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Methods used in the collection, preparation and interpretation of narrow-barred Spanish mackerel *(Scomberomorus commerson)* otoliths for a study of age and growth in Western Australia

P. D. Lewis and M. Mackie

Cover picture: Adult Spanish mackerel, courtesy of the Food and Agriculture Organisation of the United Nations (ref. No. A47/2000); FAO Fisheries Synopsis No. 125, Vol. 2 (1983) Scombrids of the world.

Department of Fisheries

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Methods used in the collection, preparation and interpretation of narrow-barred Spanish mackerel *(Scomberomorus commerson)* otoliths for a study of age and growth in Western Australia

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Abstract

The narrow-barred Spanish mackerel (Scomberomorus commerson) *is wide ranging throughout the Indo-West Pacific region where it has been the subject of various age and growth studies. Methods used in these studies have varied considerably and in some cases may have led to misleading conclusions. The purpose of this report, therefore, is to document in detail the methods used in determining the age and growth during a FRDC funded stock assessment of the species in the waters off Western Australia. These methods are considered to optimise the collection, processing, accuracy of microincrement counts, and interpretation of the annuli in both whole and sectioned sagittal otoliths from* S. commerson. *Thus this report serves as a reference for the methods used and a guide for future cost effective monitoring of the age and growth of* S. commerson *and similar species.*

1.0 Introduction

The narrow-barred Spanish mackerel (*Scomberomorus commerson*) is an important commercial, recreational and artisanal species that inhabits coastal waters of the Indian Ocean and the north coast of Australia. Because of its importance and wide distribution there have been several studies into the age and growth of *S. commerson* using length or otolith based approaches. More commonly, analysis of whole otoliths has been used (Devaraj, 1981; McPherson, 1992; Govender, 1994; Buckworth, 1999; Dudley *et al.,* 1992). However, two studies also examined sectioned otoliths of *S. commerson,* which suggested that annual opaque bands or annuli were not consistently visible on unsectioned otoliths (Dudley *et al.,* 1992), and that on average age estimates were one year higher for sectioned otoliths (Buckworth, 1999). Sectioning the otoliths of *S. commerson* may therefore provide a more reliable estimate of the age and growth of this species.

Nevertheless, the study by Dudley *et al.* (1992) was based on only 38 sectioned otoliths and that of Buckworth (1999) was hampered by the poor readability of the otoliths. It has also been reported that the interpretation of *S. commerson* otolith structure for age estimation is difficult due to features such as irregular sub-annual increments and opaque material obscuring large parts of the otolith (Devaraj, 1981; McPherson, 1992; Govender, 1994; Buckworth, 1999). Difficulties in validation of the temporal periodicity of opaque zone (annuli) formation in the otoliths of this species have also resulted in conflicting interpretations. For instance Devaraj (1981) suggested that up to four opaque zones a year may be formed in the otoliths of *S. commerson* collected off India, whereas Govender (1994) considered two opaque zones per year were formed in waters of South Africa. Yet other studies (McPherson, 1992; Buckworth, 1998) have concluded that one primary opaque zone forms per year and that sub-annual increments may sometimes form but are often closely associated with the primary zone and together can be counted as a single annulus. Further, only the studies by McPherson (1992) and Buckworth (1999) analysed the data for males and females separately. As distinct differences were found in the size at age and growth of each sex it is therefore likely that conclusions drawn from other studies may be misleading. The interpretation of ageing structures on whole and sectioned otoliths from adult *S. commerson* is therefore still somewhat ambiguous, despite the fact that a large number of the otoliths from the similar congeneric species *S. cavalla* were sectioned and reliably aged by Devires and Grimes (1997).

Several studies have also attempted to describe the growth of juvenile *S. commerson* from analysis of their otolith microincrement structure (Brothers and Mathews, 1987; McPherson, 1992; Dudley *et al*., 1992). These again have been based on few individuals, (n=24, 7 and 3 respectively) and the temporal periodicity of microincrements in *S. commerson* otoliths remains unvalidated (although they can be assumed to be daily based on the results obtained by Peters & Schmidt (1997) and Shoji *et al.* (1999) on the related species *S. maculatus* and *S. niphonius*).

Detailed analysis of the microstructure in juvenile *S. commerson* otoliths is important as it may help resolve some of the conflict with the interpretation of the adult otolith structure. This is particularly the case with identification of the first annual opaque zone, which in a preliminary test amongst experienced otolith readers at the Western Australian Department of Fisheries was shown to be ambiguous.

Description of the age and growth characteristics of *S. commerson* was an important objective of a recent Fisheries Research and Development Council (FRDC) funded stock assessment of this species in Western Australian waters (FRDC Project # 1999/151). This research report provides a detailed description of the methods that achieved a high degree of success in the collection and preparation of *S. commerson* sagittal otoliths for the observation of microincrements and annuli. It also details results from the microincrement counts used to assist with the interpretation of the first annuli formation in adult otolith sections, and provides other details on the interpretation of both whole and sectioned otoliths of *S. commerson* for age and growth studies. Thus, this report can be utilised as a guide for future economical and consistent monitoring of the age structure of the *S. commerson* catch in Western Australian waters and may be applicable to assessing the age and growth of other similar species.

2.0 Collection of otoliths

Sagittal otoliths were collected from *S. commerson* caught in west and north Western Australian waters by commercial mackerel fishers, recreational anglers and research staff. Where possible data on fork length (FL), total length (TL), head length (HL), upper jaw length (JL) (all in mm), total weight (kg), sex, gonad weight (g) and gonad macroscopic stage were also obtained. Refer to Mackie & Lewis (2001) for details of gonad staging and reproductive analysis. Terminology used in this report to describe otoliths is taken from Secor *et al.* (1995).

S. commerson sagittae are relatively small, elongate and fragile with a long and narrow rostrum and a short anti-rostrum (Figure 1). The rostrum in particular is quite fragile and easily broken. Care and use of the correct technique is therefore required for the extraction of whole otoliths, particularly if data from whole sagittal otoliths (otolith weight and length) are required or they are to be used for comparative studies (stable isotope analysis or shape analysis). Despite their fragile nature the methods described obtained at least one whole sagittae from 96.7% of the 2970 pairs that were collected, weighted and measured. The *S. commerson* collected during the study ranged in size from 58 to 1650 mm FL and whole otoliths were removed from these both in the field and laboratory situations. During the project whole otoliths were even removed from fish that had been repeatedly hit over the head, in order to be subdued, and those that had been killed by the ike jime method, provided this was done by a single stab with a knife. The most significant cause of breakage was by attempting to remove otoliths from heads that were still partially frozen and in most cases where otoliths were broken it was only the rostrum that fractured and the otolith could usually still be sectioned and successfully aged.

