Age validation of Deepwater Flathead from the Great Australian Bight Trawl Fishery

Kyne Krusic-Golub, Leanne Gunthorpe and Simon Robertson

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1. Non-Technical Summary

2005/008	**Age validation of Deepwater Flathead from the Great Australian Bight Trawl Fishery**

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Objectives:

1. Validate the periodicity of zone formation from marginal zone data and daily age estimation to the first opaque zone.
2. Determine the counts of presumed daily rings between the primordium of the otolith and the outside edge of the first opaque zone of young fish caught in different locations in the GAB.
3. Implement standards on the age estimation of this species based on the timing of zone formation and the variability in position and appearance of the first annulus.
4. Understand the variability in growth rates of juvenile Deepwater Flathead.

Non-Technical Summary:

OUTCOMES ACHIEVED TO DATE

1) Determined the timing of first increment formation and subsequent increment formation in age classes of Deepwater Flathead to asymtopic length (where samples are available).

2) Established there were temporal differences in the timing of increment formation and juvenile growth from samples collected from the Great Australian Bight Trawl Fishery (GABTF).

The number of daily rings was determined between the primordium of the otolith and the first opaque zone of fish from three Zones within the GAB: the Central, the Eastern and the Western Zones.

These data confirms the known spawning variability within the Zones of GAB. The mean daily zone counts were significantly different indicating that Deepwater Flathead in the Western Zone spawn before the fish in the Central or Eastern Zones.

Measurements indicated that there is little difference between the average position of the first opaque zone in samples collected from different Zones of the GAB. Even though fish from the Western Zone spawn earlier than those from the Eastern and Central Zones, the formation of the opaque zone occurs at the same time for all fish across all Zones.

3) Developed a standard for the interpretation of age for Deepwater Flathead, which will lead to more accurate and precise age estimates of this species.

An ageing protocol was developed for Deepwater Flathead.

The results of this study will improve the stock assessments, which will assist managers in meeting the objectives for the sustainable use of the resource.

This project had a further objective; understand the variability in growth rates of juvenile Deepwater Flathead. This objective was not fully met because there were insufficient samples available from juvenile Deepwater Flathead for analysis. The analyses that were done indicated that the Deepwater Flathead in the Western GAB have a slower otosomatic growth rate than the Deepwater Flathead from the Eastern or Central Zones. The implication of this with relation to somatic growth rates could not be examined due to sample size.

The growth data obtained from this study from the juvenile Deepwater Flathead allowed more biologically 'sensible' growth parameters to be estimated (t_0, K,L_∞).

The Deepwater Flathead fishery is managed though a stock assessment that uses an age-structured model to estimate biomass (unexploited biomass) and to indicate whether the yield from the fishery is sustainable. One of the assumptions of the age-structure model used in the stock assessment is that the age estimates are accurate (Anon 2004). If the age data are in-accurate, management may be making decisions based on an estimated fishable biomass 30% higher (assuming 2 year bias) than the true fishable biomass. The effect of this would be unsustainable levels of total allowable catch (TAC) from the Deepwater Flathead fishery in the Great Australian Bight Trawl Fishery (GABTF).

The lack of validated ages for Deepwater Flathead was discussed at the GAB Fishery Assessment Group (GABFAG) meeting held in June 2004. Validating age estimates was seen as a component of the stock assessment model that could be improved relatively easily, given the otolith collections made for the FRDC project 2003/003 and by the Integrated Scientific Monitoring Program (ISMP).

The main objectives of this project were directed towards obtaining a greater understanding of growth zone formation. To do this, this study:

- Compared the bias in zone counts derived from whole otoliths and sectioned otoliths
- Validated the position of the first growth increment using daily age counts
- Analysed periodicity of the annual increment
- Developed a standardised ageing protocol
- Compared growth, age composition and mortality derived from the validated protocol with previous estimates
- Audited the historical ageing.

The study found:

- The most accurate method for examining age and growth of Deepwater Flathead is through counts of annuli on transverse otolith sections.
- The daily zone estimates could be used effectively to determine the location and timing of the formation of the first opaque zone.
- Daily zone counts from the hatch mark to the start of the first opaque zone (corresponding usually to the end of the diffuse nucleus) support the assumption that the first opaque zone starts to form in winter.
- Measurements taken from the primordia to the first opaque zone indicated that there is little difference between the average position of the first opaque zone in samples collected from different zones of the GAB and that a proxy measurement for the first opaque zone would be a useful tool in the ageing of this species.
- Spawning dates when back calculated from daily rings were consistent with peak spawning periods which further supported the assumption that micro-increments counted in this study were formed daily.

- The first annual zone is located 0.460 mm on the dorsal side of the sulcus and 0.373 mm on the ventral side of the sulcus. These distances can be used as proxies for the position of the first annual zone.
- One increment (one translucent and opaque zone) is formed each calendar year.
- Increments can be confidently used to age Deepwater Flathead.
- An ageing protocol was developed from these results to standardise the interpretation of otolith microstructure for the routine ageing of Deepwater Flathead.
- The growth of Deepwater Flathead using the developed protocol is similar to those from previous studies. The mortality of female Deepwater Flathead is slightly higher than the total mortality in males.
- An audit of the historical ageing using the validated protocol showed the majority of the historical age estimates:
    - Had adequate levels of precision
    - Are consistent with the validated ageing protocol developed in this study.
- Errors detected in the historical ageing data are below the margin of error (± 2 years) that would invalidate current biomass estimates (Wise and Tilzey, 2000).
- Since 2007, age estimates have been undertaken using the validated Deepwater Flathead ageing protocol, developed in this study. No bias has been detected and age estimates show an acceptable level of precision (CAF unpublished data, FAS unpublished data).

Keywords: *Neoplatycephalus conatus*, Deepwater Flathead, Great Australian Bight Trawl Fishery, otoliths, age estimation, marginal zone analysis

2. Acknowledgments

The authors would like to thank the observers working on fishing vessels responsible for the large amount of work in measuring and recording the catch and removing otoliths. Acknowledgment must also be given to the fishers and fishing industry that allows research to be carried out on their vessels.

Special thanks to Lauren Brown (Integrated Scientific Monitoring Program) for opportunistically collecting the majority of small samples of discarded Deepwater Flathead. Lauren also provided valuable information on the fishing practices for Deepwater Flathead. Joe Restall (Fisheries Victoria) assisted in weighing otoliths, entering biological data and the sectioning of otoliths. Thanks to Brent Wise (BRS, now WA Fisheries) for running the equilibrium models. Thanks to Therese Bruce for reading, sample preparation and registration of otoliths. We would like to thank Corey Green for reading, sample preparation for both annual and daily age estimates and providing the plates of the daily preparation.

We would also like to thank for the following people for providing helpful comments on drafts of this report:

- Dr James Andrews and Mr Jon Presser, Fisheries Victoria
- Dr Carolyn Stewardson, FRDC
- The anonymous reviewer.

FINAL REPORT

2005/008	**Age validation of Deepwater Flathead from the Great Australian Bight Trawl Fishery**

3. Background

Deepwater Flathead

Deepwater Flathead (*Neoplatycephalus conatus*) is endemic to southern coastal waters of Australia between the western edge of Bass Strait and south coast of Western Australia. This species is found at depths of 70–360 m (Gomon *et al.*, 1994).

Deepwater Flathead are a species, that:

- Grows to a length of 94 cm for females and 62 cm for males
- Weigh over 4 kg and 2 kg, respectively
- Lives for more than 20 years (Newton *et al.*, 1994).
  Un-validated age estimates have produced a maximum age of 33 years for females and 28 years for males (Krusic-Golub and Stokie, 2008).

Deepwater Flathead are the dominant commercial species caught within the designated zones of the Great Australian Bight Trawl Fishery (GABTF) of the Southern and Eastern Scalefish and Shark Fishery (SESSF) (Figure 1).

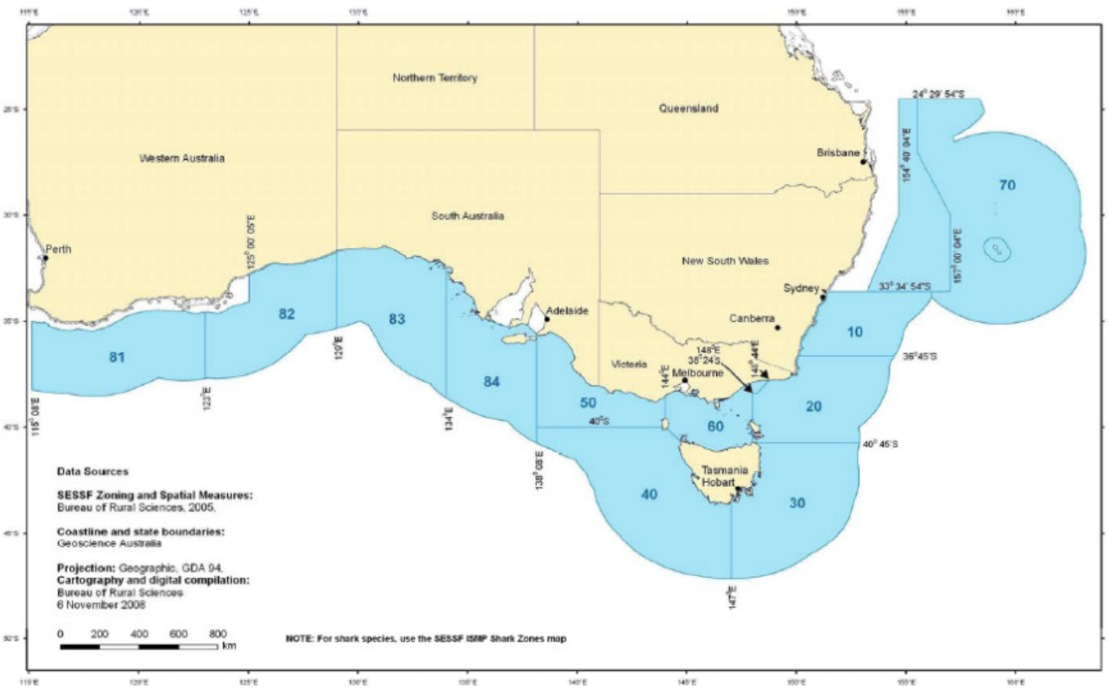


Figure 1. The Southern and Eastern Scalefish and Shark Fishery (SESSF) showing the zones of the Great Australian Bight Trawl Fishery (GABTF): zones 81 (far west), 82 (west), 83 (central) and 84 (east).
Source AFMA ISMP Progress Report (September 2009)

The fishery for Deepwater Flathead

A brief history of the GABTF is shown in Table 1. The fishery began in 1912 and continued with intermittent commercial trawling until 1986. It was not until the development of the orange roughy (*Hoplostethus atlanticus*) fishery in the late 1980s that the GABTF underwent significant development. The GABTF is predominantly a demersal otter trawl fishery. This fishery reportedly harvests 226 species of which Deepwater Flathead is one of the main targeted species (Bromhead and Bolton, 2005).

Ten vessels have quota entitlements allowing fishing in the GABTF. These vessels operate year-round on the continental shelf and upper continental slope in depths to about 250 m.

Catch characteristics

Integrated Scientific Monitoring Program (ISMP) data for the GABTF indicated that for the period September 2000–October 2006, the majority of the total Deepwater Flathead catch was between 45 and 60 cm in length. Very few individuals less than 35 cm are caught. Of the 3.3% of Deepwater Flathead caught under 35 cm, less than 1% was discarded (Figure 2).

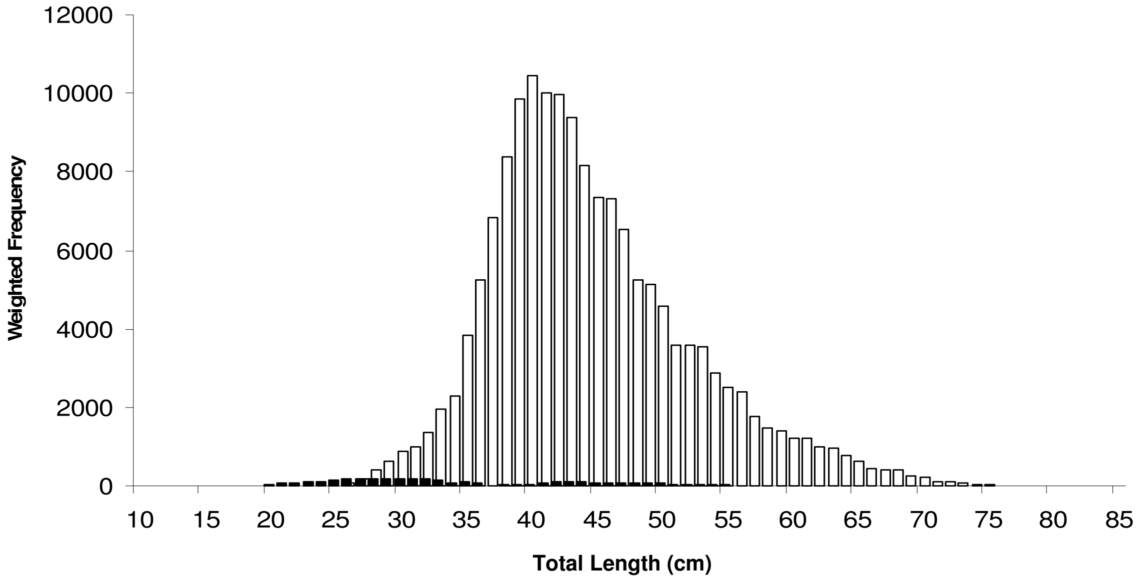


Figure 2. Length frequency (weighted) of Deepwater Flathead retained and discarded for period September 2000 to October 2006. Solid column indicate the number of fish discarded. Open columns indicate the percentage of fish retained.

Table 1. Brief history of the GABTF fishery.

Year	Activity	Vessels active	Reference
1912	Fishery commenced – demersal trawling intermittently on the continental slope shelf to depths of 1000 m		Larcombe & McLoughlin, 2007
1985	Annual catch of Deepwater Flathead 30 tonnes		Lynch & Garvey, 2003
1986	Regular targeting of orange roughy commenced – operating on the continental shelf (fishing activity in depths of 200 m)		
1988	The main target species of the GABTF begins to shift away from orange roughy (49 % of catch) to Deepwater Flathead (18 %)	17	Lynch & Garvey, 2003
1989	Annual catch of Deepwater Flathead 430 tonnes		
1990	GABTF activities shifted to the continental shelf		
1993	GABTF the first fishery managed under the (Commonwealth) Fisheries Management Act 1991.	10	Tilzey & Wise, 1999 Larcombe & McLoughlin, 2007
1994	A shift in the targeting by GABT to Deepwater Flathead completed (56 % of catch by weight *cf* orange roughy 5 %)	6	Lynch & Garvey, 2003
1995	Doubling of total GABTF catch Peak of Deepwater Flathead catch - 61% of total catch (to date) Catch rates for Deepwater Flathead the highest on record (to date)	12	Anon, 2004
1997	Deepwater Flathead remains target species Proportion of Deepwater Flathead in total catch decreases to 44%		
1998	Increase in effort within the GABT over 3 years peaked at 170%	10	
1999	Effort increased remained at 150% higher than that of 1995 Total catch of GABTF was around 2,700 t Proportion of Deepwater Flathead in catch reduced to 20%		Lynch & Garvey, 2003
2000	Deepwater Flathead catch begins to increase and peaks at 2,304 t Fishing effort on continental shelf declines		
2003	Effort increases (from 79% above 2002 total and 77 % increase in the shelf fishery) minor increase in Catch per unit effort (CPUE) 5 % increase in mean annual catch rate		Anon, 2004
2005	Annual Deepwater Flathead catch fallen to 1,728 t (36% of total catch)		Koopman *et al.*, 2006
2006	Mean annual catch of Deepwater Flathead landed in GABTF 1,326 tonnes (2000–06) Quota management introduced for Deepwater Flathead. Total Allowable Catch set at 3,000 t for the SESSF global catch	10	Walker *et al.*, 2006 Anon, 2006
2007	Quota for SESSF global TAC set at 2,190 t		Anon, 2007

Length

The length-frequency distribution of the Deepwater Flathead catch between September 2000 and October 2006 is shown in Figure 3.

Female Deepwater Flathead were significantly larger than male Deepwater Flathead (Stokie and Talman, 2003). Males ranged in length from 31 to 63 cm, while females ranged from 34 to 78 cm in length. Males and females are reproductively mature after reaching 43 cm TL (Anon, 2006). Up to the age of 6 years, the length-at-age of male and female Deepwater Flathead is similar.

Age

The age composition of Deepwater Flathead collected by the Integrated Scientific Monitoring Program from the GABTF between 1995 and 2005 showed an age distribution between 1–19 years (Figure 4). The majority of the catch was between 5 and 8 years (54%), and more than three quarters of the catch comprised of age-classes between 4 and 10 years (77%).

Deepwater Flathead fully recruit to the fishery at 4 to 5 years for males and females (Stokie and Krusic-Golub, 2005) (Figure 4). In commercial catches, up to 80% of the Deepwater Flathead are mature fish (Brown and Sivakumaran, 2007).

An analysis of the age composition data of Deepwater Flathead in the early fishery catches (1988 and 1989) compared with that in 1994 showed the proportion of fish aged 9 years and older had decreased. Further analysis of the age composition of the catch from 1996 compared with that from 2003 indicated the proportion of 9-year-old fish in the catch had declined significantly from 57% to 19%. The dominant age-classes of Deepwater Flathead caught in 2006 were between 5–8 years (Krusic-Golub and Stokie, 2008).

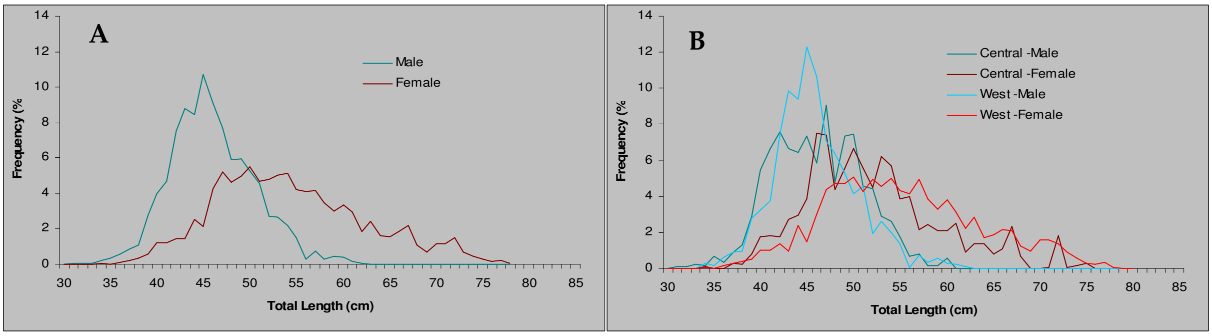


Figure 3. Length-frequency distribution of Deepwater Flathead caught from GABTF showing A) the size difference among sexes and B) the size difference among sexes between zones, for period September 2000 to October 2006 .

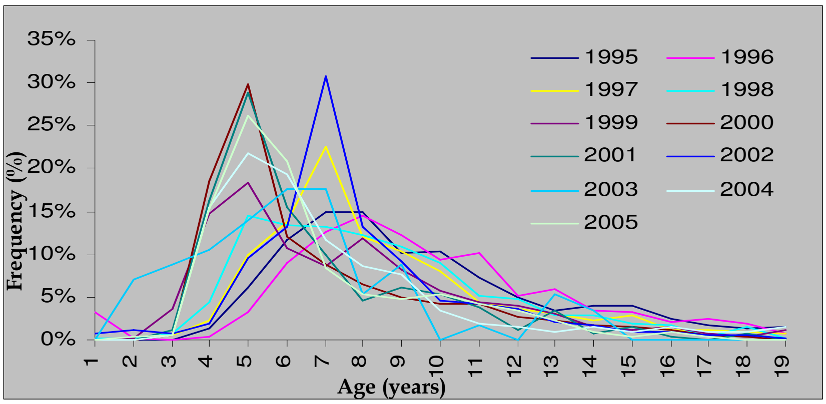


Figure 4. Percentage age frequency composition estimates of Deepwater Flathead for 1995 through to 2005.

Historical Ageing

Since 1995, the Central Ageing Facility (CAF) provided age estimates for Deepwater Flathead to AFMA for use in modelling, a critical component of the stock assessment for this species. Age is estimated from counts of opaque growth zones on transversely sectioned otoliths from Deepwater Flathead sampled from the GABTF commercial catch.

The routine ageing of a species requires that:

- Zones in the otolith microstructure reflect biological ageing of the species (i.e. one opaque zone reflects one year)
- Interpretation of the microstructure is consistent across and between readers.

The ageing method for Deepwater Flathead has assumed one opaque and one translucent zone are formed each year in the sagittal otoliths. This assumption is consistent with the assumptions inherent in ageing methods used to age other species from the Platycephalidae family (Hyndes *et al.* 1992; Jordan, 1998; Masuda, 2002).

While the timing and periodicity of zone formation has been validated in several Australian species of platycephalidae, e.g. Yank Flathead (Hyndes *et al.*, 1992), Dusky Flathead (Gray *et al.*, 2002), and Sand Flathead (Koopman, 2004), no validated method incorporating direct evidence of timing and position of the annuli formation has been undertaken for Deepwater Flathead.

The age estimation of Deepwater Flathead from counting growth zones in sections of sagittal otoliths is considered difficult (Krusic-Golub and Robertson, 2001) because the otolith microstructure is difficult to interpret.

The sections of many Deepwater Flathead otoliths exhibit a large opaque centre that obscures the microstructure, making identification of the first few zones difficult. Additionally, a large number of fine irregular zones in the inner region of the otolith complicate the identification of the first zone. These fine irregular zones are presumed to be sub-annual.

These interpretation difficulties are compounded by the fact the Deepwater Flathead fishery is not characterised by the presence of a few strong year classes, which move through the fishery. The presence of strong year classes can be used to support the accuracy of the age estimates.

Deepwater Flathead is a multiple spawner that spawns over eight months from October to May (Brown and Sivakumaran, 2007). The length of the spawning season influences the position of the first annuli (Shepherd and Grimes, 1983; Allman *et al.*, 2005; Karlou-Riga, 2000). The length of the spawning period and the relative growth rates of fish in each of the first few years of life complicate the interpretation of where the first annulus lies. This, coupled with the absence of strong age-classes moving through the fishery to provide a validation of age, has the potential to lead to inaccurate ages used for stock assessment.

4. Need

The Deepwater Flathead fishery is managed though a harvest strategy with reference to a stock assessment that uses an age-structured model to estimate biomass (unexploited biomass) and to indicate whether the yield from the fishery is sustainable. One of the assumptions of the age-structure model used in the stock assessment is that the age estimates are accurate (Anon, 2004).

The setting of annual total allowable catch of Deepwater Flathead is reliant on age estimates (catch–at-age data), as other biological data are limited. These data provides an indicator of the strength of recruitment into the fishable biomass and the loss of older year classes from the population.

It has been suggested that catches of Deepwater Flathead are near or above estimated sustainable limits (Anon, 2004). In response to increasing fishing pressure on the continental shelf, it is critical to obtain accurate estimates of biomass and yield to ensure the fishery is sustainable.

A simple equilibrium model was run to demonstrate the effect that uncertainty in ageing has on maximum sustainable yield (MSY) using the Deepwater Flathead biological parameters (Wise and Tilzey, 2000). This sensitivity analysis was based on the current ageing versus ageing which assumes that fish are in reality 2 years older. The analysis showed that there is a 30% decrease in available biomass if the current ageing is shown to be biased. A bias of 2 or more years in the age estimation could be a reality for this species due to the uncertainty in timing and the position of the first and subsequent zones. Accurate (i.e. validated) ageing is therefore essential.

In the absence of accurate age data, management may be making decisions based on an estimated fishable biomass 30% higher (assuming 2 year bias) than the true fishable biomass. The effect of this would be unsustainable levels of total allowable catch (TAC) from the Deepwater Flathead in the GABTF.

Un-validated age estimates have produced a maximum age of 33 years for females and 28 years for males. Age at which Deepwater Flathead recruit to the fishery is at approximately 3–5 years of age. It is generally the youngest and the oldest Deepwater Flathead that are the most difficult to age accurately. These age groups have the most influence on the estimates of growth, mortality and longevity. These parameters are required to determine the sustainability of the fishery. Accurate and defined age estimates of young and old age groups are essential for the stock assessment model.

The lack of validated ages for Deepwater Flathead was discussed at the GAB Fishery Assessment Group (GABFAG) meeting held in June 2004. This was seen as a component of the stock assessment model that could be improved relatively easily, given the otolith collections for the FRDC project 2003/003 and by the ISMP. Improvement of the stock assessment model was given a high priority by GABFAG.

A greater understanding of growth zone formation and growth is the main objective of this project. The increase in accuracy of the age estimates will facilitate an increase in the precision of the age estimates, which in turn will assist the accuracy of stock assessments for this species. Outcomes will be extended by the continued inclusion of the validated age estimates into the stock assessment models.

5. Objectives

The project objectives in the original application were:

1. Validate the periodicity of zone formation from marginal zone data and daily age estimation to the first opaque zone.
2. Determine the counts of presumed daily rings between the primordium of the otolith and the outside edge of the first opaque zone of young fish caught in different locations caught in the GAB.
3. Implement standards on the age estimation of this species based on the timing of zone formation and the variability in position and appearance of the first annulus.
4. Understand the variability in growth rates of juvenile Deepwater Flathead.

After review and subsequent consultation with FRDC the projects objectives were revised as follows:

1. Validate the periodicity of zone formation from marginal zone data and daily age estimation to the first opaque zone.
2. Determine the counts of presumed daily rings between the primordium of the otolith and the outside edge of the first opaque zone of young fish caught in different locations caught in the GAB.
3. Implement standards on the age estimation of this species based on the timing of zone formation and the variability in position and appearance of the first annulus.
4. Understand the variability in growth rates of juvenile Deepwater Flathead.

6. Methods

Approach

Whole versus sectioned otoliths

Interpretation of otolith microstructure is influenced by the preparation and reading method selected.

In the early 1990s, most species from Commonwealth managed fisheries were aged using whole otoliths viewed under a dissection microscope using reflected light. This protocol was applied to Deepwater Flathead otoliths. As the science of fish ageing evolved, protocols were modified to improve the accuracy of the age estimates. A major shift in ageing methods was to read the patterns in otolith microstructure, using transverse sections of sagittal otoliths examined microscopically using transmitted light. Within the past 10 years, the method used for ageing Deepwater Flathead otoliths was changed from reading whole otoliths to reading sectioned otoliths.

Before validating the position, timing and periodicity of zone formation, the appropriate otolith preparation and reading method needed to be determined. Chapter 7 Section 7.1 examines the bias inherent in using whole or sectioned otolith preparation for estimating the age of Deepwater Flathead.

Validation

Direct Validation

Direct validation is a term used to describe methods which determine the temporal deposition of material within the otoliths over a known time scale. These studies typically use chemical marking of the otoliths using flourochromes, tag recapture experiments, radiocarbon analyses and tank rearing experiments. Typically a combination of techniques is used (e.g. mark recapture with oxytetracycline marking) and provide absolute age data or data on the frequency of increment deposition.

While absolute age should be the preferred goal of any age validation study (Campana, 2001), direct validation was not possible for Deepwater Flathead within the constraints of this project. During the initial stages of this project, direct validation of Deepwater Flathead age was proposed through chemical marking and captive rearing.

A trial was conducted in which 20 juvenile Deepwater Flathead were held in a flow through tank onboard a commercial vessel. After one hour in the tank all fish had died. Based on this observation it was unlikely that the transfer of live Deepwater Flathead from commercial GABTF vessels to tanks in a laboratory was feasible.

The suitability of other direct validation procedures such as a mark and recapture experiment and bomb radiation was also considered. These procedures were rejected because they were too costly or not applicable to this species.

Indirect Validation

An alternative to direct validation is indirect validation techniques. These are used where direct methods are prohibitively expensive or are species inappropriate. Included in this suite of methods are marginal increment analyses, marginal state analyses and counting daily growth zones. The use of strong modes of cohorts moving through the fishery over time can also be used to determine whether increment counts are consistent with the population biology.

Three approaches of indirect validation were used to determine the timing and periodicity of zone formation in this project. These were:

1. Counts of daily growth increments in the juvenile Deepwater Flathead otoliths to determine the timing of first opaque increment formation
2. Marginal increment and marginal state analysis to determine periodicity across the age range that constituted the majority of the catch. The analysis focused on the year classes 4 to 8 years old, which dominate the commercial catches of the Deepwater Flathead fishery
3. Compare the ages of discrete length modes of an unusually large sample containing small Deepwater Flathead of less than 35 cm to determine if the modes correspond to age-classes.

Standard protocol

Based on the evidence derived from examination of the biases in otolith preparation method and the indirect validation, a manual for ageing Deepwater Flathead has been developed and is included in Appendix 7.

Accuracy of age estimates

The newly defined protocols were then applied to sub-samples of otoliths from each year of the archival collection held by the CAF and which had previously provided age data for the assessment model.

This analysis was undertaken to determine whether previous age estimates contained systematic bias and what implications, if any, this had on the reliability of the estimates produced for the stock assessment of this species.

6.1 Comparison of bias in zone counts from whole otoliths and sectioned ototliths

A comparison between zone counts estimated from whole and sectioned Deepwater Flathead otoliths was undertaken.

Morphometric measurements

Onboard observers working on commercial vessels operating in the GABTF since 1992 have routinely collected Deepwater Flathead otoliths. A sub-sample of 381 otoliths pairs was selected from the Deepwater Flathead otolith archive. This sub-sample represented the length distribution of the landed catch from the Deepwater Flathead trawl fishery.

One otolith from each pair was weighed to the nearest 0.001 g. One otolith was selected for measurement and age reading. Whole otoliths were immersed in water in a black dish and viewed with a Leica Z5 dissecting microscope under reflected light at 6.3x magnification. The length of the otolith was measured along an axis through the primordium. The width of the otolith was measured along an axis through the primordium from the distal to the proximal edge. The distal surface of the otolith was used to measure length and width.

Otoliths were rotated onto the dorsal edge and held with Blue-Tac™ . The otolith thickness was measured along an axis from the distal to proximal side through the approximate position of the primordium (Figure 5). Measurements were collected using the image analysis software Optimas® to the nearest mm.

The relationships between the fish length and the following otolith morphometric data were plotted and regression lines fitted:

- Otolith length
- Otolith width
- Otolith thickness
- Otolith weight.

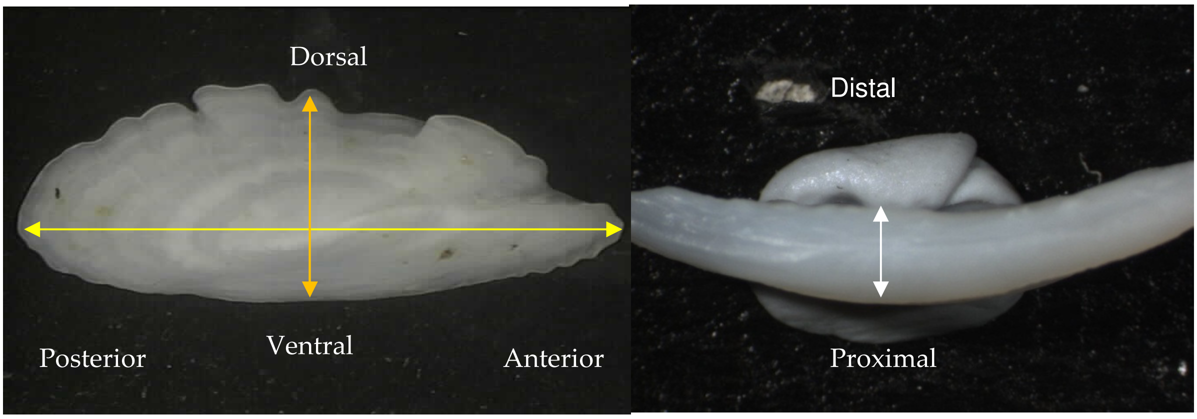


Figure 5. Whole otolith images showing the measurement axis. Orange indicates width axis, yellow indicates length axis and white indicates thickness axis.

Ageing

Whole otoliths were prepared for ageing by immersing the otolith in water against a black background. The otolith was viewed with a Leica Z5 dissecting microscope under reflected light at 6.3x magnification. Age was estimated by counting opaque growth zones (which appear white under reflected light) along a transect from the primordium to the otolith edge towards the anterior tip (Figure 6). Zone positions were measured using a customised image analysis program (Optimas®) and the data recorded in MS Excel ™.

The sister otolith was prepared using a thin transverse section method previously described by Stokie and Krusic-Golub (2005). Otolith sections were examined with a dissecting microscope using transmitted light at 12.5x magnification. Age was assigned by counting opaque growth zones (which appear dark under transmitted light) along a transect form the primordium to the otolith margin on the ventral side of the sulcus (Figure 7). All otoliths were read by the same reader without prior knowledge of length, sex, otolith weight, date and location of capture.

Analysis of data

The bias and precision of zone counts were compared between reading methods using paired t tests and age bias plots (Campana *et al.,* 1995; Campana, 2001). An age difference table was used to examine whether bias was systematic by age-class. The direct zone counts from each method were plotted.

The index of average percent error (IAPE) between reading methods was also estimated. The IAPE is a common method for quantifying this variation (Beamish and Fournier 1981) and is calculated by the equation

$$[IAPE] = \frac{100}{N} \sum_{j=1}^{N} \left(\frac{1}{R} \sum_{i=1}^{R} \frac{\left| X_{ij} - X_j \right|}{X_j} \right)$$

where N is the number of fish aged, R is the number of times fish are aged, Xij is the ith determination for the jth fish, and Xj is the average estimated age of the jth fish.

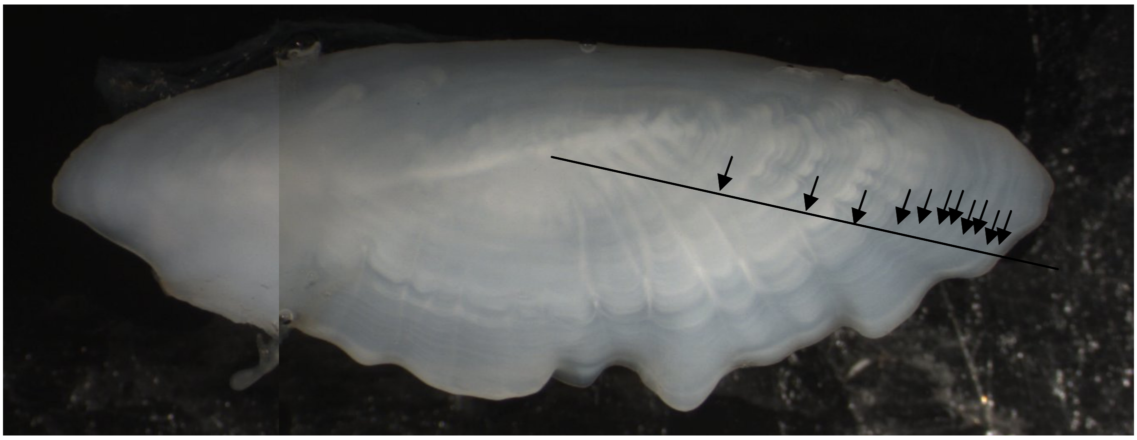


Figure 6. Whole otolith under reflected light. Black line indicates the ageing transect and the black arrows indicate the zone counts. Fish length 57 cm TL, estimated age = 11 years.

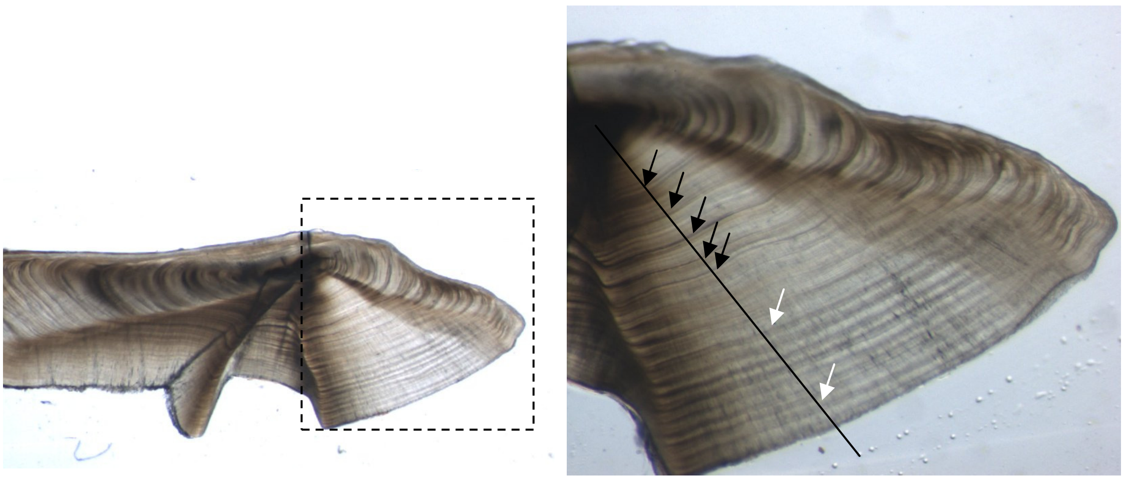


Figure 7. Transverse section of the sister otolith as in Figure 6, indicating the ageing transects and the zone counts. Black arrows indicate the first five years and white arrows indicate the 10th and 15th zone. Estimated age = 16 years.

To establish confidence intervals, a bootstrap technique was employed on the individual error estimates (Efron and Tibshirani 1993). Five thousand samples of error estimates (each the same size as the original) were randomly taken with replacement from the paired readings, and a new IAPE calculated for each. The mean of these replicate IAPEs is the mean bootstrap IAPE. The bootstrap procedure exaggerates any bias present in the original estimate, so it is necessary to correct for this by adding the difference between the original statistic and the bootstrap mean, to the original estimate. The bias-corrected bootstrapped IAPE is calculated as:

Bias-corrected IAPE = Original IAPE + (Original IAPE– Mean Bootstrap IAPE)

The 95% confidence interval was calculated as:

95% C.I. = Bias-corrected IAPE ± (1.96 Standard deviation of Mean Bootstrap IAPE)*

The distribution of the differences between the reading methods were plotted and provided an indicator of ageing error and bias. Regression analysis between [sectioned – whole zone count] and [sectioned zone count] to determine the statistical difference between the two methods (whole vs. sectioned) was undertaken.

