that helps to identify whether it is the first true opaque zone Figure 5. If the presence of radial striae cannot be seen in the primary reading area, then the secondary reading area is used to help identify its occurrence (Figure 6).

Because grey mackerel may have an extended spawning period, the distance between the primordium and the first opaque zone may vary considerably between otoliths for this species. The first true opaque zone is well defined and is predominantly seen as a dense thin opaque band that tends to coincide with a break in the radial striae.

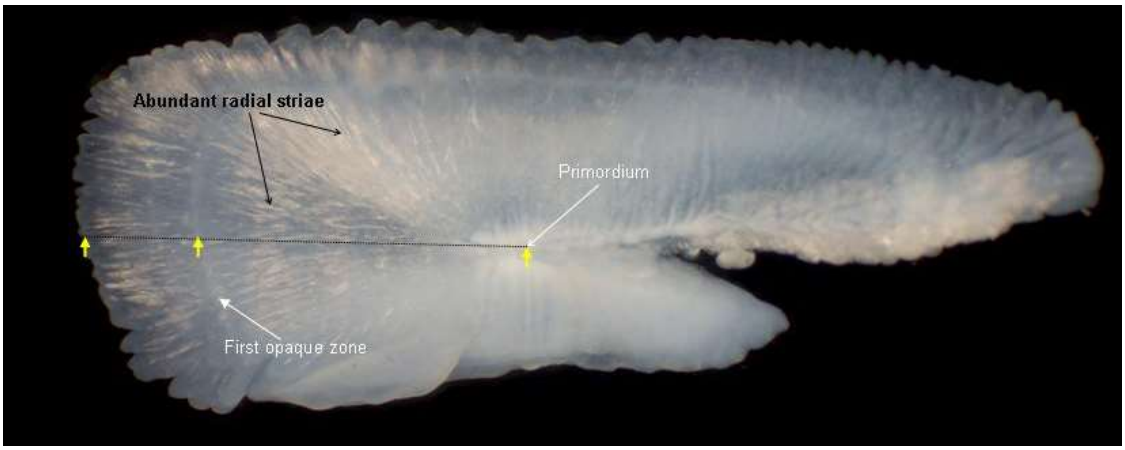


Figure 5 Distal view of a whole grey mackerel otolith with 2 increments, illustrating abundant radial striae prior to the first opaque zone. Yellow arrows represent the distance between primordium and opaque zones.

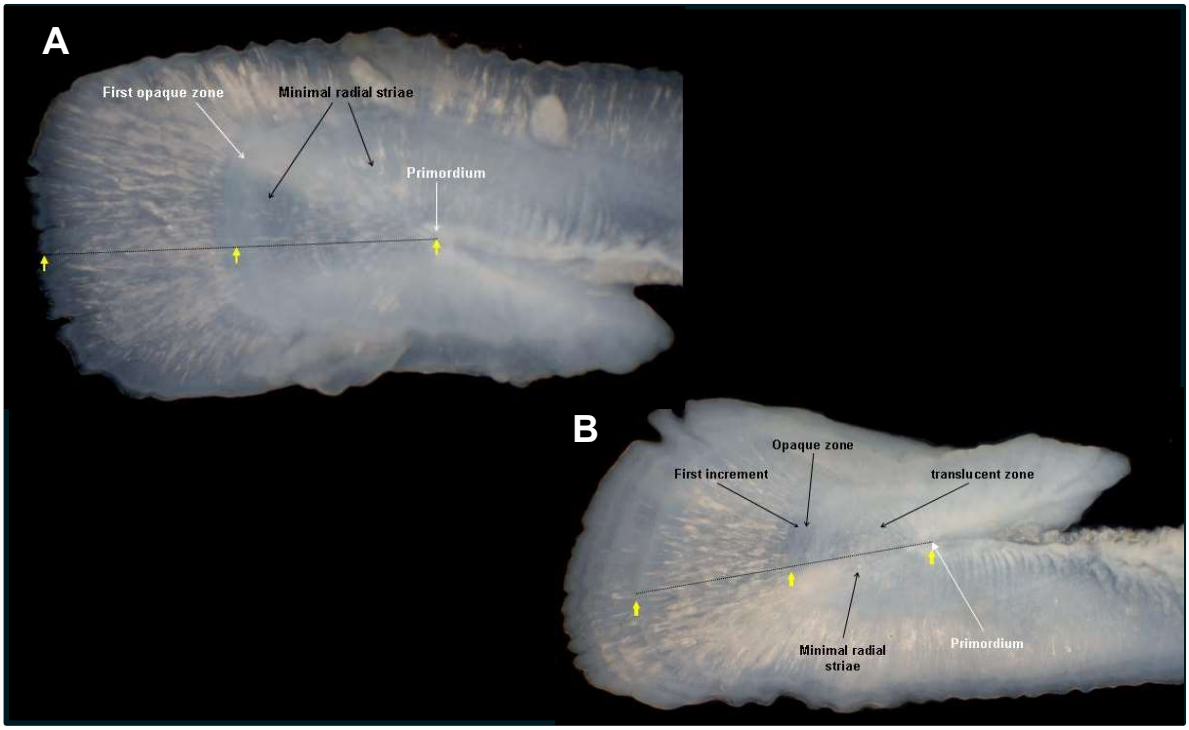


Figure 6 A & B Distal view of whole grey mackerel otoliths. The image on left illustrates minimal radial striae prior to the first opaque zone, on right minimal radial striae in an older fish. Yellow arrows represent distance between primordium and first two opaque zones.