The sagittal otoliths of *S. commerson* were removed using the up through the gills method (Secor *et al.,* 1992).

The details of this method include;

- Separate the head from the body, if not already done to facilitate head measurements. This can be done quite easily, even on large fish, by cutting down either side at the operculum junction then from underneath around the posterior margin of the gills and through the oesophagus. The head can then be easily snapped backwards to break the spine at the junction of the skull and the first vertebra. A final rotation of the head and cut may be required to completely remove the head.

- Cut the gills away from the skull and move to one side or remove completely.
- Cut away the basioccipital or base section of the skull with bonecutters. In this project we used 27 cm double action bone cutting forceps (Liston Stille Model No. 30.70.27) made from surgical grade stainless steel. Rope shears are a cheaper alternative (C. Bryce, *pers. comm*.) and are adequate for the majority of fish using the first method described below.

The base of the skull can be cut away using 2 different methods, depending upon the size of the specimen:

1. For average sized fish (head length between 150 and 250 mm).
   Holding the head ventral-side up, a single cut vertically down with bonecutters (from the anterior side) at the widest point of the exposed parasphenoid bone, which forms the base of the skull, (Figure 2a) to the full depth possible, and lever the posterior section of skull off to expose the otolith cavities (Figure 2b).
2. For smaller and larger fish (head length less than 150 mm or greater than 250 mm). Again with the head ventral side up firstly cut vertically down with bonecutters to the full depth possible at the anterior of the exposed parasphenoid bone (anterior to where the gills were attached Figure 2c).
   Secondly, cut down at an angle of approximately 45 degrees from the posterior end of the skull using the two large nerve openings in the exoccipital bone as a guide to the depth required (Figure 2d). For larger fish, there may need to be a series of cuts to achieve this. The whole base of the skull is then levered up and off to expose the otoliths (Figure 2b). Occasionally the otoliths will remain in the removed base section of the skull.

If the otoliths were not fully exposed the soft cartilaginous nature of *S. commerson* bones allowed the remaining base of the skull to be carefully sliced away with a knife. The otoliths were removed from their cavity and membrane with forceps, rinsed in freshwater, allowed to dry, and stored in ependorf vials within labelled envelopes. For transport, a wad of dry tissue paper was placed in the vials to prevent otolith breakage.

The otoliths of the smallest individuals (FL $<$ 150 mm) required the used of a dissecting microscope for the successful application of this method of removal. The otoliths from these and other small individuals (FL $<$ 700 mm), which were to be processed for microincrement counts, often required further cleaning. These were either immersed in a weak 10% bleach solution for 30 – 60 seconds to dissolve the proteinaceous material, or left in a 70% glycerol solution for 12 hours, to loosen the adhered material for subsequent mechanical removal. The otoliths were then thoroughly rinsed in freshwater and dried for storage. It is essential that otoliths are thoroughly dried over a few days prior to further analyses as the weight and appearance of opaque annuli are affected by moisture content (see Section 4.3).

3.0 Otolith preparation and sectioning

In the laboratory each sagittal otolith pair were examined and the state of each recorded (broken or unbroken). One whole otolith of the pair (noting which one) was weighed (to 0.0001 g), and the overall length, posterior tip to anti-rostrum length, dorso-ventral width at the widest section and distal-proximal thickness at the core region, measured with digital vernier callipers (to 0.01 mm) (see Figure 1 for orientation of sagittal otolith). For sectioning and ageing it was not necessary for the otoliths to be whole as long as the region around the core was intact. Sectioning methods follow those of Jenke (2002). Where possible the left otolith was selected and mounted in a block of casting or epoxy resin with an identity label, using ice cube trays as moulds which enabled numerous otoliths to be embedded at the same time. Firstly, a thin bed of resin (approximately 3-5 mm thick) was set in the block 2-24 hours prior to the otoliths being embedded to prevent the otolith from settling to the bottom of the resin block. A small amount (approximately 0.5 ml) of freshly mixed resin was put onto the base and the otolith inserted into this so as to prevent an air bubble forming in the sulcus of the otolith, which could cause the otolith to vibrate and fracture during sectioning. The otoliths were positioned towards one end of the block with the rostrum pointing towards that end with the sulcus facing down. Sufficient resin was added to cover the otolith and the blocks left for at least 48 hours, to allow the resin to cure completely before sectioning.

Prior to being sectioned, the core region of each otolith was located and marked on the surface of the block by examining the blocks under a dissecting microscope. In the smaller otoliths the core usually appeared as a small circular translucent area just posterior to the posterior dorsal origin of the rostrum and posterior ventral origin of the anti-rostrum (Figure 1). However, in larger otoliths the translucent core region was often not obvious but the general area in which it was located, in the centre of the otolith near the posterior origins of the rostrum and anti-rostrum, could be identified from experience and its position in the smaller otoliths, albeit somewhat subjectively initially. The location of the core was marked on the surface of the block with a fine point pen for the adults, and with a needle for those to be processed for microincrements. To further guide the sectioning a line perpendicular to the longitudinal axis of the otolith (See Figure 1) was marked transversely across the otolith through the core using a glass slide with crosshairs under the section as a guide, again using a fine pen for adult otolith sections and a needle for microincrement otoliths. The needle was used for otoliths requiring microincrement counts to give a fine accurate mark defining the location of the core so a transverse section containing the core of the otolith could be confidently cut from the block.

3.1 Sectioning of otoliths for microincrement counts

The methods described below achieved a 96.5% success rate at locating the primordium (at the centre of the core) in the *S. commerson* sagittal otoliths that were sectioned and processed for microincrement counts (N=85). Thus the full compliment of microincrements from the primordium to the proximal margin of the otolith could be counted in these sections.

A single 300-450 μm thick section containing the core of the otolith was cut from the block using a low speed jewellery saw (Isomet) with a diamond-wafering blade (Beulher). The block was mounted in the chuck of the saw so the blade was perfectly aligned with the needle line on the resin block. To ensure an exact cut, the chuck was raised and the trueness of the saw blade visually checked by starting the saw and monitoring the path of the blade. If the blade course was true, the chuck was lowered to re-check the alignment of the blade with the needle line prior to sectioning. However, if there was a significant variation in the path of the blade, the saw was disassembled, cleaned and re-checked or the blade replaced. After final alignment of the saw the micrometer of the chuck was wound back 400 µm (anticlockwise 40 units) and the alignment checked to ensure the cut would miss the needle line. The first cut was made at this setting and for the final cut, on the inside of the needle line, the micrometer was wound up 800 µm (clockwise 80 units or 1 full revolution and 30). The block was then re-checked from above to ensure that the needle line would be included in this section before the final cut was made. Ideally this would produce a section 467 µm thick, due to the Beuhler blade thickness of 333 µm, but in reality the section thicknesses varied due to saw inaccuracies and the extra width of the actual cut (see Jenke, 2002).