6.2 Validation of the position of the first growth increment

Sampling

Juvenile Deepwater Flathead were collected from the central (n=6) and western (n=10) regions of the fishery in April 2003 and between June and September 2005 respectively (Table 2). Integrated Scientific Monitoring Program staff working onboard commercial vessels fishing within the GABTF collected these samples opportunistically. Samples were collected from waters less than 300 m deep.

Small Deepwater Flathead were not collected from the eastern region of the GAB because of the low incidence of specimens less than 30 cm (TL) in the catch. As no juvenile samples were available from the eastern region, otoliths from Deepwater Flathead ≤50 cm in length from this region were chosen to be ground down to their cores (n=12) (Table 2). These samples had previously been aged by the CAF and were selected because the otolith exhibited a relatively clear first opaque zone.

Sample preparation

To reveal daily growth zones, otoliths were prepared to achieve transverse sections approximately 30 µm thick.

Using thermoplastic glue (Crystalbond) each otolith was attached to a slide. The otolith was positioned so that its posterior end was projecting over the slide and the primordium in line with the slide edge. Using 400 grit glass paper, the posterior side was ground away until the primordial region was exposed. A finer grade paper (1200 grit) was used to grind down to the primordium and remove deep scratches. The slide was reheated and the remaining half-otolith removed.

The ground face of the otolith was then glued to a second slide using Crystalbond. The anterior side of the otolith was ground, using the methods described above, until growth zones were visible from the primordium to the outer edge of the otolith. During this stage, the otolith was continually checked to prevent over-grinding.

To improve zone clarity and definition and reduce surface refraction, immersion oil was used to cover the preparation before reading.

Daily age estimation

Daily zone counts and otolith measurements, together with knowledge of the approximate time of annual zone formation, allow the timing and position of first zone formation to be determined.

Daily age estimates were determined by counting micro-zones apparent on the transversely ground face of the sagittal otolith. The method for determining daily growth zones from other growth checks in the otolith preparations follows Stevenson and Campana (1992). When viewed under a compound microscope at up to 1000x magnification, the daily growth zones within the opaque nucleus appeared as wide and translucent, whereas the discontinuous growth zones were relatively new and opaque. A daily zone was defined as the completion of a single translucent and opaque zone.

Opaque zones were counted from the primordium to the first obvious metamorphic mark (M1) and from the metamorphic mark to the presumed first winter zone (O) along the clearest count path. Since daily increments often become difficult to interpret during the winter months, (Francis *et al.*, 1992) daily zones were counted from the metamorphic mark to the start of the presumed first opaque winter zone (O) rather than to the end. Total zone count for the first annulus (A1) was the sum of the two counts. Features associated with the prepared otolith and the terminology used in this report is shown in Figure 8.

The position of the first annulus in otoliths that have been ground is often difficult to locate, as the preparation is very thin and translucent. Otolith preparations of the sister otolith previously prepared for annual age estimation were used to define the position of the first annual zone. Once defined, the location of the first annulus in the daily preparations could be more easily determined.

The distance from the primordium to the beginning of the first opaque zone was measured through the *crista* inferior on the dorsal side and the *crista* superior on the ventral side (Figure 9). The diameter of the metamorphic mark was measured from the primordium to the start of the first opaque zone on both the ventral and dorsal side. Annual age of the specimen was estimated from the sister otolith.

Analysis

For each region the:

- Mean daily zone count was estimated (±2 SE)
- Mean width of daily zones on both the dorsal and ventral side was estimated
- Mean distance from the primordium to the first annual zone was estimated.

Analysis of variance (ANOVA) was used to test differences between areas for these three factors.

6.3 Increment analysis

Otolith collections

Biological information

The sex of the individual was recorded and total length (to the nearest 0.5 cm) was measured. Fish weight (to the nearest 10 grams) was recorded for some of the samples. Otoliths were removed, dried and stored in envelopes marked with collection and biological information such as fish length, sex, date of collection, vessel and gear type.

Otolith archiving

Otoliths were assigned to batches according to a unique combination of location of capture, date of capture and vessel and gear type. Each sample was allocated a unique identification number based on species, batch number and specimen number.

Monthly sample collection

Samples of Deepwater Flathead were collected on a monthly basis in 2001 as part of the ISMP and FRDC project 2003/003. Samples were collected in all but three months: July, September and October 2001. The number of samples collected varied each month (from 100 to 24).

To ensure reasonable numbers of samples in each month were available for marginal increment analysis, a composite year was constructed using samples sourced from different years represenative of each month. Samples were sourced from archival otolith collections from 1998, 1999, 2000 and 2002 (Tables 3 & 4).

Juvenile data set

A large number of juvenile Deepwater Flathead <25 cm TL (n=137) were collected opportunistically during an observer trip in October 2005 from onboard a vessel fishing at 250 metres. The sex, total length and date of capture were recorded on the otolith envelope.

These samples were combined with otoliths from Deepwater Flathead collected opportunistically from the GABTF (n=36).

Table 2. Zone counts and measurements, and biological data for Deepwater Flathead otoliths aged for daily zone counts.

Specimen	Zone count Primordia to M1	Primordia to 1st Opaque zone (Ventral) (mm)	Primordia to 1st Opaque zone (Dorsal) (mm)	M1 Diameter	Zone count Primordia to A1	Mean increment width (μm) ventral	Mean increment width (μm) dorsal	Estimated annual age (yrs)	Fish Length TL (cm)	Sex	DOC	Zone
480015	17	0.33	0.42	0.12	145	2.28	2.90	8	50	M	22-Aug-97	EAST
480019	17	0.42	0.46	0.13	140	3.00	3.29	7	41	J	22-Aug-97	EAST
480032	19	0.48	0.51	0.14	165	2.91	3.09	16	44	M	22-Aug-97	EAST
490003	16	0.33	0.34	0.15	125	2.64	2.72	7	50	F	6-Sep-97	EAST
490004	17	0.33	0.43	0.15	120	2.75	3.58	8	43	F	6-Sep-97	EAST
490005	17	0.33	0.39	-	140	2.36	2.79	6	42	M	6-Sep-97	EAST
490007	-	0.34	0.39	0.16	-	-	-	8	46	F	6-Sep-97	EAST
490015	17	0.41	0.44	0.15	155	2.65	2.84	7	43	F	6-Sep-97	EAST
490016	17	0.36	0.51	0.13	150	2.40	3.40	7	45	F	6-Sep-97	EAST
490026	20	0.32	0.36	0.16	155	2.06	2.32	6	45	M	6-Sep-97	EAST
490028	17	0.4	0.44	0.15	150	2.67	2.93	7	45	M	6-Sep-97	EAST
490029	17	0.42	0.5	0.15	160	2.63	3.13	8	46	F	6-Sep-97	EAST
116011	17	0.36	0.39	0.12	130	2.77	3.00	1	20	J	04-Apr-03	CENTRAL
116013	17	0.39	0.44	0.14	140	2.79	3.14	1	20	J	04-Apr-03	CENTRAL
116024	17	0.37	0.44	0.17	135	2.74	3.26	2	28	J	04-Apr-03	CENTRAL
116028	17	0.43	0.63	0.15	145	2.97	4.34	1	20	J	04-Apr-03	CENTRAL
116031	17	0.34	0.47	-	135	2.52	3.48	1	16.5	J	04-Apr-03	CENTRAL
116034	17	0.34	0.51	0.13	140	2.43	3.64	0	12.5	J	04-Apr-03	CENTRAL
182012	17	0.41	0.44	0.15	165	2.48	2.67	1	21	J	07-Jul-05	WEST
182016	17	0.42	0.53	0.13	270	1.56	1.96	1	18	J	07-Jul-05	WEST
182017	16	0.38	0.46	0.13	235	1.62	1.96	1	17	J	07-Jul-05	WEST
182018	17	0.34	0.42	0.16	200	1.70	2.10	1	15	J	07-Jul-05	WEST
182019	16	0.42	0.57	0.12	230	1.83	2.48	1	16	J	07-Jul-05	WEST
183002	17	0.32	0.53	0.13	155	2.06	3.42	1	15.5	J	06-Jun-05	WEST
183003	17	0.37	0.46	0.17	140	2.64	3.29	1	18.5	J	06-Jun-05	WEST
183004	17	0.33	0.4	0.12	135	2.44	2.96	1	17	J	06-Jun-05	WEST
183009	19	0.33	0.41	0.14	150	2.20	2.73	1	14	J	06-Jun-05	WEST
184001	18	0.41	0.46	0.13	230	1.78	2.00	1	17	J	6-Sep-05	WEST

Legend: - Blanks indicate data were not recorded, - indicates no data, M1 indicates the metamorphic mark, A1 indicates the first annulus, DOC indicates Date-of-Capture M indicates male, F indicates female, J indicates juvenile.

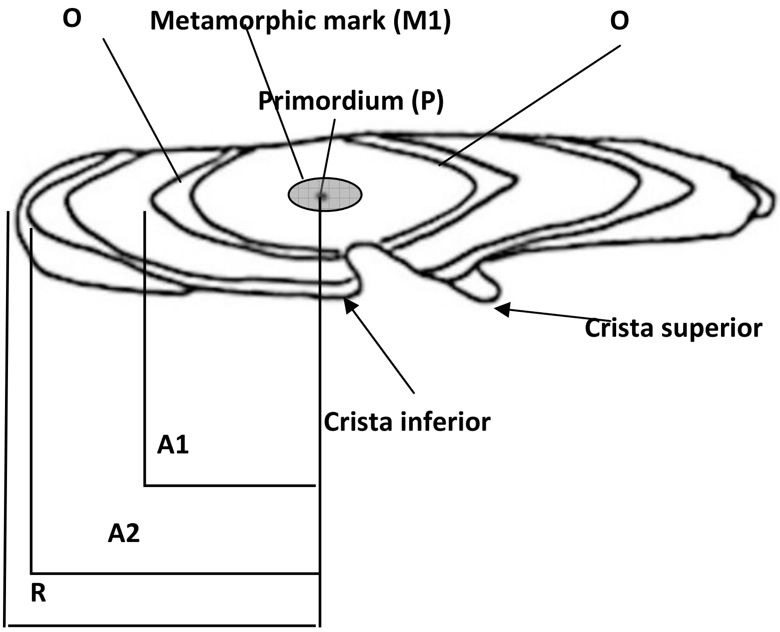


Figure 8. Illustration of transversely ground juvenile Deepwater Flathead otolith, indicating otolith features and terms used in this Section. A1 = 1st annulus, A2 = 2nd annulus, R = Radius. O = presumed first annual zone.

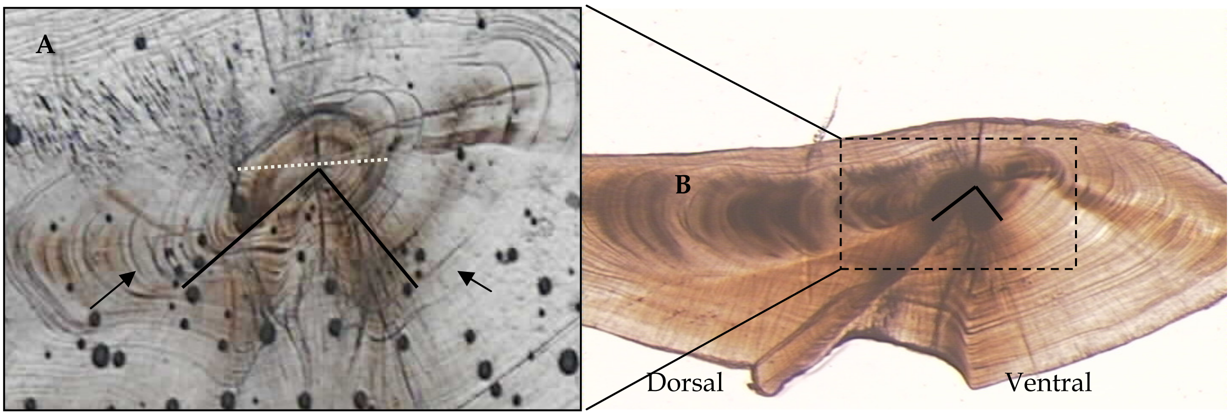


Figure 9. Transversely ground Deepwater Flathead otolith (A) indicating the measurement plane for the metamorphic mark diameter (white broken line) and the primordium to A1 measurement (black solid line) on both the dorsal and ventral side. Relative position is shown on the sister otolith section (B).

Table 3. Number of samples aged and measured for marginal increment analysis on the dorsal surface.

Year	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*	*N*
1998					69		49		111		96		**325**
1999				84		116							**200**
2000							42		43				**85**
2001	100	90	25	100	24	80		82			74	55	**630**
2002			55		56		56			84		107	**358**
N	**100**	**90**	**80**	**184**	**149**	**196**	**147**	**82**	**154**	**84**	**170**	**162**	**1598**

Table 4. Number of samples aged and measured for marginal increment analysis on the ventral surface.

Year	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*	*N*
1998					61		49		105		88		**303**
1999				67		87							**154**
2000							40		40				**80**
2001	108	90	27	99	25	81		82			74	56	**642**
2002			55		56		53			82		106	**352**
N	**108**	**90**	**82**	**166**	**142**	**168**	**142**	**82**	**145**	**82**	**162**	**162**	**1531**

Validation of the periodicity and timing of opaque zone formation

Marginal increment analysis was undertaken on otolith samples that dispayed a clear structure and that were aged between 3 and 9 years, inclusive. This age range was chosen for analysis because it included both juvenile samples and the dominant age-classes in the Deepwater Flathead fishery.

The structure of the otolith margin was investigated at a magnification of 40x.

The periodicity of zone formation was examined by calculating the mean index of completion (*C*). Indices of completion were calculated using the formula:

$C = Wn/Wn\text{-}1$

Where *Wn* is the width of the marginal increment (distance from the start of the last opaque zone to the marginal edge) and *Wn-1* is the width of the previously completed annulus (the distance of the start of the second most outer opaque zone to the distance of the last opaque zone) (Ewing *et al.,* 2003). Measurements were taken using the same digitized computer system as described in the ageing section.

The measurements was measured along a transect from the primordia to the proximal edge adjacent to the sulcus on both the dorsal and ventral side (Figure 10). Mean monthly marginal zones (± 1 standard error) were calculated for separate age-classes, or pairs of age-classes where sample sizes were low, combining sexes and areas, and pooling data for the same months in different years. These data were examined to determine if there was an annual cycle in the timing of zone formation.

The timing of opaque zone formation was also examined by recording the marginal state (W= wide, N=new or I=intermediate) on the proximal margin of the same sub-sample of otoliths (see Appendix 6 for examples of edge type classification). This was recorded for both the dorsal and ventral side of the otolith section. The percentage of otoliths with an opaque zone on the growing edge was plotted according to month of sampling. Separate plots were produced for each age-class and for the dorsal and ventral side. Separate plots for combined age-classes were also produced for each sampling year.

Assignment of age

A birth date of 1 July was chosen as it corresponded to the beginning of the spawning period as determined by Brown and Sivakumaran (2007) and the onset of opaque zone formation. Ages were assigned to every individual according to the number of opaque zones and the date of capture. A new opaque zone found on the otolith of a fish sampled before 1 July was not considered as an annulus in age assignment; when a fish sampled after the assumed birth date had no new opaque zone, an annulus that was supposed to form was considered in age estimation. The time elapsed from June to the sampling month was also considered in assignment of ages.

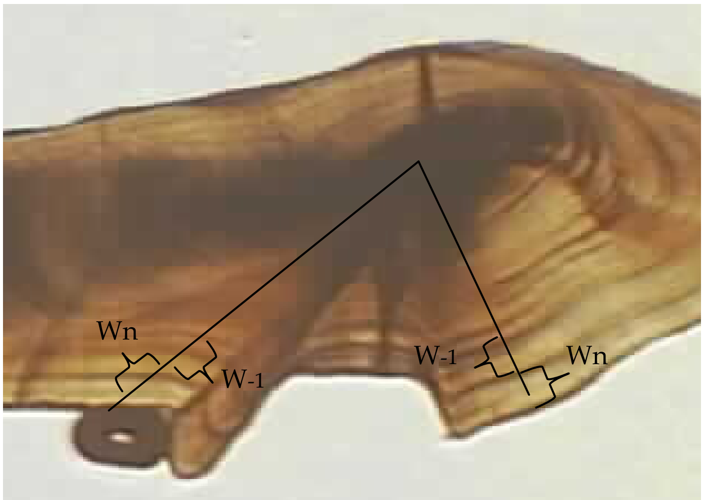


Figure 10. Deepwater Flathead otolith section viewed at 40x indicating the measurements taken for edge analysis.

6.4 Growth, age composition and mortality

Age and length frequency

The ageing data were combined with biological and capture data (otolith weight, fish length and sex, location and date of capture).

Length and age frequency distributions were shown for males, females and males, females and juvenile combined. Distributions were produced for each year and combined years. Age length keys for males, females and combined sexs (including juveniles) were produced for each year (Appendix 5).

Length and age-frequency distributions of male and females were compared. Shapiro–Wilks tests were used to determine the normality of the length and age distributions by sex. Subsequent tests for differences between distributions of length and age by sex were non-parametric, and tested using the Kruskal–Wallis ANOVA (Zar 1974). Analyses were performed using Analyse-it® software in Microsoft™ Excel.

Precision of the age estimates

Precision of the age estimates was determined using standard methods (see Chapter 6 page 14).

Growth

The von Bertalanffy growth curve (VBGC) model was fitted to length-at-age. The VBGC model is expressed as:

$$L_t = L_\infty\left(1 - e^{-k(t-t_0)}\right)$$

where L_t is the length at age t, L_∞ is the asymptotic length, K is the growth coefficient and t_0 is the theoretical age at length 0.

The von Bertalanffy growth curves were fitted using a non-linear, least-squares procedure. Growth curves were calculated for males, females and both sexes combined. Data from immature fish were randomly allocated to the male and female data sets.

Mortality

Total instantaneous mortality (Z) was estimated by catch curve analysis of the Deepwater Flathead age composition for males, females and combined sexes.

Estimating Z from catch curves requires the assumption of constant recruitment over time, and equal selectivity to gear for all age-classes fully recruited to the fishery. The age-length key used for these analyses are presented in Appendix 5.

The slope of $ln(A_t)$ [the estimated number of fish of age t in the age sample], plotted over the age range 5 to 25 years was used to estimate Z for the combined data. The age range 5 to 19 years was used for males and 7 to 21 years used for females.

Total mortalities for males, females and both sexes combined were also estimated for the age range that accounted for ~90% of the age composition (5 to 13 years for combined, 5 to 13 years for males and 7 to 13 years for females).

6.5 Audit of historical ageing data

Historical data

Age estimates have been provided routinely since 1991 for use in the Deepwater Flathead stock assessment. Some of ageing data obtained from otoliths collected prior to 1991 were also used in the stock assessment process. These data were provided by the Central Ageing Facility. In total 7,232 age estimates of Deepwater Flathead have been provided for use in the stock assessment.

Historical Precision

Since 1991, four readers have been used to provide age estimates (Table 5). The CAF reported IAPE's ranging from 3.07% to 4.62% for age estimates provided for analysis (Table 6). No estimates of precision between readers have been reported for this species.

Audit of previous ageing

To audit historical age estimates a sub-set of the historical otoliths were re-read. A minimum of 20 otoliths were selected from each year of collection.

Reader 4 aged the historical samples using the validated protocol. As edge type was not recorded in ageing data prior to 1999, comparisons of the re-aged data were made with the historical age assignment.

To determine accuracy and precision estimates, the methods described for the comparison of age estimates in Section 6.1 (comparison between whole and sectioned ages) were followed. IAPE values should be less than 5% (Morison *et al.*, 1998). Age estimates were considered to be appropriate where the calculated IAPE was less than 5% and the age-bias plots indicated a mode of zero between the original age estimates and the re-reads.

To examine differences between the historical and audited samples, age-difference tables and age-difference distribution plots were used. These were produced for each year.

Table 5. Number of samples aged per year, sub-sample numbers for age error estimation and the IAPE values for each year 1998–1990 and 1993–2006. Source: Central Ageing Facility.

Year	*Reader 1*	*Reader 2*	*Reader 3*	*Reader 4*	*N*
1988		191			**191**
1989		414			**414**
1990	187				**187**
1993	249				**249**
1994	210	240			**450**
1995		454			**454**
1996	287	248			**535**
1997	346		247		**593**
1998			979		**979**
1999			568		**568**
2000			340		**340**
2001			260		**260**
2002				555	**555**
2003				87	**87**
2004				208	**208**
2005				685	**685**
2006				477	**477**
N	**1279**	**1547**	**2394**	**2012**	**7232**

Table 6. Reported Index of Average Percent Error (IAPE) from Deepwater Flathead samples aged at the Central Ageing Facility.

IAPE Reported	*Source*
4.62%	*Anon, 1996*
3.32%	*Anon, 1998*
3.58%	*Krusic-Golub and Morison, 1999*
3.53%	*Krusic-Golub, 1999*
4.02%	*Krusic-Golub and Robertson, 2001*
3.07%	*Stokie and Talman, 2003*
3.23%	*Stokie and Krusic-Golub, 2005*

7. Results and Discussion

This Chapter is structured as follows:

- 7.1. Comparison of bias in zone counts from whole otoliths and sectioned otoliths
- 7.2. Validation of the position of the first growth increment
- 7.3. Increment analysis
- 7.4. Growth, age composition and mortality
- 7.5. Audit of historical ageing data

Full methods that relate to each particular section are described in detail within Chapter 6.

7.1. Comparison of bias in zone counts from whole otoliths and sectioned ototliths.

Introduction

It is generally accepted that sectioned otoliths provide more reliable estimates of age than whole otoliths. Many age and growth studies have demonstrated the under estimation of age from reading growth zones on whole otoliths (Dwyer *et al.*, 2003; Campana, 2001; Beamish and Fournier, 1981). Otoliths do not grow linearly in length, width and depth throughout the fish's life history. As a fish ages, growth in the length and depth of the otolith slows (viewed *in vivo*), and in many cases, ceases, while the otolith continues to thicken. This results in a stacking of growth increments in the sagittal plane between the otolith core and proximal surface. When this occurs, ages determined from whole otoliths will underestimate the zone count as these outer growth increments cannot be viewed in the whole otolith. These zones can only be observed by taking a thin section through the otolith.

This pattern of otolith growth has been observed in other platycephalids. Hyndes *et al.* (1992) determined that differences in age estimates derived from reading whole and sectioned *P. speculator* otoliths increased with age. Age was underestimated by as much as 6 years in some samples when whole otoliths were used. Jordan (1998) used sectioned otoliths to obtain validated age estimates of *P. bassensis* and produced longevity estimates far greater than estimated by Brown (1997), who derived age estimates by reading whole otoliths. While no comparison between whole and sectioned age estimates were made for *P. fuscus*, Gray *et al.* (2002) noted that from preliminary examination, whole otoliths were more difficult (and thus less accurate) to interpret than sectioned otoliths.

Since 2000, Deepwater Flathead have been aged by counting opaque growth zones on transversely sectioned otoliths. While estimates of Deepwater Flathead age are considered to be within acceptable precision levels (Krusic-Golub and Stokie, 2008; Morison *et al.,* 1998), comparisons between age estimates made from reading whole versus sectioned otoliths has not been presented for this species.

While it is likely that ages estimated from sectioned otoliths will provide a more robust, accurate and precise measurement than ages determined from reading whole otoliths, the difference between age estimates produced by both methods can be small. The influence of small differences in age composition often has little impact on estimates of growth rate for some fish species (Marriot *et al.,* 2006). This is not the case for Deepwater Flathead where preliminary modelling suggested that a bias of two years in the age estimates would affect the biomass projections by up to 30% (Wise and Tilzey, 2000).

It is appropriate when developing age estimation protocols for that species to compare the accuracy and bias of age estimates derived from both whole and sectioned otoliths.

Results

Otolith morphology

The otolith length–fish length relationship was linear for the range of lengths examined (r^2=0.83; Figure 11A). The otolith width-fish length relationship is also linear for the range examined (r^2=0.70; Figure 11B). The otolith depth–fish length relationship is poorly represented by a linear model (r^2=0.34; Figure 11C). A linear model was fitted for comparative purposes only. The relationship is essentially curve linear and shows a marked decrease in otolith depth at a fish length of approximately 45 cm (Figure 11C).

The otolith length–otolith weight relationship was linear for the range of samples examined (r^2=0.81; Figure 12A). The otolith width–otolith weight relationship was linear for the range of samples examined (r^2=0.62; Figure 12B). The otolith depth–otolith weight relationship was linear for the range of samples examined (r^2=0.61; Figure 12C).

Growth zones

Distinct growth zones, which were presumed to be annual, could be observed for the first 8–10 years in both the whole and thin-sectioned otoliths (Figure 6 and Figure 7; page 15). Zones after 10 years in whole otoliths were more difficult to observe as the opaque zones on the outer edge of the otolith became more translucent and difficult to distinguish from the translucent zones. The spacing between zones in whole otoliths also narrowed with age. Whole otoliths from old fish (large otoliths) also were characterised by a large and diffuse centre. In sectioned otoliths, the centre was more defined and the opaque zones after the first 5–7 zones were distinct from translucent zones and evenly spaced compared with those of whole otoliths (Figure 7).

Zone counts ranged from 3 to 14 for whole otoliths and 3 to 22 for sectioned otoliths (Figure 13A and B). Fish length increased with zone counts from examining both whole and sectioned otoliths. The maximum zone count from the examination of whole otoliths was 14, while the maximum zone count from sectioned otoliths was 22.

Von Bertalannfy (VB) curves were fitted to zone count from whole otoliths *vs.* fish length and zone count from sectioned otoliths vs. fish length. The asymptotic length for sectioned zone count *vs*. fish length was 57.9 cm, k= 0.26 and T_0 =-1.6. The asymptotic length for whole zone count *vs*. fish length was 116.9 cm, k= 0.04 and T_0 =-9.08. The negative T_0 for the whole zone count *vs*. fish length was less than -7 indicating a lack of samples in the younger age-classes. The T_0 for the sectioned zone count *vs*. fish length was -1.6. These low T_0 values are a reflection of the length frequency of the catch. The fitted parameters for the VB curve for section zone count *vs*. fish length are biologically reasonable, indicating the model was suitable to describe fish growth. This was not case for the whole zone count *vs*. fish length relationship.

Zone count and otolith morphology

The relationships between otolith weight and zone counts derived from sectioned and whole otoliths were linear (Figure 14 A and B). The correlation between zone count from whole otoliths and otolith weight was r^2 = 0.57 while the correlation between zone count from sectioned otoliths and otolith weigh was r^2 = 0.50. Both correlations were weak indicating a variable relationship between otolith length and zone counts for both methods.

Zone count and method

Zone counts from both methods were compared (Figure 15). A line of unity (x=y) was drawn to indicate parity between the two methods. Paired zone counts from sister otoliths collected from the same fish differed by 16 zones. The correlation between whole and sectioned zone counts from sister otoliths was weak (r^2 = 0.32). The slope (slope=0.35) indicates that whole otoliths underestimate sectioned zone counts.

Zone counts from sectioned otoliths tended to be lower than those obtained from whole otoliths up to and including the first 6 zones (Table 7). After 7 zones, more zones can be seen on sectioned otoliths than on whole otoliths taken from the same fish. After 13 zones, 100% of the samples had a higher zone count using sectioned otoliths (Table 7, Figure 16).

The frequency distribution of differences between zone counts from both whole and sectioned otoliths shows a mode on zero (Figure 17). The distribution was positive indicating higher zone counts were obtained using sectioned otoliths.

A paired t test showed a significant difference between zone counts estimated from paired reading between whole and sectioned otoliths ($t=7.74$; $p<0.05$; Appendix 3). From the regression analysis of whole *vs*. sectioned zone counts, the 95% confidence intervals for the slope and intercept indicated that they were significantly different from unity to zero, respectively, indicating a bias for whole zone counts (Appendix 3). Zone counts from sectioned otoliths agreed with whole zone counts in 21% of cases and 51% were within 2 zones. The distribution of differences indicated that ages derived from sectioned otoliths compared to whole otoliths had a consistent negative bias up to six zones and a large positive bias after 10 zones (Table 7 and Figure 17).

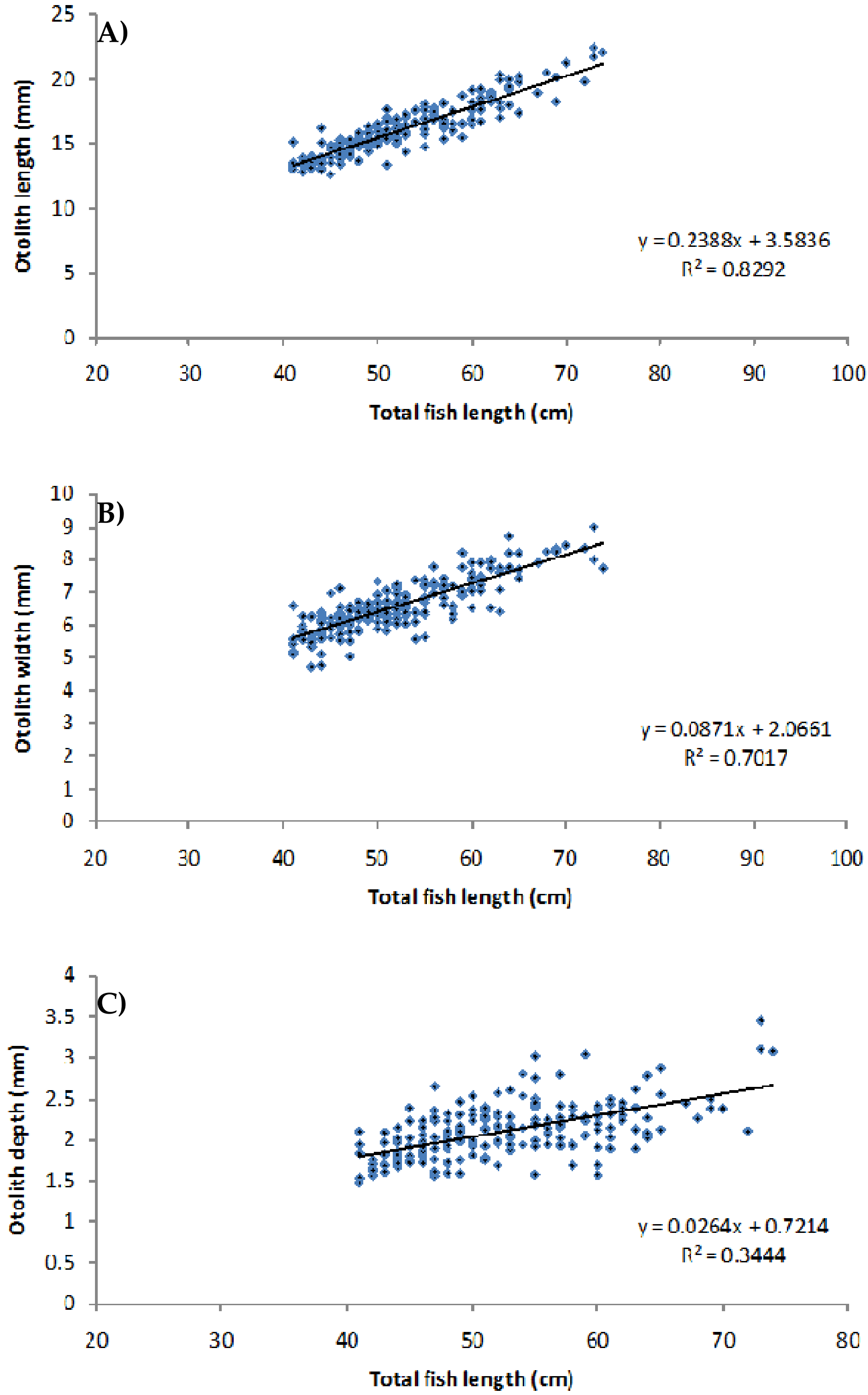


Figure 11. Relationship between fish length (mm TL) and A) otolith length (mm), B) otolith width (mm) and C) otolith depth (mm).

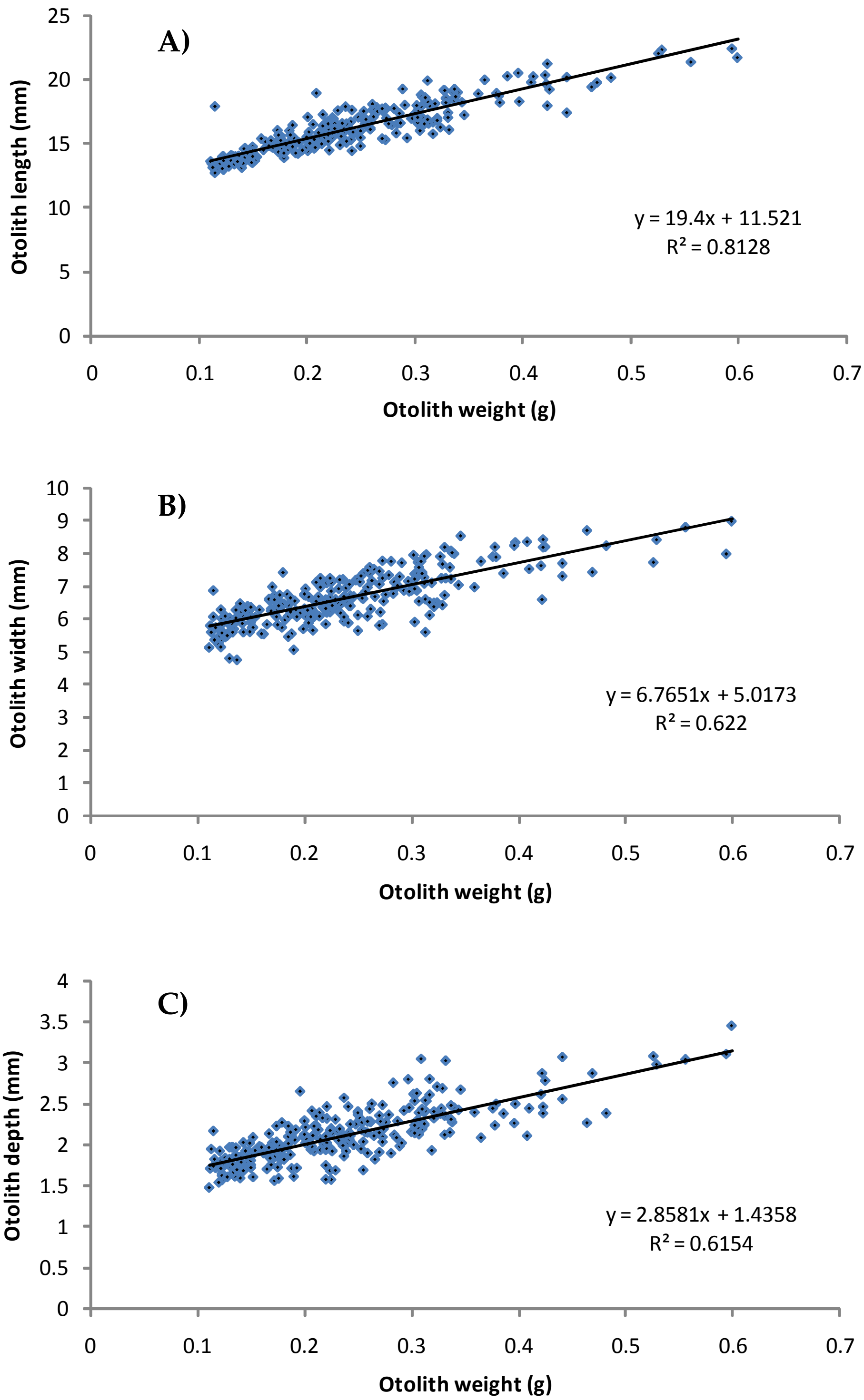
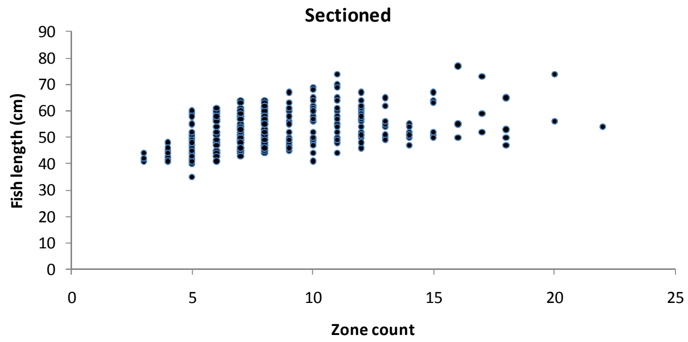


Figure 12. Relationship between otolith weight (g) and A) otolith length (mm), B) otolith width (mm) and C) otolith depth (mm).

A)



B)

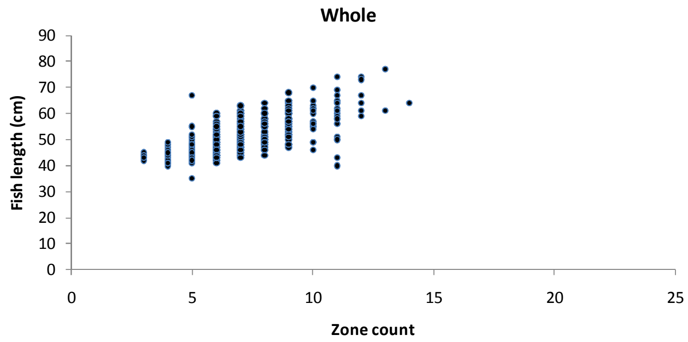
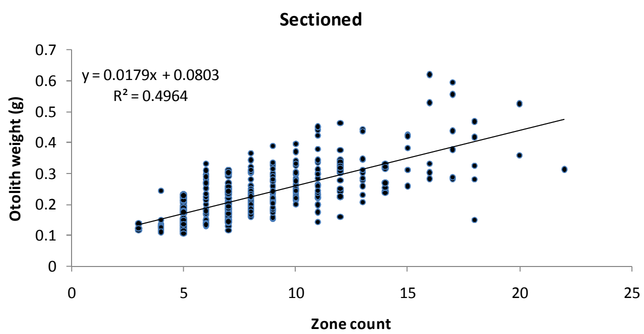


Figure 13. Relationship between fish length and zone counts derived from A) sectioned otoliths and B) whole otoliths (sister otoliths).