False banding – before 1st increment

Occasionally a false band (or juvenile check) will form within the translucent zone of the first increment (Figure **7**). This band of opaque material is considered to have formed early in the life of the fish but could be misidentified as the first increment. The false band is associated with the dense core and contains little or no radial striae present between it and the primordium. When this formation is identified the reader should not include it in the increment count.

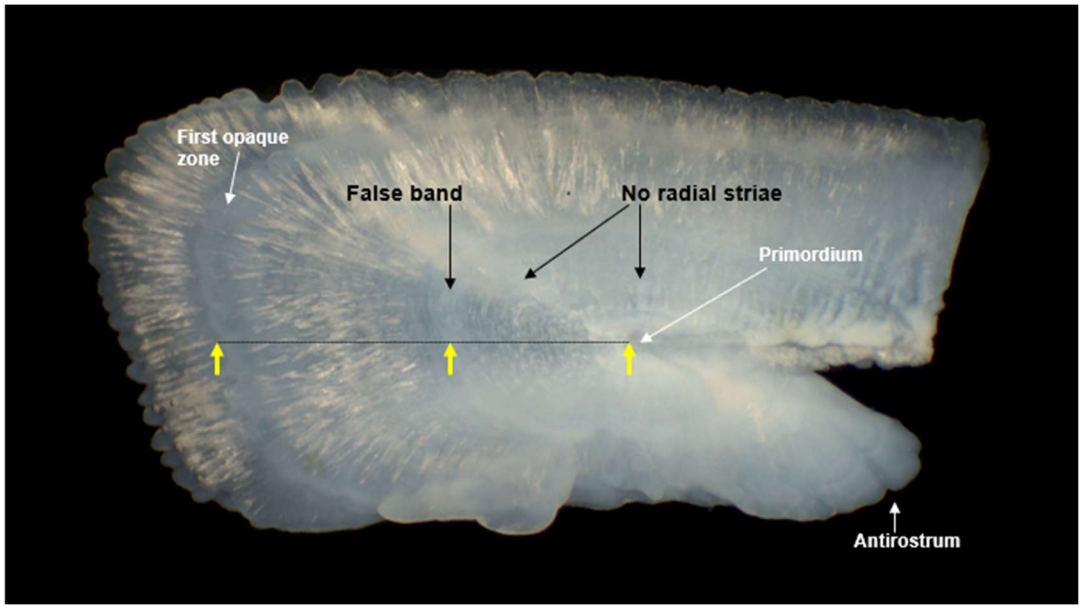


Figure 7 Distal view of whole grey mackerel otolith illustrating a false check (juvenile check) before the 1st increment shows no radial striae between the primordium and the opaque material. The yellow arrows show that the distance between the primordium and the juvenile check is less than the distance between the juvenile check and the first true opaque zone.

In otoliths with multiple increments, it is useful to consider the distance between all the opaque zones when deciding if the 1st opaque zone is an annual increment. In some cases, there may be a small amount of radial striae present before the 1st opaque zone even if it is not considered to be the annual band. In this case the distance between the primordium and first opaque zone is considered. If the width of the 1st translucent zone is less than the width of the 2nd translucent zone, then the 1st opaque zone is considered a false band (see Figure 8 and Appendix 1).