To reduce the amount of polishing required, the resin sections were cut down to approximately 10 mm square with a scalpel, taking care not to fracture the section by cutting too close to the otolith. Each side of the section was polished for approximately 30 seconds on wet 7 µm polishing paper to remove the cutting marks of the saw. The polished section was mounted on a slide, in clove or immersion oil, and each side examined at varying magnifications with transmitted light on a phase contrast compound microscope. Occasionally, the core (consisting of the primordium which appeared as a very small opaque point surrounded by 9-10 circular rings, Figure 3a) could be located by focusing down into the section and adjusting the contrast. However, this was often not possible on either side of the section and further polishing was required. The oil was cleaned off and each side polished for a further 60 seconds on polishing paper before being re-examined. Often this process had to be repeated many times until the core could be located. Generally, the core was located when the section was viewed from the side cut posterior to the core. The microincrements to the posterior of the core were circular as opposed to the two sets of converging rings formed by the rostrum and anti-rostrum on the anterior side. This posterior ring structure was clearer and hence the primordium and core could be located deeper within the section from this side. Additionally, when focussing down into the section it was possible to follow the converging rings, which gave an indication of the depth of the primordium, even when it could not be seen.

Once the primordium was located clearly from one side of the section, the top right hand corner of the section was cut off, to clearly identify the correct side to polish. The amount of polishing required could be roughly determined by noting the readings on the focus dial of the microscope at the surface of the section and at the primordium. At X 200 magnification every 10 units difference required approximately 2 minutes of polishing to remove. However, this depended upon the state of the polishing paper, the pressure applied and the method of the polisher. Once the primordium and core region of the otolith was very close to the surface of the section it was regularly polished for short periods of 30 seconds or less, and re-examined to ensure the primordium was not removed.

With the primordium at or very close to the surface of the good side of the section the reverse side was regularly polished for 2 minutes and the good side re-examined until it was thin enough for the microincrements to be clearly visible. The majority of otolith sections were polished to a thickness of approximately 150 µm, which allowed the microincrements immediately surrounding the core region to be just visible while the increments towards the proximal margin also remained clearly visible. However, some sections were polished very thin, less than 100 µm and down to approximately 50 µm, in order to clearly see the microincrements immediately surrounding the core. In cases where the thickness of the section was ≤ 100 µm, the section was reinforced with thermoplastic (Crystal Bond) to prevent the section from cracking or the otolith section falling out when it was further polished. To do this the section was placed on a clean glass slide on a hotplate set at approximately 80°C and a small piece of Crystal Bond (3 mm diameter) melted onto the side of the section that did not require further polishing. When some otoliths were polished very thin, ≤ 100 µm, the microincrements towards the proximal margin became very faint and were only just discernible.

The finished sections were semi-permanently mounted on a labelled slide without a cover slip using Crystal Bond. The section was completely immersed in the heated Crystal Bond, using a hot scalpel, and left on the hotplate for approximately 5 minutes to allow any air bubbles to clear. Higher temperatures, above 80°C, tended to discolour the resin but had little effect on the visibility of the microincrements in the otolith section.

3.2 Sectioning of otoliths for annuli counts

To determine the best method of sectioning adult *S. commerson* otoliths a series of trials were conducted on the 3 variables thought to influence the clarity of the opaque zones in the otolith sections. These were the axis of the otolith along which sections were cut, the treatment of the cut sections before mounting and the thickness of the sections. The overall results indicated that the readability of *S. commerson* otolith sections was not significantly influenced by any of these factors, although the thickness did influence the interpretation of the internal opaque zone structure with the optimum thickness varying between otoliths. Consequently, three sections of different thicknesses (320, 370 and 420 µm) were cut transverse to the longitudinal axis of the otolith (Figure 1) from each otolith block with the same saw used for the microincrement otoliths. The block was mounted in the chuck of the saw so the blade was perfectly aligned with the line denoting the location of the core marked on the surface of the resin block. As with the microincrement sectioning, the saw was tested for accuracy before the micrometer of the chuck was wound back 900 µm (anticlockwise 90 units) and the first cut was made. The micrometer of the chuck was then wound up 650, 700, 750 µm (clockwise 65, 70 and 75 units) between the next 3 cuts resulting in 3 sections of slightly differing thicknesses and the middle section containing the core region of the otolith. Each side of the sections were polished for 5 seconds on wet 7µm polishing paper before being labelled A, B, and C respectively. The finished sections were mounted on a glass slide with a cover slip using casting or epoxy resin and the sample identification number of the fish written on the slide.

4.0 Interpretation of otolith structure

4.1 Microincrements

A phase contrast compound microscope fitted with an eyepiece micrometer was used to count the microincrements of the mounted otolith sections. Using X400 magnification, each section was read on two occasions. For each reading the section was first examined under low magnification and the best axis or axes, from the primordium to the proximal margin, along which the microincrements were most clearly visible was determined (Figure 3b). The most accurate method to count the microincrements was to move the section across the field of view and count the microincrements, with a hand counter, as they moved past a point on the eyepiece micrometer. This method allowed the reader to easily keep track of which microincrements had been counted and also facilitated the axis of the count to be easily changed when the microincrements became faint by tracking the last clear microincrement to the new axis.

If the first two independent microincrement counts differed by ≥ 10% a third count was done. For all otoliths the final microincrement count assigned was the average of the two counts that differed by less than 10%. If the three counts differed by more than 10% the otolith was excluded from the study and aged by the annuli count only (see Section 4.2). To be incorporated into the adult otolith data the juvenile otoliths were assigned a final age based on their final microincrement count as a portion of a year, assuming that the microincrements had daily periodicity.

Generally, the *S. commerson* otolith sections had a clear series of microincrements (Figures 3a and b), with each microincrement consisting of an opaque D-zone and a translucent L-zone. The first 9-10 microincrements forming the core region immediately around the primordium of the otolith were very close together (Mean width =4.35 µm, SE= 0.97, N=10), spherical to oval in shape and easily discerned (Figure 3a) in most sections. The following opaque zone required a higher light intensity for the microincrements to be clearly visible. This region was composed of 15-20 microincrements that were much broader (Mean width= 9.7 µm, SE= 1.7, N=10) that were not clearly defined in the region dorsal and ventral to the core but could be counted proximal to the core (Figure 3a). During this period the deposition or growth of the otolith was predominantly in dorso-ventral width where numerous secondary increments appeared to be underlying the regular pattern of microincrements but there was also some proximal growth in which the pattern of microincrements was more clearly visible. After this opaque zone had formed, the first translucent zone of otolith deposition occurred which was associated with the formation of radial striae or argonite needles (Panella, 1980). These were particularly evident in the portion of the section ventral to the sulcus acusticus (ventral lobe) whilst the microincrement structure remained distinct near the sulcus (Figure 3b). The growth of the otolith during this period was both in dorso-ventral width and distal-proximal thickness.