A)



B)

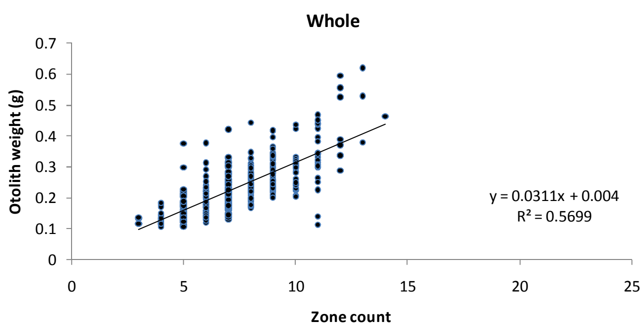


Figure 14. Relationship between otolith weight and zone counts derived from A) sectioned otoliths and B) whole otoliths.

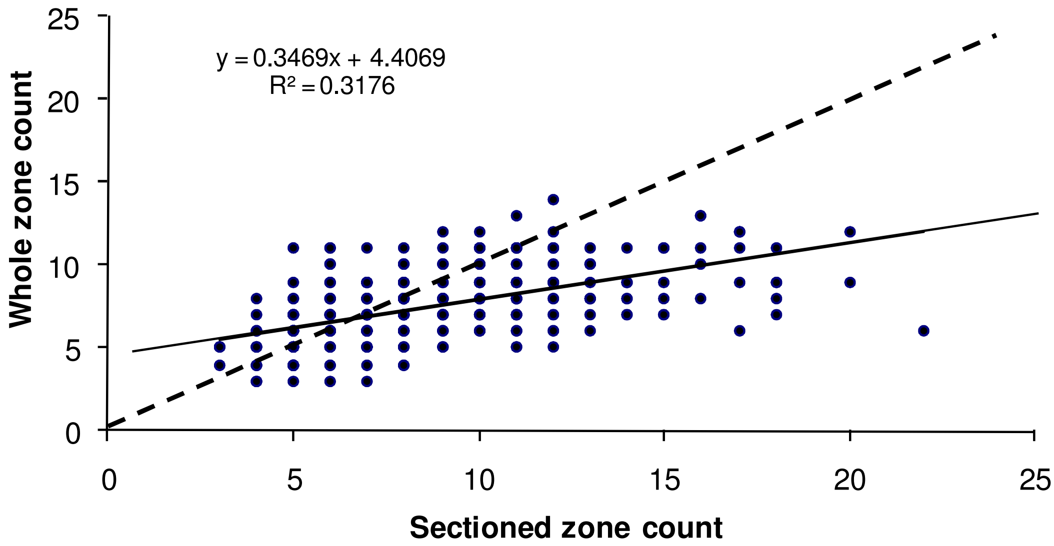


Figure 15. Linear comparison of from whole and sectioned zone counts from the same sample. Black line is the regression line. The dotted line shows the Y=X relationship.

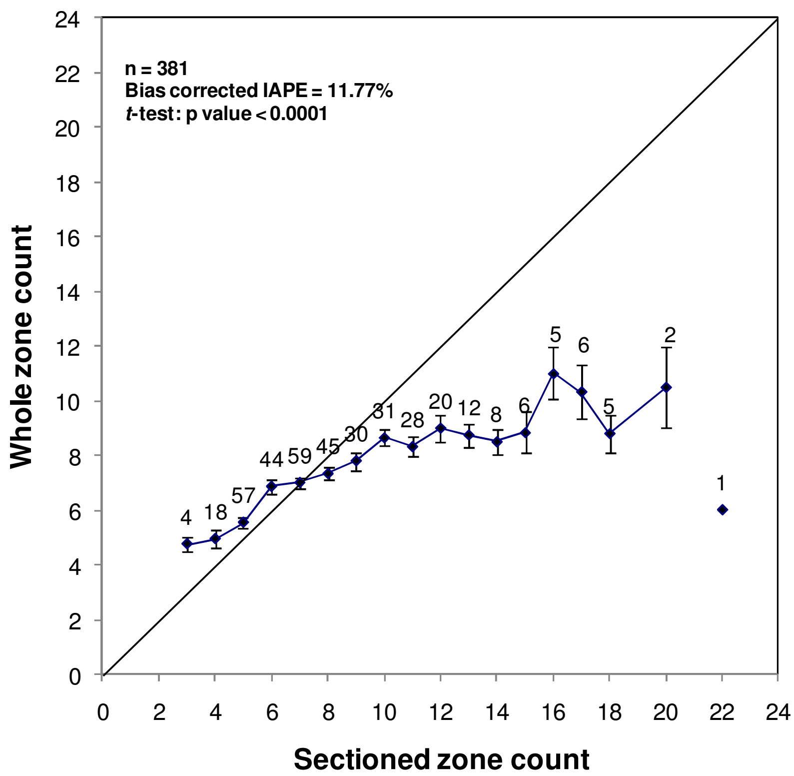


Figure 16. Age bias plots comparing zone counts for whole and sectioned otoliths. Error bars are standard error. Black line indicates the X=Y relationship.

Table 7. Age difference table between Deepwater Flathead sectioned and whole age estimates.

Difference	Sectioned Age																				
(section-whole)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	22	N	% Agreed	% ±1
-10																						
-9																						
-8																						
-7																						
-6				1																1		
-5					2															2		
-4			1	1	3	1														6		
-3			1	2	3		1	1												8		
-2		3	3	8	5	9	3	1	1	1	1									35		
-1		1	7	13	11	8	5	4	4											53		
0	**0**	**0**	**3**	**19**	**11**	**21**	**12**	**3**	**5**	**4**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**79**	**21%**	**51%**
1			3	12	5	13	11	7	6	2	3									62		
2				1	3	5	8	7	5	5	2	1								37		
3					1	1	3	4	5	6	6	4	1		2					33		
4						1	2	3	4	5	2	1		2						20		
5										3	2	4	3		1	3				16		
6										2	2	1	2	1	1	1				10		
7											1	1	2	1			1			6		
8														2	1	1		1		5		
9																	2			2		
10+																1	2	1	1	1		
N		4	18	57	44	59	45	30	30	28	20	12	8	6	5	6	5	2	1	380		

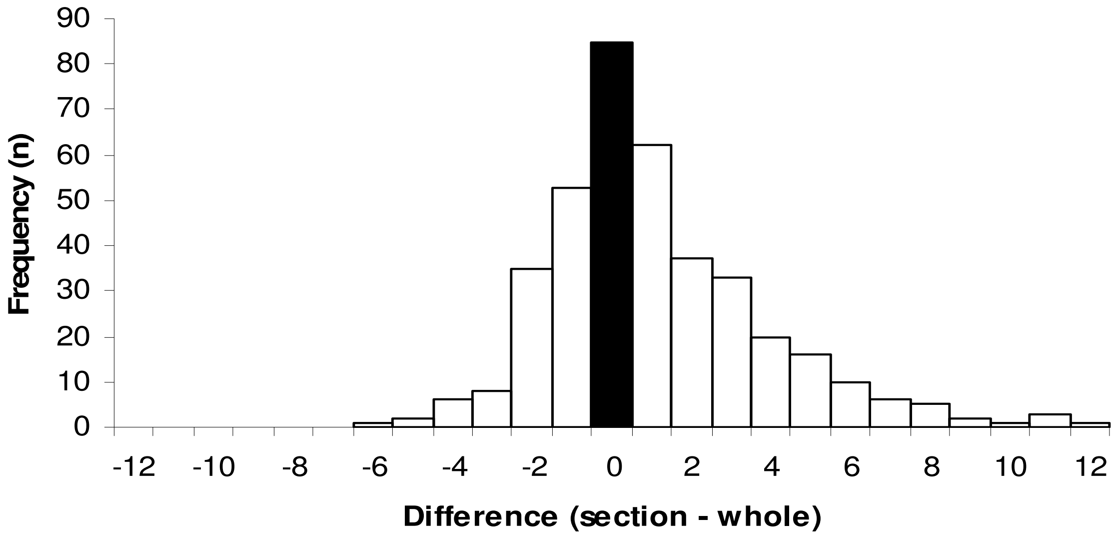


Figure 17. Zone count difference plot of sectioned zone count minus whole zone count.

Discussion

Zone counts estimated from whole and sectioned sister otoliths were significantly different. This indicates that the method of ageing can significantly affect the derived age structure of Deepwater Flathead.

A systematic bias of the underestimation the older fish was identified in ages derived from whole otoliths. Zone counts from whole otoliths ranged from 3 to 12 compared with 3 to 22 from sectioned otoliths. The extent of the underestimation from whole otoliths was large; in excess of 10 years and all zone counts derived from whole otoliths after 13 were underestimated.

There is a tendency for zones counts derived from whole otoliths to be lower than of sectioned otoliths. The outer zones on the distal surface of the whole otolith become very opaque and tightly spaced near the edge. The resolution between opaque and translucent zones was poor and interpretation was difficult. These results indicate that whole otoliths provide varied zone counts and have the potential to underestimate age estimates compared to sectioned otoliths. Similar observations have been observed in other age estimation studies; for example, Kimura and Lyons (1991) and Dwyer *et al.* (2003) also observed this bias when comparing zone counts from whole and sectioned otoliths from Yellowtail Flounder (*Limanda ferruginea*).

Underestimation of the age of Deepwater Flathead has profound implications for the sustainable management of the Deepwater Flathead fishery. Modelling, assuming that fish are two years older than estimated ages, indicated there would be a 30% decrease in available biomass. The level of uncertainty in estimated zone counts derived from whole otoliths is greater than a bias of ± 2.

Un-validated age estimates suggest the maximum age for Deepwater Flathead to be 33 for females and 28 for males. Ages estimated from whole otoliths do not provide sufficient accuracy to be used to populate the age-based models that underpin the Deepwater Flathead stock assessment.

In contrast, sectioning of Deepwater Flathead otoliths enhanced the ability to interpret the opaque zones from translucent zones as the distance and appearance of the opaque and translucent zones were consistent. The potential for the underestimation of zone counts is greatly reduced.

A description of growth derived from fish length versus time (estimated from zone counts) found the parameters of a VB curve derived from zone counts estimated from sectioned otoliths provided more biologically reasonable estimates of L_∞ and T_0 (58 and -1.6 respectively). These parameters were biologically unfeasible (L_∞ = 116 and $T_{0=}$ -9.0) when derived from ages estimated from whole otoliths.

While sectioned otoliths provide greater clarity of the outer increments, estimates of the first six zone counts made from sectioned otoliths were consistently lower than those obtained from whole otoliths. This study has shown that 69% of the estimates of the first six zone counts from both whole and sectioned otoliths were within ± 1, and a further 20% within ± 2. While not large, underestimation of zone counts in otoliths from these young fish is also problematic.

In the Deepwater Flathead fishery, the majority of the catch is comprised of 4 to 8 year-old-fish and age-at-recruitment is at approximately 3–5 years. Accurate and precise (repeatable) age estimates for these age-classes are also essential for the ecologically sustainable development of the fishery, as the age estimates of these young fish can influence the outcomes of the model estimates for growth, mortality and longevity.

The inaccuracies of the estimates of the first six zones are most likely a consequence of the incorrect interpretation of the position of the first annual zone. The inaccuracies arise because of the microstructure of the Deepwater Flathead otolith. Deepwater Flathead otoliths exhibit a large opaque centre that obscures the microstructure, making identification of the first few zones difficult. Additionally a large number of fine irregular zones in the inner region of the otolith complicate the identification of the first zone. These fine irregular zones are presumed to be sub-annual.

These interpretation difficulties are compounded by the fact the Deepwater Flathead fishery is not characterised by the presence of a few strong year classes, which move through the fishery. The presence of strong year classes can be used to support the accuracy of the age estimates.

Validation of the position of the first annual zone will improve the accuracy of estimates of the first six annual zones.

Conclusion

The most accurate method for examining age and growth of Deepwater Flathead is through counts of annuli on transverse otolith sections.

Validation of the position of the first annual growth zone will improve the accuracy the ageing, especially the younger fish.

Section 7.2 aims to validate the position of the first annual zone indirectly using daily age counts.

7.2. Validation of the position of the first growth increment.

Introduction

Deepwater Flathead have traditionally been aged assuming one opaque and one translucent zone is formed each year on the sectioned face of sagittal otoliths. This is consistent with the methods used to age other species from the Platycephalidae family (Hyndes *et al.*, 1992; Jordan, 1998; Masuda, 2002). While the timing and periodicity of zone formation has been validated in several Australian flathead species (for example, Yank Flathead (Hyndes *et al.*, 1992), Dusky Flathead (Gray *et al.*, 2002), and Sand Flathead (Koopman, 2004)), no method has been described to show the timing and position of annuli formation for Deepwater Flathead.

While zone counts estimated from sectioned otoliths were shown to be preferable to those taken from whole otoliths (Chapter 7 Section 7.1), uncertainties in the position of the first few growth zones remains an issue. The area identified as causing interpretation difficulties is the position of the first opaque zone (annulus), which represents the end of the first year of growth. The interpretation of the position of this zone is confounded by the presence of a large opaque and dense primordium and the presence of irregular zones in the centre of most Deepwater Flathead otoliths. The irregular zones are presumed to represent sub-annual growth.

Deepwater Flathead is a multiple spawner that spawns over eight months from October to May (Brown and Sivakumaran, 2007). The length of the spawning season influences the position of the first annuli (Shepherd and Grimes, 1983; Allman *et al.*, 2005; Karlou-Riga, 2000). This variation in spawning period complicates the interpretation of the first annulus.

Another difficulty with estimating the age of Deepwater Flathead is the absence of strong age-classes moving through the fishery, which can be used as a validation tool for ageing.

While analysis of discrete length frequency modes can provide validation for age-classes, without knowledge of the timing and position of the first increment it is possible that the earliest length mode will not correspond with the first year class.

Very few juvenile Deepwater Flathead are caught by trawlers operating in the GABTF. The rate of discards of small fish is low (less than 1% 2000–2006) as only 3.3% of Deepwater Flathead caught are under 30 cm. Without adequate samples of small fish, it has not been possible to determine accurately the growth over the full life history of Deepwater Flathead.

In 2005 a small number of juvenile Deepwater Flathead <20 cm were caught by a commercial fishing vessel operating in the GAB. Otoliths from these fish were obtained and provide an opportunity to investigate the microstructure of the otoliths to validate the position of first opaque zone formation.

The microstructure of otoliths describes the daily growth of larval fish (Radtke, 1984). This microstructure can be used to identify the position of the first annual zone. The use of otolith microstructure to verify the first annulus has widely been applied to other species (Taniuchi *et al.*, 2004; Wright, 2002; Itoh, 1996). Koopman (2004) used daily zone counts from Sand Flathead (*P. bassensis*) otoliths and determined that the first annulus was formed at approximately 370 days old.

This Section describes the timing and position of first annulus formation from daily zone counts.

Results

Samples

The clearest reading path was along a non-linear transect on the ventral side. There was no difference in the position and timing of the metamorphic mark between each region (central, eastern and western GAB) (Figures 18 and 19). Appendix 4 contains example images of the otoliths used to determine daily ages.

Daily zone counts

Zone counts from the primordium to the first annual zone were determined for 27 of the 28 otoliths prepared (Table 8, summary data are presented in Appendix 4). An estimate was unable to be determined for one sample due to preparation failure.

Daily zone counts from the hatch mark to the first annual zone ranged from:

- 135 to 270 days for the western zone (n=10)
- 135 to 145 days for the central zone (n=6)
- 120 to 165 days for the eastern zone (n=11; Table 8).

The otolith samples collected from the western region of GAB in June and September 2005 all exhibited an opaque zone just inside or on the otolith margin. One sample, caught in early April 2003 from the central region was estimated at 138 days old. In this sample the first opaque zone had not yet formed.

The mean daily zone count (± 2 SE) from the primordium to the first zone was:

- 191.0 (± 30.3) for 10 samples from the western zone
- 137.5 (± 3.7) for 6 samples from the central zone
- 145.9 (± 8.1) for 11 samples from the eastern zone of the GABTF (Figure 20).

The mean daily zone count was significantly different across the three zones of the GABTF (f=7.8, F_{crit}=3.4, p<0.05, n-=26).

The mean width of the daily growth increment on the ventral side was:

- 2.03 µm (± 0.25) for the western zone
- 2.70 µm (± 0.16) for the central zone
- 2.58 µm (± 0.17) for the eastern zone (Figure 21).

The mean width of the daily growth increment on the dorsal side was:

- 2.56 µm (± 0.35) for the western zone
- 3.48 µm (± 0.39) for the central zone
- 3.00 µm (± 0.21) for the eastern zone (Figure 21).

The mean width of the daily growth increment varied significantly across the three zones of the GABTF (ventral f=11.4, F_{crit}=3.4, p<0.05, n=26; dorsal f=7.6, F_{crit}=3.4, p<0.05, n=26).

The mean distance from the primordium to the first annual zone on the ventral side adjacent to the sulcus was:

- 0.37 mm (±0.026) [range 0.32–0.42] for the western zone
- 0.37 mm (±0.028) [range 0.34–0.43] for the central zone
- 0.37 mm (±0.030) [range 0.32–0.48] for the eastern zone (n=12) (Figure 22).

The mean distance from the primordium to the first annual zone on the dorsal side adjacent to the sulcus was:

- 0.47 mm (±0.04) [range 0.40–0.57] for the western zone
- 0.48 mm (±0.07) [range 0.39–0.63] for the central zone
- 0.43 mm (±0.03) [range 0.34–0.51] for the eastern zone (Figure 22).

The mean distances from primordium to the first annual zone were similar across the three zones of the GABTF (ventral f=0.001, F_{crit}=3.38, p>0.05, n=27; dorsal f=1.45, F_{crit}=3.38, p>0.05, n=26).

Table 8. Zone counts and measurements, and biological data for Deepwater Flathead otoliths aged for daily zone counts.

Specimen	Zone count Primordia to M1	Primordia to 1st Opaque zone (Ventral) (mm)	Primordia to 1st Opaque zone (Dorsal) (mm)	M1 Diameter	Zone count Primordia to A1	Mean increment width (μm) ventral	Mean increment width (μm) dorsal	Estimated annual age (yrs)	Fish Length TL (cm)	Sex	DOC	Zone
480015	17	0.33	0.42	0.12	145	2.28	2.90	8	50	M	22-Aug-97	EAST
480019	17	0.42	0.46	0.13	140	3.00	3.29	7	41	J	22-Aug-97	EAST
480032	19	0.48	0.51	0.14	165	2.91	3.09	16	44	M	22-Aug-97	EAST
490003	16	0.33	0.34	0.15	125	2.64	2.72	7	50	F	6-Sep-97	EAST
490004	17	0.33	0.43	0.15	120	2.75	3.58	8	43	F	6-Sep-97	EAST
490005	17	0.33	0.39	-	140	2.36	2.79	6	42	M	6-Sep-97	EAST
490007	-	0.34	0.39	0.16	-	-	-	8	46	F	6-Sep-97	EAST
490015	17	0.41	0.44	0.15	155	2.65	2.84	7	43	F	6-Sep-97	EAST
490016	17	0.36	0.51	0.13	150	2.40	3.40	7	45	F	6-Sep-97	EAST
490026	20	0.32	0.36	0.16	155	2.06	2.32	6	45	M	6-Sep-97	EAST
490028	17	0.4	0.44	0.15	150	2.67	2.93	7	45	M	6-Sep-97	EAST
490029	17	0.42	0.5	0.15	160	2.63	3.13	8	46	F	6-Sep-97	EAST
116011	17	0.36	0.39	0.12	130	2.77	3.00	1	20	J	04-Apr-03	CENTRAL
116013	17	0.39	0.44	0.14	140	2.79	3.14	1	20	J	04-Apr-03	CENTRAL
116024	17	0.37	0.44	0.17	135	2.74	3.26	2	28	J	04-Apr-03	CENTRAL
116028	17	0.43	0.63	0.15	145	2.97	4.34	1	20	J	04-Apr-03	CENTRAL
116031	17	0.34	0.47	-	135	2.52	3.48	1	16.5	J	04-Apr-03	CENTRAL
116034	17	0.34	0.51	0.13	140	2.43	3.64	0	12.5	J	04-Apr-03	CENTRAL
182012	17	0.41	0.44	0.15	165	2.48	2.67	1	21	J	07-Jul-05	WEST
182016	17	0.42	0.53	0.13	270	1.56	1.96	1	18	J	07-Jul-05	WEST
182017	16	0.38	0.46	0.13	235	1.62	1.96	1	17	J	07-Jul-05	WEST
182018	17	0.34	0.42	0.16	200	1.70	2.10	1	15	J	07-Jul-05	WEST
182019	16	0.42	0.57	0.12	230	1.83	2.48	1	16	J	07-Jul-05	WEST
183002	17	0.32	0.53	0.13	155	2.06	3.42	1	15.5	J	06-Jun-05	WEST
183003	17	0.37	0.46	0.17	140	2.64	3.29	1	18.5	J	06-Jun-05	WEST
183004	17	0.33	0.4	0.12	135	2.44	2.96	1	17	J	06-Jun-05	WEST
183009	19	0.33	0.41	0.14	150	2.20	2.73	1	14	J	06-Jun-05	WEST
184001	18	0.41	0.46	0.13	230	1.78	2.00	1	17	J	6-Sep-05	WEST

Legend: - Blanks indicate data were not recorded, - indicates no data, M1 indicates the metamorphic mark, A1 indicates the first annulus, DOC indicates Date-of-Capture M indicates male, F indicates female, J indicates juvenile.

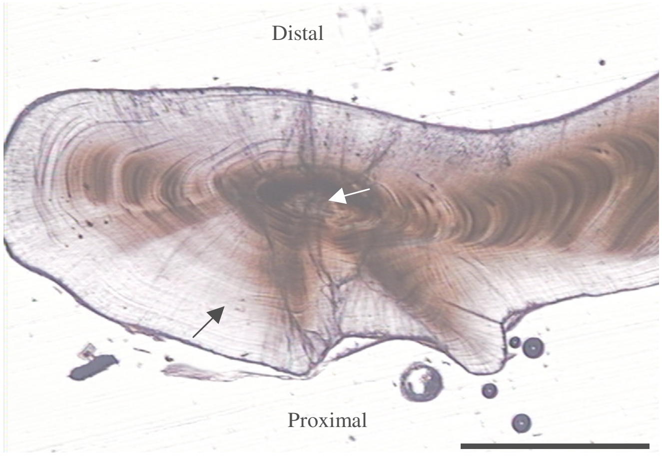


Figure 18. Transverse section of a juvenile Deepwater Flathead otolith. White arrow indicates position of metamorphic mark. Black arrow indicates position of first annual zone. Estimated age to the first annual zone is 230 days. Scale bar 500 μm.

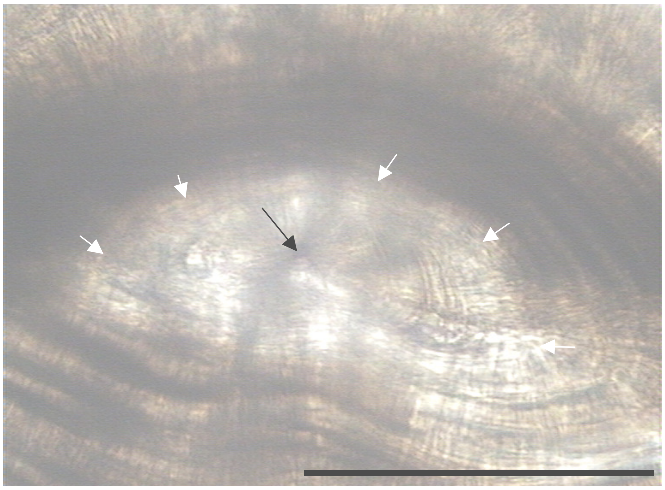


Figure 19. A magnified (400x) view of a juvenile Deepwater Flathead otolith revealing daily zones and metamorphic mark. Black arrow indicates position of primordium. White arrow indicates position of settlement mark. Scale bar 100 μm.

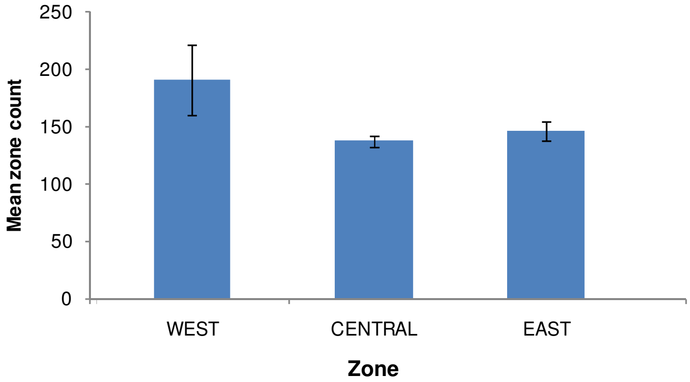


Figure 20. Mean zone count (± 2 SE) for samples within each zone region.
(Sample size n=10 West zone; n=6 Central zone; n=12 Eastern zone).

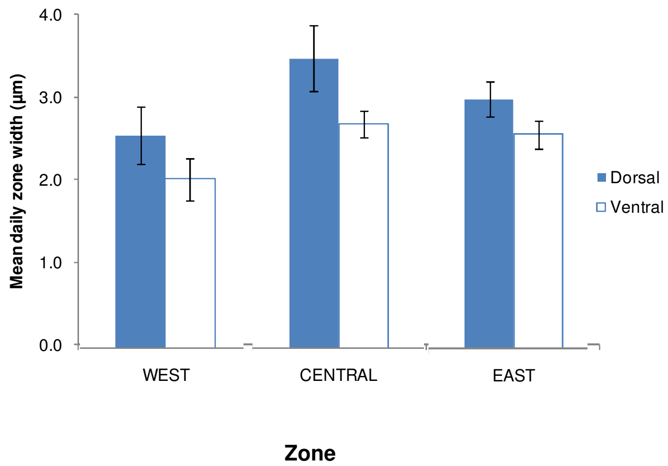


Figure 21. Mean width of the daily increment (± 2 SE) from the primordium to the outer edge of the first opaque zone.
(Sample size for both ventral and dorsal; n=10 West zone; n=6 Central zone; n=11 Eastern zone).

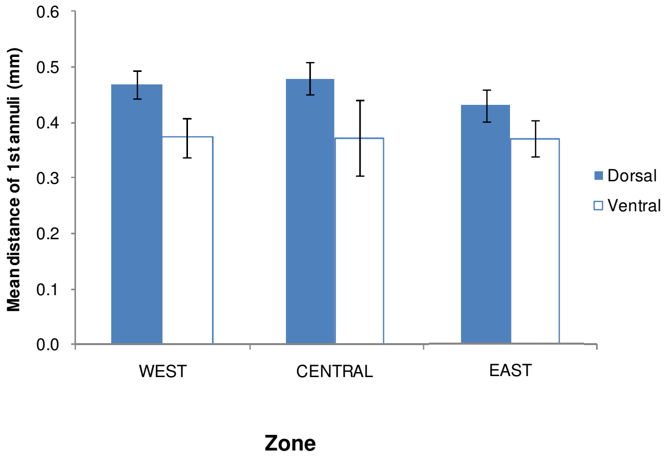


Figure 22. Mean distance from the primordium to the first annular zone (±2SE).
(Sample size for both ventral and dorsal; n=10 West zone; n=6 Central zone; n=11 Eastern zone).

Discussion

Although the microstructure in the sagittal otoliths for Deepwater Flathead has not been validated, the microstructure and zones observed in this study are similar to that described by Pannella (1971, 1974) and Campagna and Neilson (1985). This study indicates that counts of daily zones can provide a reliable method to determine the position of the first annual zone in Deepwater Flathead.

Using the data available on spawning times (Brown and Sivakumaran, 2007) and the timing of opaque zone formation, it can be assumed that the amount of growth between the primordium and the outer edge of the opaque nucleus would represent less than one year of growth. As zones could not be observed past the start of the first opaque zone, presently it can only be assumed that the end of the first translucent zone succeeding the opaque nucleus represents approximately 1-year's growth.

The daily zone counts between the three Zones (Western, Central and Eastern) ranged between 120 days and 270 days before the formation of the first annual increment. Given the assumption that the time of zone formation between the zones is consistent, then the spawning may vary between regions by up to 150 days for the samples examined. The largest variation in spawning was seen in the Western Zone where spawning was between 135 and 270 days. The Central Zone had a difference of only 10 days before formation of the first annual increment. The Western Zone was more variable than the Central Zone with a difference of 45 days before the formation of the first annual zone. The mean daily zone counts were significantly different indicating that Deepwater Flathead in the Western Zone spawn before the fish in the Central or Eastern Zones. Opaque zone formation may occur at different times between regions within the GAB. It is unlikely that the difference between opaque zone formation would be greater than the 150 days demonstrated in this study.

The mean width of the daily zones before the formation of the first annual increment was also significantly different with the samples in the Western Zone having the narrowest width. The mean width of daily zones in samples from the Eastern and Western Zones was similar. This suggests that although more daily increments are present in the samples from the Western Zone, the width of each daily zone is smaller. The distance from the primordium to the first annual increment is approximately equal between the Western, Central and Eastern Zones in the GABTF.

The spawning period was elucidated by the back-calculated time of spawning for the six specimens where daily zone counts could be made to the edge of the otolith (no formation of the first annual zone). These data indicate the major spawning occurred between December–January. Due to the protracted spawning season, the number of daily zones and the relative distance from the primordium to the first opaque zone would vary between individuals. Depending on spawning time, the sample may be 120–270 days old at the time of first opaque zone formation. While the first zone does not represent a full year of growth, if a 1 July birthday is adopted, all fish spawned in a season would be allocated to the same year class. From the observations made from the few juvenile samples available with opaque margins in June and July, it appears zone formation commences in winter.

Based on these data, 1 July is an date to allocate fish to a spawning year. This birth date assignment should be used in the routine ageing of Deepwater Flathead.

The assignment of 1 July an appropriate birthday assignment to separate fish into their cohorts for the purpose of stock assessment as all fish from a given year's spawning event (October to May) will be placed into the same cohort.

In routine ageing, the development of protocols that allow for the accurate and repeatable interpretation of the first annulus is essential. Since the average distance of the opaque zone is consistent between samples from the three regions, identification of the first zone can be made using the proxy measurement of 0.460 mm on the dorsal side and 0.373 mm on the ventral side regardless of the appearance of the inner structure.

While the differences observed in the daily age to the first annual zone has little importance on the ageing process, understanding juvenile growth and somatic growth rates can be beneficial. Studies have shown that larval growth can predict recruitment success and in turn, can predict the variability in recruitment in a fishery or stock (Bergenius *et al.*, 2002; Meekan *et al.*, 1998; Butler and Nishimoto, 1997; Campana, 1996). For these types of studies to be effective, a large number of larval otoliths from the spatial and temporal range being studied, needs to be collected to achieve the resolution between strong and weak year classes in the estimated age composition. The low number of juvenile Deepwater Flathead samples obtained in the past 10 years from observing and monitoring commercial catches and the stable age structure observed over time suggests that this species is probably not suited to this type of study.

Conclusion

The daily zone estimates could be used effectively to determine the location and timing of the formation of the first opaque zone.

Zone counts from the hatch mark to the start of the first opaque zone (corresponding usually to the end of the diffuse nucleus) support the assumption that the first opaque zone starts to form in winter.

Measurements taken from the primordia to the first opaque zone indicated that there is little difference between the average position of the first opaque zone in samples collected from different zones of the GAB and that a proxy measurement for the first opaque zone would be a useful tool in the ageing of this species.

Spawning dates, when back calculated from daily counts, were consistent with peak spawning activity, which further supported the assumption that micro-increments counted in this study were formed daily.

The key to successful routine ageing is to understand where the first annual zone is located. This Section provides strong evidence that the location of this zone has been identified. It is located 0.460 mm on the dorsal side of the sulcus and 0.373 mm on the ventral side of the sulcus. These distances can be used as proxies for the position of the first annual zone.

The next question with respect to routine ageing is the periodicity of the zone formations after the first annual zone. The periodicity of zone formation is explored in Section 7.3.

7.3. Increment analysis.

Introduction

Determing the annual growth zones in sectioned otoliths from Deepwater Flathead is difficult. The structure of the annual growth zones can be complicated consisting of double or multiple translucent zones. Some otoliths can display clear growth zones on both ventral and dorsal sides. It has been observed that often growth zones on the dorsal side are easier to read in the larger samples (Stokie and Talman, 2003).

Understanding how and when the structure of the annual growth zones are deposited would assist in the intrepretation of age. While otoliths have been routinely collected from the GAB Bight since 1993, they rarely were collected on a monthly basis. The timing and periodicity of zone formation could not be investigated using marginal increment analysis.

Since 2004, two sources of data with the potential to validate the periodicity of annual growth zones in Deepwater Flathead have become available:

- A collection of otoliths sampled monthly between January 2001 and December 2001
- A set of otoliths that exhibit three distinct juvenile modes.

The examination of the marginal increment will determine the periodicity and timing of zone formation in Deepwater Flathead sectioned otoliths and the discrete length modes will allow the testing of the validity of the presumed annuli as age indicators. The ageing of a larger number of samples < 30 cm TL will allow for growth estimates to be re-estimated that more accurately represents the growth of Deepwater Flathead over their complete length range.

The periodicity of zone formation is explored in this Section.

Results

Samples

A total of 2238 otoliths collected in 1998, 1999, 2000, 2001 and 2002 were available. Of these 1598 were analysed for marginal increment on the dorsal surface while marginal increment analysis was undertaken on the ventral surface of 1531(Table 9 and Table 10). The difference in the numbers of otoliths analysed was due to differences in the clarity of the microstructure between the ventral and dorsal reading planes.

Juvenile length sample

A total of 173 juvenile otoliths were available for ageing. Of these, 137 otoliths were sampled from one trip in October 2005 and 36 were provided over a long time period of opportunistic collections. The length frequency histogram indicated distinct length modes at 12 cm, 19 cm and 30cm (Figure 23). These discrete length modes represented the 0+ to 4 + age-classes.

Table 9. Number of samples aged and measured for marginal increment analysis on the dorsal surface.

Year	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*	*N*
1998					69		49		111		96		**325**
1999				84		116							**200**
2000							42		43				**85**
2001	100	90	25	100	24	80		82			74	55	**630**
2002			55		56		56			84		107	**358**
N	**100**	**90**	**80**	**184**	**149**	**196**	**147**	**82**	**154**	**84**	**170**	**162**	**1598**

Table 10. Number of samples aged and measured for marginal increment analysis on the ventral surface.

Year	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*	*N*
1998					61		49		105		88		**303**
1999				67		87							**154**
2000							40		40				**80**
2001	108	90	27	99	25	81		82			74	56	**642**
2002			55		56		53			82		106	**352**
N	**108**	**90**	**82**	**166**	**142**	**168**	**142**	**82**	**145**	**82**	**162**	**162**	**1531**

Periodicity of increment formation

Marginal Increment Analysis

The mean monthly indices of marginal increment completion was examined for ages 5 to 8 separately (age 3 and 4 were not presented separately because of low sample size) and for combinations of ages 5 & 6, 7 & 8, 4 to 8 and all ages. These are presented for measurements taken on both the ventral and dorsal side of the sulcus (Figures 24 and 25).

The mean monthly marginal increment analysis on the dorsal side shows a sinoidal correlation with a frequency of one cycle per annum . In most ages and age groups considered there was a clear pattern in the mean monthly marginal increment. The lowest marginal increment was usually between July and October for both individual age-classes and grouped age-classes. The maximum marginal increment value was ~80% and the minimum value was ~40%.

For both individual age-classes and the grouped age-classes there was only one cycle of deposition per calendar year.

Edge Type Analysis

The percentage edge type, by month, was examined for ages 5 to 8 separately and for combinations of ages 5 & 6, 7 & 8, 4 to 8 and all ages. These are presented for both the ventral and dorsal side of the otolith (Figures 26 and 27). The highest proportion of new edge types was observed between July and October for both indivual age-classes and the grouped age-classes. The highest proportion of wide edges was observed between January and April.

Overall the pattern of the zones was clearer on the dorsal side.

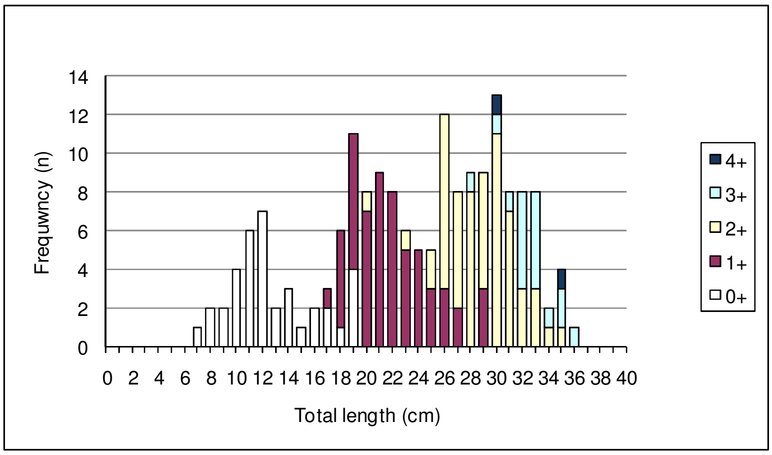


Figure 23. Juvenile length frequency histogram of Deepwater Flathead sampled opportunistically from the GABTF.