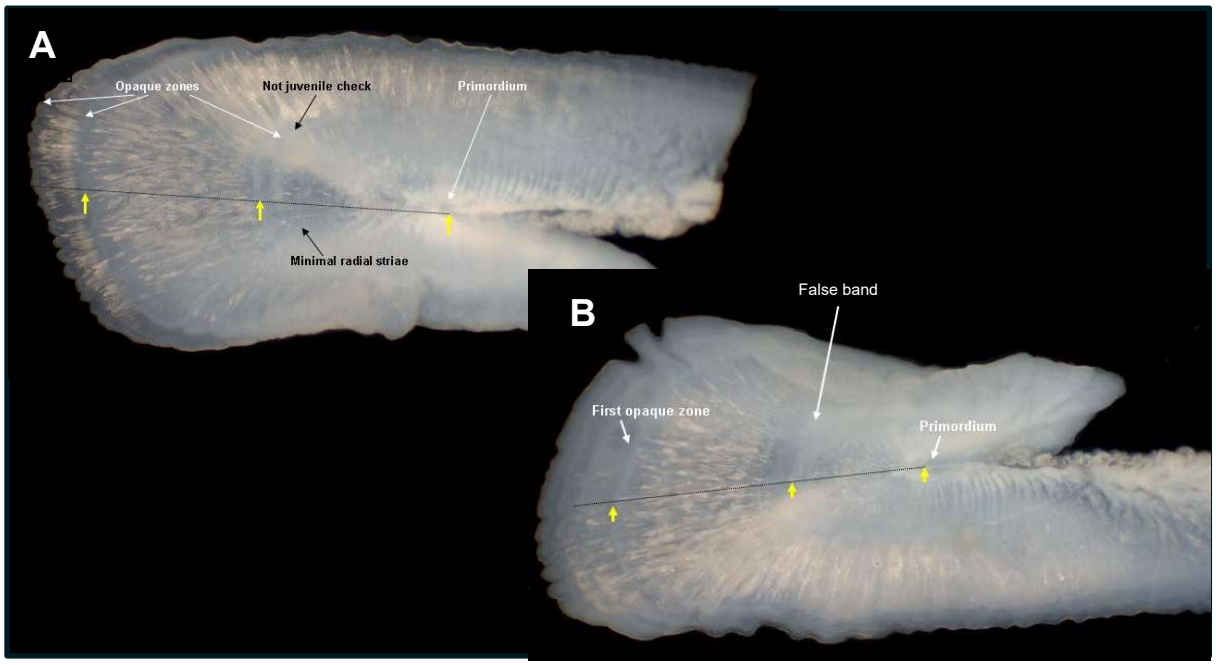


Figure 8 A & B Distal view of whole grey mackerel otolith illustrating using distance to determine if the 1st opaque zone is an annual band. A - distance between primordium and 1st opaque zone (yellow arrows) is equal to or larger than the distance between 1st opaque zone and the next opaque zone, therefore 1st opaque zone is 1st increment. B - distance between primordium and 1st opaque zone is smaller than distance between 1st opaque zone and 2nd opaque zone, therefore it is a false band (juvenile check).

Identification of subsequent increments

Subsequent opaque zones form parallel to the posterior margin, are generally dense and well defined, and tend to be continuous throughout the reading plane. The width of subsequent translucent zones generally decreases towards the posterior margin. It is common to see large translucent zones that are characterised by light refracting radial striae between the primordium and the first two increments (Figure 9). They tend to coincide with a break in radial striae however small amounts of radial striae may continue through the opaque zone. Once seen in the first opaque zone often seen in all subsequent opaque zones (Figure ***10***).

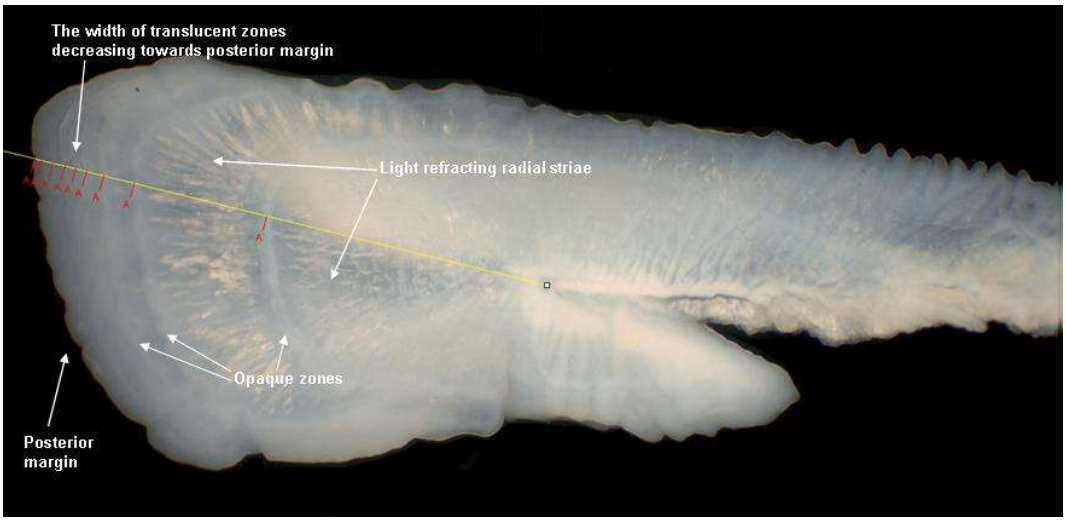


Figure 9 Distal view of a whole grey mackerel otolith with 9 increments, illustrating decreasing width of translucent zones between opaque zones toward the posterior margin on older otoliths. Red marks represent annual increments.