Figure 3b clearly shows the occurrence of radial striae in the ventral lobe of the otolith section. During this period of growth after the initial opaque zone microincrements became progressively closer together (Mean width= 4.35 µm, SE=0.34, N=10) and were often very faint, particularly in the thinner sections. These could be made clearer by lowering the light intensity and adjusting the phase contrast platform of the microscope to provide more contrast. At the end of the translucent zone containing radial striae there was a marked change in the structure of the otolith. Counts showed this change occurred after the first 117 to 142 microincrements at which point the deposition of microincrements had become much narrower (Mean width= 1.95 μm, SE= 0.37, N=10).

This distinct change in otolith structure and microincrement width can be clearly seen in the sections of all adult *S. commerson* otoliths (see Section 4.2). However, some adult otolith sections showed that the deposition of radial striae could occur in subsequent regions of the otolith, to a lesser degree. After the first 150 microincrements, the deposition of subsequent microincrements is not always visible around the entire proximal margin of the section, and often increments were only discernible along one axis. This was particularly the case in the otolith sections of the larger individuals processed for microincrement and counts were only possible on the ventral margin of the section. Thus, microincrement counts which were greater than 200 were likely to be an underestimate of the actual number and hence age in days.

During the second reading the dimensions of each juvenile otolith section were measured using the eyepiece micrometer, for reference to the adult sections. The overall dorso-ventral width and distal-proximal thickness of the otolith section were measured along with the width and thickness from the core of the dorsal and ventral lobes at the sulcus. All measurements were recorded in eyepiece units (epu) at X40 magnification and converted to millimetres (mm) using the following conversion.

At X40 magnification 1epu = 0.0273 mm

A plot of the otolith section distal-proximal thickness with microincrement count, for the range of juveniles processed, provides an indication of the thickness at 1 year of age (Figure 4), assuming the microincrements in *S. commerson* otoliths are formed on a daily basis like those observed in *S. maculatus* and *S. niphonius* (Peters & Schmidt, 1997 and Shoji *et al.,* 1999). Hence, the otolith distal-proximal thickness at 1 year of age was determined to be approximately 0.72 mm and the opaque zone that often occurred at the change in otolith deposition or completion of the distinct translucent zone containing radial striae was discounted as the first annual opaque zone and the true first annual opaque zone occurred soon after this (see Section 4.2). However, this otolith distal-proximal thickness at 1 year of age is only true for a transverse section that contains the core of the otolith and the thickness at 1 year of age for sections anterior or posterior to this were considerably less and more than 0.72 mm.

4.2 Annuli

A number of otolith sections with clearly defined opaque zones or annuli were used as reference specimens to regularly recalibrate the readers (Buckworth, 1999). To remove any bias the otolith slides were read blind with no knowledge of the date of capture, specimen ID or fork length. The primary reader (PL) examined each otolith slide on two independent occasions and the secondary reader (MM) examined a sub-sample of the slides once (N=200). At each reading the slides were selected randomly from the storage box and readings were done with a phase contrast compound microscope, fitted with an eyepiece micrometer, at X40 magnification. For each slide the three sections (A, B and C) were examined using both transmitted light and reflected light and the best section, on which the reading was based, was noted and given a readability index category (Table 1), a marginal increment category (Table 2) and a count of the visible annuli. Note that an opaque zone was counted as an annuli even if it had not completely formed at the margin of the otolith (i.e. no translucent zone was required proximal to it). In addition, the axis of the clearest reading and the relative widths of annuli (narrow or broad) were recorded along with notes on best light source (reflected or transmitted) and any difficulties or explanations of the reading.

Table 1. Readability index (RI) categories for *S. commerson* (after Buckworth (1999)).

RI Category	Annuli Readability
1	Unreadable
2	Poor
3	Fair
4	Good
5	Perfectly Readable

Table 2. Otolith marginal increment (MI) category index for *S. commerson*.

MI Category	Otolith section margin appearance
1	Opaque zone at margin
2	1-50% of previous translucent zone
3	51-100% of previous translucent zone

Examination of the three different otolith sections on each slide improved the readability of each otolith sample. Often the annuli were obscured in one section but clearly visible in another due to the slightly different thickness or the location of the section relative to the core. However, sections cut posterior and anterior of the core (usually sections A and C) had to be interpreted differently to those that contained the core (usually section B); see Table 3. Thus, the description given below applies predominantly to the section containing the core of the otolith.

Table 3. Variations of sections posterior and anterior (Sections A and C) from the section that contains the core (Section B) for *S. commerson* otoliths.

	Posterior section	Anterior section
Distal-proximal thickness at 1yo	Thicker than 0.72 mm	Thinner than 0.72 mm
1st translucent zone containing radial striae	Radial striae can extend to 1st annulus	Radial striae much smaller not always present
Annuli appearance	Relatively broad and widely separated	Narrower and closer together

As observed in the *S. commerson* otolith sections processed for microincrements, the transverse sections of adult otoliths exhibited an initial distinct translucent zone containing radial striae, particularly in the portion of the otolith ventral to the sulcus acusticus (Figure 5a). This was followed by a marked change in the deposition of the otolith and there was often an opaque zone associated with this change, which was determined from the juvenile counts to be a sub annual increment or secondary opaque zone forming at approximately six months of age. The growth of the otolith was found to slow and the first true annulus began soon after this (Figures 5b and e). However, the location of the first annulus was often indistinct, appearing as a broad opaque zone after the translucent zone containing radial striae, which incorporated both the secondary opaque zone and the first annulus (Figure 5a). In fact, identification of the first annuli was the most significant problem encountered when reading *S. commerson* otolith sections with most being much less distinct than illustrated in the figures provided. Measurements of juvenile otolith sections and microincrement counts were used to determine the approximate location of the first annulus in each section (Section 4.1). This was achieved using the eyepiece micrometer on the microscope as a guide to the location of the first annulus. Subsequent annuli were generally much more distinct, resulting in easier age determination of older individuals (Figures 5b). The annual periodicity of these annuli, subsequent to the first indistinct annuli, in the sagittal otoliths of *S. commerson* has been validated by categorical marginal increment analysis and shown to roughly coincide with the spawning period of the species (Mackie *et al., unpublished data*).