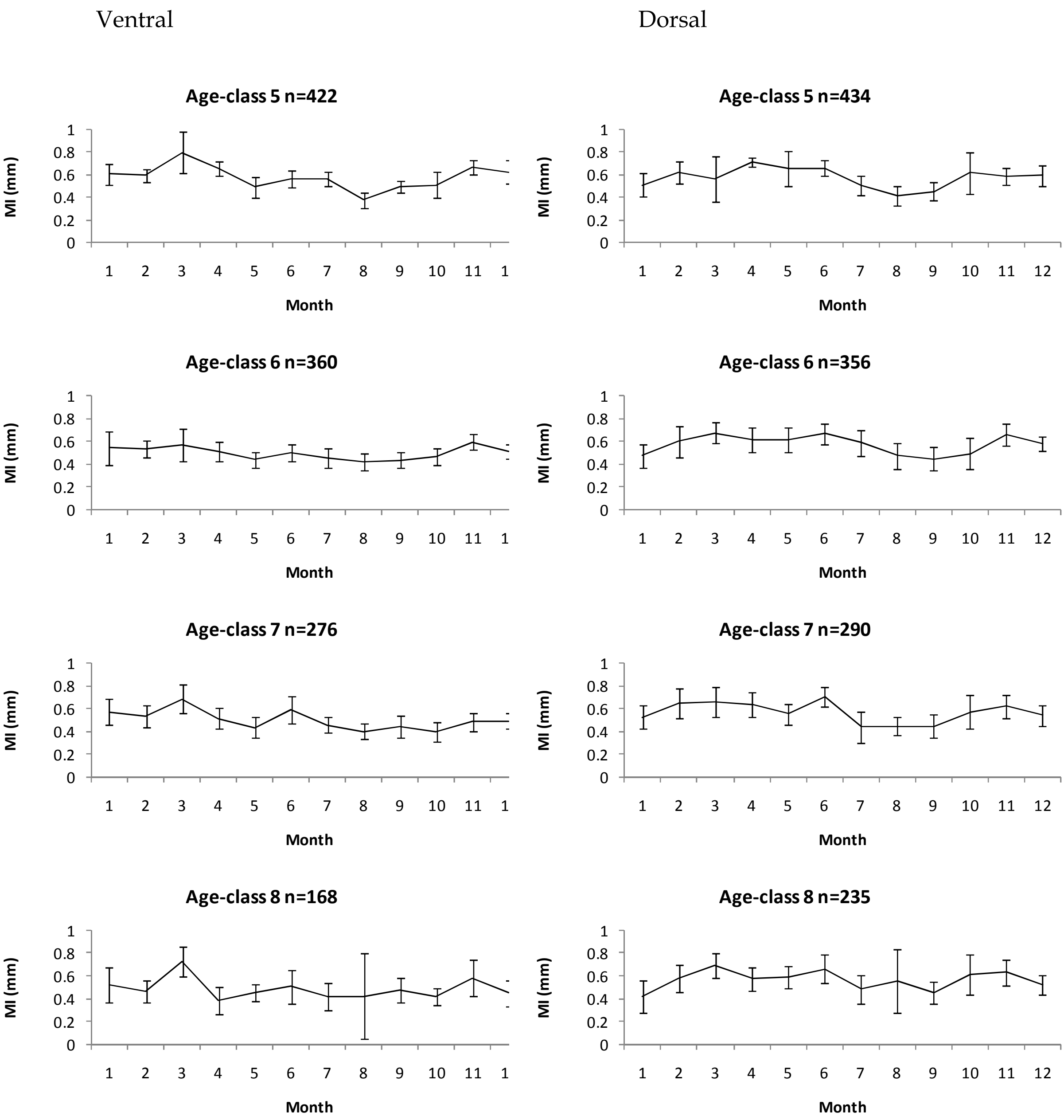


Figure 24. Mean marginal zone (± 2 SE) by month for ages 5–8 on the ventral and dorsal side of the sulcus (combined years).

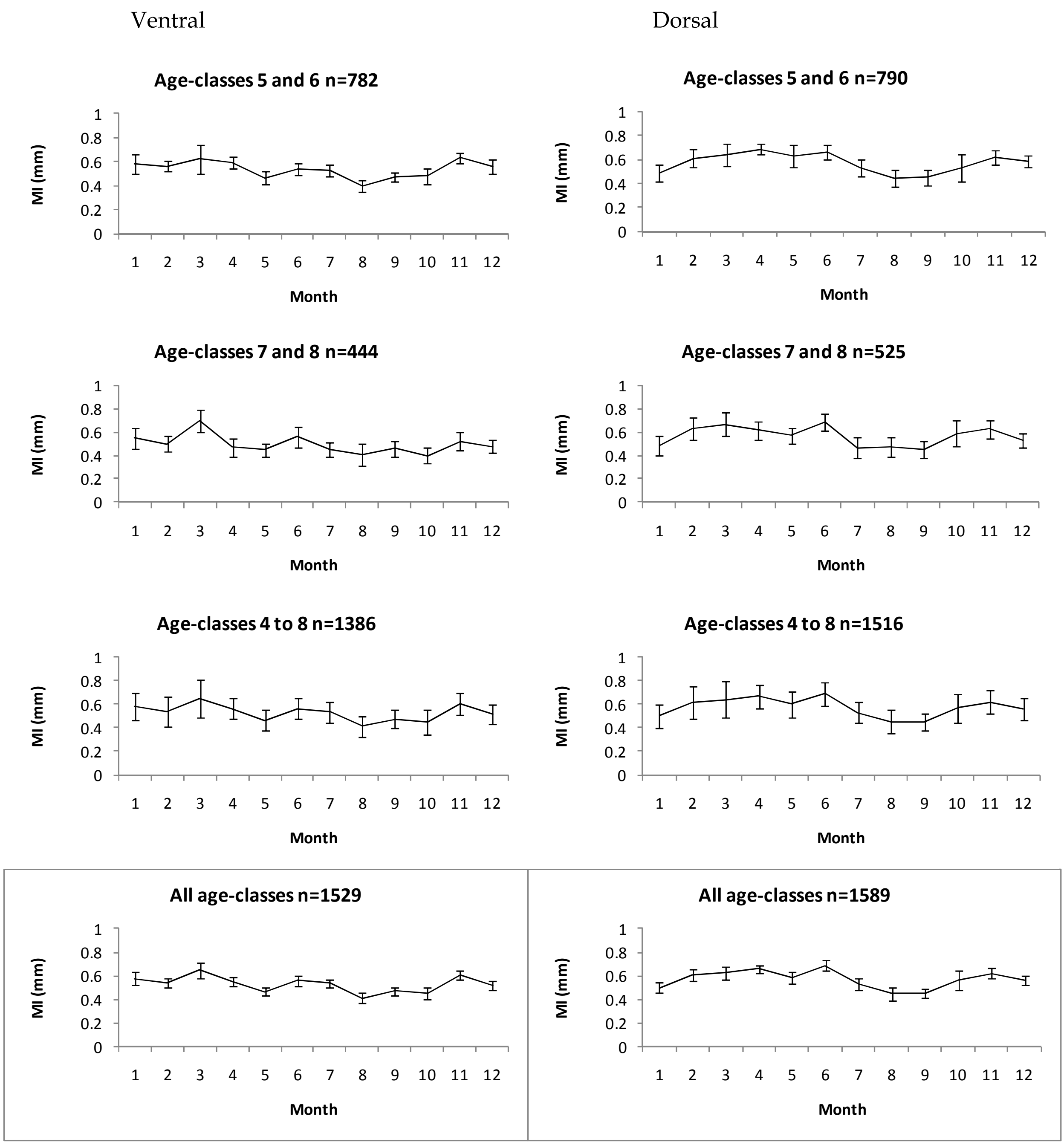


Figure 25. Mean marginal zone (± 2 SE) by month for combined ages, 5 and 6, 7 and 8, 4 to 8 and all ages on the ventral and dorsal side of the sulcus (combined years).

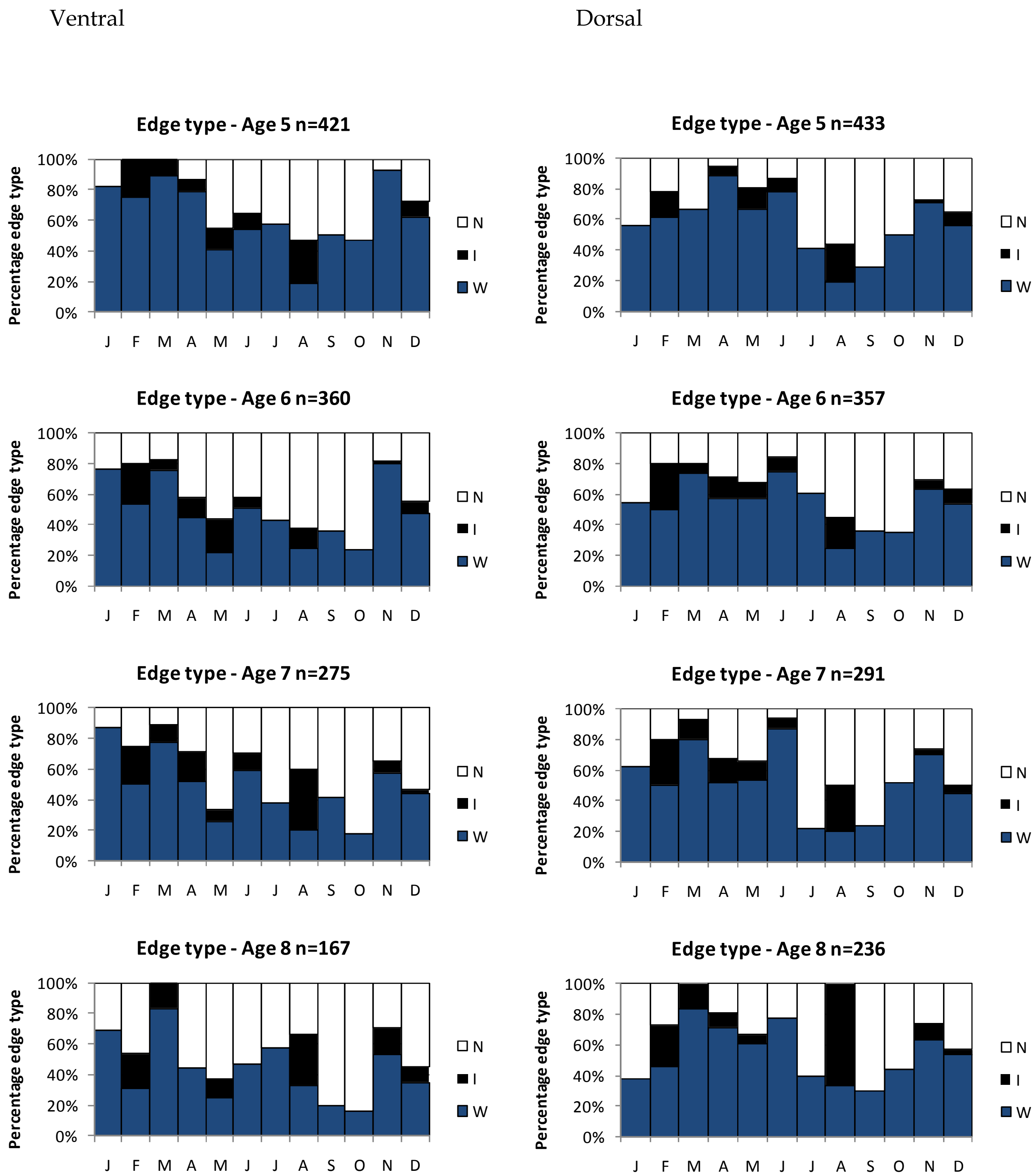


Figure 26. Percentage edge type, by month (years combined), ages 5-8 separately for the ventral and dorsal sides of the sulcus.

Legend: N= narrow; I= intermediate; W= wide

Ventral Dorsal

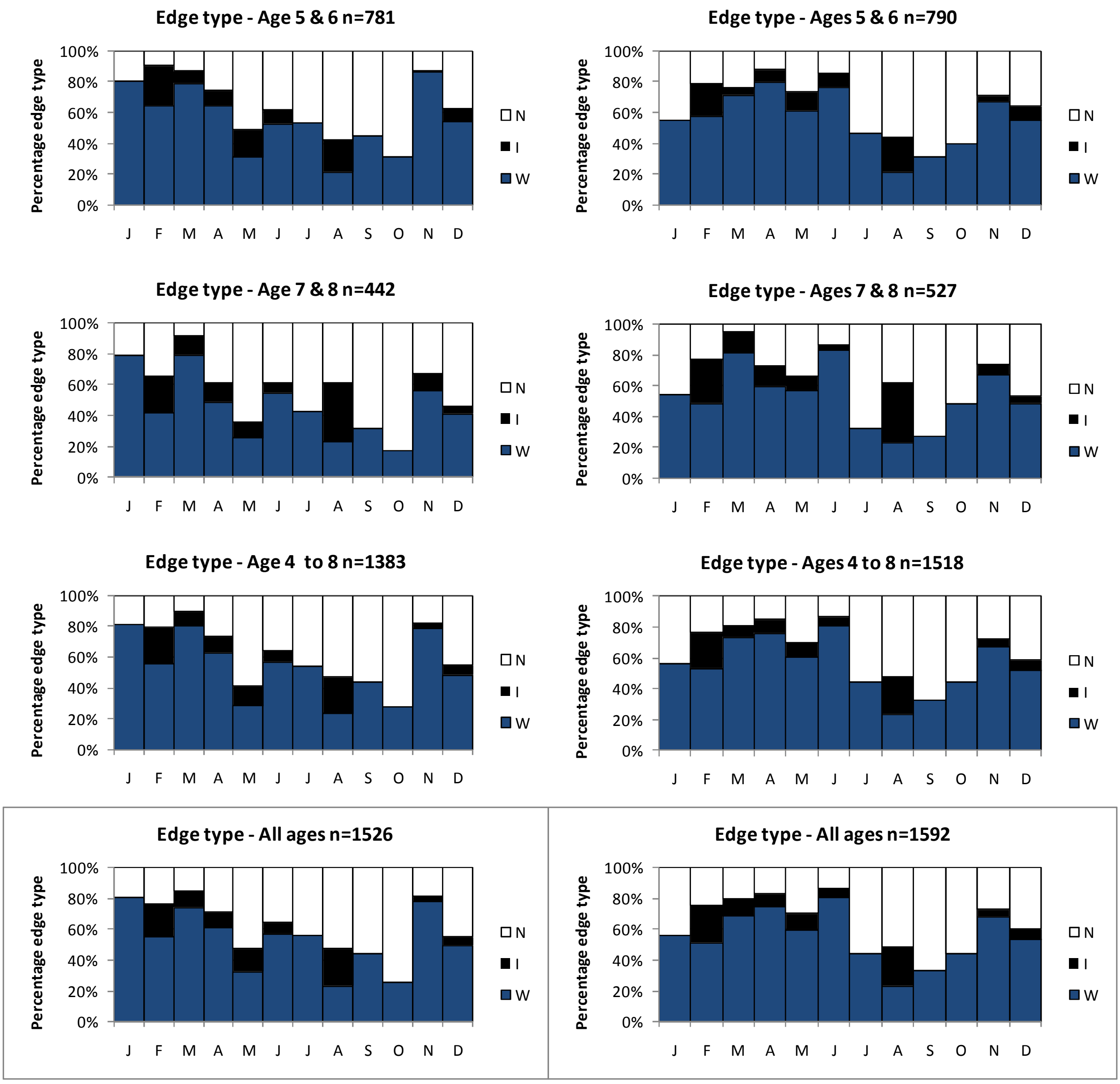


Figure 27. Percentage edge type by month (years combined), ages 5 and 6, 7 and 8, 4 to 8 and all ages combined for the ventral and dorsal sides of the sulcus.
Legend: N= narrow; I= intermediate; W= wide

Discussion

Age validation is critical to the accuracy of the stock assessment process where catch-at-age models are used to assess the health of a fishery resource. The health of the resource is maintained through being able to correctly interpret the dynamics of the fish population (Dwyer *et al.*, 2003). Validation of all age-classes in the fishery is a very difficult task to accomplish. Beamish and McFarlane (1983) examined 500 papers and noted that 66% of these papers mentioned the need for validated ages; only 4% provided validated ages over the range of ages within the fishery. When ages are under-estimated, the results can lead to a serious over-exploitation of the stock (Saborido-Rey *et al.*, 2003). It is necessary to validate the age-classes over the full range of age-classes within the fishery as growth rates vary over time through mechanisms such as density dependency and on-set of maturity (Dwyer *et al.*, 2003).

To validate Deepwater Flathead in this study, three techniques in combination were used:

- A set of otoliths from juveniles showing three distinct modes of 0+, 1+, 2+, 4+ and 3+. These three distinct modes comprised of five age-classes (0+ to 4+). The 3+ and 4+ components of this distribution comprised a low proportion of the sample.
- Marginal increment analysis
- Marginal state analysis.

A composite year of samples was required to provide enough samples of each age-class for the marginal state and marginal increment analysis.

Both the marginal state and marginal increment analysis showed the same sinoidal pattern with a frequency of one cycle per year. This pattern was observed in the single age-classes and the combined age-classes for both the marginal state and marginal increment analyses. For both these techniques the pattern is produced when one opaque and one translucent zone are deposited each year. The three discrete length modes from the juvenile data set were sectioned and zone counts were consistent within modes, and increased by one in each consecutive mode. This indicates that the modes represent age-classes. This provided validity to the results of the marginal increment analysis and edge state analysis that one opaque and one translucent zone is formed annually in the otoliths of Deepwater Flathead.

Using these three techniques in combination provided strong evidence that the deposition of one translucent and one opaque zone was being deposited over one calender year.

The validation of the zone formation in Deepwater Flathead now allows accurate descriptions of age compositions, growth and mortality. These are discussed in Section 7.4.

Conclusion

The conclusion of this aspect of study demonstrated that:

- One translucent and one opaque zone was deposited each calendar year through the use of:
  - Small fish showing distinct cohorts
  - Marginal state analysis
  - Marginal increment analysis.
- Increments can be used confidently to age Deepwater Flathead.

An ageing protocol for Deepwater Flathead has been developed using the results described in Chapter 7 Sections 7.1, 7.2 and 7.3 (Appendix 7).

7.4. Growth, age composition and mortality.

Introduction

Deepwater Flathead are the dominant species caught in the GABTF comprising approximately 40–50% of landings. The majority of the Deepwater Flathead caught range between 45 and 60 cm TL. Very few individuals less than 35 cm TL are caught.

An understanding of age and growth in aquatic animals is crucial to an understanding of the dynamics of populations and the impacts of exploitation (Smith, 1992). Determining the age composition of Deepwater Flathead in the GABTF was considered an important part of the present study.

Age and growth of Deepwater Flathead sampled from the GABTF was estimated Krusic-Golub (1999) and Stokie and Krusic-Golub (2005) by counting opaque growth zones on the ventral side of sagittal otolith sections. These studies found:

- Females grow faster and obtain a larger maximum length (maximum length 82 cm TL) than males (maximum length 59 cm TL)
- Males reach a higher maximum age (maximum age 26 years) than females (maximum age 16 years).
- Age-classes 5–9 comprised the significant proportion of the sampled catch
- Growth parameters were also estimated but were considered unrealistic as no samples less than 30cm TL were available.

The updated 2006 assessment model by Klaer and Day (2007) for Deepwater Flathead uses biological parameters obtained from three different sources:

- Analysis of biological samples collected during the 2004 GAB reproductive study
- Length and age samples collected between 2000–2003
- Length samples collected during the 2001 FRDC reproduction project since they contained a number of smaller discarded samples.

Estimates of growth parameters (k, L_∞, t_0) and mortality (Z) have been based on unvalidated zone counts obtained from sectioned otoliths.

This section presents the analysis of growth, age composition and mortality using data derived from the validated ageing method developed in Chapter 7 Section 7.3.

Results

Sex ratio

The sex ratio of the Deepwater Flathead samples aged was 54% male, 41% female. The remaining 5% were either juveniles or sex information was not recorded.

Length composition

The length frequency distributions for combined sexes for each year are shown in Figure 28. Fish length ranged between:

- 28–82 cm with a modal length class of 45 cm for 1998
- 33–79 cm with a modal length class of 42 cm for 1999
- 29–82 cm with a modal length class of 41 cm for 2001
- 17–83 cm with a modal length class of 55 cm for 2002.

Length frequency distributions of male, female and juvenile Deepwater Flathead by year are shown in Figure 29. The length frequency distributions are similar for each year except 2002, where there are a higher proportion of females greater than 50 cm in length.

Overall the female and male length frequency distributions were not normally distributed (female, $W_{0.05,930} = 0.98$, $p < 0.05$; male, $W_{0.05,1179} = 0.98$, $p < 0.05$). The length frequency distributions were significantly different between males and females ($\chi^2_{0.05,1} = 823.8$, $p < 0.05$). Females are larger than males.

Age composition

The age frequency distributions for combined sexes for each year are shown in Figure 30. Age ranged from:

- 1 to 28 years with a modal age-class of 5 years for 1998
- 3 to 23 years with a modal age-class of 5 years for 1999
- 0 to 20 years with a modal age-class of 5 years for 2001
- 1 to 26 years with a modal age-class of 7 years for 2002.

Age frequency distributions of male, female and juveniles by year are shown in Figure 31. The age frequency distributions for males were similar for years 1998–2001, with a dominant modal age-class of 5 years. The age frequency distribution for males in 2002 contained a smaller proportion of samples less than 6 years of age.

The age frequency distributions for females were similar for years 1998, 2001 and 2002, with a modal age-class of 7 years. The female age frequency distribution for 1999 indicated a modal age of 5 years and contained few samples greater than 8 years of age.

The number of juvenile samples was low for all years except 2001, where a higher proportion of 1 year olds were sampled.

The female and male age frequency distributions were not normally distributed (female, $W_{0.05,930} = 0.85$, $p < 0.05$; male, $W_{0.05,1179} = 0.86$, $p < 0.05$). Significant differences were detected between males and females age frequencies ($\chi^2_{0.05,1} = 98.29$, $p < 0.05$).

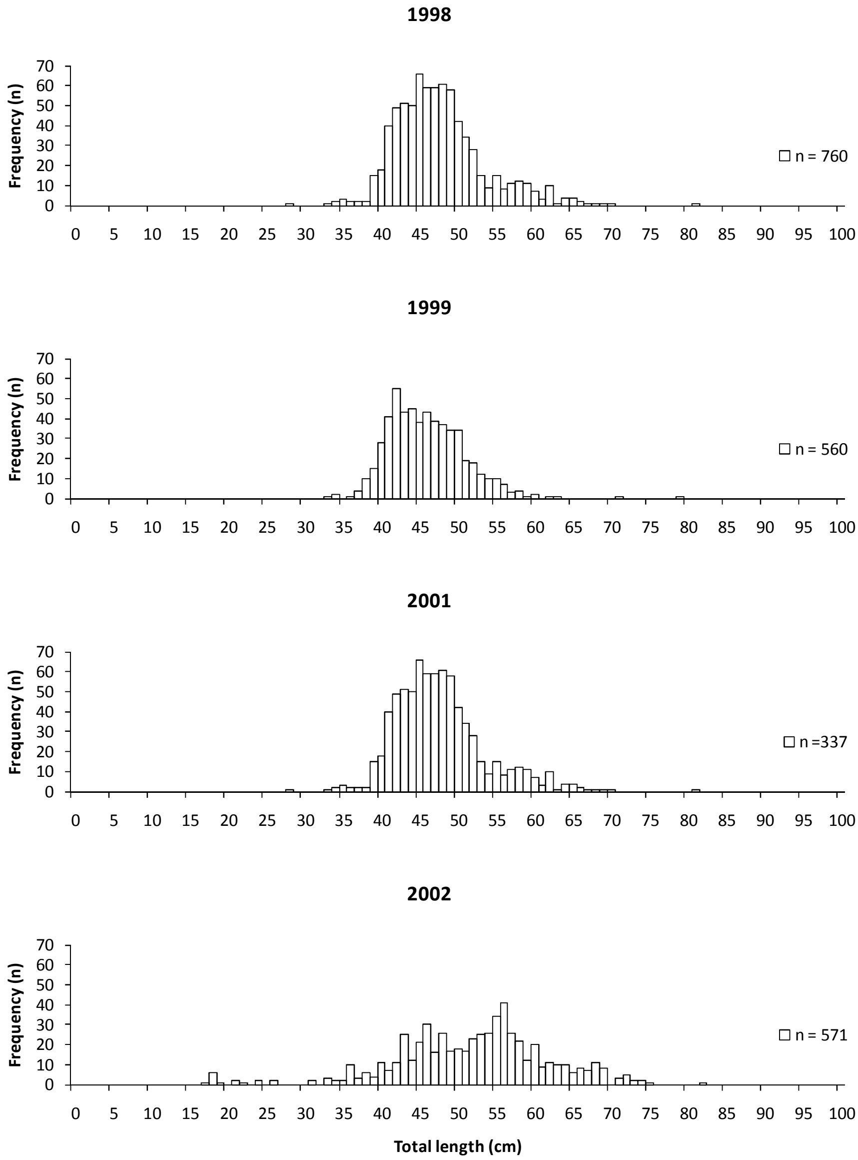


Figure 28. Length frequency distributions for Deepwater Flathead (combined sexes) sampled in 1998, 1999, 2001 and 2002.

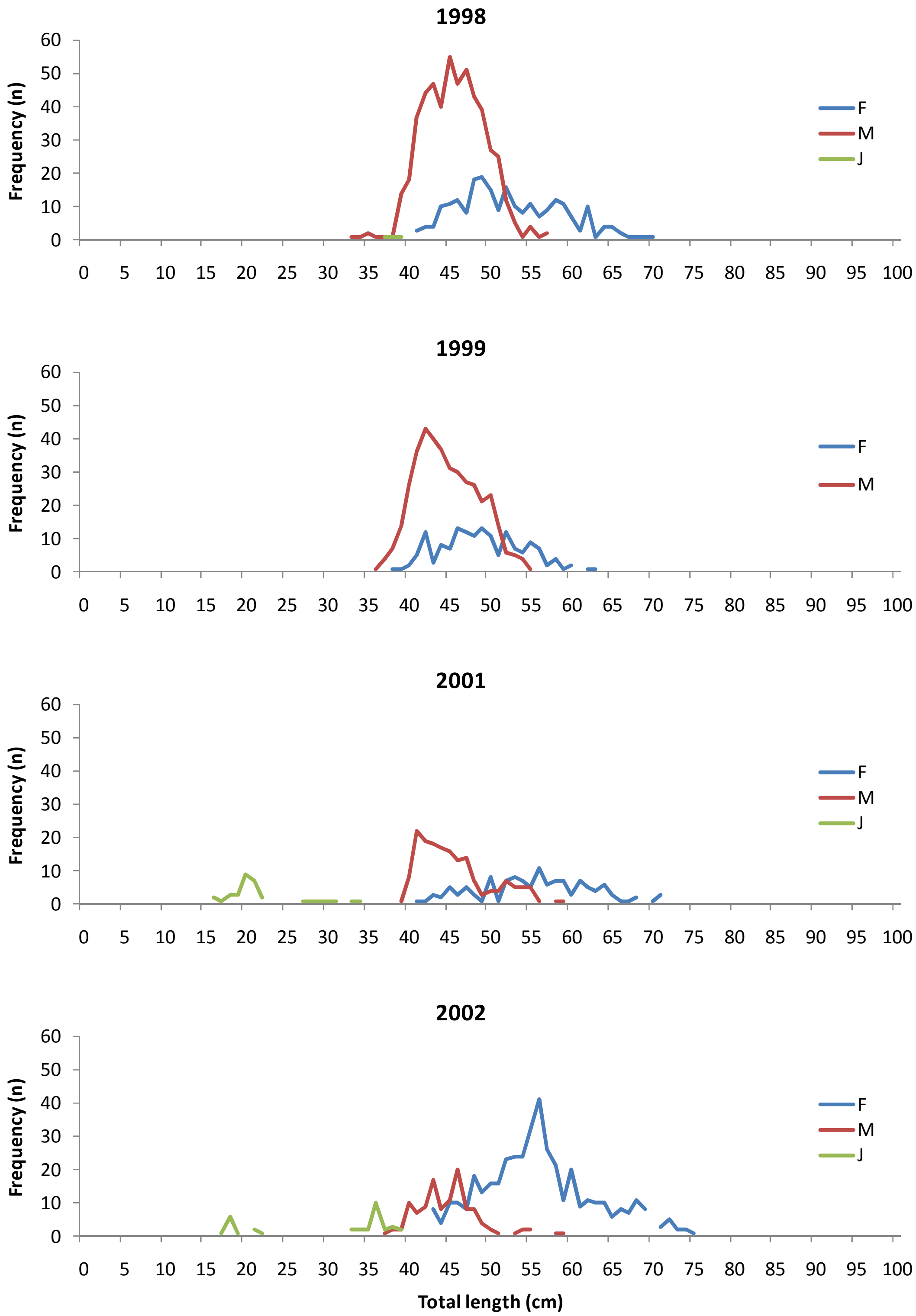


Figure 29. Female, male and juvenile length frequency distributions for Deepwater Flathead sampled in 1998, 1999, 2001 and 2002.

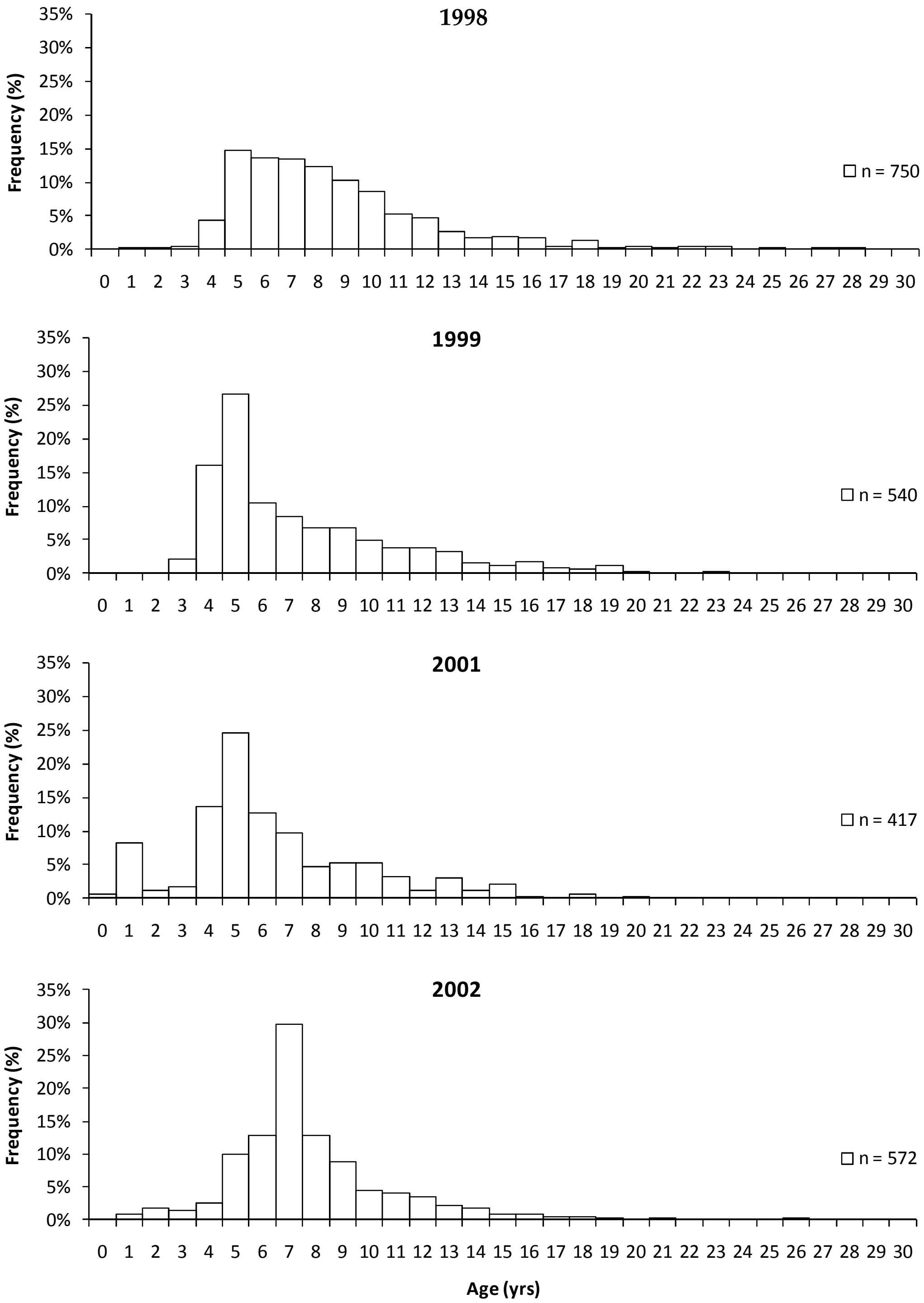


Figure 30. Age frequency distributions for Deepwater Flathead (combined sexes) sampled in 1998, 1999, 2001 and 2002.

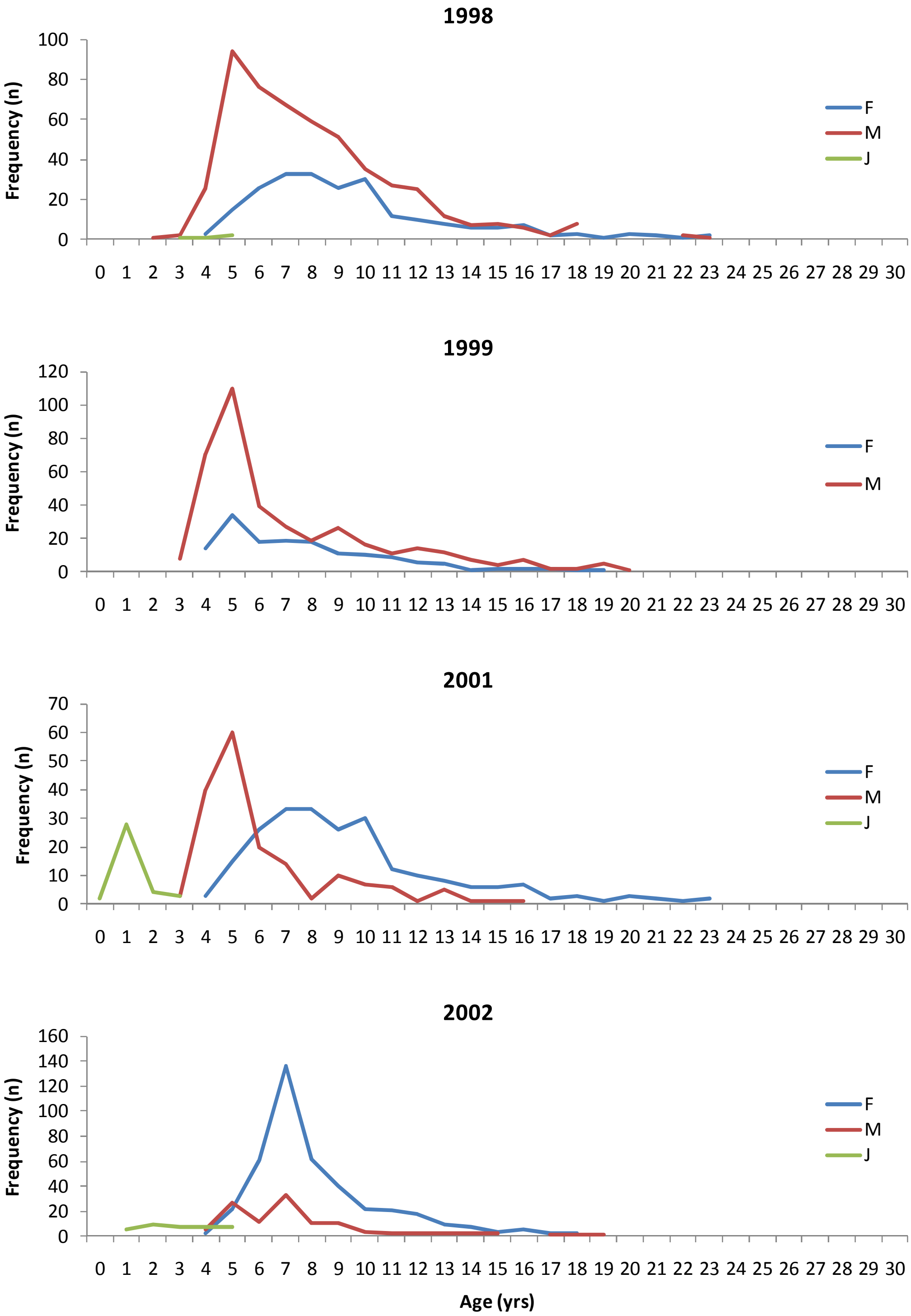


Figure 31. Female, male and juvenile age frequency distributions for Deepwater Flathead sampled in 1998, 1999, 2001 and 2002.

Growth

Female Deepwater Flathead grew faster (Table 11) and attained a greater maximum length and age compared to males (Table 12). For fish greater than 4 years old, the estimated mean length-at-age was consistently greater for females than for males.

The largest female sampled was 83 cm TL, and was estimated at 15 years of age (Figure 32). The oldest female was estimated at 28 years of age and was 65 cm TL.

The largest male sampled was 59 cm TL, and estimated at 7 years of age (Figure 32). The oldest male was estimated to be 27 years old and was 52 cm TL. There was considerable variation in length-at-age for both sexes.

The parameter estimates of the von Bertalanffy growth curves shown in Figure 33 and Table 11. The growth equation for males was:

$Lt = 49.50(1-e^{-0.358(t-0.568)})$.

Males grew rapidly until about age 4 years, before growth asympoted. The maximum age observed for males was 27 years.

The growth equation for females was:

$Lt = 63.00(1-e^{-0.230(t-0.708)})$).

Females grew rapidly until about age 4 years, before growth asymtoped. The asymtopic length was larger for females than for males.

The growth equation for the combined sexes was:

$Lt = 56.73(1-e^{-0.262(t-0.708)})$.

The observed differences between sexes was tested and shown to be significantly different ($p<0.0001$). The females were older than males and reached a larger length.

A comparison of growth curves from this study and that of Klaer and Day (2007) are shown in Figure 34. Growth curves for males are similar, while growth curves for females in the Klaer and Day study show a higher asymtopic length.

Table 11. Von Bertalanffy growth parameter estimates, for different different studies on Deepwater Flathead

Species	Parameter	Males	Females	Combined	Source
Platycephalus conatus	L_∞ (cm TL)	49.50	63.00	56.73	This study
	K (y^{-1})	0.358	0.230	0.262	
	t_0 (y)	-0.568	-0.708	-0.708	
	Max age				
Platycephalus conatus	L_∞ (cm TL)	50.56	69.82	N/A	Klaer and Day, 2007
	K (y^{-1})	0.346	0.195	N/A	
	t_0 (y)	-0.47	-0.637	N/A	
	Max age				

Table 12. Mean length-at-age for male, female and juvenile Deepwater Flathead.