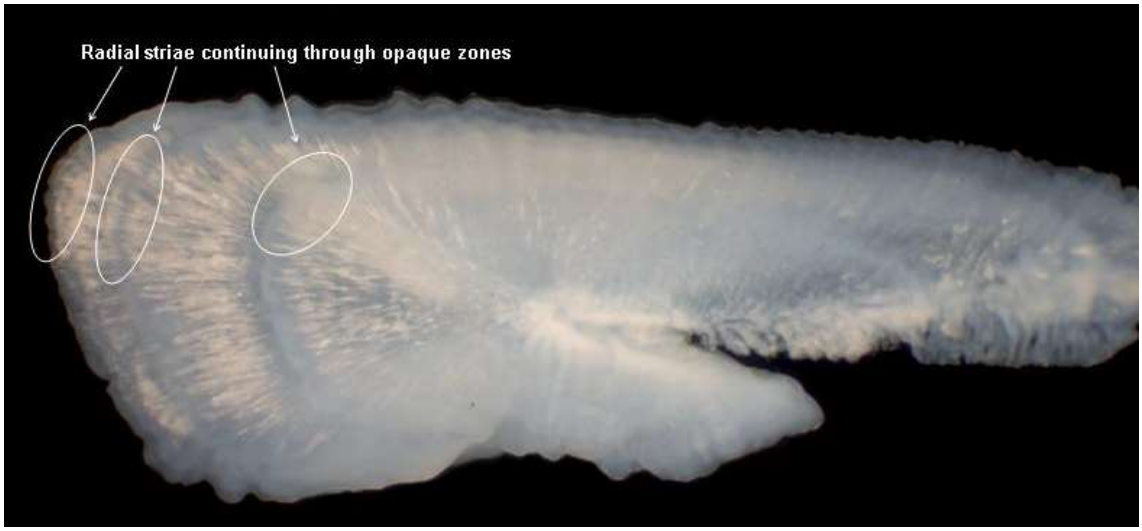


Figure 10 Distal view of a whole grey mackerel otolith with 4 increments showing radial striae continuing through opaque zones (circled).

The occurrence of closely spaced increments as they form towards the margin can reduce the contrast seen between opaque zones (Figure 11). To assist in increment interpretation, particularly helpful for older samples, a reader may use the following points to help:

1. adjust the focus for enhanced definition of increments towards the margin;
2. adjust the light source for enhanced definition of opaque and translucent zones;
3. rotate the otolith to obtain a different perspective of the reading area;
4. conduct multiple increment counts in different sections of the reading area.

False banding – after 1st increment

Occasionally false opaque bands may also be present after the 1st increment, in the translucent zone or split off from the true opaque band. These false bands are usually thinner and less distinct than the true opaque bands and may or may not occur with a break in radial striae (Figure 11).

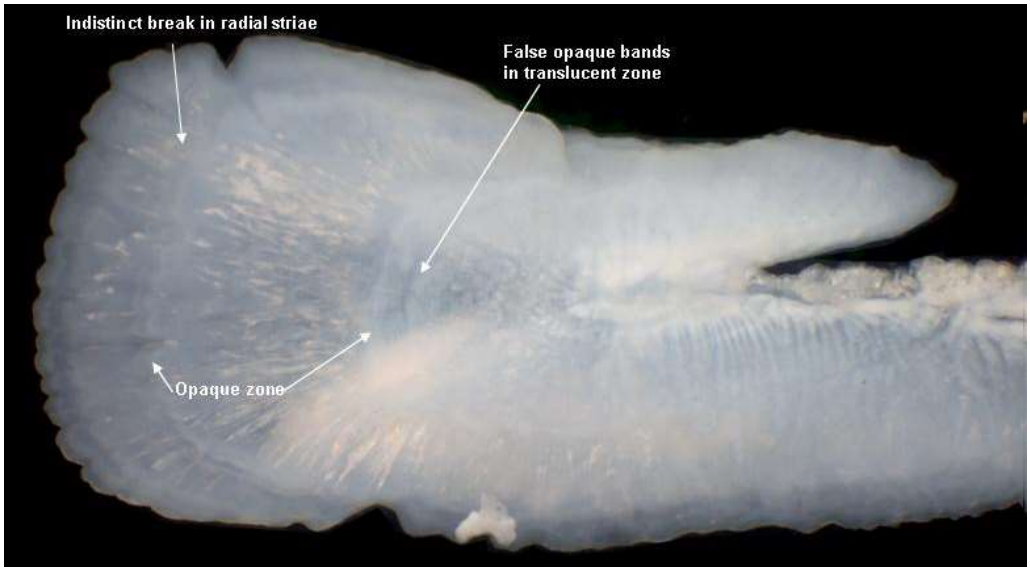


Figure 11 Distal view of whole Grey mackerel otolith with 4 increments, showing false banding.

On rare occasions, an opaque zone can be made up of two opaque bands. These parallel bands form close together and can appear to converge near the dorsal or ventral sides of the reading area (Figure 12). The occurrence of double banding may not be seen throughout the formation of subsequent increments and is therefore generally not considered when defining the margin.

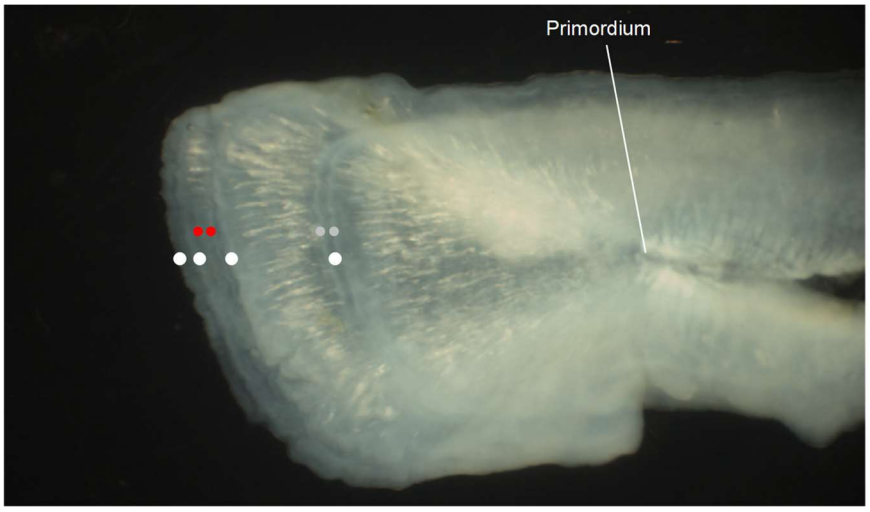


Figure 12 Distal view of whole grey mackerel otolith with red dots illustrating two false bands present between increments and grey dots illustrating double bands identified as the first increment. White dots represent increments.