The majority of otolith readings and assessment of the marginal increment were made ventral to the sulcus acusticus, with the annuli often clearly visible along an axis close to the sulcus acusticus (Figures 5a to e). Counts of the annuli along other axes and on the portion of the otolith dorsal to the sulcus were also often possible and allowed for verification (Figures 5a and c). Figure 5b shows 12 distinct annuli along this axis with the 13th starting to form at the proximal margin of the section. It also illustrates how the translucent zone between the first 2 annuli is broader than that between subsequent annuli and how these narrow considerably towards the proximal margin of sections this age. This is particularly true in the otolith sections from females older than 12 years of age and males (Figure 5a). It was found that male *S. commerson* have a smaller sagittal otolith size at age (Mackie *et al*., *unpublished data*) and hence annuli that are much narrower and closer together.

The interpretation of adult *S. commerson* otolith sections was also affected by the common occurrence of secondary opaque zones or sub annuli (Figure 5e). These could appear as finer bands that did not extend through the section as clearly as the annual zones but also appeared as narrow opaque zones immediately before the broader annuli. However, in some sections they could easily be counted as annuli, particularly in the younger otoliths where the regular pattern of annuli was not well established or obvious. This can be seen in Figure 5d, where 6 or 7 annuli can easily be counted on the detailed ventral portion, if all opaque zones are counted, but when the complete section is viewed there are 4 clear broad annuli on both sides of the sulcus.

Other problems encountered when reading the sections of *S. commerson* otoliths included the presence of broad areas of opaque material, especially in the thicker sections (Figures 5a, ventral portion), which obscured the underlying annuli structure. Additionally, the translucent zones were not always obvious in many otolith sections, particularly for samples from the Kimberley region, with the annuli often appearing as broad opaque areas instead of distinct zones (Figure 5e). This made the assessment of the marginal increment category difficult. Other less frequent problems included the abnormal deposition of the otolith resulting in outgrowths (Figure 5c) and the rare occurrence (4 in 2907) of a very abnormal “bubbly” otolith structure, both of which could hamper the interpretation of the annuli. However, in both these cases the remaining otolith of the pair did not usually have the same structure and could be sectioned and successfully aged.

4.3 Whole adult otoliths

The distal surface of whole sagittal otoliths from *S. commerson* were examined under a dissecting microscope with reflected light, immersed in 70% glycerol solution on a black background. The otoliths used were selected to obtain approximately 100 from each of the three main sectioned otolith readability categories (2 - poor, 3 - fair, and 4 - good) and approximately even numbers from each of the three regions (Kimberley, Pilbara and Gascoyne). The primary reader examined the distal surface of each whole otolith on two separate occasions, each time counting the annuli, assessing the marginal increment category (see Table 1) and giving the otolith a readability index category (see Table 2). Additionally, during the second reading the radius of the first three annuli and margin from the core of the otolith were measured along an axis from the core to the posterior tip (Figure 6a) with an eyepiece micrometer at X 10 magnification. As with the sectioned adult otoliths, if the ages from the two initial readings differed the otoliths were read again and discarded if there was no agreement or assigned an age equal to the final reading when it agreed with a prior reading.

McPherson (1992) observed clear translucent and opaque zones on the distal surface of whole *S. commerson* otoliths from Queensland waters. It was also noted that the first 3 translucent zones were characterised by light refracting radial striae that were not evident in subsequent translucent zones, where the translucent zone appeared as a raised ridge. This was also the case for most whole *S. commerson* otoliths examined from Western Australia with the majority also exhibiting distinct opaque and translucent zones (Figure 6a), although, in some otoliths the radial striae were present along the posterior axis to the fifth translucent zone (Figure 6b). In those otoliths without a clear annuli pattern (Figure 6c) only subtle changes in the density of radial striae and faint annuli could be discerned.

On the distal surface of most whole *S. commerson* otoliths examined, the first 3 annuli were clearly visible and separated by translucent zones containing radial striae while subsequent annuli became progressively closer together (Figure 6a). A very narrow translucent zone, much narrower than the annuli, separated the later annuli in older individuals often making the annuli difficult to distinguish. Generally, the annuli were more widely separated and hence the most reliable count came from an axis near or dorsal to the sulcus axis (Figures 6a to e). However, the annuli and indeed the posterior margin of the otolith were occasionally deposited unevenly (Figure 7a) and the axis along which all of the annuli can be readily discerned varied among otoliths. In addition to these axes the annuli could often be seen on the rostrum, antirostrum (Figure 6a) and along the sulcus acusticus, when viewing the proximal surface of the otoliths. Thus, counting the annuli in other areas of the otolith is required to confirm or reject the count obtained along the sulcus axis, with the highest distinct count used.

For all whole otoliths the marginal increment category was assessed along an axis near the sulcus axis on the posterior margin. However, as the axis along which the annuli were clearly visible often varied, there were difficulties in obtaining consistent measurements of the margins for marginal increment analysis (MIA). Use of the categorical system rather than actual measurements of the margin is therefore considered superior in MIA of whole *S. commerson* sagittal otoliths.

The initial occurrence of a translucent zone containing radial striae during the first 6 months and the broad first opaque zone consisting of the first annulus and the secondary opaque zone observed in the sections of *S. commerson* otoliths also occurred on the distal surface of most whole otoliths (Figure 6a). Additionally, ventral to the core, where the transverse section is taken, the initial translucent zone with radial striae was followed closely by the first annulus and no subsequent radial striae were observed, as seen in the adult otolith sections.

For the majority of whole *S. commerson* sagittal otoliths, the first translucent zone with radial striae was considerably larger than subsequent translucent zones (Figures 6a-c). However, in some otoliths the second translucent zone was larger than the first (Figure 7b). This otolith exhibits a translucent zone without radial striae immediately after the core followed by a relatively short translucent zone with radial striae and a broad but faint first opaque zone, which represents the first annulus. The second zone of radial striae (translucent zone) is considerably larger than the initial zone and is followed by a distinct second annulus. This may be an example of a late or early spawned individual whose initial growth and hence otolith deposition is not typical of the majority examined.

Mean diameters of the first 3 annuli from the core along the post-rostrum axis (Table 4) for each sex in each region show that there is a clear separation between the first and second annuli with the first ranging from 4.87 to 5.59 mm and the second from 6.43 to 7.14 mm. However, it must be noted that the mean diameters vary considerably between regions and sexes. For each region males have a smaller diameter otolith at the completion of the 2nd and 3rd annuli than females and *S. commerson* from the Kimberley region have a noticeably larger diameter otolith than those from the Pilbara or West coast, although the numbers measured are low. Thus, even though whole otolith size at age differs between regions and sexes, particularly after the first annulus, the data shows there are relatively distinct otolith diameters for the first 2 annuli.