	Sex							
	Male			Female			J	
Age-class	Mean	StdDev	N	Mean	StdDev	N	Mean	StdDev
0							13.00	1.41
1							19.56	1.81
2							24.30	4.22
3	41.50	0.58	4				33.29	2.06
4	42.07	2.36	46	43.75	6.45	8	35.75	0.50
5	43.64	2.75	88	48.05	4.13	44	38.25	2.87
6	43.92	2.24	37	51.08	4.17	86		
7	45.85	3.75	48	53.92	4.32	157		
8	45.53	2.47	15	56.23	3.90	73		
9	48.00	3.45	22	58.06	4.25	50		
10	50.80	3.55	10	62.17	5.41	29		
11	51.75	4.23	8	63.12	4.26	26		
12	47.67	1.53	3	65.00	5.86	20		
13	50.57	3.46	7	64.29	5.19	17		
14	53.25	2.22	4	63.90	7.91	10		
15	49.33	4.16	3	65.17	5.98	6		
16	48.00	!	1	65.20	2.68	5		
17	46.00		1	72.50	0.71	2		
18	55.00	0.00	2	68.00	14.00	3		
19	59.00		1					
20				56.00		1		
21				72.50	2.12	2		
26				69.00		1		

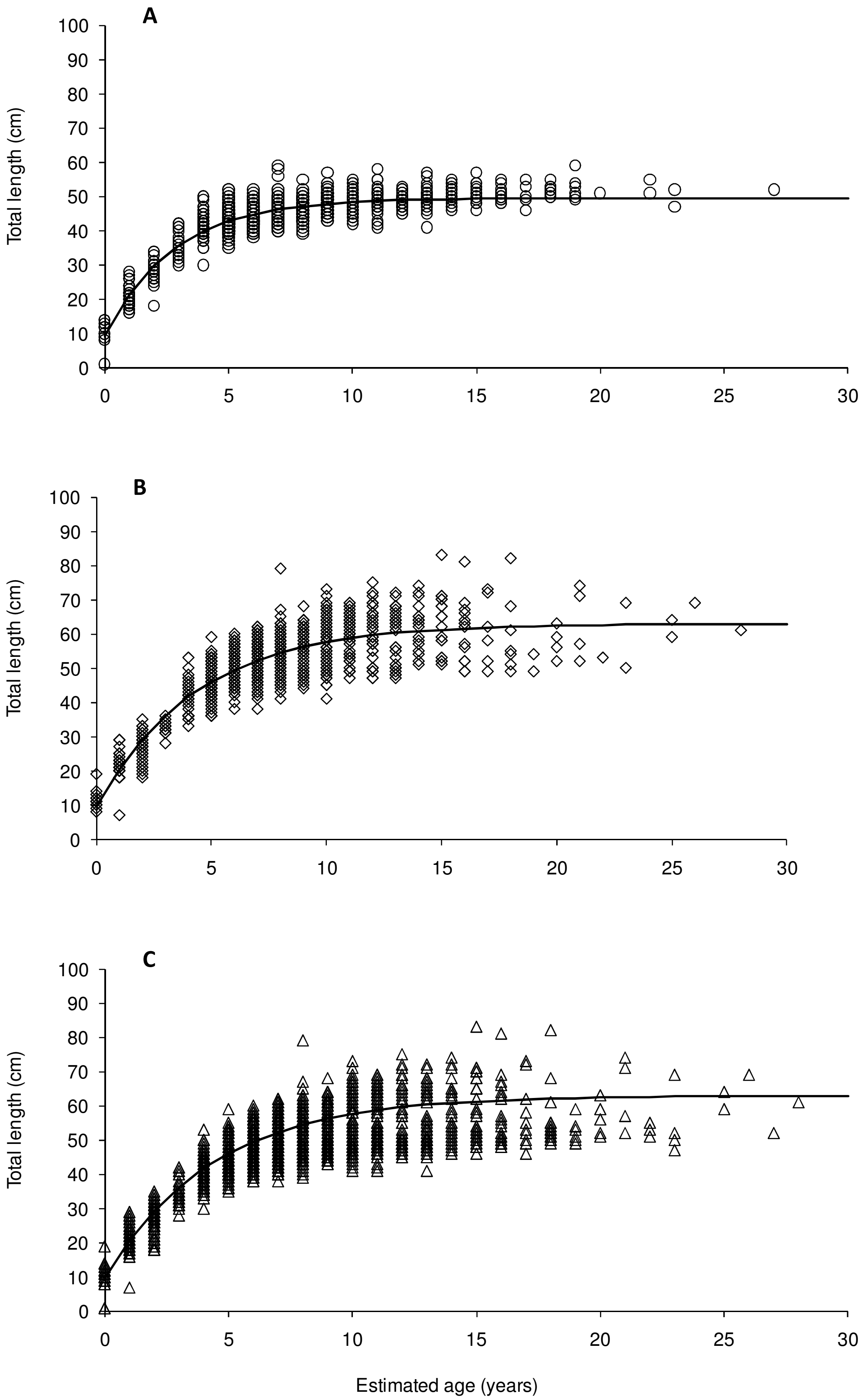


Figure 32. Estimated growth for A) males (n = 1290), (B) females (n = 1161) and (C) all data (n=2421) Deepwater Flathead.

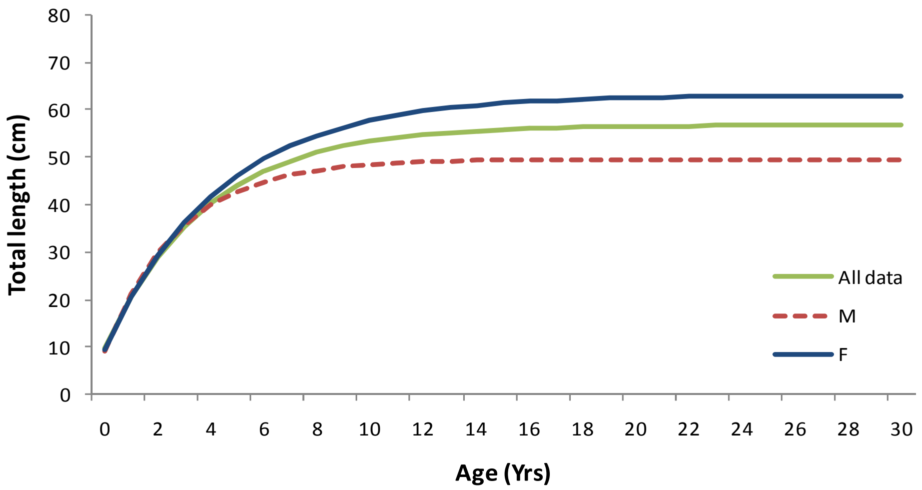


Figure 33. Comparison of Von-Bertalanffy growth models for male (red line), female (blue line) and combined data (green).

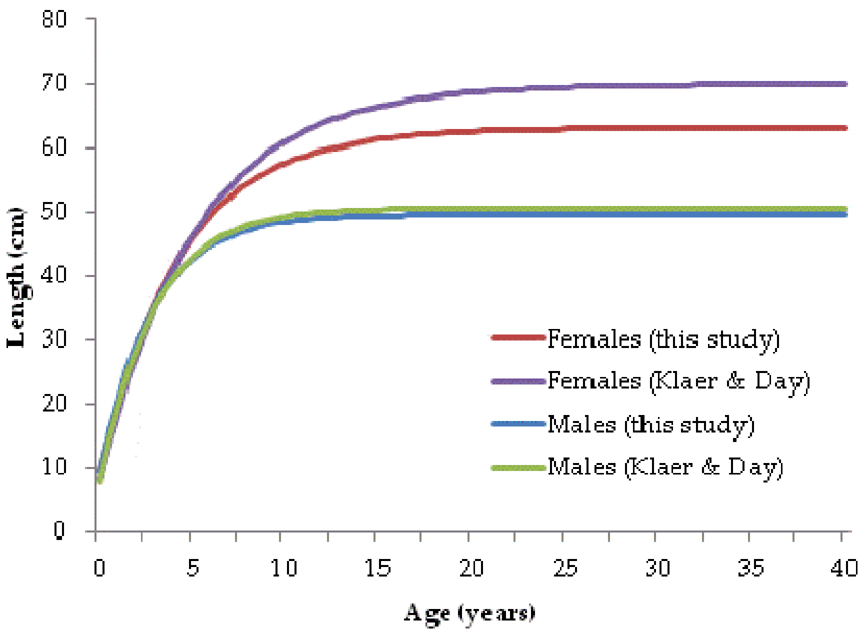


Figure 34. Von-bertalanffy growth curves for Deepwater Flathead shown from this study and from Klaer and Day (2007).

Mortality

Total instantaneous mortalities (Z) were estimated using the catch curve of the age frequency. The catch curve method assumes uniform recruitment (Vetter, 1988). The plots of log age at time t (ln(At)) suggest that recruitment is relatively uniform (Figure 35).

Mortality was estimated for age ranges:

- 5 to 19 years and 5 to 13 years for males
- 7 to 21 years and 7 to 13 years for females
- 5 to 25 years and 5 to 13 years for combined sexes.

The contracted range was chosen to cover 90% of the data past the modal age-class.

The mortality (Z) was estimated to be:

- 0.11 for males (across both ranges)
- 0.13 to 0.14 for females.
- 0.11 to 0.12 for combined sexes (Table 13, Figure 35).

Table 13. Estimates of total mortality derived from age-based catch curves of fished stocks

Sex	Age range (years)	Z (yr-1)
Combined	5–25	0.11
	5–13	0.12
Males	5–19	0.11
	5–13	0.11
Females	7–22	0.13
	7–13	0.14

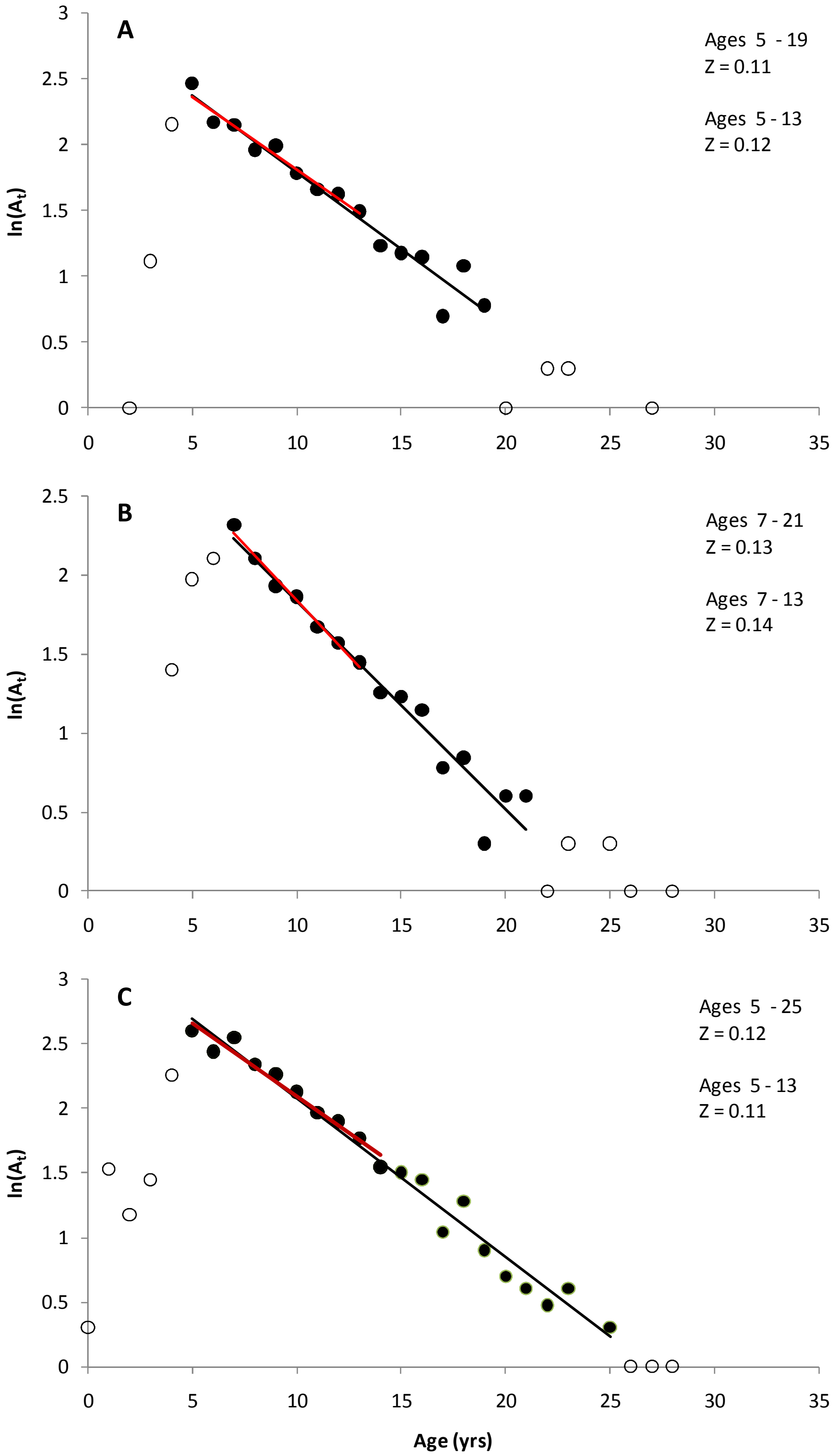


Figure 35. Mortality estimates for (A) males, (B) females and (C) combined sexes, by combined years. The red line indicates the contracted range and the black line indicates all available data. White circles indicate unused data.

Discussion

The findings from the previous two Sections (7.2 and 7.3) validate the ageing, enabling robust measures of age composition, growth and mortalities to be made.

The length and age compositions over the years 1998, 1999, and 2001 were relatively stable with modes of 40–50 cm for length and 5 years for length and age respectively. The compositions of age and length for 2002 are quite different. The length frequency shows a higher mode of about 55 cm and a modal age-class of 7 years. The reasons for this discrepancy are unknown but maybe a function of a change in fishing areas or other unknown causes. The 2001 age frequency composition shows a large cohort of 1 year-olds entering the fishery.

The revised growth curves for the Deepwater Flathead using validated ageing methods developed in this project are similar to the previous study by Klaer and Day (2007). The parameters for the males in this study are very close with the L_∞ (within 1 cm) and almost identical to values for K and t_0 produced by Klaer and Day. The parameters for the females were different to those from the previous study with a L_∞ difference of approximately 7 cm. This change may be due to the sampling strategies used to select samples for the study.

The mean length-at-age shows that males are smaller than females for a given age and reach a smaller asymptotic length. The high variance described for the older age-classes is predominately driven by low sample size. These fish account for a low proportion of the population. Even when using composite years, acquiring an adequate sample size is difficult.

The growth curves show that there is considerable variance for any given length-at-age. The estimates for growth from this Section are robust due to the relatively large sample sizes.

Mortality estimates provided in this Section also are considered the most accurate estimates of instantaneous mortality provided for this species. The estimates of mortality suggest that the mortality is slightly higher for females than males. Mortality estimates, using a subset of the available data (age-classes from the mode to 13 year-olds), produced similar total mortalities.

The results from this component of the study, along with the validation of the deposition of growth zones allows the historical age data to be examined and any issues arising from these data identified.

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7.5. Audit of historical ageing data.

Introduction

The annual stock assessment of Deepwater Flathead is heavily reliant on age estimates, as other biological data are limited. Accurate and precise (repeatable) age estimates are essential for the ecologically sustainable management of this fishery.

This study has demonstrated the periodicity of zone formation observed in sectioned otoliths. Before this study, the ageing protocol for Deepwater Flathead had been not validated and reader interpretation of the otolith microstructure may have been quite different. Given the reliance on the ageing in the stock assessment process, it is necessary to audit a sample of previously aged fish to determine if historical estimates were accurate.

If historical age estimates were in error by more than two years, the biomass estimates of this fishery could be in error by up to 30% (Wise and Tilzey, 2000). If biomass estimates are incorrect, then the current settings may be inappropriate to sustainably mange the fishery.

This Section audits the historical age estimates against the validation protocol. To do this a sub-sample of otoliths from each year were re-read according to the validated protocol. The new ages were then compared with the historical age data.

Another benefit of re-ageing a subsample of the historical collection was to help determine:

- Estimates of precision and bias
- Reader drift over time between and within readers
- When identified, whether bias was systematic or random.

Results

Audit Sample

Audit samples (n=370) were obtained from each year of the historical collection. The numbers of samples re-aged for each year ranged from 19 to 30.

Precision

A total of 7,232 otoliths have been aged by the CAF from samples collected between 1988 and 2006. For each year that CAF age data is available IAPEs were calculated. The IAPEs for each year ranged from 1.86% to 4.06% (Table 14). The IAPE for each year's data were below the criterion of 5%, indicating the required level of precision for stock assessment had been achieved.

The IAPE of the re-aging of audit sub-sample and the historical sample was 4.65%, indicating an overall acceptable degree of precision.

Comparision of audit and historical samples

The age-difference distribution plots indicated no overall systematic bias (Figure 36) between the audit and historical ageing.

The age-difference table indicated that the differences were evenly distributed around zero (Table 15). The overall distribution suggested that when age estimates did not agree there was a higher likelihood of underestimating the age than over-estimating the age.

Readings between the audit set and each of the readers varied between:

- 5.56 to 10.79% for Reader 1
- 2.55 to 5.65% for Reader 2
- 2.31 to 4.50% for Reader 3
- 2.52 to 4.35% for Reader 4 (Table 15).

Unacceptable IAPE's were detected for the 1990 (10.97%), 1993 (9.94%), 1994 (5.56%) and 1995 (5.65%) sub-samples. For all other years, the IAPE was less than 5% (Table 16).

A negative bias (historical age lower than the audit age) was detected for the 1989 audit even though the IAPE was less than 5%. Positive bias (audit age lower than the historical age) was detected for the 1990 and 1993 audit (Figure 36). The distribution of differences between audit and historical ages for 1994 showed an equal distribution on zero and one. For all other years, the distribution of differences between audit and historical ages showed a mode of zero, indicating reasonable agreement between historical and audit age estimates.

Bias between readers was examined in Tables 17 to 20. Results of the audit indicate a mode of zero, demonstrating an agreement between audit age and historical age. The difference in age estimates was greatest between Reader 4 (audit) and Reader 1 (historical age). Reader 1 biases age readings by underestimating the historical age usually by 1 year, though the distribution of differences between audit and historical ages showed a mode of zero (Table 17). The maximum difference between the audit sample and historical age was 8 years for a fish of 24 years of age.

Table 14. Calculated IAPE for each year that re-age data could be found for Deepwater Flathead ageing data (Source CAF). N/A indicates that no data were found.

Year	*IAPE (%)*	*Year*	*IAPE (%)*
1988	N/A	1998	3.91
1989	N/A	1999	3.58
1990	4.06	2000	4.05
1993	2.31	2001	N/A
1994	3.62	2002	3.98
1995	N/A	2003	1.86
1996	3.32	2004	3.43
1997	3.31	2005	2.46
		2006	3.58

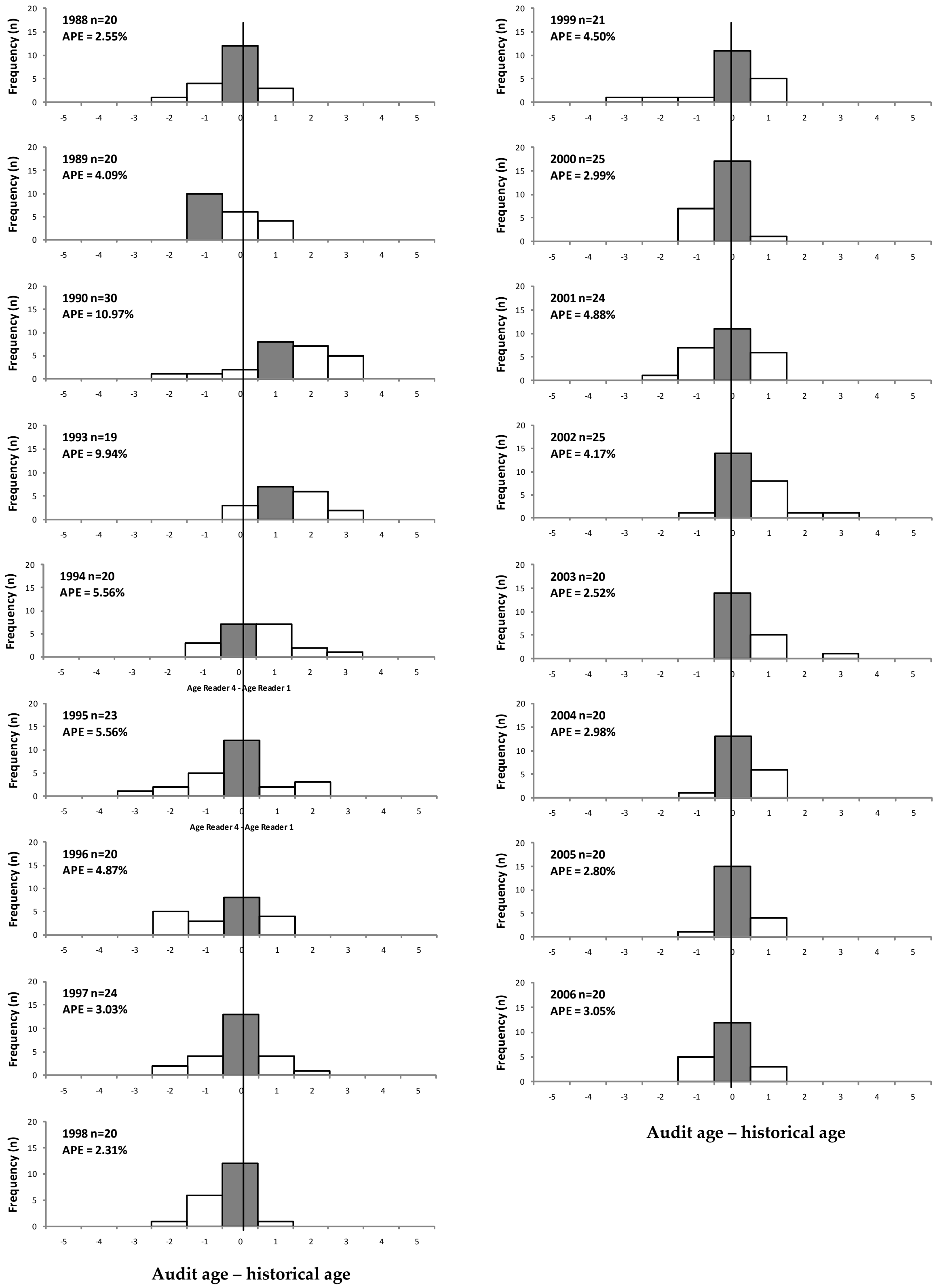

Figure 36. Age-difference distribution between the audit sub-sample and the historical age. Data presented separately for each year.

Table 15. Age-difference table between audit ages and historical age. Readers combined.

Age Reader	All																							
	Age																							
Difference	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	24	N
-5																								0
-4																								0
-3								1		1														2
-2							4	3	1	3	2			1										14
-1					7	11	6	8	8	2	2	6	1	5	2	1						1		60
0	**0**	**0**	**0**	**8**	**14**	**30**	**32**	**27**	**23**	**6**	**13**	**5**	**3**	**5**	**2**	**0**	**0**	**3**	**3**	**0**	**2**	**0**	**0**	**176**
1			1		5	14	11	7	10	6	9	7	4	2	3	1	2		2	1		1		86
2							3	4	2	1	2	1	4	1	1	1					1			21
3									2	2	1	3			1									9
7																						1		1
8																							1	1
N	0	0	1	8	26	55	56	50	46	21	29	22	12	14	9	3	2	3	5	1	3	3	1	370

Table 16. Details for each year that ageing is available for previously aged Deepwater Flathead and the comparison diagnostics. Unacceptable IAPE values are highlighted in red.

Year	*Total number of aged available*	*batch re-aged*	*Month of capture*	*Reader*	*APE between reader 4(sub-sample) and original reader*	*Comments*
1988	191	27	June	Reader 2	2.55	Acceptable precision
1989	414	30	Jan	Reader 2	4.09	Acceptable precision
1990	187	1	July	Reader 1	10.79	Reader 1's ages are less than Reader 4's ages by one year for most fish. DOC in July so perhaps caused by edge difference
		3	Nov	Reader 1	(Combined with above)	Reader 1's ages are less than Reader 4's ages by one year or several years
1991			No samples			
1992			No samples			
1993	249	6	Nov	Reader 1	9.94	Reader 1's ages are less than Reader 4's ages
1994	450	9	Jan	Reader 1	5.56	IAPE over 5%, high proportion younger by 1 year
1995	454	18	Feb	Reader 2	5.65	IAPE over 5%, poor precision but no bias
1996	535	40	Dec	Reader 1	4.87	Acceptable precision
1997	593	42	July	Reader 1	3.03	Acceptable precision
1998	979	54	Jan	Reader 3	2.31	Acceptable precision
1999	568	70	Jan	Reader 3	4.5	Acceptable precision
2000	340	81	July	Reader 3	2.99	Acceptable precision
2001	260	95	Mar	Reader 3	4.88	Acceptable precision
2002	555	113	Oct	Reader 4	4.17	Acceptable precision
2003	87	115	Apr	Reader 4	2.52	Acceptable precision
2004	208	128	Nov	Reader 4	2.98	Acceptable precision
2005	685	129	Feb	Reader 4	2.80	Acceptable precision
2006	477	185	Oct	Reader 4	3.05	Acceptable precision

Table 17. Age difference table between Reader 4 and Reader 1.

Age Reader	1																						
	Age																						
Difference	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	24	N
-3																								
-2							3	1	1	2				1										8
-1						2		1	2			2		1	2							1		11
0	**0**	**0**	**0**	**0**	**0**	**3**	**4**	**7**	**5**	**2**	**5**	**3**	**2**	**1**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**34**
1					1	2	5	2	4	5	4	3	4				2					1		33
2							2	3	1	1	2	1	4	1	1						1			17
3									2	2	1	3												8
7																						1		1
8																							1	1
Grand Total	0	0	0	0	1	7	14	14	15	12	12	12	10	4	4	0	2	1	0	0	1	3	1	113

Table 18. Age difference table between Reader 4 and Reader 2.

Age Reader	2																							
	Age																							
Difference	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	24	N
-3								1																1
-2								1		1	1													3
-1					1	1	2	5	4	1	1	2		1		1								19
0	**0**	**0**	**0**	**0**	**1**	**2**	**2**	**4**	**9**	**2**	**2**	**1**	**0**	**2**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**0**	**0**	**28**
1							1	1	1			1		1	1				2	1				9
2							1	1	1															3
3																								0
N	0	0	0	0	2	3	6	13	15	4	4	4	0	4	1	1	0	1	3	1	1	0	0	63

Table 19. Age difference table between Reader 4 and Reader 3.

Age reader	3																							
	Age																							
Difference	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	24	N
-3										1														1
-2							1	1			1													3
-1					6	5	1	2	2			1	1	3										21
0	**0**	**0**	**0**	**2**	**6**	**13**	**8**	**6**	**5**	**1**	**5**	**1**	**0**	**1**	**1**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**	**51**
1					2	4	2	2				2			1									13
2																								0
3																								0
N	0	0	0	2	14	22	12	11	7	2	6	4	1	4	2	0	0	0	1	0	1	0	0	89

Table 20. Age difference table between Reader 4 and Reader 4.

Age Reader	4																							
	Age																							
Difference		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	24	N
-3																								0
-2																								0
-1						3	3			1	1	1												9
0	**0**	**0**	**0**	**6**	**7**	**12**	**18**	**10**	**4**	**1**	**1**	**0**	**1**	**1**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**63**
1			1		2	8	3	2	5	1	5	1		1	1	1								31
2																1								1
3															1									1
N	0	0	1	6	9	23	24	12	9	3	7	2	1	2	2	2	0	1	1	0	0	0	0	105

Discussion

The audit of historical age data, overall, indicated:

- Overall there was no systemic bias in the historical data
- Historical interpretation of otolith microstructure was generally consistent with validated protocol developed in this study
- Reader interpretation for the majority of the historical samples was consistent
- Reader precision was also consistent and within the acceptable standard for stock assessments.

Where bias was detected, the historical bias was for:

  - Underestimation of age generally by one year and present in data from 1989
  - Overestimation of age generally by one year and present in data from 1990 and 1993 only.

The bias detected in the age estimates for the years 1989, 1990 and 1993 may have arisen because edge type was not considered. The bias may have arisen because at that time no thought was given to birth-date adjustment. It is likely that the bias observed could be explained by the absence of adjusted age data. The adjustment of age by ±1 year depends on:

- The assigned edge type
- Whether the date of capture was before or after the nominated birth-date.

The observed differences in assigned age between the audit and historical samples were usually less than one year.

Additionally this bias could have arisen from the mis-classification of the position of the first opaque zone in the historical samples. At the time of the historical ageing, this position had not been validated.

Bias was limited to samples from three years, and in these years age was under estimated or over estimated by one year. This bias is not likely to affect the validity of the stock assessment and subsequent management actions because:

- Ageing in most years complied with the now validated protocol
- Ageing in most years showed adequate levels of precision
- Historical age estimates in 1989, 1990 and 1993 were in error by one year. This is below the margin of error (± 2 years) that would invalidate current biomass estimates (Wise and Tilzey, 2000)
- The bias was evident in samples collected approximately 20 years ago and the effect of the ageing is obviated by time as these fish have been removed from the fishery by mortality.

This study has highlighted the need to include date of capture and adjustment period when selecting sub-samples for ageing. Ideally, if resources exist, samples should be selected throughout the year including samples around the period where edge-type adjustment is used. It has also highlighted the importance of taking ancillary data such as edge type and otolith readability scores even if they are not used currently in the routine ageing process.

Conclusions

The majority of the historical age estimates:

- Have adequate levels of precision
- Are consistent with the validated ageing protocol developed in this study.

Errors detected in the historical ageing data are below the margin of error (± 2 years) that would invalidate current biomass estimates (Wise and Tilzey, 2000). Since 2007, age estimates have been undertaken using the validated Deepwater Flathead ageing protocol, developed in this study. No bias has been detected and age estimates show an acceptable level of precision (CAF unpublished data, FAS unpublished data).

8. Benefits and Adoption

The benefits of this research are:

- A standard for interpreting age from Deepwater Flathead has been developed
- Industry and Management assured that management settings are based on the biological age of Deepwater Flathead and are scientifically sound, ensuring the fishery will be managed within ESD guidelines
- An improved understanding of the age structure of Deepwater Flathead in the GAB.

These benefits have been realised through:

- The adoption of the validated ageing protocol for the routine ageing of Deepwater Flathead since 2007. This protocol ensures the most accurate and precise techniques are used to assess the age of Deepwater Flathead
- Verbal updates of the ageing process and validated protocol have been presented to the GABFAG and GABMAC
- The age estimates of Deepwater Flathead based on the validated methods have informed the setting of fishery controls through the stock assessment process
- Industry standards implemented and report finalised.

The techniques and methods developed from this project will be made available to other organisations through FRDC ensuring consistent interpretation and accurate ageing between institutions in Australia.

9. Further Development

Activities and other steps that may be undertaken to further develop or disseminate the results of the research include:

- Analysis of the spatial and temporal changes in the zone counts.

This analysis may provide further information on the stock structure of Deepwater Flathead and represents an avenue for further research. The existence of spatial variation of zone counts may explain any regional differences in zone formation or growth. The latter may also provide evidence of separation of stocks within the GAB. The existence of separate stocks of Deepwater Flathead if found may require a different strategies for managing the resource.

This project has fully met its objectives. The validated ageing protocol has been adopted for the routine ageing of Deepwater Flathead. The ageing protocol could be adopted as an exemplar for other species of fish routinely aged within the SESSF.

Data

The CAF ceased to operate in 2009 and all AFMA ageing data were transferred to FAS Pty Ltd. FAS Pty Ltd has been contracted by AFMA to routinely age and provide ageing data to the fisheries. Under this contract FAS Pty Ltd archive and maintain all AFMA ageing project.

FAS Pty Ltd will store the data obtained from this project. This data will complement existing Deepwater Flathead SESSF and GABTF data maintained by FAS Pty Ltd as part of FAS's contractual obligations to AMFA.

The data will be maintained and curated according to FAS Data storage and maintenance protocols.

Data will be available upon application to FAS Pty Ltd following written approval from FRDC.

The report will be available publically on the FRDC website, will be lodged in selected libraries and will be distributed to key stakeholders.

The ageing protocol will be available in electronic form on application to FAS Pty Ltd.

10. Planned Outcomes

The objectives of this study were to:

1. Validate the periodicity of increment formation from marginal increment data and daily age estimation to the first opaque zone.
2. Determine the count of presumed daily rings between the primordium of the otolith and the outside edge of the first opaque zone for young fish caught in different locations within the GAB.
3. Implement standards on the age estimation of this species based on the timing of increment formation and the variability of 0+ age to the first increment formation
4. Understand the variability in growth rates of juvenile Deepwater Flathead.

The planned outcomes were:

1. To determine the timing of first increment formation and subsequent increment formation in age classes to asymptotic length (where samples are available).
2. Determine if there are temporal differences in the timing of increment formation and juvenile growth from samples collected from the GABTF.
3. Develop a standard for the interpretation of age for Deepwater Flathead, which will lead to more accurate and precise age estimates of this species.

The project output (a validated ageing protocol) has contributed to the planned outcomes as follows:

- **The periodicity of increment formation was shown to be annual**.
  This was achieved through marginal increment analysis coupled with marginal state analysis.

  The first opaque zone forms between 120 and 270 days following spawning. This information is consistent with the known spawning period for Deepwater Flathead and indicates the opaque zone starts to form in winter.

- **Determine the count of presumed daily rings between the primordium of the otolith and the outside edge of the first opaque for young fish caught in different locations within the GAB**.
  The number of daily rings was determined between the primordium of the otolith and the first opaque zone of fish from three zones within the GAB: the Central, the Eastern and the Western Zones.

  Measurements indicated that there is little difference between the average position of the first opaque zone in samples collected from different zones of the GAB.

  The daily zone counts between the three zones ranged between 120 days and 270 days. The largest variation in time of spawning was seen in the Western Zone where daily zone counts ranged between 135 and 270 days. The Central Zone had a difference of only 10 days before formation of the first annual increment. The Western Zone was more variable than the Central Zone with a difference of 45 days before the formation of the first annual zone. The mean daily zone counts were significantly different indicating that Deepwater Flathead in the Western Zone spawn before the fish in the Central or Eastern Zones.

  The mean of the daily zones before the formation of the first annual increment was also significantly different with the samples in the Western Zone having the narrowest width. The mean width of daily zones in samples from the Eastern and Western Zones was similar. This suggests that although more daily increments are present in the samples from the Western Zone, the width of each daily zone is smaller. The distance from the primordium to the first annual increment is approximately equal between the Western, Central and Eastern Zones in the GABTF.

- **Implement standards on the age estimation of this species based on the timing of increment formation and the variability of 0+ age to the first increment formation**
  An ageing protocol was developed for Deepwater Flathead based on the following results:
  - The most accurate method for examining age and growth of Deepwater Flathead is through counts of annuli on transverse otolith sections.
  - Daily zone counts from the hatch mark to the start of the first opaque zone (corresponding usually to the end of the diffuse nucleus) support the assumption that the first opaque zone starts to form in winter.
  - Measurements taken from the primordia to the first opaque zone indicated that there is little difference between the average position of the first opaque zone in samples collected from different zones of the GAB and that a proxy measurement for the first opaque zone would be a useful tool in the ageing of this species.
  - The first annual zone is located 0.460 mm on the dorsal side of the sulcus and 0.373 mm on the ventral side of the sulcus. These distances can be used as proxies for the position of the first annual zone.
  - One increment (one translucent and opaque zone) is formed each calendar year.
  - Increments can be used confidently to age Deepwater Flathead.

- **Understand the variability in growth rates of juvenile Deepwater Flathead**
  This objective was not fully met because there were insufficient samples available from juvenile Deepwater Flathead for analysis. The analyses that were done indicated that the Deepwater Flathead in the Western GAB have a slower otosomatic growth rate than the Deepwater Flathead from the Eastern or Central Zones. The implication of this could not be examined due to sample size. The growth data from the juvenile Deepwater Flathead allowed more biologically 'sensible' growth parameters to be estimated.

Performance indicators for this project were:

1. Determine the timing of first increment formation and subsequent increment formation in age classes to asymptotic length (where samples are available).
   Achieved – see Chapter 7 Sections 7.1 and 7.2
2. Determine if there are temporal differences in the timing of increment formation and juvenile growth from samples collected from the GABTF.
   Achieved – see Chapter 7 Sections 7.1 and 7.2
3. Develop a standard for the interpretation of age for Deepwater Flathead, which will lead to more accurate and precise age estimates of this species.
   Achieved – see Chapter 7 Sections 7.1, 7.2, 7.3, 7.4 and 7.5. The ageing protocol is presented in Appendix 7.

11. Conclusions

- The most accurate method for examining age and growth of Deepwater Flathead is through counts of annuli on transverse otolith sections.
- The daily zone estimates could be used effectively to determine the location and timing of the formation of the first opaque zone.
- Daily zone counts from the hatch mark to the start of the first opaque zone (corresponding usually to the end of the diffuse nucleus) support the assumption that the first opaque zone starts to form in winter.
- Measurements taken from the primordia to the first opaque zone indicated that there is little difference between the average position of the first opaque zone in samples collected from different zones of the GAB and that a proxy measurement for the first opaque zone would be a useful tool in the ageing of this species.
- Spawning dates when back calculated from daily zone counts were consistent with peak spawning periods which further supported the assumption that micro-increments counted in this study were formed daily.
- The first annual zone is located 0.460 mm on the dorsal side of the sulcus and 0.373 mm on the ventral side of the sulcus. These distances can be used as proxies for the position of the first annual zone.
- One increment (one translucent and opaque zone) are formed each calendar year.
- Increments can be used confidently to age Deepwater Flathead.
- An ageing protocol was developed from these results to standardise the interpretation of otolith microstructure for the routine ageing of Deepwater Flathead.
- The growth of Deepwater Flathead using the developed protocol is similar to those from previous studies. The mortality of female Deepwater Flathead is slightly higher than the total mortality in males.
- An audit of historical ageing using the validated protocol was undertaken. The audit showed the majority of the historical age estimates:
    - Had adequate levels of precision
    - Are consistent with the validated ageing protocol developed in this study.
- Errors detected in the historical ageing data are below the margin of error (± 2 years) that would invalidate current biomass estimates (Wise and Tilzey, 2000).
- Since 2007, age estimates have been undertaken using the validated Deepwater Flathead ageing protocol, developed in this study. No bias has been detected and age estimates show an acceptable level of precision (CAF unpublished data, FAS unpublished data).