Edge classification

Edge classification involves assigning a value to the otolith margin as per Section 1 of the Fish Ageing Protocol (Fisheries Queensland 2018). Edge classification is assessed along the otolith margin within the Primary Reading Area. This process helps to determine the stage of the increment formation. It has implications in the allocation of age to individual fish (see the Age estimation section below). The classification of intermediate and wide edges is based on the relative stage of completion of the marginal translucent zone. The classification of a new edge is based on the formation of a new marginal opaque zone. The following paragraphs describe how each of these edge classifications are defined.

New

An otolith with a “new” classification has opaque material visible on the otolith margin (Figure 13(A). The opaque material does not need to be continuous around the entire edge of the reading area Figure 13(B).

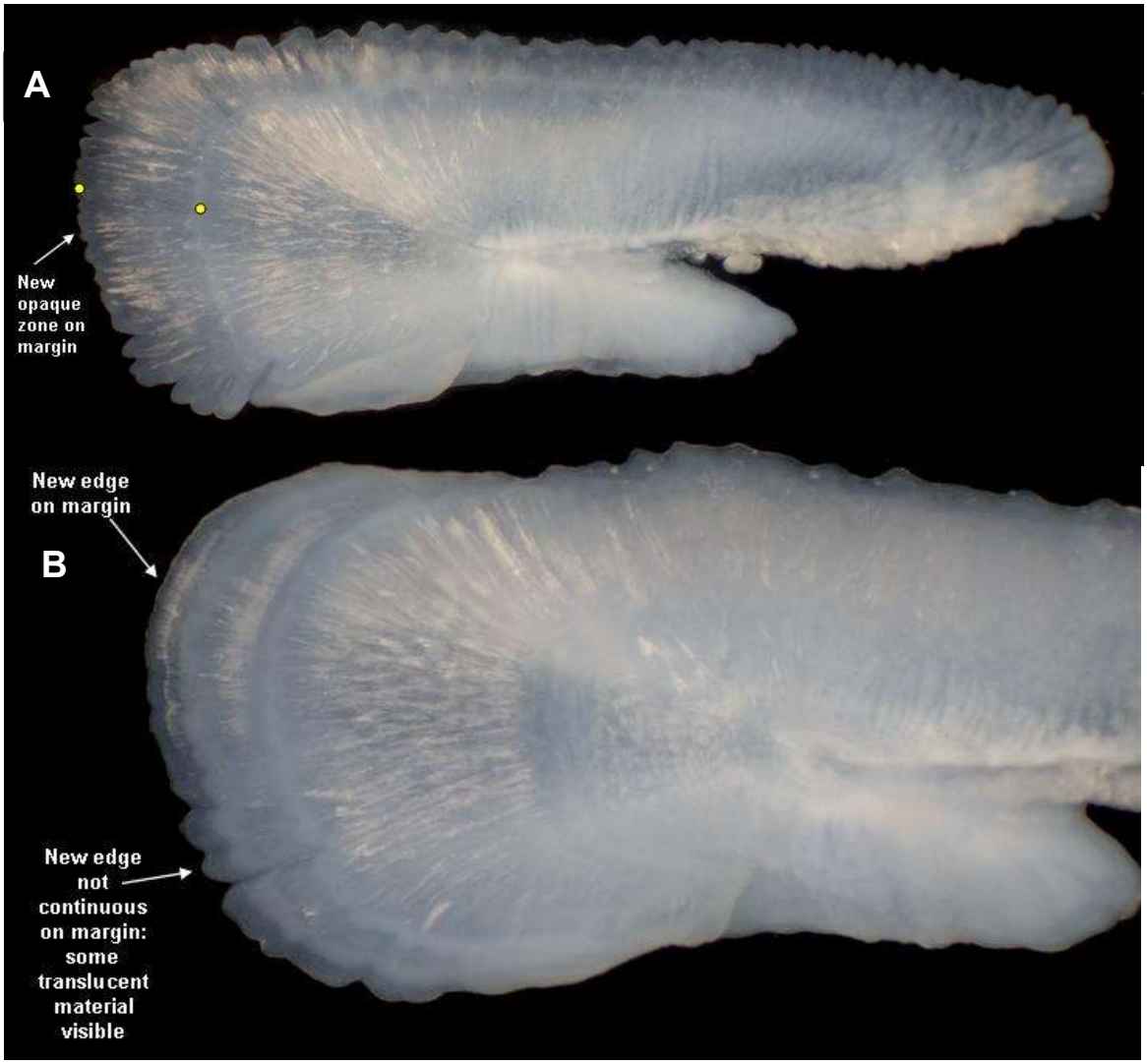


Figure 13 Distal view of whole grey mackerel otoliths with a new opaque margin. A - Yellow dots represent opaque zones of increments. B - New edge not continuous on margin

Intermediate

The “intermediate” edge classification is applied when there is a continuous band of translucent material visible on the margin of the Primary Reading Area of the otolith. This marginal translucent zone is less than two thirds complete. This is assessed by comparing the marginal translucent zone to the width of the last fully complete translucent zone, or by comparing it to the expected width of the translucent zone (Figure 14). For otoliths with two or less increments, readers can also use the density of the preceding radial striae to assist in determining the completeness of a translucent zone.