The influence of completely drying the otoliths on the visibility of the opaque annuli was noted by McPherson (1992). This was confirmed during the present study when whole *S. commerson* sagittal otoliths examined immediately after removal, rinsing and blotting dry had no obvious opaque annuli but did so after 3 days of drying (Figures 8a and b). Furthermore weight of these otoliths had reduced by 5% over this period, presumably due to moisture loss. It should be remembered that in some instances otoliths stored in airtight containers (such as sealed ependorf vials used in the present study) may not be properly dried when removed for analyses.

Table 4. Mean diameters (in mm), with standard errors, of whole *S. commerson* sagittal otoliths at the completion of 1st, 2nd, and 3rd annuli for each region and sex. Diameter measured from the core along the post rostrum axis.

	1st annulus			2nd annulus			3rd annulus		
Region / Sex	**Count**	**Mean diam (mm)**	**St. error**	**Count**	**Mean diam (mm)**	**St. error**	**Count**	**Mean diam (mm)**	**St. error**
Kimberley males	43	5.27	0.16	17	6.91	0.12	6	7.48	0.22
Kimberley females	39	5.03	0.14	25	7.14	0.08	6	7.94	0.20
Pilbara males	100	4.87	0.06	87	6.43	0.06	73	7.04	0.07
Pilbara females	108	4.98	0.06	90	6.61	0.06	77	7.27	0.06
West coast males	19	5.26	0.16	18	6.62	0.13	15	7.30	0.13
West coast females	48	5.59	0.09	27	6.65	0.08	16	7.44	0.12

5.0 Conclusions

This report has documented in detail the methods used to obtain estimates of the age and growth of *S. commerson* as part of a FRDC funded stock assessment project on this species in Western Australia (FRDC Project # 1999/151). It has provided the following details for future study into the age and growth of this and similar species in Western Australia.

- Methods for the successful removal and storage of sagittal otoliths.
- Illustrated the importance of allowing the otoliths to completely dry.
- Description of adult and juvenile otolith sectioning methods.
- Notes for the interpretation of the microincrement structure in juvenile sagittal otolith sections.
- Details the use of microincrement results to determine the location and depth of the first annuli in adult otolith sections.
- Notes for the consistent interpretation of annuli in both whole and sectioned adult sagittal otoliths.

However, it is noted that validation of the temporal periodicity of either annuli or microincrement structure by chemical marking of otoliths has not been achieved to date in this species. Thus far only validation by monthly MIA has been achieved in Western Australia. Additionally, the differences in otolith size at age between sexes and regions require further investigation.

6.0 Acknowledgements

The authors wish to acknowledge the Fisheries Research and Development Corporation (FRDC) for providing the funding which enabled this project to commence. We would also like to thank the numerous commercial and recreational fishers who contributed to the collection of samples, without whose help we would never have achieved the sample numbers. In particular we would like to thank Hayden Webb, John Higgins, Andy Gilchrist, Jeff and Tony Westerberg, Ian Lew and Pam Canney, Shane, Phil and Peter Moore, Dion Hipper, Eric Mustoe, Ian Stewart, Jamie Waite and Barry Paxman for their contributions and Len Vertigan who put us onto the juvenile *S. commerson* in the Dampier region. Thanks also to the fellow research staff, including Lee Higgins and Justin King who sectioned a large number of otoliths, Jerry Jenke who provided advice on sectioning otoliths, Rod Lenanton, Dan Gaughan, Steve Newman, Graeme Baudains, Tim Leary, Ron Mitchell, Jeff Norriss, Craig Skepper, and Fiona Webster who helped on field trips and Dan Gaughan, Rod Lenanton, Suzy Ayvazian and Steve Newman who reviewed the draft manuscript, providing numerous useful comments.

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8.0 Figures

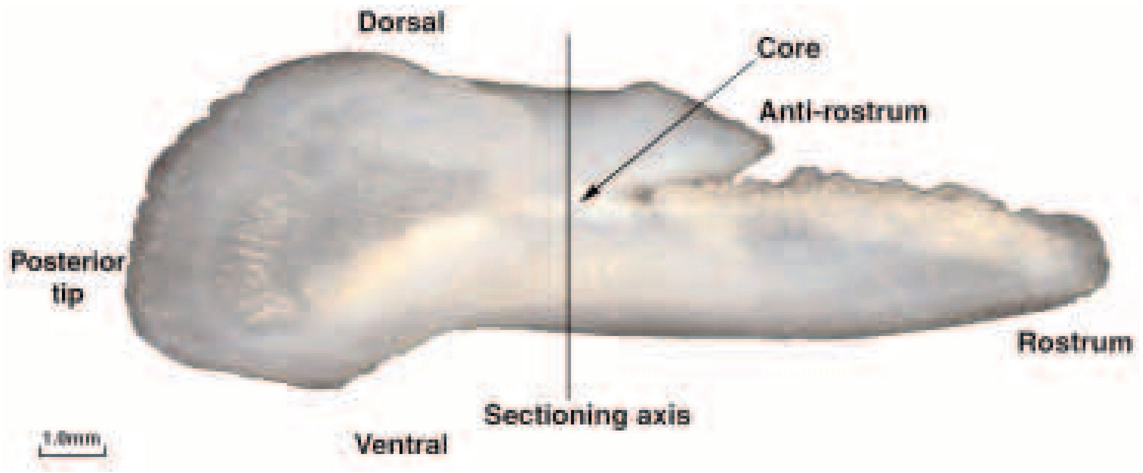


Figure 1. Distal view of right sagittal otolith from *S. commerson* (Male, fork length (FL): 1032 mm) showing orientation, location of core and sectioning axis.