12. References

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Appendix 1: Intellectual Property

No intellectual property has arisen from the research that is likely to lead to significant commercial benefits, patents or licences. Intellectual property associated with the information produced from this project will be available to all stakeholders in the GABTF and will be shared equally by the Fisheries Research Development Corporation and the Victorian department of Primary Industries.

Appendix 2: Staff

Mr Kyne Krusic-Golub - Principal Investigator FAS Pty Ltd

Leanne Gunthorpe – Co-investigator DPI

Simon Robertson – Co-Investigator FAS Pty Ltd

Appendix 3: Summary statistics for comparison of the reliablity of reading whole vs sectioned otoliths

t-Test: Paired Two Sample for Means

	Sectioned age	*whole age*
Mean	8.467191601	7.343832021
Variance	11.71273657	4.436731593
Observations	381	381
Pearson Correlation	0.563567686	
Hypothesized Mean Difference	0	
df	380	
t Stat	7.740695334	
P(T<=t) one-tail	4.51742E-14	
t Critical one-tail	1.648873399	
P(T<=t) two-tail	9.03483E-14	
t Critical two-tail	1.966226323	

REGRESSION SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.789109396
R Square	0.622693638
Adjusted R Square	0.621698107
Standard Error	1.742290482
Observations	381

ANOVA

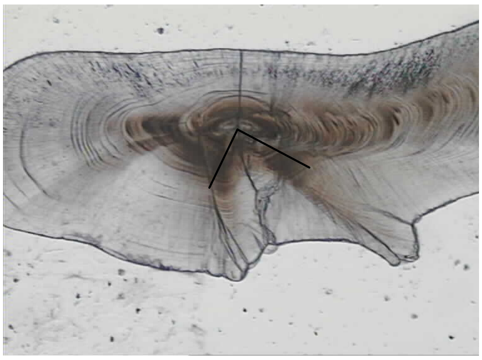
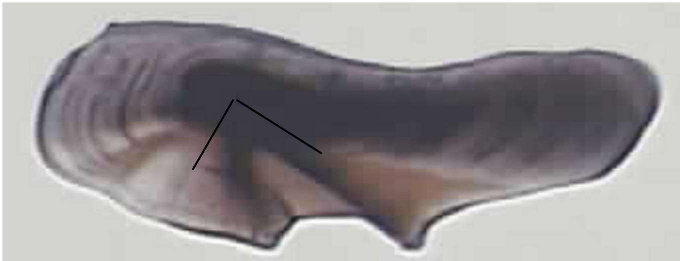
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1898.718749	1898.71875	625.48876	3.14808E-82
Residual	379	1150.48335	3.03557612		
Total	380	3049.2021			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-4.4069408	0.238461546	-18.4807189	7.933E-55	-4.875814122	-3.938067478	-4.875814122	-3.938067478
X Variable 1	0.653144589	0.026115574	25.0097733	3.148E-82	0.601795027	0.704494151	0.601795027	0.704494151

Appendix 4: Daily otolith samples

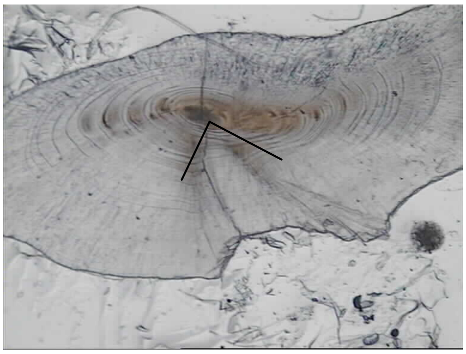
Central

611_116_011 (200 mm TL)

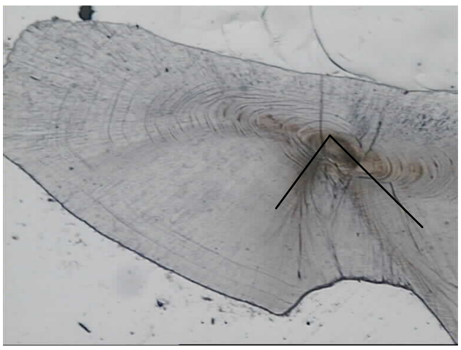
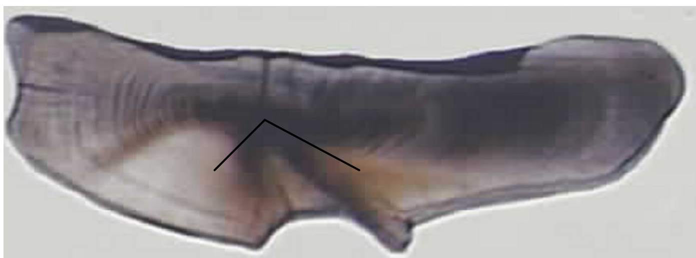
Ventral line 0.36 mm, dorsal line 0.39 mm. Transect used for measuring zones in annual age estimation

611_116_013 (205 mm TL)



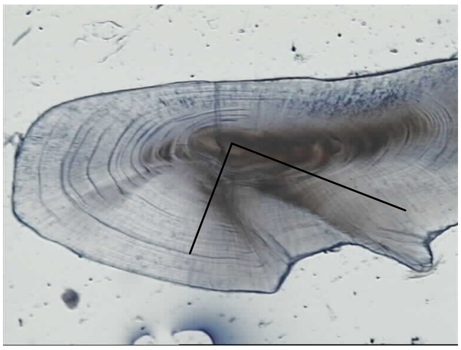

Ventral line 0.39 mm, dorsal line 0.44 mm.

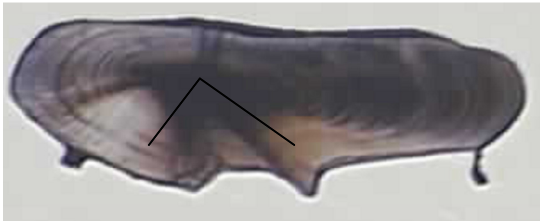
611_116_024 (280 mm TL)

Ventral line 0.37 mm, dorsal line 0.44 mm.

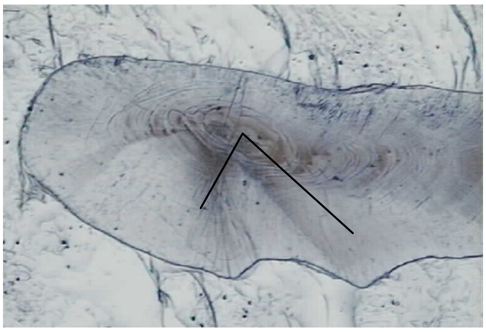
611_116_028 (200 mm TL)

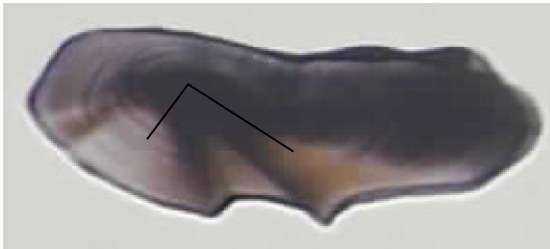




Ventral line 0.43 mm, dorsal line 0.63 mm.

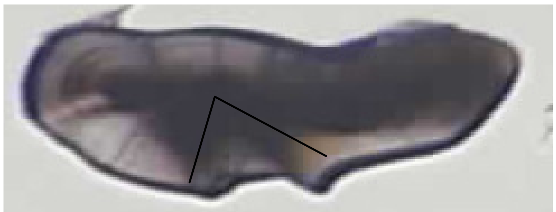
611_116_031





Ventral line 0.34 mm, dorsal line 0.47 mm.

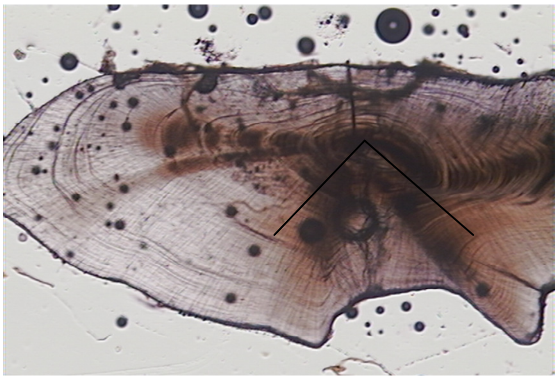
611_116_034 (125 mm TL)

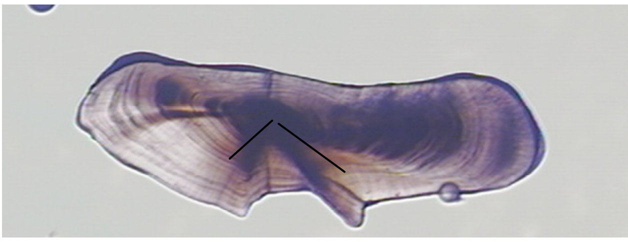


Ventral line 0.34 mm, dorsal line 0.51 mm.

West

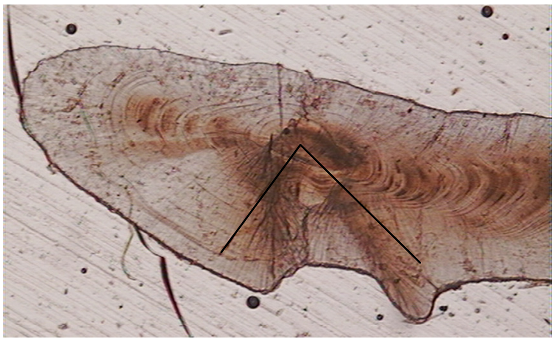
611_182_012 (120 mm TL)

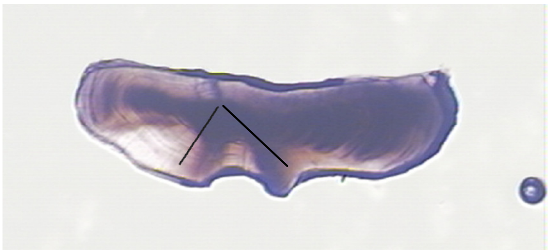




Ventral line 0.41 mm, dorsal line 0..44 mm.

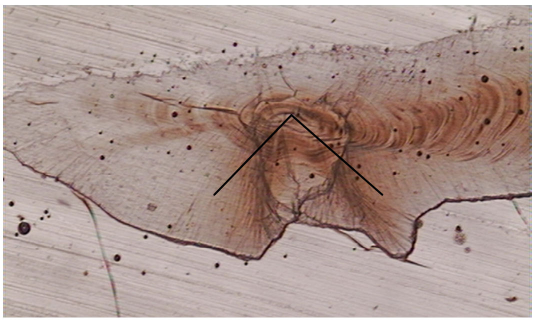
611_182_016 (180 mm TL)

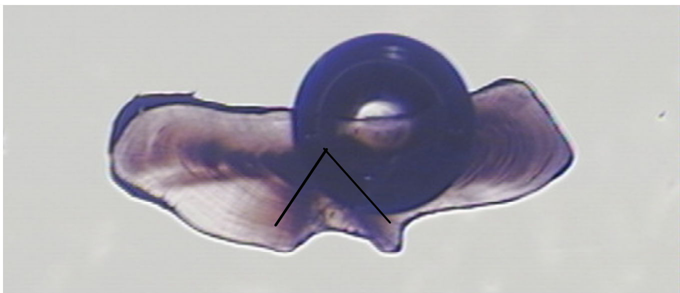




Ventral line 0.42 mm, dorsal line 0.53 mm.

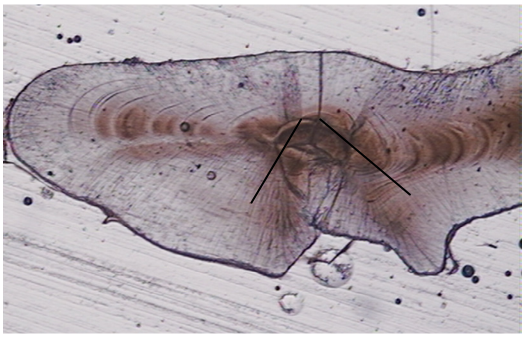
611_182_017 (170 mm TL)

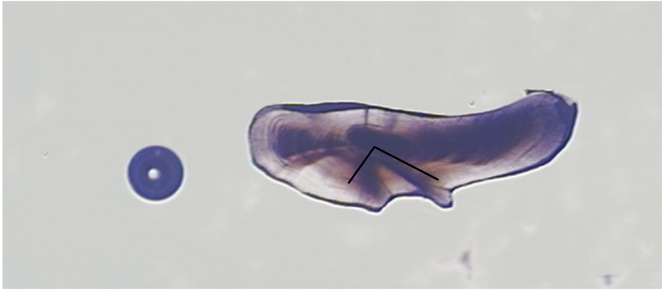




Ventral line 0.38 mm, dorsal line 0.44 mm.

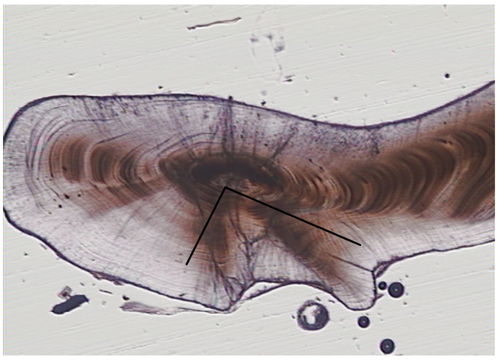
611_182_018 (150 mm TL)

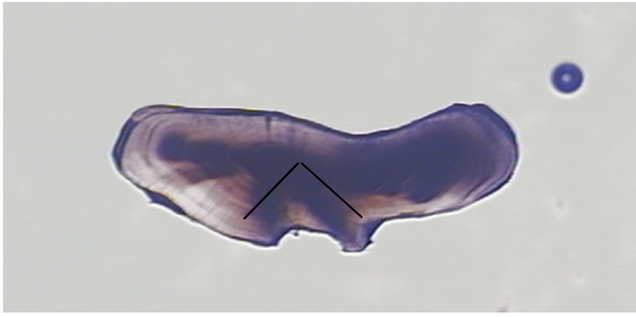




Ventral line 0.34 mm, dorsal line 0.42 mm.

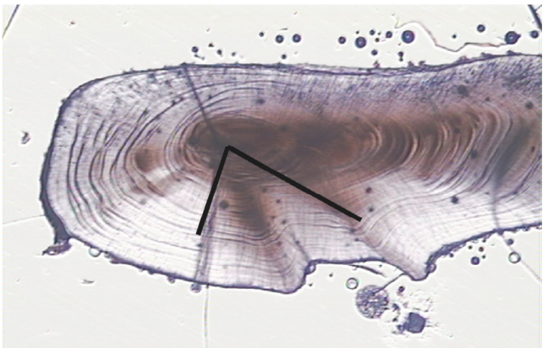
611_182_019 (160 mm TL)

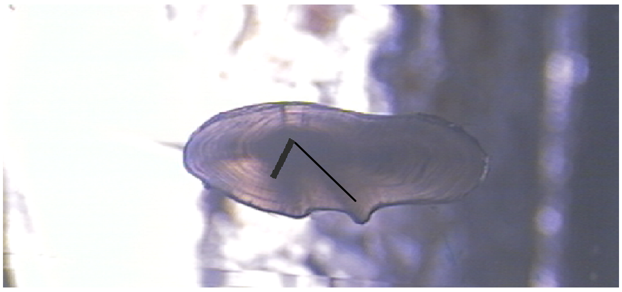




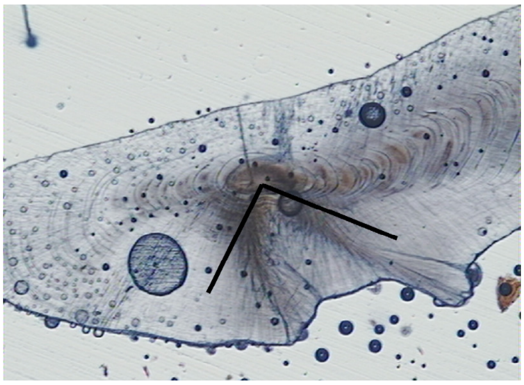
Ventral line 0.42 mm, dorsal line 0.57 mm.

611_183_002 (170 mm TL)



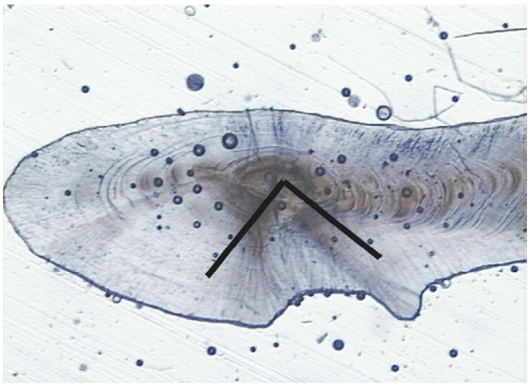


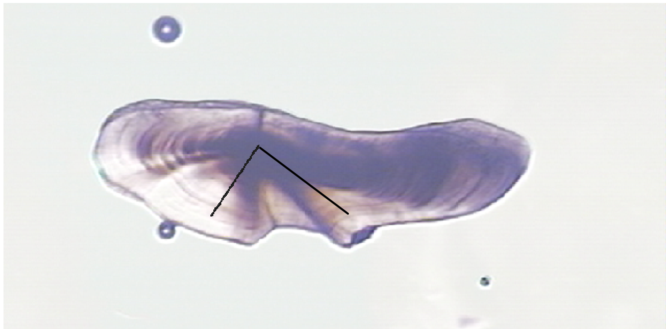
611_183_003 (185 mm TL)



Ventral line 0.37 mm, dorsal line 0.46 mm.

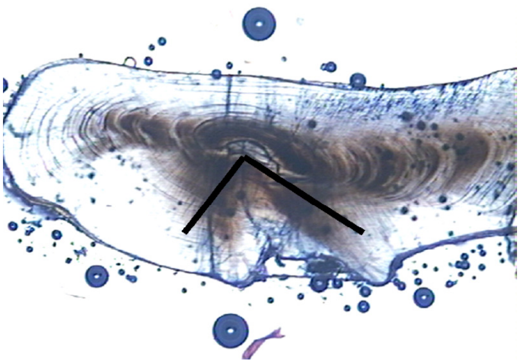
611_183_004 (170 mm TL)

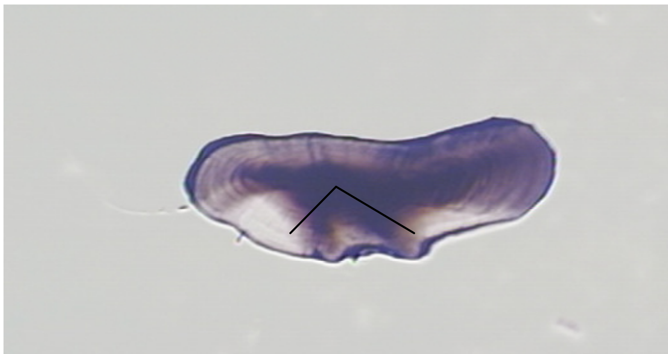




Ventral line 0.33 mm, dorsal line 0.4 mm.

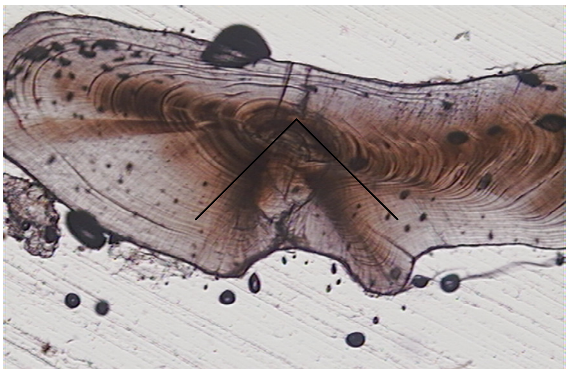
611_183_009 (170 mm TL)

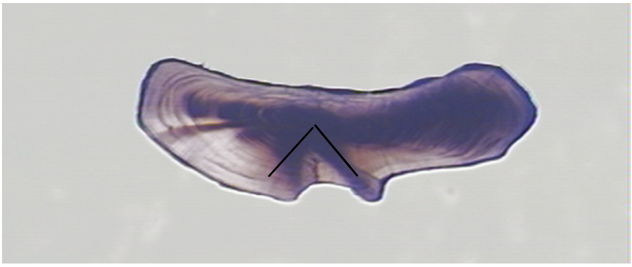




Ventral line 0.33 mm, dorsal line 0.39 mm.

611_184_001 (170 mm TL)

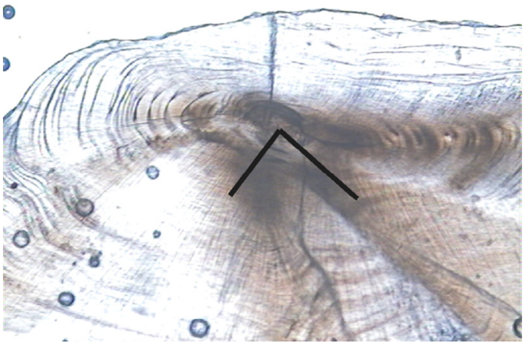


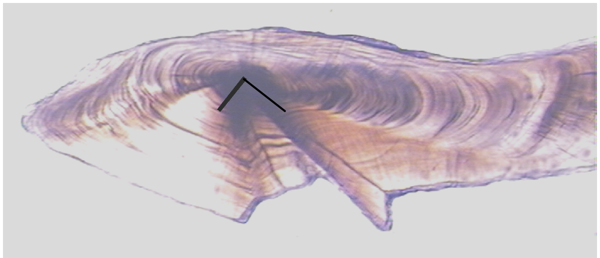


Ventral line 0.41, dorsal line 0.46 mm.

East

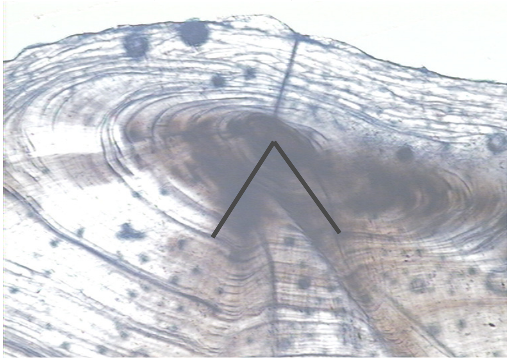
611-048-015 (500 mm TL)

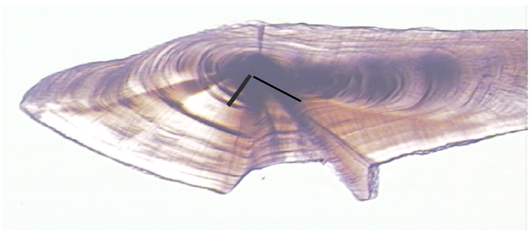




Ventral line 0.33 mm, dorsal line 0.42 mm.

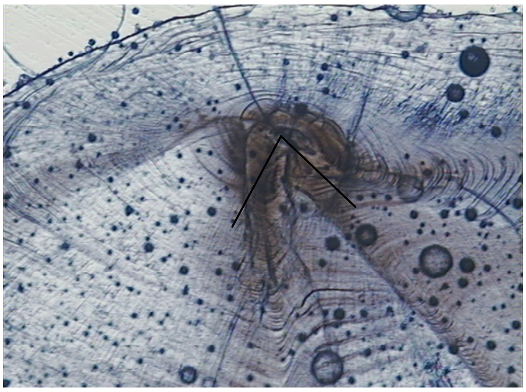
611-048-019 (410 mm TL)





Ventral line 0.42 mm., dorsal line 0.46 mm.

611-048-032

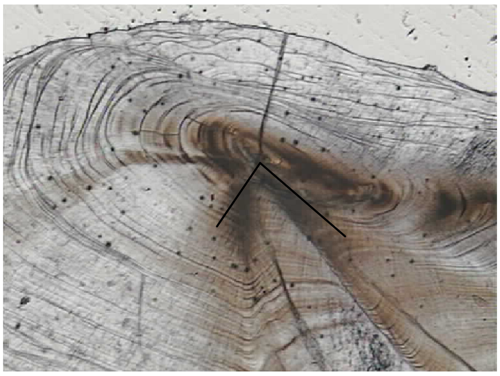


Ventral line 0.48 mm, Dorsal line 0.51 mm.

611_049_003 (500 mm TL)

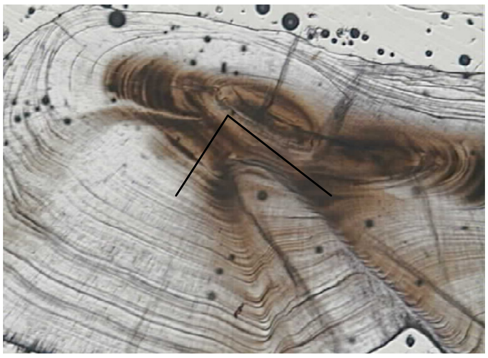
Ventral line 0.33, dorsal line 0.34 mm.

611_049_004 (430 mm TL)



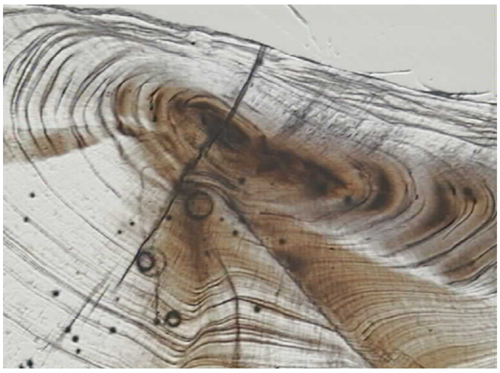
Ventral line 0.33, dorsal line 0.43 mm

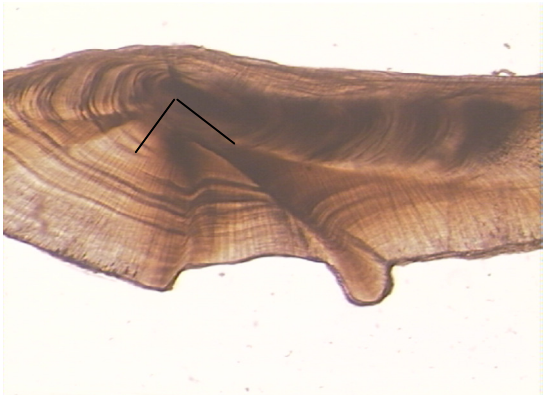
611_049_005 (430 mm TL)



Ventral line 0.33 mm, dorsal line 0.39 mm.

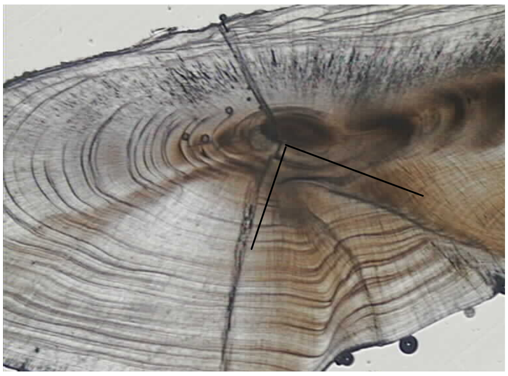
611_049_007 (460 mm TL)

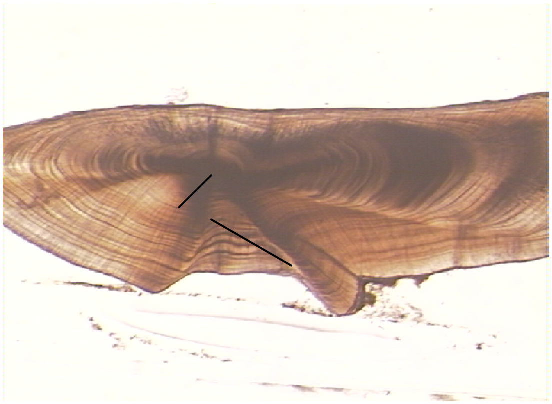




Ventral line 0.34 mm, dorsal line 0.39 mm.

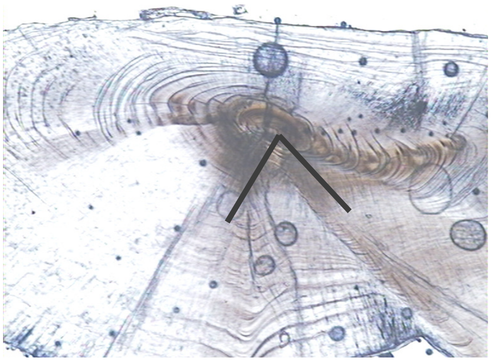
611_049_015 (430 mm TL)

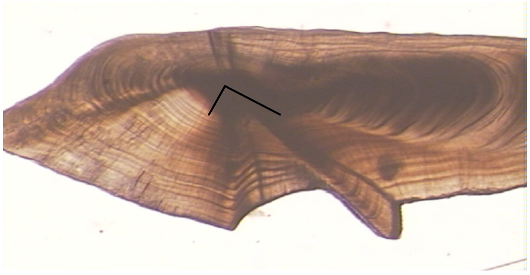




Ventral line 0.41 mm, dorsal line 0.44 mm.

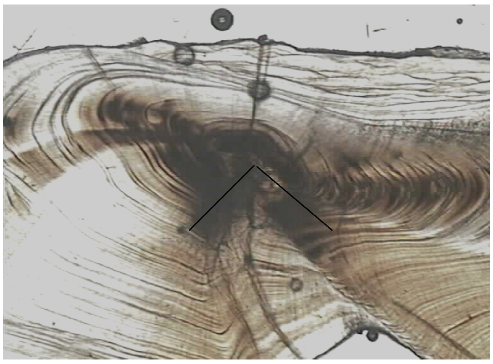
611_049_016 (460 mm TL)

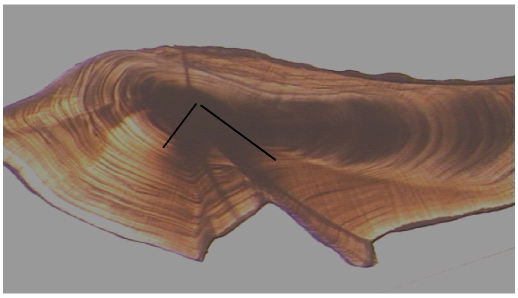




Ventral line 0.36 mm, dorsal line 0.51 mm.

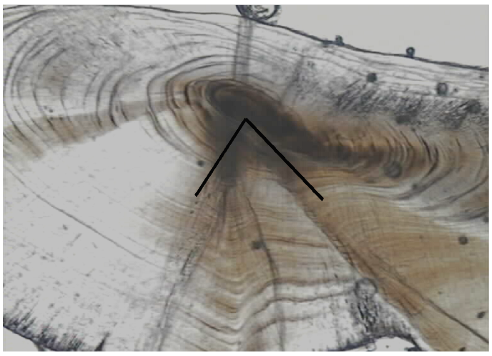
611_049_026 (450 mm TL)

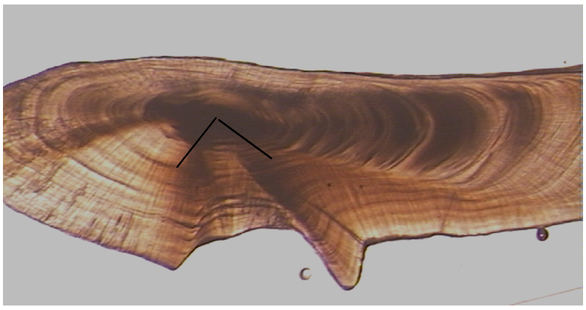




Ventral line 0.32 mm, dorsal line 0.36 mm.

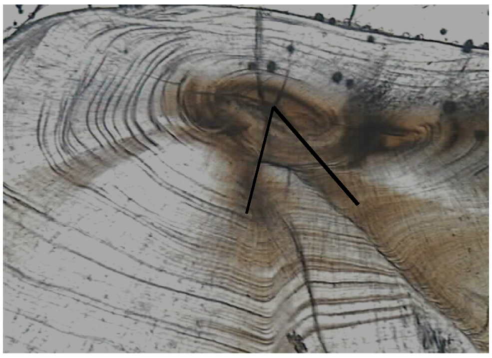
611_049_028 (450 mm TL)



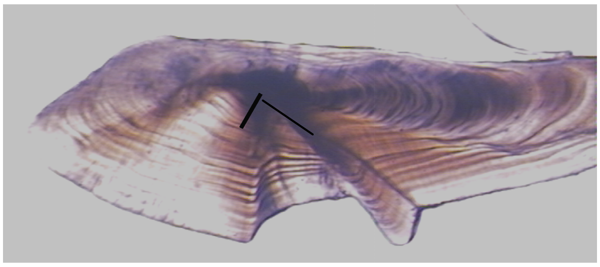


Ventral line 0.40 mm, dorsal line 0.44 mm.

611_049_029 (450 mm TL)



Ventral line 0.42 mm, dorsal line 0.45 mm.