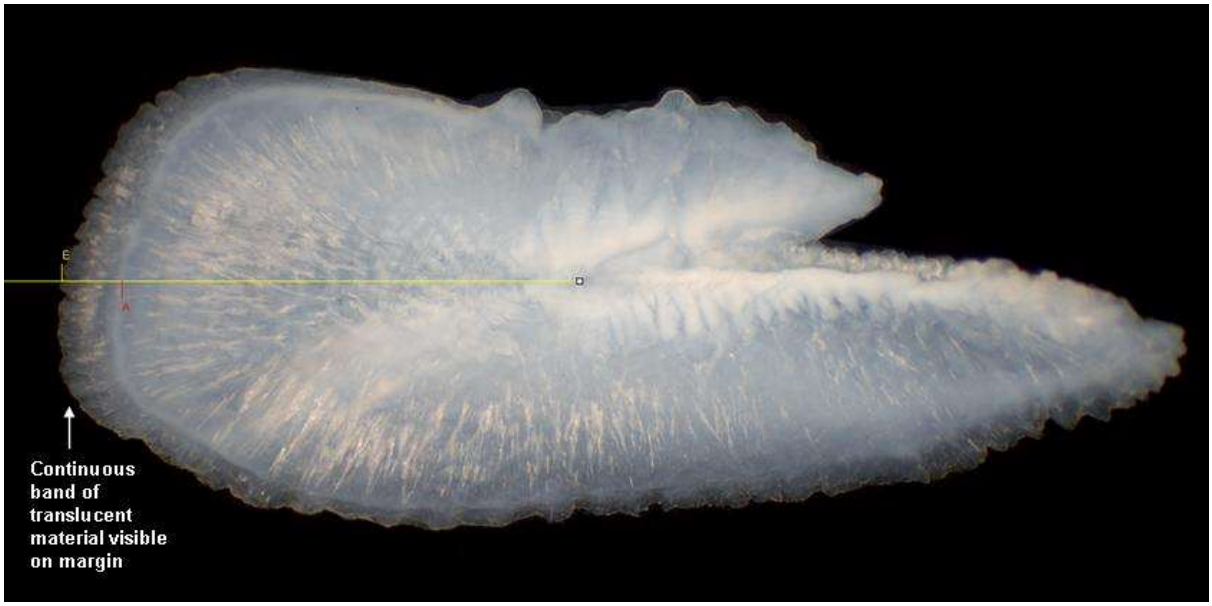


Figure 14 Distal view of a 1 increment whole grey mackerel otolith with intermediate margin. The marginal translucent zone is continuous throughout the readable area of the primary reading area and is considered to be less than two thirds complete by using the width of the last fully complete translucent zone as a guide. Red mark represents the opaque zone of the increment.

Wide

An otolith with an edge classification of “wide” also has a continuous band of translucent material visible on the edge of the otolith. However, the marginal translucent zone is more than two thirds complete. This is established by comparing the marginal translucent zone to the width of the last fully complete translucent zone (Figure 15 and Figure 16) or by comparing it to the expected width of the translucent zone for otoliths with an increment count of zero.
The distinction between an “intermediate” and a “wide” edge classification becomes less clear for otoliths with 6 or more increments.