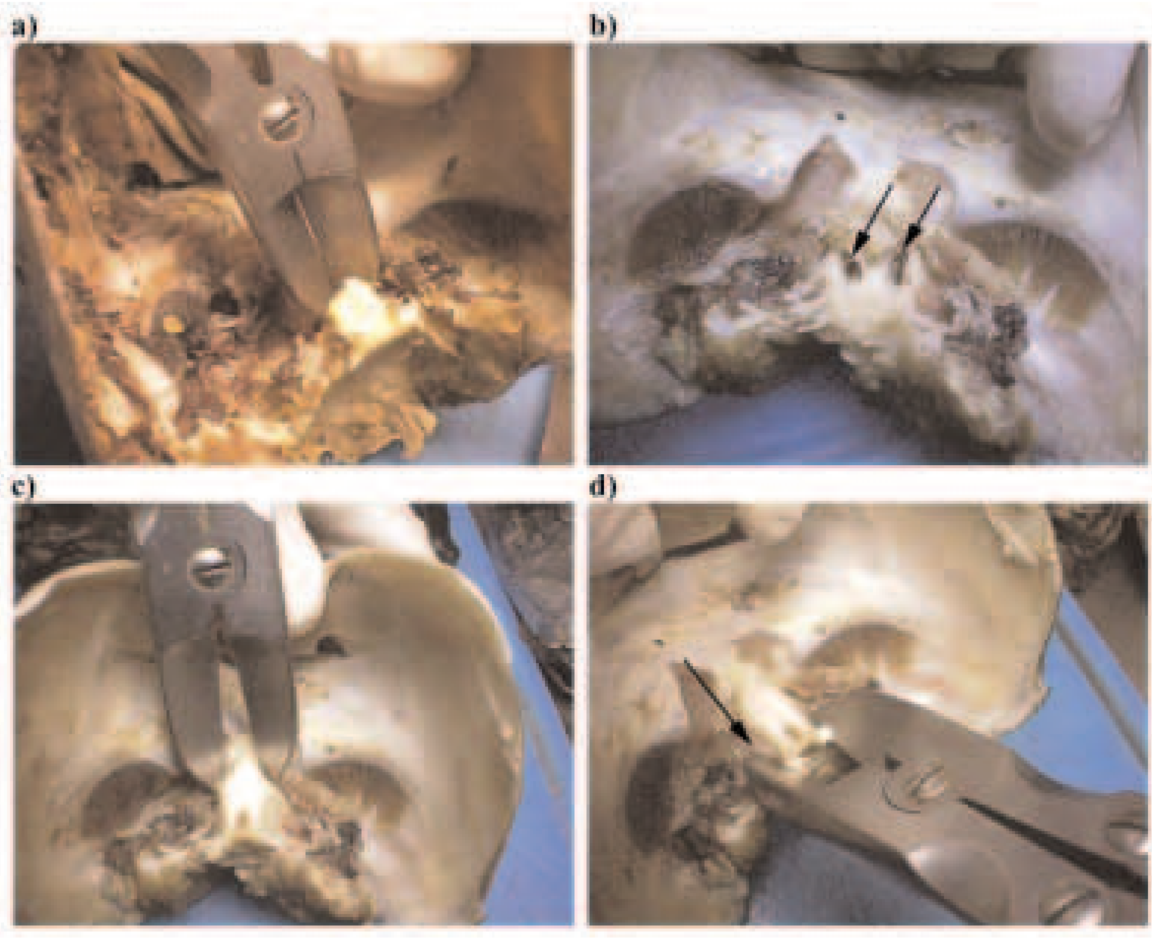
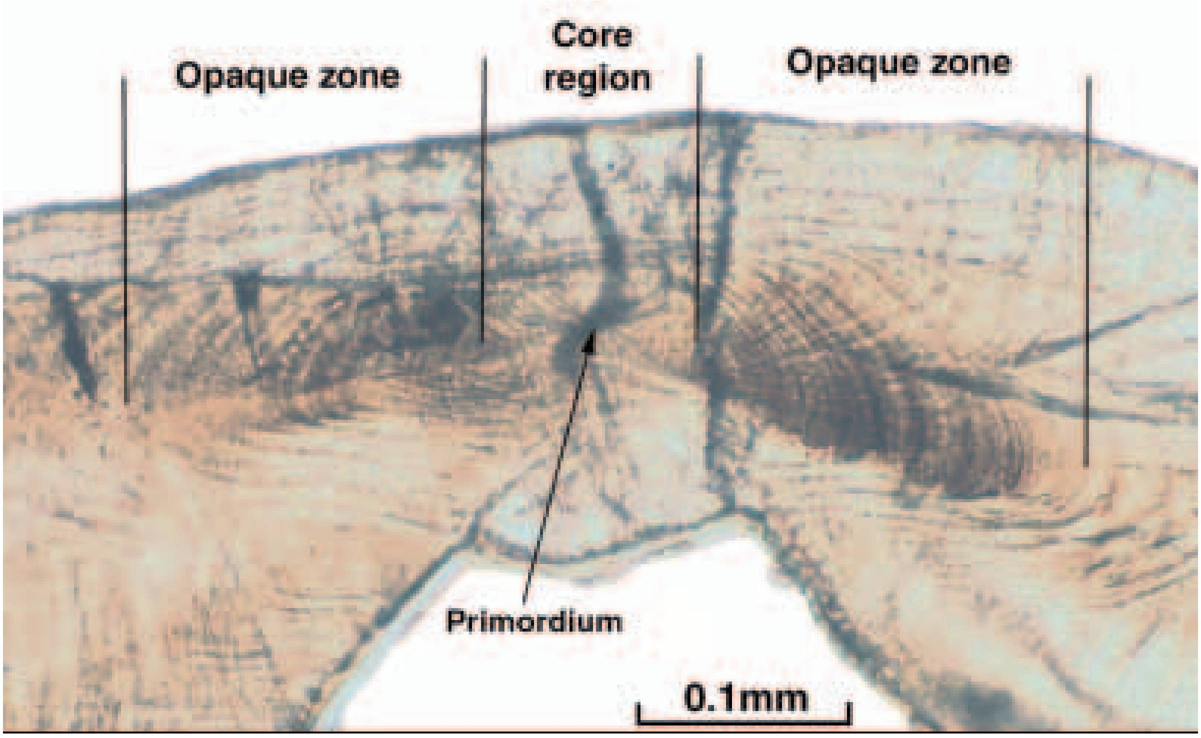


Figure 2. Illustrations of methods used to remove the sagittal otoliths from *S. commerson*, a) location of cut at base of skull for method 1 of otolith removal, b) base of skull removed, arrows indicating exposed otolith cavities, c) location of first cut in method 2, d) location of second cut for method 2, arrow indicates the nerve pores.

a)



b)