Summary Data

Mean zone count from primordia to the outer edge of the first opaque (A1)

	Zone		
Data	CENTRAL	EAST	WEST
Mean	137.50	145.91	191.00
SD	5.24	13.93	47.95
N	6	11	10
2SE	4.28	8.40	30.32

Mean increment width (μm) for daily zones out to the edge of the fist opaque zone on the ventral side

	Zone		
Data	CENTRAL	EAST	WEST
Mean (v)	2.70	2.58	2.03
SD (v)	0.20	0.28	0.39
N (v)	6	11	10
2SE (v)	0.16	0.17	0.25

Mean increment width (μm) for daily zones out to the edge of the fist opaque zone on the dorsal side

	Zone		
Data	CENTRAL	EAST	WEST
Mean (d)	3.48	3.00	2.56
SD (d)	0.48	0.35	0.55
N (d)	6	11	10
2SE (d)	0.39	0.21	0.35

Mean distance (mm) from the primordia to the outer edge of the first opaque zone (A1) on the ventral

	Zone		
Data	CENTRAL	EAST	WEST
Mean (v)	0.372	0.373	0.373
SD (v)	0.034	0.052	0.041
N (v)	6	12	10
2SE (v)	0.028	0.030	0.026

Mean distance (mm) from the primordia to the outer edge of the first opaque zone (A1) on the dorsal

	Zone		
Data	CENTRAL	EAST	WEST
Mean (d)	0.480	0.433	0.468
SD (d)	0.083	0.056	0.057
N (d)	6	12	10
2SE (d)	0.07	0.03	0.04

Appendix 5: Age length keys

Combined

Sex	(All)
Year	(All)

	Age																												
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
12	1																												1
14	1																												1
16		2																											2
17		2																											2
18		7	2																										9
19		3	1																										4
20		9																											9
21		7	2																										9
22		2	1																										3
24		1	2																										3
25			2																										2
27			1																										1
28		1	1																										2
29			1																										1
30			1																										1
31				3																									3
33				5	1																								6
34			1	4	2																								7
35				1	3	1																							5
36				1	7	5																							13
37				4	4	2																							10
38				2	8	3	2	1																					16
39					14	15	4		1																				34
40				2	22	29	5	4	1																				63
41				2	30	54	11	6	2		1	1		1															108
42				4	29	60	21	16	1		1	1																	133
43					22	53	34	16	9	2	1	1																	138
44					15	51	27	14	8	6	2																		123
45					8	37	27	30	16	13	6	1	2	1															141
46					4	24	25	33	17	21	11	2	2	1	1	1		1											143
47					5	14	16	22	19	17	12	8	12	4	2									1					132
48					1	12	16	22	19	18	18	8	6	7	1	3	2												133
49					1	4	15	17	11	14	6	11	10	5	4	2	5	2	1	2									110
50					3	8	12	18	10	11	9	3	10	8	2	2	3	1	2	1				1					104
51						4	7	12	7	10	3	9	3	5	2	3	3		3	1	1		1						74
52						9	15	17	7	3	5	4	3	1	3	3	3	1	3		1	1		1			1		81
53					1	5	11	11	11	4	3	4	2	3	1	2		1	3	1			1						64
54						3	4	16	13	3	4	3		2	3	2	1		1	2									57
55						1	6	18	15	8	4	4	2	1	2		1	1	3				1						67
56							7	29	12	10	4			3			1				1								67
57							5	19	4	10		1	2	1	1	1	1					1							46
58							3	9	10	4	9	5	2		1	2		1											46
59						1	1	7	4	2	7	2	3		1	1				1	1				1				32
60							1	5	10	9	3	4																	32
61								5	2	4	3	1	1	1					1									1	19
62								3	3	7	3	3	3	1	1	1	1	1											27
63									3	1	2	3	1	2	1		2				1								16
64										5	3	3	1	3	3		1								1				20
65									1		3	2	2	1	2	2													13
66											1	4	4	1			1												11
67									1		5	1		1			1												9
68										1	2	2	1	4	1	2			1										14
69											1	2	3				1							1		1			9
70																2													2
71											1		1	1	1	2						1							7
72													2	1	1			1											5
73											1							1											2
74															1							1							2
75													1																1
79									1																				1
81																	1												1
82																			1										1
83																1													1
N	2	34	15	28	180	395	275	350	218	183	134	93	79	59	35	32	28	11	19	8	5	4	3	4	2	1	1	1	2199

Sex	(All)
Year	1998

															Age														
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
12																													
14																													
16																													
17																													
18																													
19																													
20																													
21																													
22																													
24																													
25																													
27																													
28		1																											1
29																													
30																													
31																													
33				1																									1
34			1		1																								2
35				1	1	1																							3
36				1	1																								2
37					1	1																							2
38					1	1																							2
39					5	5	4																						14
40					6	8	2	1	1																				18
41					4	24	3	4	2			1		1															39
42					4	22	10	10	1		1	1																	49
43					2	16	15	6	7	2	1	1																	50
44					1	12	15	9	8	3	1																		49
45					1	9	14	16	8	8	5	1	1	1															64
46					2	4	10	14	9	10	7	1			1														58
47					1	1	6	8	11	10	6	5	8	3															59
48						1	6	5	10	11	13	5	4	3	1	2													61
49						3	7	3	9	11	5	5	6	2	1	2	2		1										57
50					1	1	5	7	7	4	3	2	4	3		2	1		1					1					42
51						1	2	4	2	4	2	7	1	2	1	1	2		3				1						33
52						1	1	1	4	1	2	1	2		2	3	2	1	3		1	1		1			1		28
53								2	3		1	2	1		1	1		1	2				1						15
54								3	2			1			1		1			1									9
55							1	2	2	2	2	1	1		1			1					1						14
56								3		2	1			1			1												8
57							1	2		3			1	1	1		1					1							11
58								1	3	2	4	1	1																12
59									1		5	1	1		1						1				1				11
60									1	2	3	1																	7
61									1										1									1	3
62										2		2	2	1		1	1	1											10
63																					1								1
64											1		1		1										1				4
65											2		1		1														4
66														1			1												2
67														1															1
68																1													1
69																								1					1
70																1													1
71																													
72																													
73																													
74																													
75																													
79																													
81																	1												1
82																													
83																													
N		1	1	3	32	111	102	101	92	77	65	39	35	20	13	14	13	4	11	1	3	2	3	3	2		1	1	750

Sex	(All)
Year	1999

															Age														
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
12																													
14																													
16																													
17																													
18																													
19																													
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21																													
22																													
24																													
25																													
27																													
28																													
29																													
30																													
31																													
33				1																									1
34				1	1																								2
35																													
36						1																							1
37				2	1	1																							4
38				2	5	2	1																						10
39					8	7																							15
40				2	11	11	2	1																					27
41				1	13	20	5				1																		40
42				2	19	26	6																						53
43					14	17	9	2																					42
44					9	25	6	3																					43
45					3	12	5	5	4	4	1		1																35
46						8	7	8	7	4	4		1																39
47					2	5	2	8	5	4	4	2	4		2									1					39
48					1	2	5	5	5	7	4	3	1	3			1												37
49						1	2	4		3	1	6	3	3	3		3	2		2									33
50							1	1	2	6	5	1	6	5	1		2	1	1	1									33
51						1	1	1	2	3		1	2	3		2	1			1	1								19
52						2	2	2	2	1	2	3	1				1												16
53						2	1		2		1			2		1			1	1									11
54						1		1	3		1	1			1	1				1									10
55								2	1	3			1				1		1										9
56							2	3	2																				7
57										1		1				1													3
58											1	1			1			1											4
59																1													1
60									1			1																	2
61																													
62										1																			1
63														1															1
64																													
65																													
66																													
67																													
68																													
69																													
70																													
71											1																		1
72																													
73																													
74																													
75																													
79									1																				1
81																													
82																													
83																													
N				11	87	144	57	46	37	37	26	20	20	17	8	6	9	4	3	6	1			1					540

Sex	(All)
Year	2001

	Age																												
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
12	1																												1
14	1																												1
16		2																											2
17		1																											1
18		3																											3
19		3																											3
20		9																											9
21		7																											7
22		2																											2
24		1																											1
25																													
27			1																										1
28			1																										1
29			1																										1
30			1																										1
31				1																									1
33				1																									1
34				1																									1
35																													
36																													
37					1																								1
38																													
39					1																								1
40					5	3																							8
41				1	11	9	2																						23
42				2	6	7	3	2																					20
43					6	8	5	1	1																				21
44					5	11	3																						19
45					4	10	5	1		1																			21
46					2	7	3	3		1																			16
47					2	7	3	1	1	3	1			1															19
48						5	2	1			1						1												10
49							2	1					1																4
50					2	5		3		1	1																		12
51						1				2	1	1																	5
52						5	2	4		1	1			1															14
53					1	2	3	1		3		2		1															13
54						1	2	1			3	1		2		1			1										12
55						1	2		1	1		3			1				1										10
56							2	2	5					2							1								12
57							3	1	1	1																			6
58							1	2	2		1	1				1													8
59						1		3	2		2																		8
60								1		2																			3
61								3		1	2	1																	7
62								2	1		1		1																5
63									2		1				1														4
64										1	1	1		2	1														6
65												1				2													3
66													1																1
67											1																		1
68											1			1															2
69																													
70																1													1
71													1		1	1													3
72																													
73																													
74																													
75																													
79																													
81																													
82																													
83																1													1
N	2	28	4	6	46	83	43	33	16	18	18	11	4	10	4	7	1		2		1								337

Sex	(All)
Year	2002

															Age														
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
12																													
14																													
16																													
17		1																											1
18		4	2																										6
19			1																										1
20																													
21			2																										2
22			1																										1
24			2																										2
25			2																										2
27																													
28																													
29																													
30																													
31				2																									2
33				2	1																								3
34				2																									2
35					2																								2
36					6	4																							10
37				2	1																								3
38					2		1	1																					4
39						3			1																				4
40						7	1	2																					10
41					2	1	1	2																					6
42						5	2	4																					11
43						12	5	7	1																				25
44						3	3	2		3	1																		12
45						6	3	8	4																				21
46						5	5	8	1	6		1	1	1		1		1											30
47						1	5	5	2		1	1																	15
48						4	3	11	4				1	1		1													25
49					1		4	9	2																				16
50						2	6	7	1						1														17
51						1	4	7	3	1					1														17
52						1	10	10	1						1														23
53						1	7	8	6	1	1		1																25
54						1	2	11	8	3					1														26
55							3	14	11	2	2			1					1										34
56							3	21	5	8	3																		40
57							1	16	3	5			1																26
58							2	6	5	2	3	2	1			1													22
59							1	4	1	2		1	2							1									12
60							1	4	8	5		2																	20
61								2	1	3	1		1	1															9
62								1	2	4	2	1			1														11
63									1	1	1	3	1	1			2												10
64										4	1	2		1	1		1												10
65									1		1	1	1	1	1														6
66											1	4	3																8
67									1		4	1					1												7
68										1	1	2	1	3	1	1			1										11
69											1	2	3				1									1			8
70																													
71														1		1						1							3
72													2	1	1			1											5
73											1							1											2
74															1							1							2
75													1																1
79																													
81																													
82																			1										1
83																													
N		5	10	8	15	57	73	170	73	51	25	23	20	12	10	5	5	3	3	1		2				1			572

Males

Sex	M
Year	(All)

															Age														
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
12																													
14																													
16																													
17																													
18																													
19																													
20																													
21																													
22																													
24																													
25																													
27																													
28																													
29																													
30																													
31																													
33				1																									1
34			1																										1
35					1	1																							2
36				1		1																							2
37				2	4	1																							7
38				1	6	2	1																						10
39					13	12	4		1																				30
40				2	21	28	4	4	1																				60
41				2	27	51	11	5	1			1		1															99
42				4	22	51	20	14	1		1	1																	114
43					21	44	30	13	8	2	1	1																	120
44					13	43	16	13	7	5	2																		99
45					5	25	22	25	12	12	4	1	2	1															109
46					3	13	14	25	14	19	11	2	2	1	1	1		1											107
47					3	7	10	16	17	14	10	7	10	3	2									1					100
48						5	7	9	11	13	14	8	6	5	1	3	2												84
49					1	2	4	6	9	10	3	7	8	4	4	2	3	1		1									65
50					2	2	2	5	5	8	6	2	7	5	2	2	3	1	2	1									55
51						1	1	1	2	9	2	9	3	4	1	2	3		2	1	1		1						43
52						2	1	2	1	1	2	2	3	1	2	1	1		3					1			1		24
53										3	1	1	1	2	1	1		1	3	1									15
54										1	3			2	2	2	1			1									12
55									1		1	3			1		1	1	2				1						11
56								1						1															2
57										1				1		1													3
58								1				1																	2
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Sex	M
Year	1998

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Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
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35					1	1																							2
36				1																									1
37					1																								1
38					1																								1
39					4	5	4																						13
40					6	8	2	1	1																				18
41					3	24	3	3	1			1		1															36
42					4	20	9	8	1		1	1																	44
43					2	13	15	6	6	2	1	1																	46
44					1	9	10	9	7	2	1																		39
45						8	12	14	6	7	4	1	1	1															54
46					1	3	7	12	6	8	7	1			1														46
47					1		4	7	10	9	5	5	8	2															51
48							5	2	7	7	9	5	4	1	1	2													43
49						1	2	1	8	9	3	3	5	2	1	2	1												38
50					1	1	2	3	3	3	3	2	3	2		2	1		1										27
51						1	1	1	1	3	1	7	1	1	1	1	2		2				1						24
52									1				2		2	1	1		3					1			1		12
53													1		1			1	2										5
54																	1												1
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57										1				1															2
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Sex	M
Year	1999

															Sex														
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
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36						1																							1
37				2	1	1																							4
38				1	4	2																							7
39					8	6																							14
40				2	10	10	2	1																					25
41				1	12	17	5																						35
42				2	13	21	6																						42
43					13	16	8	2																					39
44					7	22	3	3																					35
45					2	9	5	5	2	4			1																28
46						3	4	5	7	4	4		1																28
47					1	1	2	6	4	3	4	1	2		2									1					27
48							2	3	3	6	4	3	1	3			1												26
49						1	1	2		1		4	2	2	3		2	1		1									20
50									2	5	2		4	3	1		2	1	1	1									22
51									1	3		1	2	3		1	1			1	1								14
52							1				1	2	1																5
53														1		1			1	1									4
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Sex	M
Year	2001

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Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
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41				1	10	9	2																						22
42				2	5	7	3	2																					19
43					6	7	4		1																				18
44					5	10	2																						17
45					3	7	4	1		1																			16
46					2	5	2	3		1																			13
47					1	5	2	1	1	2	1			1															14
48						4		1			1						1												7
49							1	1					1																3
50					1	1		1			1																		4
51										2	1	1																	4
52						2		2		1	1			1															7
53										3		1		1															5
54											2			2		1													5
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Sex	M
Year	2002

														Age														
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
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39						1			1																				2
40						7		2																					9
41					2	1	1	2																					6
42						3	2	4																					9
43						8	3	5	1																				17
44						2	1	1		3	1																		8
45						1	1	5	4																				11
46						2	1	5	1	6		1	1	1		1		1											20
47						1	2	2	2			1																	8
48						1		3	1				1	1		1													8
49					1			2	1																				4
50								1							1														2
51										1																			1
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83																													
N					5	27	12	33	11	11	3	2	2	2	2	2		1	1	1									115

Females

Sex	F
Year	(All)

															Age														
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
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39						1																							1
40					1	1	1																						3
41					3	3		1	1		1																		9
42					7	7	1	1																					16
43					1	9	4	3	1																				18
44					2	8	11	1	1	1																			24
45					3	12	5	5	4	1	2																		32
46					1	11	11	8	3	2																			36
47					2	7	6	6	2	3	2	1	2	1															32
48					1	7	9	13	8	5	4			2															49
49						2	11	11	2	4	3	4	2	1			2	1	1	1									45
50					1	6	10	13	5	3	3	1	3	3										1					49
51						3	6	11	5	1	1			1	1	1			1										31
52						7	14	15	6	2	3	2			1	2	2	1			1	1							57
53					1	5	11	11	11	1	2	3	1	1		1							1						49
54						3	4	16	13	2	1	3			1				1	1									45
55						1	6	18	14	8	3	1	2	1	1				1										56
56							7	28	12	10	4			2			1				1								65
57							5	19	4	9		1	2		1		1					1							43
58							3	8	10	4	9	4	2		1	2		1											44
59						1	1	6	4	2	7	2	3		1	1					1				1				30
60							1	5	10	9	3	4																	32
61								5	2	4	3	1	1	1					1									1	19
62								3	3	7	3	3	3	1	1	1	1	1											27
63									3	1	2	3	1	2	1		2				1								16
64										5	3	3	1	3	3		1								1				20
65									1		3	2	2	1	2	2													13
66											1	4	4	1			1												11
67									1		5	1		1			1												9
68										1	2	2	1	4	1	2			1										14
69											1	2	3				1							1		1			9
70																2													2
71											1		1	1	1	2						1							7
72													2	1	1			1											5
73											1							1											2
74															1							1							2
75													1																1
79									1																				1
81																	1												1
82																			1										1
83																1													1
N					25	94	128	207	127	85	73	47	37	28	18	17	14	6	7	2	4	4	1	2	2	1		1	930

Sex	F
Year	1998

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Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
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42						2	1	1																					4
43						3			1																				4
44						3	5		1	1																			10
45					1	1	2	2	2	1	1																		10
46					1	1	3	2	3	2																			12
47						1	2	1	1	1	1			1															8
48						1	1	3	3	4	4			2															18
49						2	5	2	1	2	2	2	1				1		1										19
50							3	4	4	1			1	1										1					15
51							1	3	1	1	1			1					1										9
52						1	1	1	3	1	2	1				2	1	1			1	1							16
53								2	3		1	2				1							1						10
54								3	2			1			1					1									8
55							1	2	1	2	2	1	1		1														11
56								3		2	1						1												7
57							1	2		2			1		1		1					1							9
58								1	3	2	4	1	1																12
59									1		5	1	1		1						1				1				11
60									1	2	3	1																	7
61									1										1									1	3
62										2		2	2	1		1	1	1											10
63																					1								1
64											1		1		1										1				4
65											2		1		1														4
66														1			1												2
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N					3	15	26	33	33	26	30	12	10	8	6	6	7	2	3	1	3	2	1	2	2			1	232

Sex	F
Year	1999

	Age																												
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
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43					1	1	1																						3
44					2	3	3																						8
45					1	3			2		1																		7
46						5	3	3																					11
47					1	4		2	1	1		1	2																12
48					1	2	3	2	2	1																			11
49							1	2		2	1	2	1	1			1	1		1									13
50							1	1		1	3	1	2	2															11
51						1	1	1	1							1													5
52						2	1	2	2	1	1	1					1												11
53						2	1		2		1			1															7
54						1		1	3			1																	6
55								2	1	3			1						1										8
56							2	3	2																				7
57										1		1																	2
58											1	1			1			1											4
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N					14	34	18	19	18	11	10	9	6	5	1	2	2	2	1	1									153

Sex	F
Year	2001

	Age																												
Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
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51						1																							1
52						3	2	2																					7
53					1	2	3	1				1																	8
54						1	2	1			1	1							1										7
55						1	2		1	1																			5
56							2	1	5					2							1								11
57							3	1	1	1																			6
58							1	2	2		1					1													7
59						1		2	2		2																		7
60								1		2																			3
61								3		1	2	1																	7
62								2	1		1		1																5
63									2		1				1														4
64										1	1	1		2	1														6
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N					6	23	23	19	14	8	11	5	3	5	3	6			1		1								128

Sex	F
Year	2002

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Length	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	N
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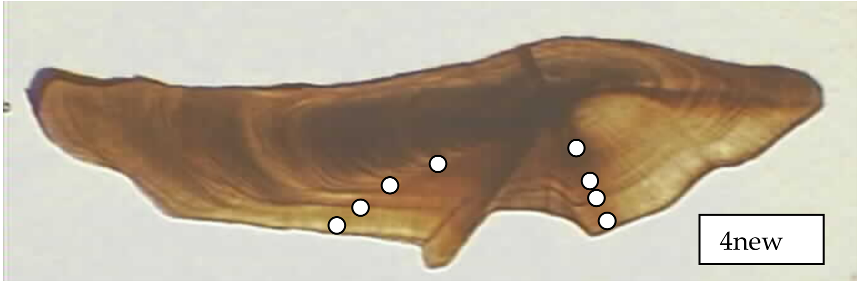
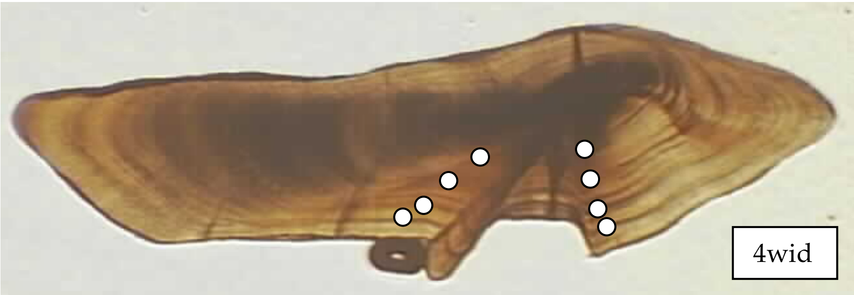
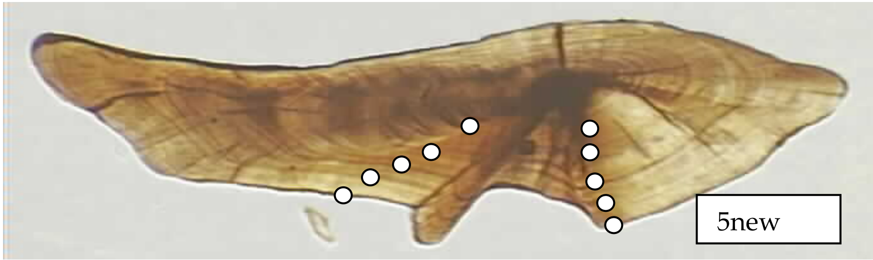
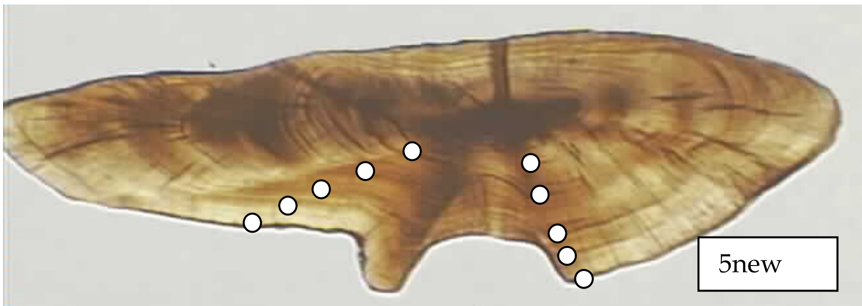
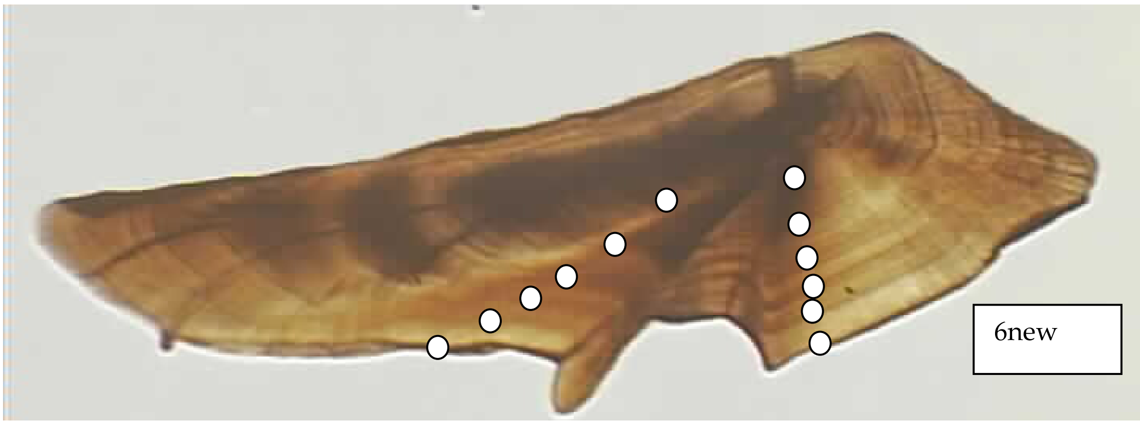
Appendix 6: Images of otolith sections from Deepwater Flathead, with age and edge type estimates.







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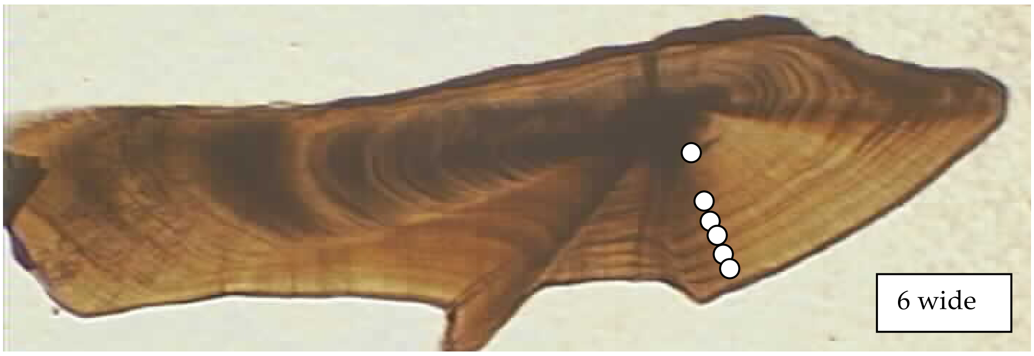
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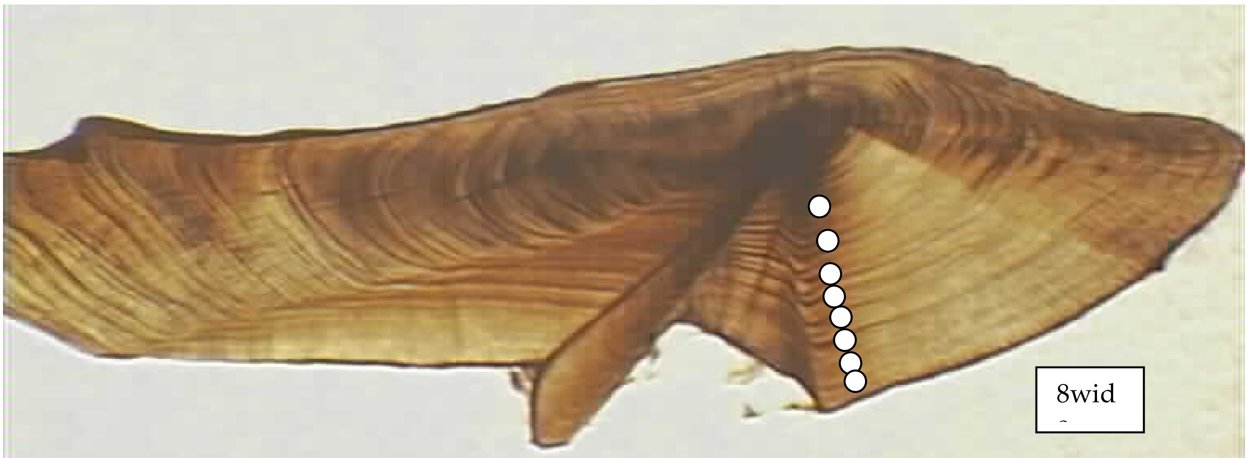
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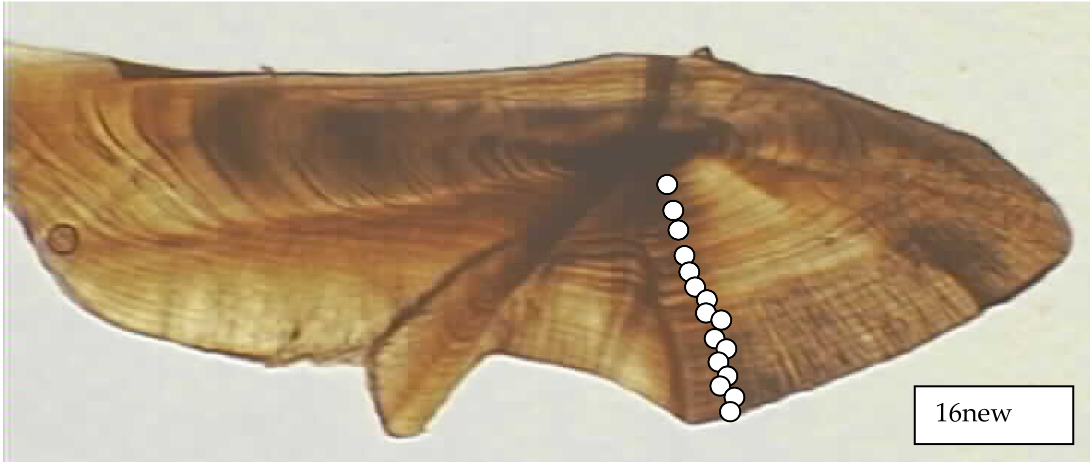
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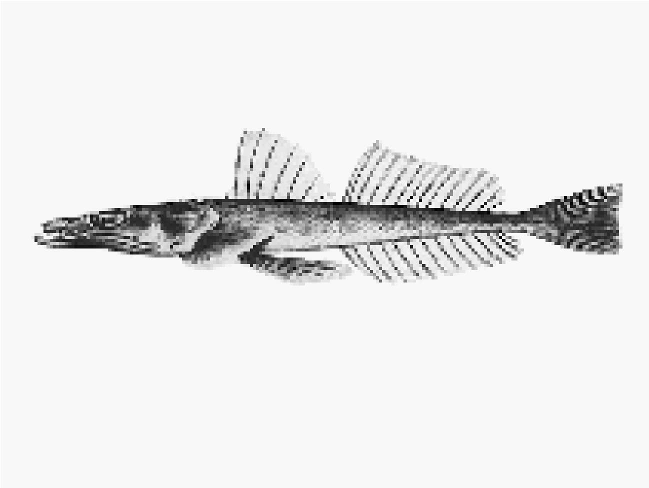
Appendix 7: Ageing protocol for Deepwater Flathead

A MANUAL FOR AGE DETERMINATION OF DEEPWATER FLATHEAD, *NEOPLATYCEPHALUS CONATUS*



Kyne Krusic-Golub, Simon Robertson, Therese Bruce, Leanne Gunthorpe and Corey Green

State Government Victoria Department of Primary Industries

Australian Government
Fisheries Research and Development Corporation

Project No. 2005/008

A MANUAL FOR AGE DETERMINATION OF DEEPWATER FLATHEAD, *NEOPLATYCEPHALUS CONATUS*

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Deepwater Flathead

Deepwater Flathead (*Neoplatycephalus conatus*) is endemic to southern coastal waters of Australia between the western edge of Bass Strait and south coast of Western Australia (Figure 1). This species is found at depths of 70–360 m (Gomon *et al.*, 1994).

Deepwater Flathead are a species, that:

- Grows to a length of 94 cm for females and 62 cm for males
- Weighs over 4 kg and 2 kg respectively
- Lives for more than 20 years (Newton *et al.*, 1994). Un-validated age estimates have produced a maximum age of 33 years for females and 28 years for males (Krusic-Golub and Stokie, 2008).

Deepwater Flathead are the dominant commercial species caught within the designated zones of the Great Australian Bight Trawl Fishery (GABTF) of the Southern and Eastern Scalefish and Shark Fishery (SESSF).

The Deepwater Flathead fishery is managed though a stock assessment that uses an age-structured model to estimate biomass (unexploited biomass) and to indicate whether the yield from the fishery is sustainable. One of the assumptions of the age-structure model is that the age estimates are accurate (Anon 2004).

The setting of annual total allowable catch of Deepwater Flathead is reliant on age estimates (catch–at-age data), as other biological data are limited. The age data provides an indicator of the strength of recruitment into the fishable biomass and the loss of older year classes from the population.

Quality assurance is an essential component of the age estimation process and a large component of quality age estimates is well developed protocols within each species.

This document is intended to :

- Document a standardised approach for routine age estimation of Deepwater Flathead otoliths.

Its purpose is to facilitate the precise age estimation over time, between readers and institutes. It is not intended to validate the age estimates produced.

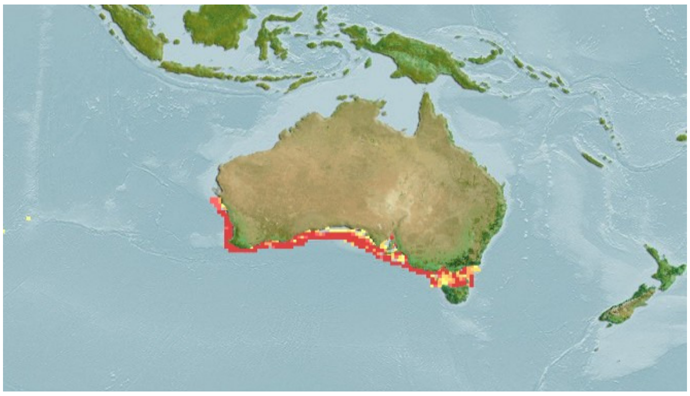


Figure 1. Distribution of Deepwater Flathead. Colours indicate probability of occurrence (red 0.80–1.00, yellow 0.20–0.39). Source: Fishbase.

The information presented in this document is based on the results of the Age validation of Deepwater Flathead from the Great Australian Bight Trawl Fishery project (FRDC 2005/008).

This major findings of this study found were:

- Sectioned otoliths provide more reliable estimates of zone counts
  Zone counts from whole otoliths were unreliable for all age classes except 7 years
- The first opaque zone in Deepwater Flathead is formed approximately 7–10 months after spawning.
  The approximate location of the start of the opaque zone was estimated on the dorsal side (0.34 mm) and the ventral side (0.41 mm). This zone must be counted when ageing Deepwater Flathead (Figure 2).
- The formation of the opaque zone begins in winter and is completed by the end of summer (Krusic-Golub *et al*, 2012) (Figure 3).

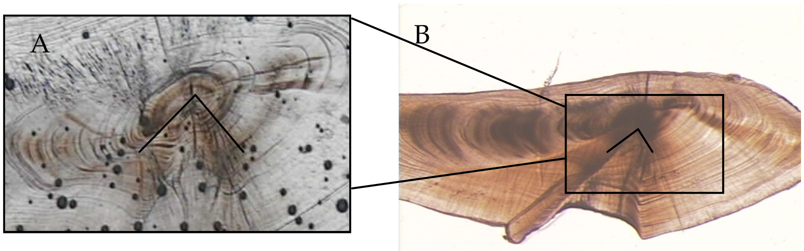


Figure 2. Transversely ground Deepwater Flathead otolith (A) indicating the primordia to first opaque zone measurement (black solid line) on both the dorsal and ventral side. Relative position is shown on the sister otolith section (B).

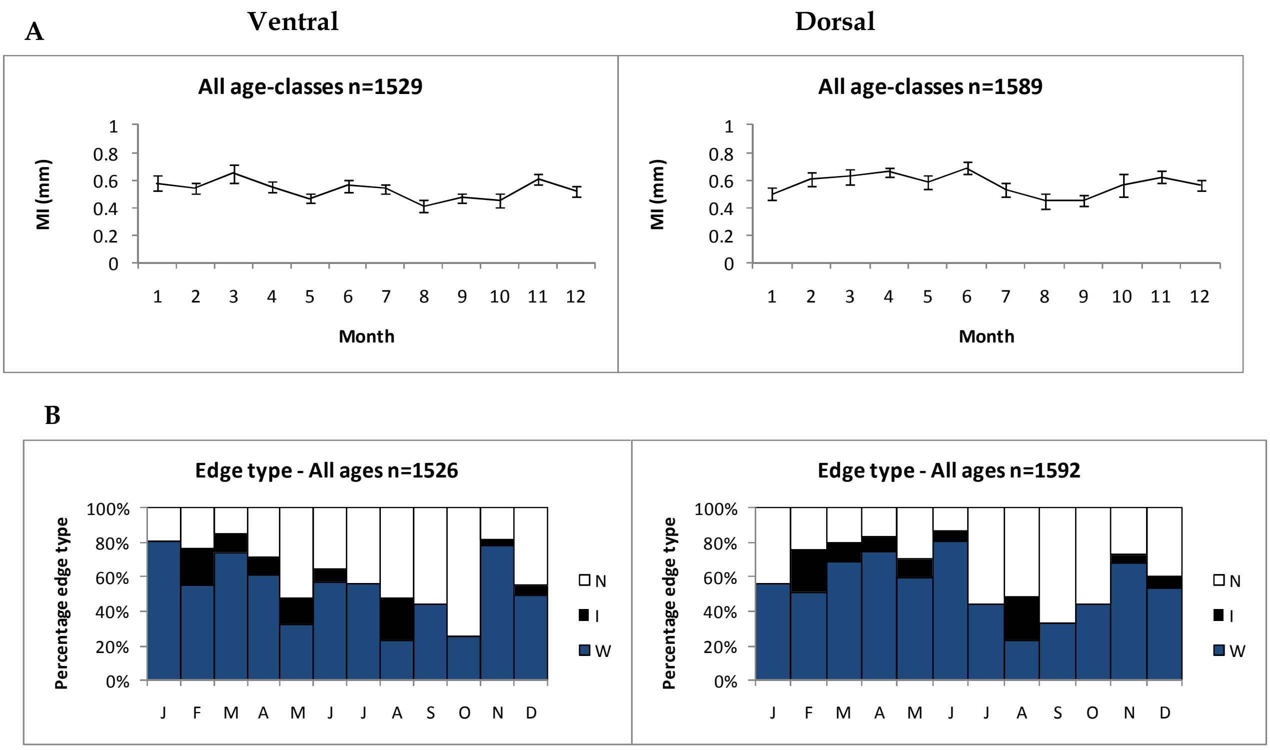


Figure 3. A) Monthly trends in marginal zone analysis, B) Marginal edge type analysis. All age classes combined.

Preparing otolith sections for age estimates

Sections appropriate for Deepwater Flathead age estimation

In Deepwater Flathead, the otolith thickens laterally mainly because material is deposited along two growth axes: dorso-medial and ventro-medial. This results in a relatively deep sulcus.

It has been found that a transverse section approximately 300–350 μm thick produced the greatest clarity of growth increments.

Sectioning method

- Clean dry otoliths are arranged in two columns of 5 and embedded in clear casting polyester resin (methyl-ethyl ketone peroxide is used as hardening agent), ensuring that the primordia of the otoliths are aligned (Figure 4).
- A minimum of 4 transverse sections approximately 250–400 μm thick are cut from the centres of the otoliths using a modified Gemasta™ lapidary diamond cutting saw fitted with a 250 μm wide diamond impregnated blade (Figure 5).
- The sections are then cleaned in water, rinsed with alcohol and dried (Figures 6 and 7).
- Sections are then mounted on numbered microscope slides using polyester resin and covered with glass cover-slips (Figures 8).
- Generally the otoliths are not baked in this process although this can be done if desired.
- Approximately 250 otoliths can be prepared per day.

Figure 4. Silicone moulds used to embed otoliths in polyester resin. Source: Central Ageing Facility, Australia.

Figure 5. Modified Gemasta™ sectioning saw in use. Source: Central Ageing Facility, Australia.

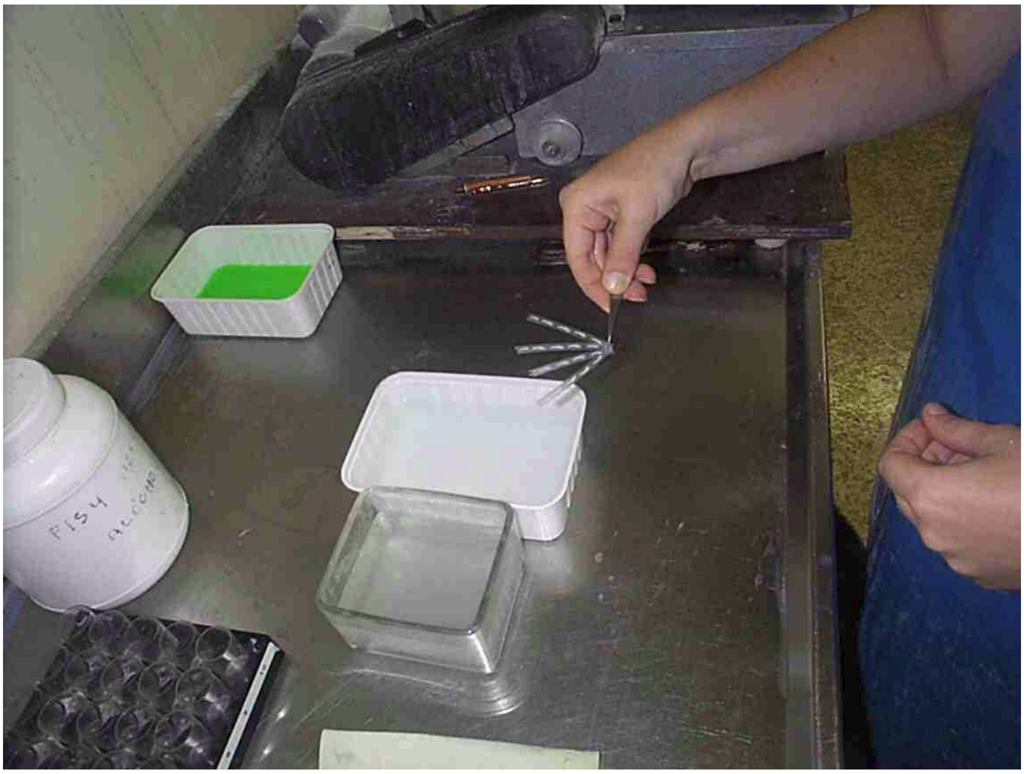


Figure 6. Cleaning sectioned otoliths. Source: Central Ageing Facility, Australia.

Figure 7. Sectioned otoliths in labelled vials. Source: Central Ageing Facility, Australia.

Figure 8. Finished preparations. Sections attached to glass slides and covered with cover-slips. Source: Central Ageing Facility, Australia.

Morphology of a sectioned otolith

The basic morphology of a sectioned otolith is shown in Figure 9.

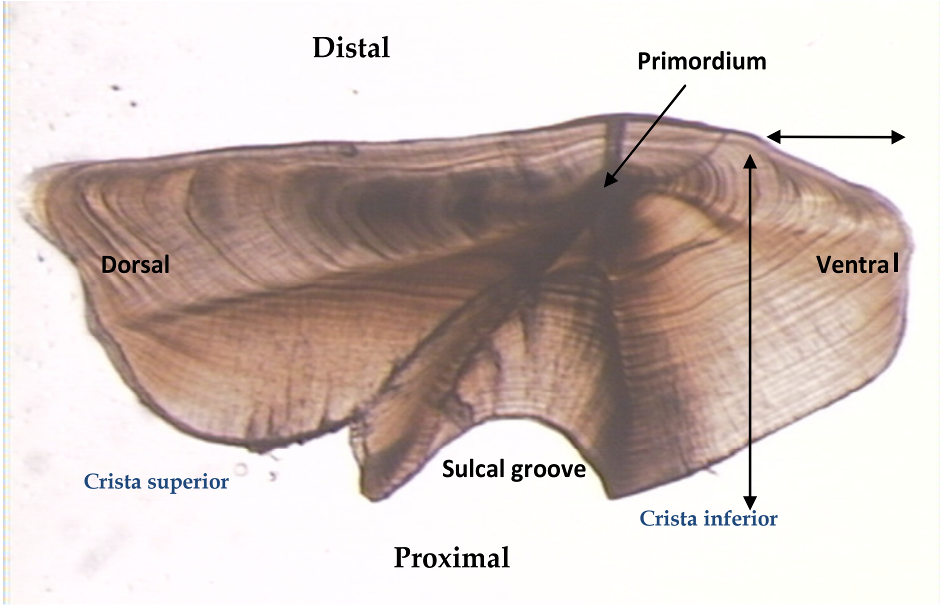


Figure 9. Basic morphology of a Deepwater Flathead otolith.