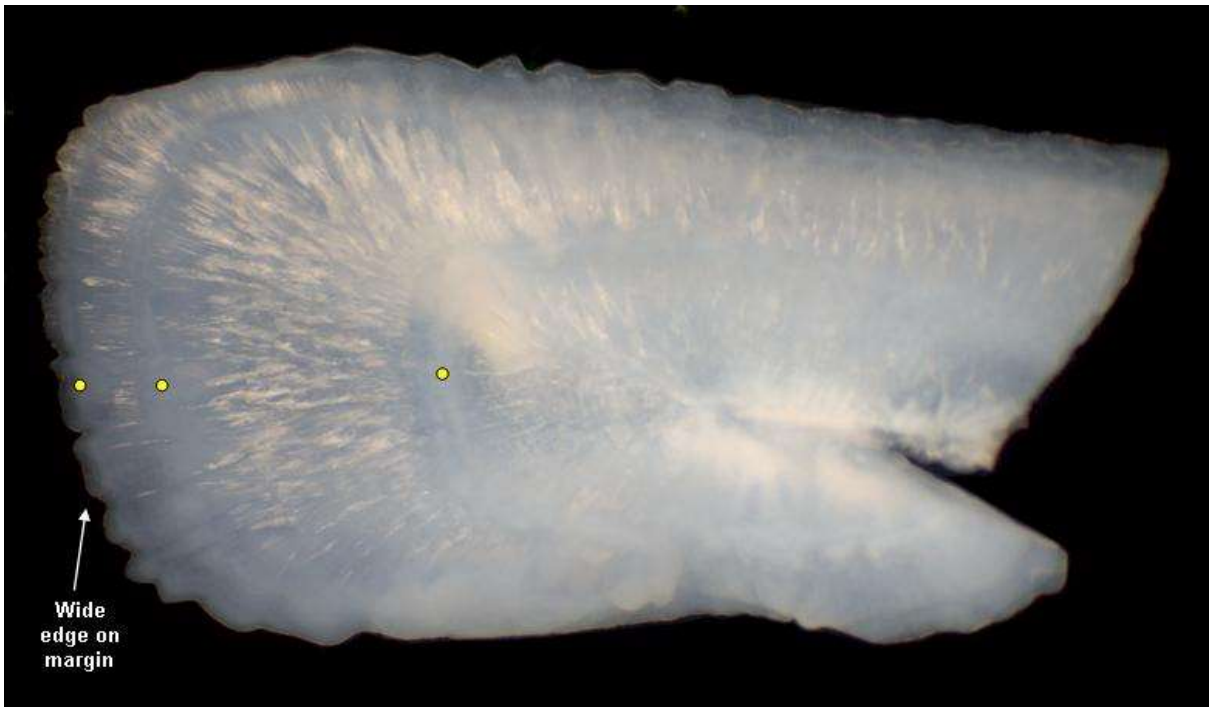


Figure 15 Distal view of a 3 increment whole grey mackerel otolith with a wide margin. The marginal translucent zone is continuous throughout the readable area of the primary reading area and is more than two thirds the expected completed width using the size of the last fully complete translucent zone, and knowledge of the usual pattern of formation as a guide. Yellow dots mark increments.

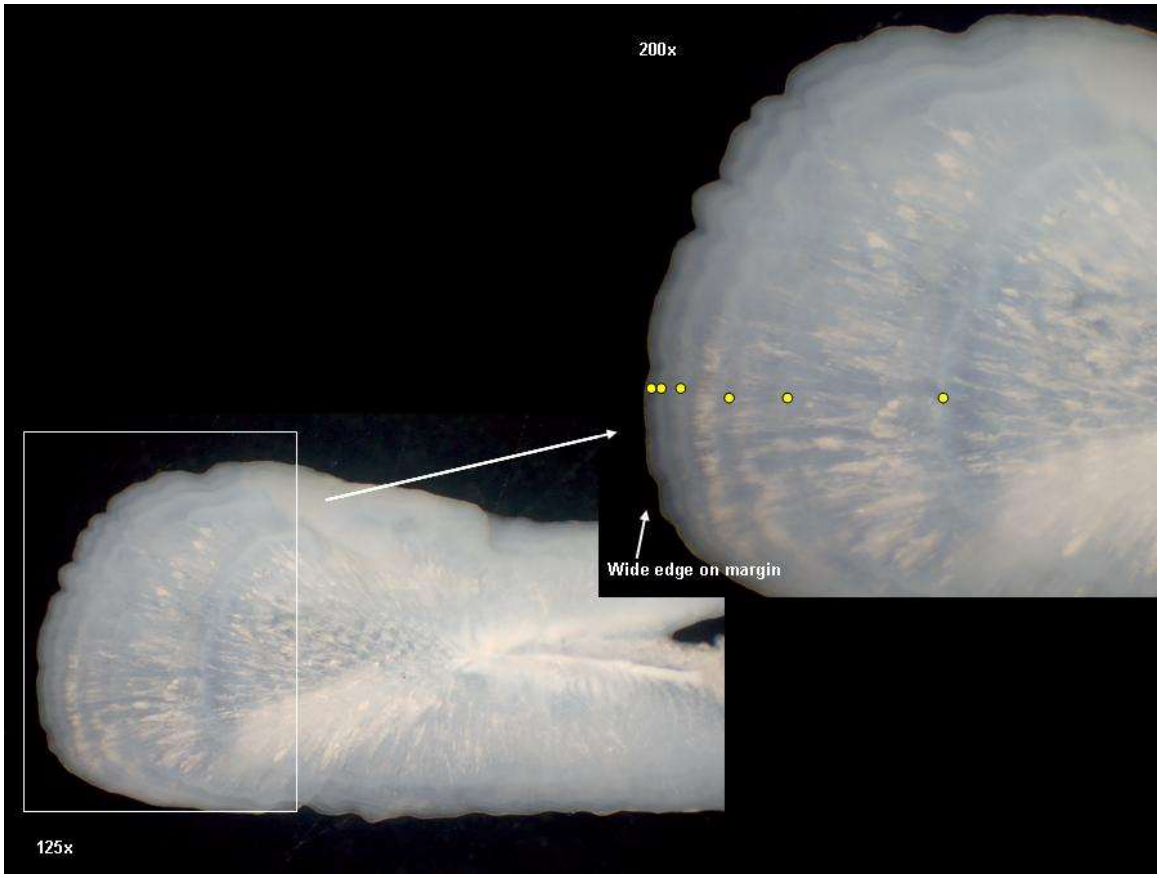


Figure 16 Distal view of a 6 increment whole grey mackerel otolith with a wide margin. The marginal translucent zone is continuous throughout the readable area of the primary reading area and is more than two thirds complete using the width of the last fully complete translucent zone as a guide.

Summary of reading procedure

Complete training and competency for grey mackerel whole otoliths (from saved images on the screen).

The following points should be followed when interpreting grey mackerel otoliths:

- ensure microscope settings are correct,
- remove the left otolith and place distal side up for viewing,
- identify the primordium, anti-rostrum, and primary reading area of the otolith,
- check focus, light and camera settings are optimal - particularly checking the clarity of the 1st increment and the closely spaced increments towards the margin,
- take image of otolith and name file with fish number,
- determine whether the otolith is readable or unreadable within the primary reading plane.
-

After determining that an otolith can be interpreted:

- consider the appearance of opaque zones before starting the interpretation of each otolith,
- translucent material with radial striae must be present between the primordium and the first opaque zone to avoid misinterpretation,
- if radial striae are not present between the primordium and the first opaque zone within the primary reading area, the secondary reading area is used to help confirm the presence of radial striae,
- assess the decreasing distances been opaque zones to decide if first opaque zone is a true increment or a juvenile /false check,
- count the number of opaque zones from the first increment out to the otolith edge,
- assign an edge classification,
- assign a readability score to the interpretation according to your level of confidence (confident or not confident) (Fisheries Queensland 2018).

Quality Control measures

Increment counts are tested for bias and precision. These quality control measures are used during reader competency and the re-read of the current sample to ensure precise and unbiased results (Fisheries Queensland 2018).

Bias

Unacceptable bias exists for a reading of grey mackerel otoliths when:

- the calculated bias is ≥ 1 increment
- this bias could exist for one or more groups of increment counts (i.e. along the x-axis)
- the calculated bias shows a divergent trend across a series of increment counts (i.e. along the x-axis).

Precision

Unacceptable precision exists for a reading of grey mackerel otoliths when:

- The calculated Index of Average Percent Error (IAPE) for counts of increments is ≥ 3%
- 80% or higher of the new edge type is interpreted correctly between Reading1/Reading2 and Competency/Reference collection.
- 50% or higher of the “intermediate” and “wide” edge types are interpreted correctly between Reading1/Reading2 and Competency/Reference collection.
- Note: Edge type criteria are only assessed for an edge type when more than 10% of the otoliths are within that specific edge type classification.

Age estimation

There is a difference between the age group, age class and biological age of fish (Fisheries Queensland 2018).

- Age group, which is expressed in whole years, is the maximum age class fish would reach during a sampling season.
- Age class, which is also expressed in whole years, is the number of birthdays a fish is assumed to have had.
- Biological age is the age class plus the time between when the fish were captured and their previous birthday. Biological age is expressed in months (e.g. 54 months).

The age group and age class of individual grey mackerel equal their otolith increment count. As data become available on the period of opaque zone formation, these calculations will be modified to include the edge classification, nominal birth date and the period of opaque zone formation.

A nominal birth date grey mackerel is set at 1 November based on the middle of the spawning period identified by Cameron and Begg (2002) and Welch et al (2009).

Otolith increment count and the month the fish was captured are required to estimate the biological age of individual fish. Biological age is the time between the capture date and the previous birthday is then added to the increment count using the following formulae (note: these formulae output the age in months):

If Cm < Bm

Am = (increment count x 12) + (Cm – Bm + 12)

If Cm > Bm

Am = (increment count x 12) + (Cm – Bm)

Where: Am = biological age; Cm = capture month; Bm = biological birth month (e.g. 11 = November).

References

Fisheries Queensland (2020). Fishery Monitoring biological sampling protocol: Grey Mackerel. Department of Agriculture and Fisheries. Brisbane, Australia.

Fisheries Queensland (2018). Fisheries Queensland Sampling Protocol – Fish Ageing. Department of Agriculture and Fisheries. Brisbane, Australia.

Cameron, D. and Begg, G. (2002). Fisheries biology and interaction in the northern Australian small mackerel fishery. FRDC Final Project Report. Department of Primary Industries, Brisbane, Brisbane 236pp.

Welch, D.J., Buckworth, R C, Ovenden, SJ, Broderick, D, Lester, RJG, Ballagh, AC, Stapley, J, Charters, RA Gribble, NA. (2009). Determination of management units for Grey Mackerel fisheries in northern Australia. FRDC Final Project Report No. 2005/010. Jaems Cook University, Townsville 158pp.

Appendix 1

False bands – before the 1st increment

A. Distal view of a whole 2 increment grey mackerel. Example of false band before the 1st increment showing lack of radial striae, the density of material between primordium and false band, and distance rule.
B. Distal view of a whole 3 increment grey mackerel with false band, showing distance rule and minimal radial striae.
C. Distal view of a whole 2 increment grey mackerel with false band. Distance rule applied.
D. Distal view of a whole 2 increment grey mackerel with false band and no radial striae. Distance rule applied.