Sectioned otoliths are characterised by:

- A large opaque centre
- Alternating translucent and opaque zones, which appear light and dark respectively when viewed under transmitted light.

The otolith can be broken into three regions:

- Inner region
- Middle region
- Outer region (Figure 10).

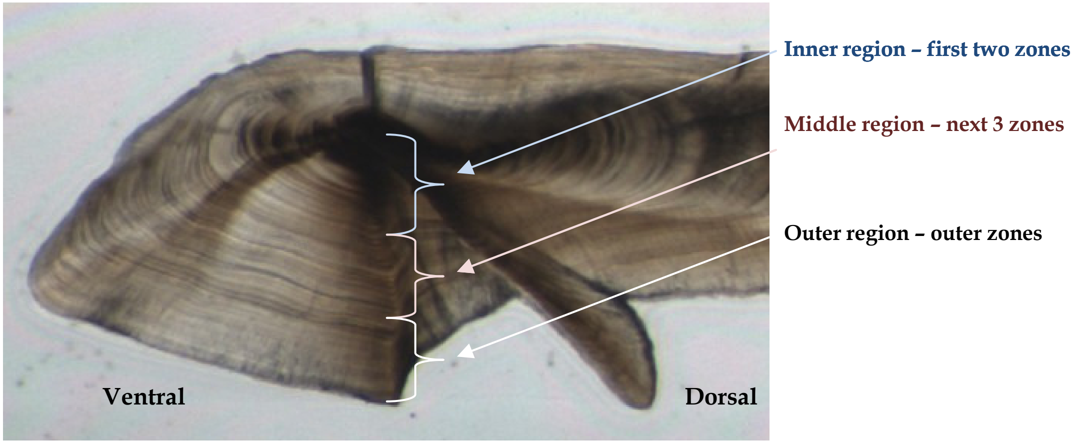


Figure 10. Sectioned otolith showing the inner, middle and outer regions.

The **inner region** contains:

- The otolith nucleus
- The first two annuli.
  - The first two opaque zones appear quite wide, particularly close to the distal surface.
  - Primordium represents the larval phase of the fish's life while the translucent zone before the first 'annual' zone represents a change in habitat.
  - The first annual zone represents the first exposure of the fish to the environmental parameters which cause the opaque zone to be deposited on the otolith.
  - The position of the first two zones can often be determined by the inflection in the subscupular meshwork fibre zone (SMF) (Figure 11) (Francis *et al*, 1992).

The **middle region** contains:

- The next 3 opaque zones.
- This area is characterised by:
  - Prominent opaque zones that sometimes appear to split.
  - The majority of sectioned otolith examined contained numerous sub-annular checks between the annual zones. This can make interpretation of the annual opaque zones in this region difficult.
  - The fine split zones are recognizable from the presumed annuli because they appeared as comparatively fine and narrow zones that were not continuous throughout the section and were unevenly spaced.
  - The distance between opaque zones is still relatively wide, especially on the dorsal side.
  - On the ventral tip the opaque zones can be closely spaced as a result of the reduction in otolith growth along the ventral distal plane.

The **outer region** contains:

- Successive evenly spaced opaque zones
- More regular the zone pattern.
- The opaque zones are generally clearer through areas adjacent to the crista inferior and the crista superior (Figure 9).

Determining age estimates

Overall approach

Age estimates are determined by counting the number opaque zones from the primordium to the edge of the otolith section.

- Otolith sections are viewed using transmitted light
- Usually a magnification of 12.5 x is use to view otoliths
- Magnification can be increased to 25 x to view the outer zones on the larger otoliths
- Both the ventral and dorsal planes of the otolith can be used to estimate reliable zone counts; however the ventral plane provides the most appropriate transect for the marking of increments
- The number of opaque growth zones (these appear dark under transmitted light) are countered.

Initial examination

Before attempting to assign age estimates, it is recommended that readers view a large number of otolith sections of different to become familiar with the otolith structure and reading protocol.

This screening allows readers to:

- Determine the most appropriate light intensity and microscope focus
- Determine whether the fish is young, medium-aged, or old taking into account:
    a. Relative size and depth of the otolith
    b. Presence or absence of regular outer growth zones near edge
    c. Size of the sulcus (becomes wider and deeper with age)
- Readily identify features of the otolith that will assist in assigned age.

Selecting the appropriate section and reading plane

Once readers are familiar with the characteristics of Deepwater Flathead otoliths, then the ageing procedure can commence.

To do this, readers must:

- Screen the four sections taken from the same otoliths and select the "best" section to age. Characteristics to select for include:
    - clarity of the otolith microstructure, including the presence and position of the subscupular
    - optimum optical density of the section (i.e. sections are not too thin or too thick as too little or too much light obscure microstructure)
    - proximity of section to the primordium (i.e. to ensure all zones are on the section)

  Select the most appropriate plane to count the growth zones, noting a combination of zones may be used (Figure 11).

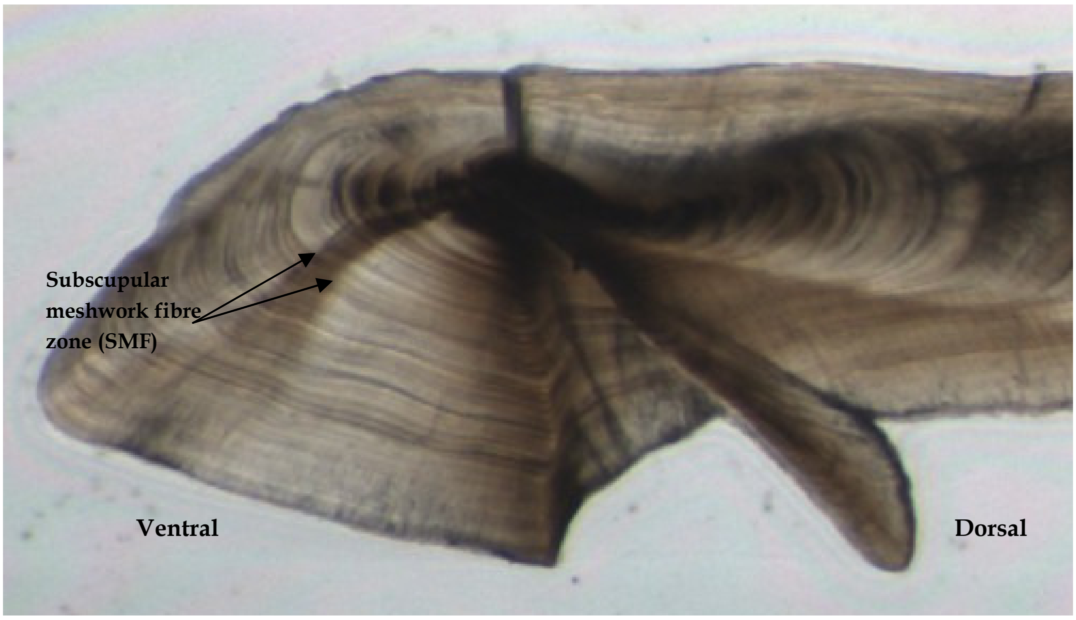


Figure 11. Otolith section indicating the subscupular meshwork fibre zone (SMF) relating to the first opaque zone.

Counting zones

The first two zones

Once the preferred section and reading planes have been determined, the reader focuses on the biological centre of the otolith to identify the position of the first two zones. To do this:

- Identify the primordium (the biological centre i.e. the dark opaque middle section)
- Identify the position of the SMF zone, if possible (Figure 11)
  - The SMF zone may change direction or be interrupted at the end of the first zone and again at the end of the second zone (though less frequent)
  - Locate the position of the inflection/break in the SMF zone (Figure 12)
  - Check the position of this inflection/break
    a. This should be located approximately 0.34 mm from the primordium on ventral plane or 0.41 mm from the primordium on the dorsal plane
    b. If it is, then this is the general area where the first opaque zone is completed
    c. If not continue searching
- Identify the end of the first opaque zone noting
  - This zone is broad and diffuse
  - The presence of multiple fine translucent and opaque sub-annual bands may confuse and must not be countered (Figure 13)
- Once the first zone has been identified, locate the end of the second opaque zone, noting
  - This zone is broad and diffuse
  - The presence of multiple fine translucent and opaque sub-annual bands may confuse and must not be countered. A good practice is to trace these lines around the otolith – sub annual zones dissipate
  - The second zone is clearer through the sulcus than the first opaque zone (Figure 14).

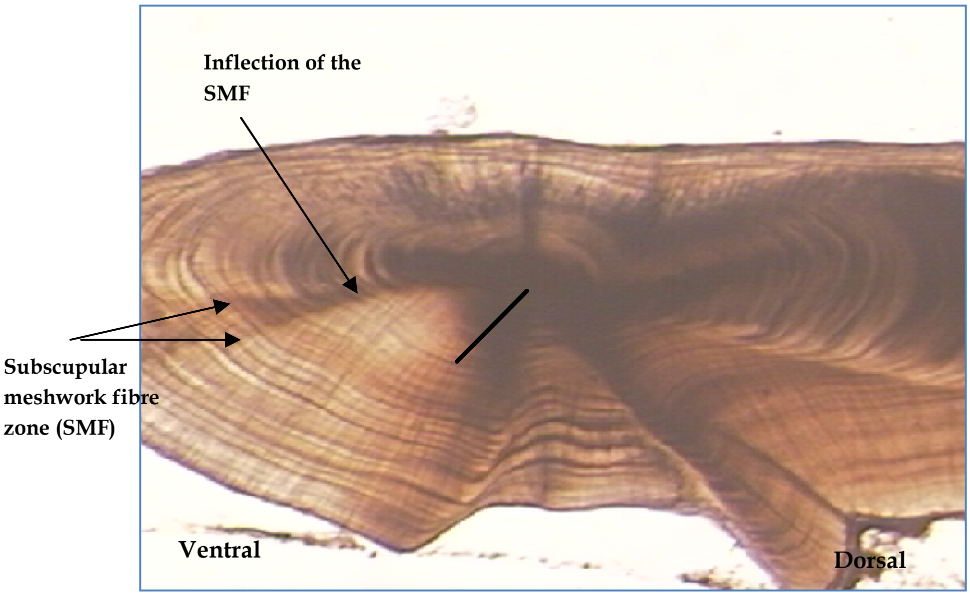


Figure 12. Deepwater Flathead otolith showing the SMF and the inflection in the SMF (black arrow) which can be used to locate the end of the first opaque zone. The black bar measures 0.34 mm from primordium, also a useful device to assist in the location of the first annual zone.

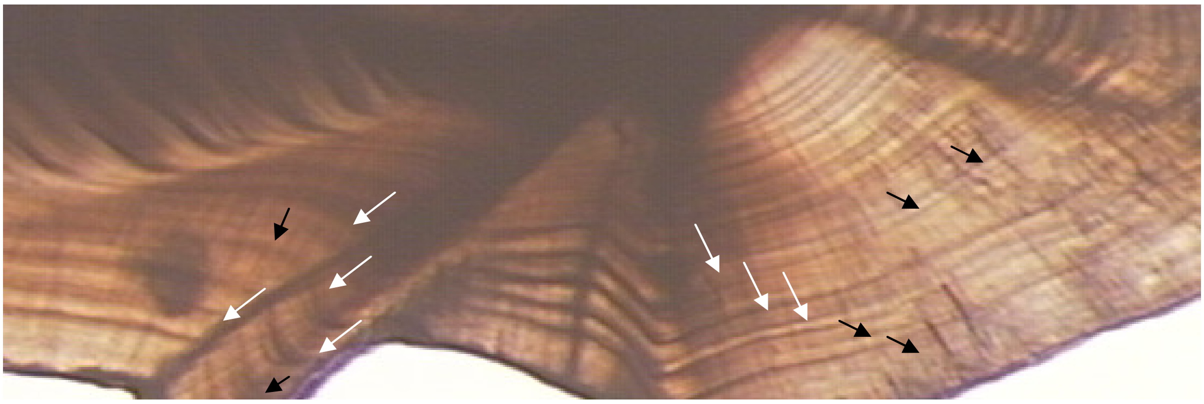


Figure 13. Deepwater Flathead otolith section indicating examples of annual zones (white arrows) and sub-annual checks (black arrows).

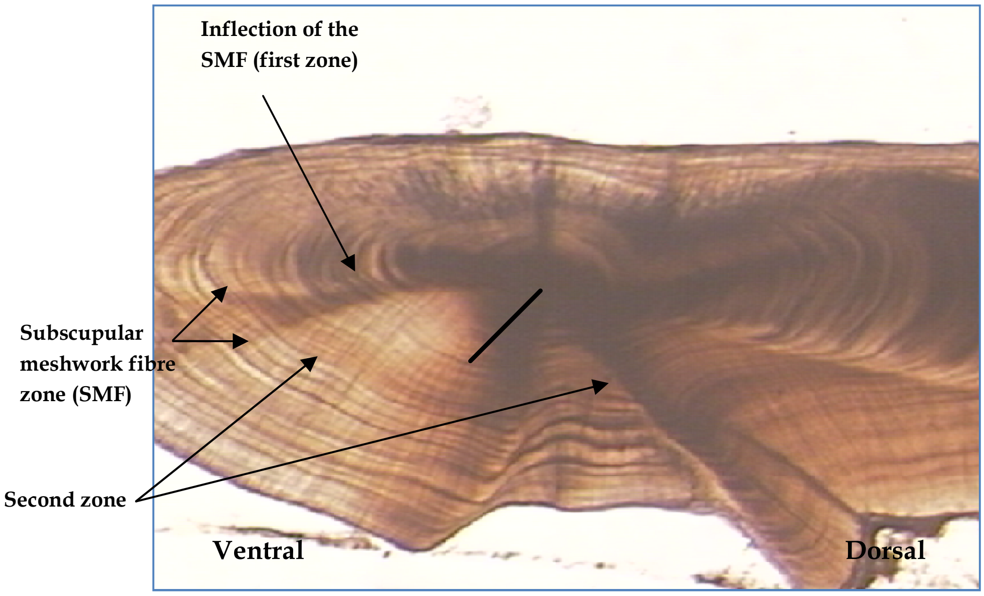


Figure 14. Deepwater Flathead otolith showing the SMF and the inflection in the SMF (black arrow) which can be used to locate the end of the first opaque zone. The black bar measures 0.34 mm from primordium, also a useful device to assist in the location of the first annual zone

The middle zones

Once the first two zones have been identified, the reader focuses on the middle of the otolith to identify the position of the zones three to five. The SMF in this middle region remains constant (i.e. there are no points of inflection)

To do this, the reader recognises:

- The middle zones:
  - Are darker and clearer than inner zones
  - Are usually regular in width and appearance
  - Spacing is narrower than that of inner zones
  - Usually consist of prominent opaque zones with many fine check marks
  - Are usually more often easier to count on the dorsal plane
  - The sulcus can provide the clearest demarcation of the zones in the middle region
  - May appear to be double. These double zones can be interpreted by using a higher magnification to determine whether:
    - o The two structures merge at the groove and/or sulcal margin (count as one zone)
    - o Are distinct throughout their length (count as two zones) (Figure 15).

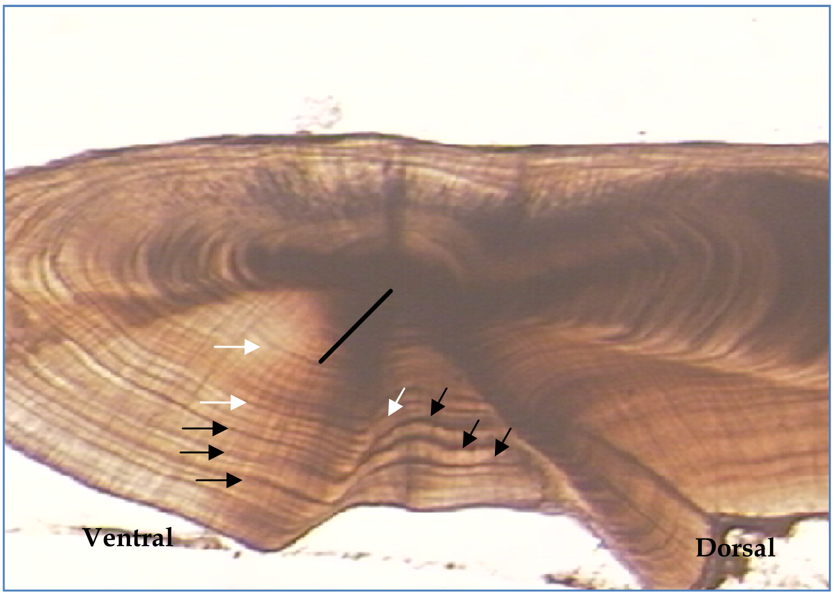


Figure 15. Deepwater Flathead otolith showing position of zones 3 to 5 (black areas) and the position of the first two zones (white arrows). The black bar measures 0.34 mm from primordium, also a useful device to assist in the location of the first annual zone.

The outer zones

Once the inner and middle zones have been identified, the reader focuses on the outer section of the otolith to identify the position of the zones six and greater.

- To do this, the reader recognises:
  - The outer zones (approx >8) generally appear clearer and are more consistent in pattern (Figure 16). Zones are often clearest on the crista inferior and crista superior
  - Higher magnification may be required for counting the closely-spaced outermost zones
  - Assess the state (opaque or translucent) of the terminal edge and record the edge type (see marginal edge section below)
  - Record the total zone count, including the edge type classification
  - Record section details and readability
  - Record any comment relating to the age estimation.

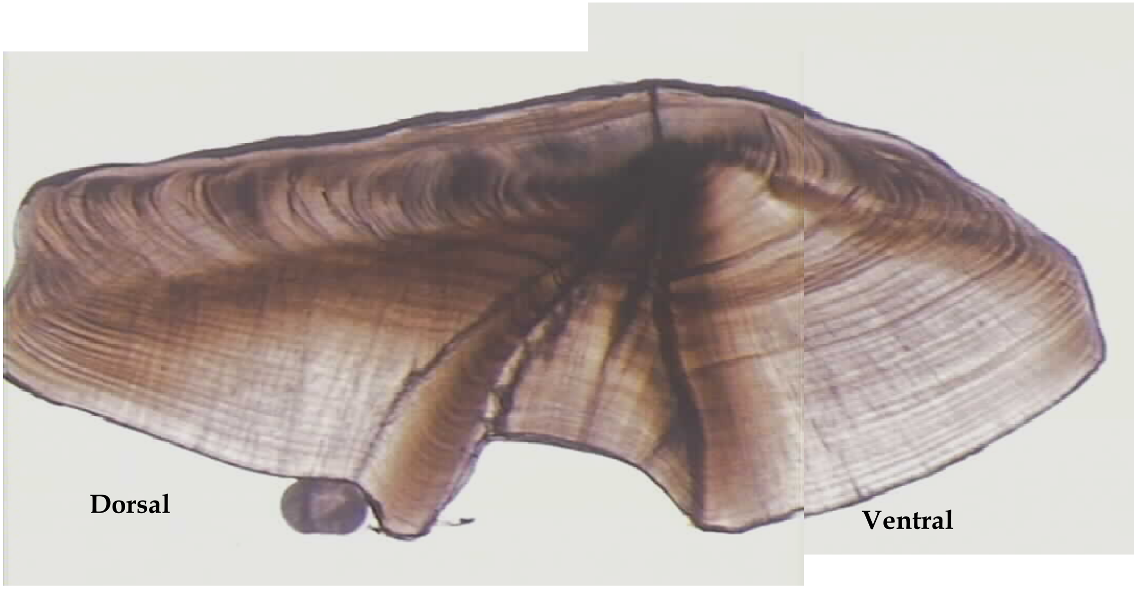


Figure 16. Classic example of a easy to read deepwater section otolith showing the regularly spaced and optically consistent outer zones.

Marginal edge classification

The edge is classified as:

- New (N)
- Intermediate (I)
- Wide (W) (Figure 17).

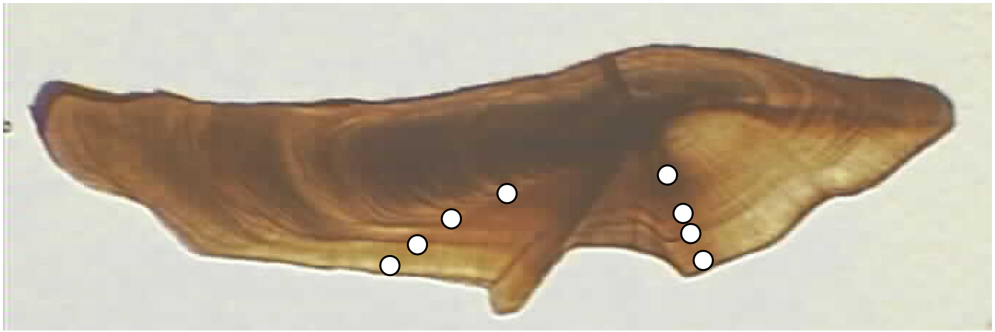
These classifications are based on the relative state of the marginal proximal edge on the ventral and dorsal planes and within the sulcus. These are interpreted by the reader at the time that zone counts are made.

The following definitions are used for each classification:

- **New**:
  - o Opaque material is present on the marginal edge.
  - o This suggests that the 'new' opaque zone has formed or is forming on the edge margin
  - o The opaque material may be visible on one side of the otolith and not the other, or only on some parts of the edge within the same side.
  - o The opaque material need not be continuous along the edge to be classified as new.

- **Intermediate**:
  - Translucent material is continuous along the otolith edge on both the dorsal and ventral sides.
  - The relative width of the translucent zone from the opaque zone to the edge margin is approximately less than 60% of the width of the previously completed translucent zone.
- **Wide**:
  - Translucent material is continuous along the otolith edge on both the dorsal and ventral sides. The marginal increment is approximated at being more than 60% complete.

A



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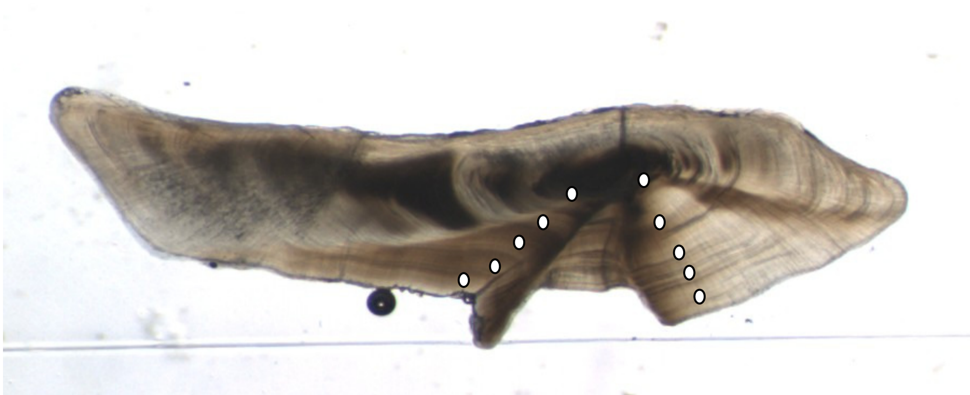

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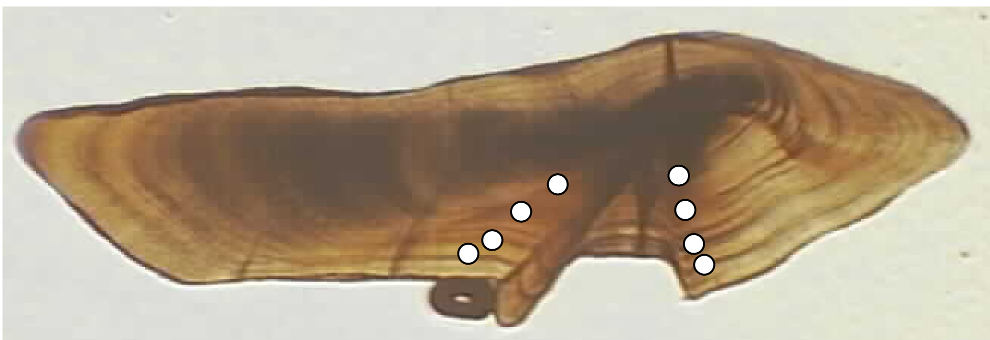

Figure 17. Example of (A) new, (B) intermediate, and (C) wide edge types in Deepwater Flathead otolith sections.

Birthdate assignment

To convert increment counts to age estimates, a theoretical birth-date needs to be determined.

The marginal state of Deepwater Flathead otoliths sampled between January 2001 and December 2002 from the Great Australian Trawl Fishery (GABTF) has been investigated. Results suggested that opaque increments were formed annually and were deposited during the winter (Krusic-Golub *et al*, 2012).

A birth-date of 1 July was chosen. This date was chosen because it preceded the start of the known spawning period for this species (October–March) (Brown and Sivakumaran, 2007).

By applying this birth-date to age estimate, readers will ensure fish spawned within a season classified to the same year class.

A 1 July birth date is consistent with the current assessment of this species. The adjustment converts the zone count to the age-class for a calendar year basis.

Converting Zone Counts to Age Estimates

In order for a final zone count to be converted to an age (and thereby determining the year class), the following additional information is required:

- Total zone count
- Edge type (N, I or W) of the otolith
- Date of capture.

The marginal state of Deepwater Flathead is usually 'new' from May to October. Even in December 40% of samples were still classified as having a new edge.

Consequently zone counts are converted to estimates of age using the following criteria which includes consideration of the edge type and the date of capture:

- If samples are collected within 2 months prior to birth-date (i.e. May and June inclusive):
  - If 'New' edge, then age = increment count - 1
  - If 'Intermediate' edge, then age = increment count
  - If 'Wide' edge, then age = increment count
- If samples are collected within 5 months the birth-date (i.e. July to November inclusive):
  - If 'New' edge, then age = increment count
  - If 'Intermediate' edge = increment count + 1
  - If 'Wide' edge, then age = increment count + 1
- For samples collected between December–April, age = increment count.
- Figure 18 illustrates the application of the Birth-date

Period of zone formation

Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
ZC	ZC	ZC	ZC	ZC	ZC	ZC	ZC	ZC	ZC	ZC-1	ZC-1
ZC+1	ZC+1	ZC+1	ZC+1	ZC+1	ZC	ZC	ZC	ZC	ZC	ZC	ZC
ZC+1	ZC+1	ZC+1	ZC+1	ZC+1	ZC	ZC	ZC	ZC	ZC	ZC	ZC

Key

Period of Zone formation

New Zone

Intermediate zone

Wide zone

ZC	Zone count
ZC-1	Zone count -1
ZC+1	Zone count +1

.

Figure 18. Illustration of the application of a birth-date to Deepwater Flathead increment counts.

Scales of readability and confidence for otolith sections

Readers should assess the "readability" of each otolith section. The term readability encompasses both:

- The clarity of the growth zones
- The confidence the reader has in the final zone count.

Separate readability assessments should be made for the inner, middle and outer regions, as these often vary in clarity and confidence.

The following scale for readability and confidence scale is well established having been in use for many years at the Central Aging Facility and at Fish Ageing Services Pty Ltd:

1. Sample is exceptionally clear with unambiguous increments
2. Sample is clear and a confident estimate can be made
3. Sample may be one year from determined age
4. Sample is difficult to interpret and subject to multiple interpretations
5. Sample is unreadable due to failed preparation or missing sample.

Quality Assurance / Quality Control

Precision and accuracy

In studies of age determination:

- Accuracy refers to the closeness of an age estimate to the true age
- Precision is a measure of the variability between individual readings, either within or between readers (Campana, 2001).

The aim is to increase both precision and accuracy.

Age estimates are still useful where estimated age is a true reflection of biological age but precision is low.

Problems arise with age data when there is bias in age estimates i.e. the accuracy is poor.

Indices of precision can also be a useful tool for comparing between readers and between methods (e.g. otoliths and vertebrae).

There is a whole suit of statistical and non statistical tests available to quantify precision and detect bias. These have been covered in detail by Campana (1995).

The Index of Average Percent Error (IAPE) is used to quantify intra- and inter-reader variability (consistency) between otolith readings and age bias plots and age difference tables to determine relative bias (Beamish and Fournier, 1981).

Average Percent Error

$$[IAPE] = \frac{100}{N}\sum_{j=1}^{N}\left(\frac{1}{R}\sum_{i=1}^{R}\frac{\left|X_{ij} - X_j\right|}{X_j}\right)$$

where *N* is the number of fish aged, *R* is the number of times fish are aged, *Xij* is the *i*th determination for the *j*th fish, and *Xj* is the average estimated age of the *j*th fish.

The acceptable level of IAPE is species specific; however, Morison *et al.* (1998) suggested that for a range of species routinely aged, levels less than 5% are acceptable. IAPEs for this species range from 3.07% to 4.02% (CAF unpublished data).

Age Bias Graphs

Age bias graphs plot one age estimate against another and can be interpreted using an equivalence line (Figure 19). When Age estimate 1 = Age estimate 2 there is perfect agreement.

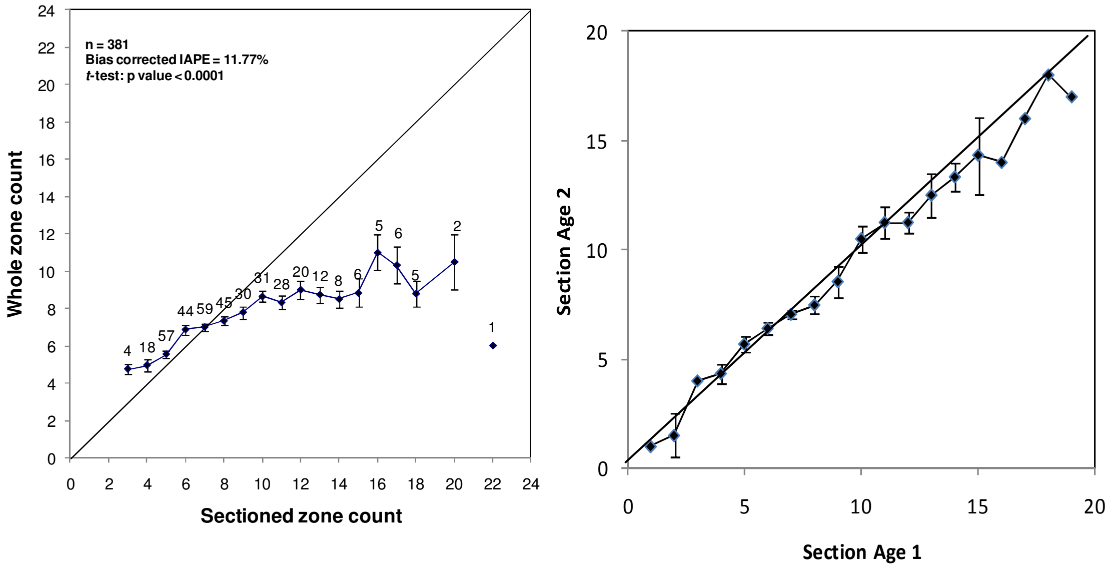

Figure 19. Age bias plot examples, (A) Comparison of Deepwater Flathead age estimates from whole and sectioned otoliths indicating bias. (B) Comparison of first age estimate and second age estimate made by the same reader for Deepwater Flathead indicating no bias. Diagonal line indicates equality of age estimates.

Age-Difference Histograms and Age-Difference Tables

Age-difference distribution indicates the distribution of differences around two age estimates (Figure 20). The distribution of errors around zero should be fairly normal. In this example, the Reader 4 has over-estimated the age compared to the Original ager. Although the mode is on zero and the IAPE is below 5%, this distribution would indicate further examination of the reasons for the differences. The age-difference distribution should be used in conjunction with the age-difference table to determine any possible systematic bias.

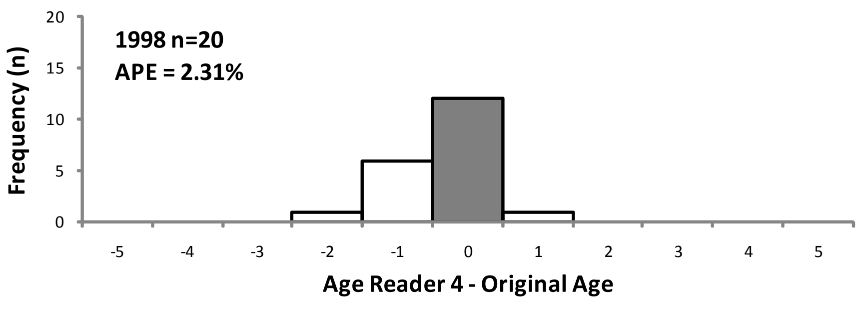

Figure 20. Example of an Age-difference distribution. In this example age-difference distribution between the audit sub-sample and the historical age.

Age-difference tables display the difference between readings for all age groups.

The use of age-difference tables is important because they have the ability to indicate systematic under ageing or over ageing bias or various age classes, even though the distribution of differences is evenly distributed around a zero difference (Age 1 = Age 2) (see Table 1).

Table 1. Example of an Age-difference table. In this example the age-difference between audit ages and historical age. Readers combined.

Age Reader	All																						
	Age																						
Difference	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	24	N
-5																								0
-4																								0
-3								1		1														2
-2							4	3	1	3	2			1										14
-1					7	11	6	8	8	2	2	6	1	5	2	1						1		60
0	**0**	**0**	**0**	**8**	**14**	**30**	**32**	**27**	**23**	**6**	**13**	**5**	**3**	**5**	**2**	**0**	**0**	**3**	**3**	**0**	**2**	**0**	**0**	**176**
1			1		5	14	11	7	10	6	9	7	4	2	3	1	2		2	1		1		86
2							3	4	2	1	2	1	4	1	1	1					1			21
3									2	2	1	3			1									9
7																						1		1
8																							1	1
N	0	0	1	8	26	55	56	50	46	21	29	22	12	14	9	3	2	3	5	1	3	3	1	370

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Glossary

This glossary has been developed to reduce confusion and aid interpretation of Deepwater Flathead otolith structure. It is not intended to describe or define all parts of the otolith, rather, it provides a standardized terminology for convenient communication between otolith readers. The definitions have been adapted from Kalish *et al* (1995), Smale *et al* (1995) and Tracey *et al* (2007).

Accuracy - The closeness of a measured or computed value to its true value.

Age estimation, age determination - These terms are preferred when discussing the process of assigning ages to fish. The term aging (ageing) should not be used as it refers to time related processes and the alteration of an organism's composition, structure, and function over time.

Age group - The cohort of fish that have a given age (e.g., the 5-year-old age-group). The term is not synonymous with year-class or day-class.

Antirostrum - The antero-dorsal corner or projection of the otolith.

Annulus (pl. annuli) - One of a series of concentric zones on a structure that may be interpreted in terms of age. The annulus is defined as either a continuous translucent or opaque zone that can be seen along the entire structure or as a ridge or a groove in or on the structure. In some cases, an annulus may not be continuous or obviously concentric. The optical appearance of these marks depends on the otolith structure.

Band - A sub-unit of a growth increment **(**See Zone)

Crista inferior - Ventral rim or margin of the sulcus.

Crista superior - Dorsal rim or margin of the sulcus

Check - A discontinuity (e.g., a stress-induced mark) in a zone, or in a pattern of opaque and translucent zones.

Core - The area surrounding the primordium and bounded by the first prominent growth zone.

Edge type - the term used to indicate either a new, intermediate or wide margin classification.

Increment - The region between similar zones on a structure used for age estimation. The term refers to a structure, but it may be qualified to refer to portions of the otolith formed over a specified time interval (e.g. sub-daily, daily or annual). Depending on the portion of the otolith considered, the dimensions, chemistry, and period of formation can vary widely. An annual increment comprises an opaque zone and a translucent zone. Increments can be complex structures, comprising multiple opaque and translucent zones.

Inflection - Change in the direction of the growth axis.

Margin – The term to describe the outer edge of the otolith when viewed as section.

Marginal increment - The region beyond the last identifiable zone at the margin of a structure used for age estimation. Quantitatively, this increment is usually expressed in relative terms, that is, as a fraction or proportion of the last complete annual or daily increment.

Microincrement - Increments that are typically less than 50 μm in width; the prefix "micro" serves to indicate that the object denoted is of relatively small size and that it may be observed only with a microscope. Often used to describe daily and sub-daily increments. See increment.

Nucleus - Originally used to indicate the primordium and core of the otolith but is now considered ambiguous and should not be used. The preferred terms are primordium and core (see definitions).

Opaque zone - A zone that restricts the passage of light when compared with a translucent zone. The term is relative, because a zone is determined to be opaque on the basis of the appearance of adjacent zones in the otolith (see Translucent zone). In transmitted light, the opaque zone appears dark and the translucent zone appears light. Under reflected light the opaque zone appears light and the translucent zone appears dark if viewed against a black background.

Precision - A measure of the variability between individual age estimates.

Primordium (pl. primordia) - The first-formed part of an otolith. It consists of granular or fibrillar material surrounding one or more optically dense nuclei from 0.5 µm to 1.0 µm in diameter.

Reflectedlight - light from above used to illuminate objects viewed under the microscope.

Rostrum - The anterior extension of the otolith.

Sagitta (pl. sagittae) - One of the three otolith pairs found in the membranous labyrinth of osteichthyan fishes.

Subcupular meshwork fibre zone – A zone of darkened optical density radiating from the primordium to the dorso-proximal edge of the otolith in a V shape found by Francis *et al*. (1992). This zone can exhibit an inflexion at the first zone which is used as an identifying feature.

Sulcus acusticus (commonly shortened to sulcus) - A groove along the medial surface of the sagitta.

Transmitted light – an illumination used from below an object used to pass through the object viewed under the microscope.

Validation - The process of demonstrating that an age estimation method is accurate, i.e. confirming the temporal meaning of the structures being counted.

Zone - Region of similar structure or optical density. Synonymous with ring. The term zone is preferred. A band is a sub-unit of a zone.