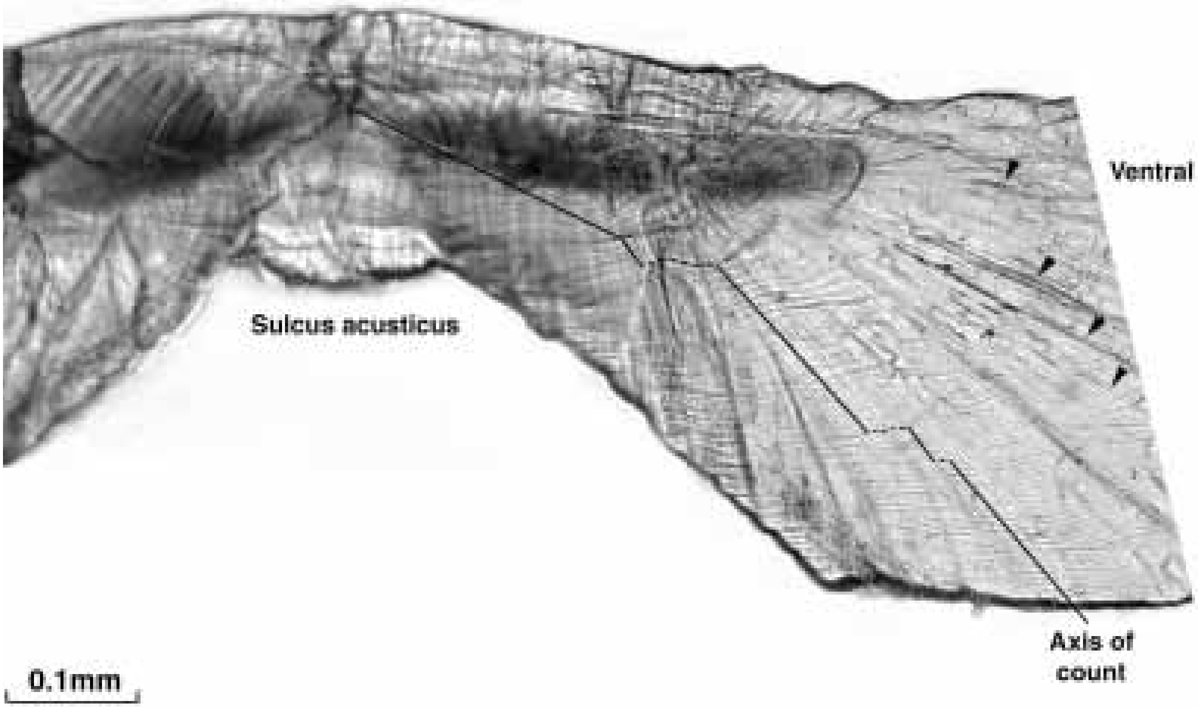


Figure 3. Photomicrographs of transverse sections of sagittal otoliths from juvenile *S. commerson* a) central region of otolith section showing location of primordium (black arrow), the core region with clear microincrements, and the initial opaque zones containing numerous secondary increments, b) from a juvenile, FL 325 mm, with a microincrement count of 86, line indicating the axis of the count and pointing out some of the radial striae (triangles).

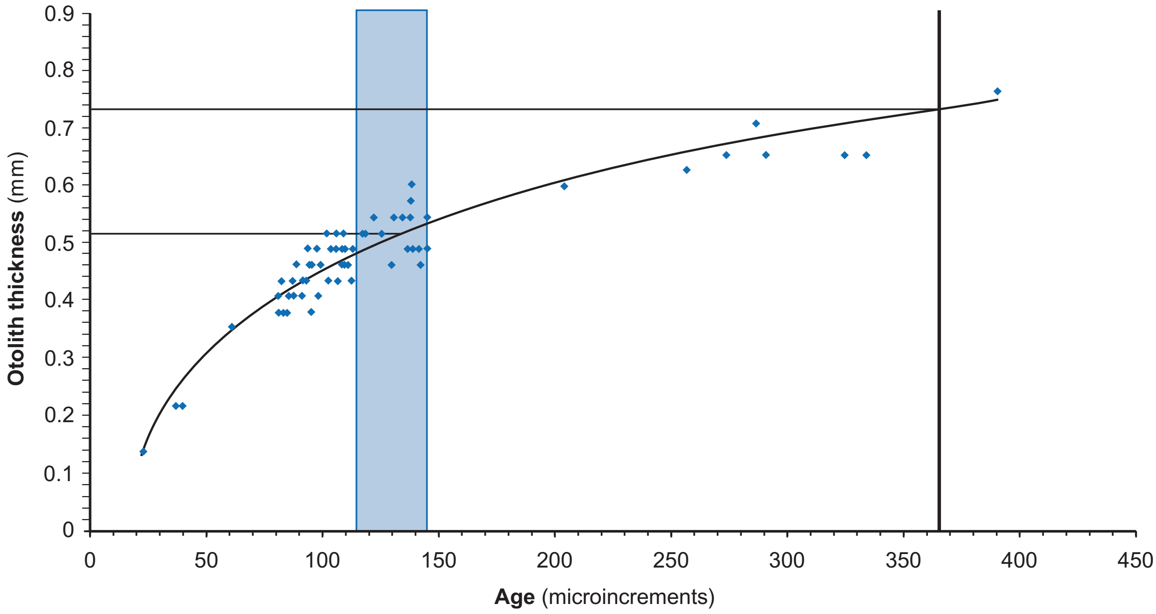
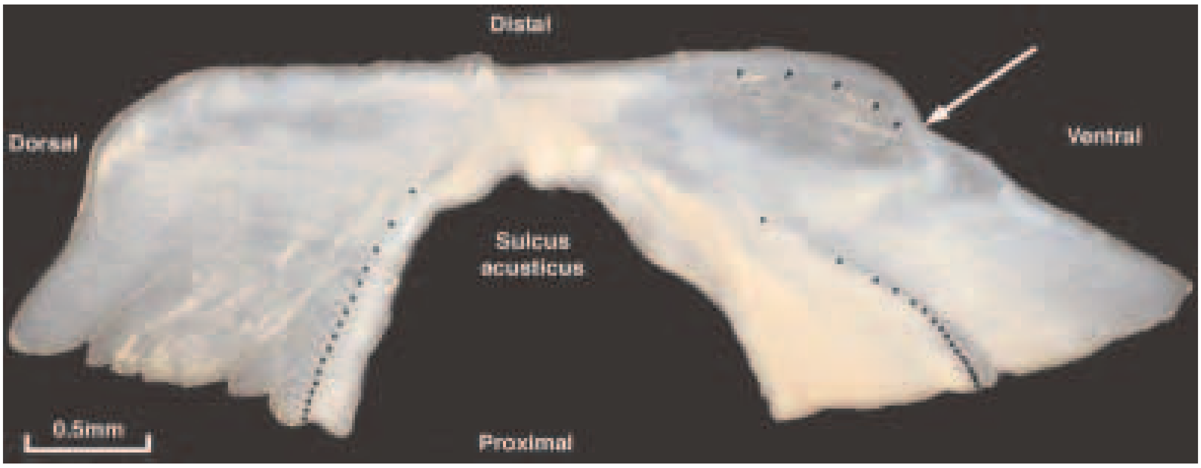


Figure 4. Distal-proximal thickness of *S. commerson* sagittal otoliths with age (microincrement count), bold line indicating one year (365 days), shaded area indicates the change in otolith deposition at the completion of translucent zone with radial striae in the transverse section containing the core and corresponding lines from fitted line (logarithmic regression line) indicate otolith thicknesses at these times.

a)



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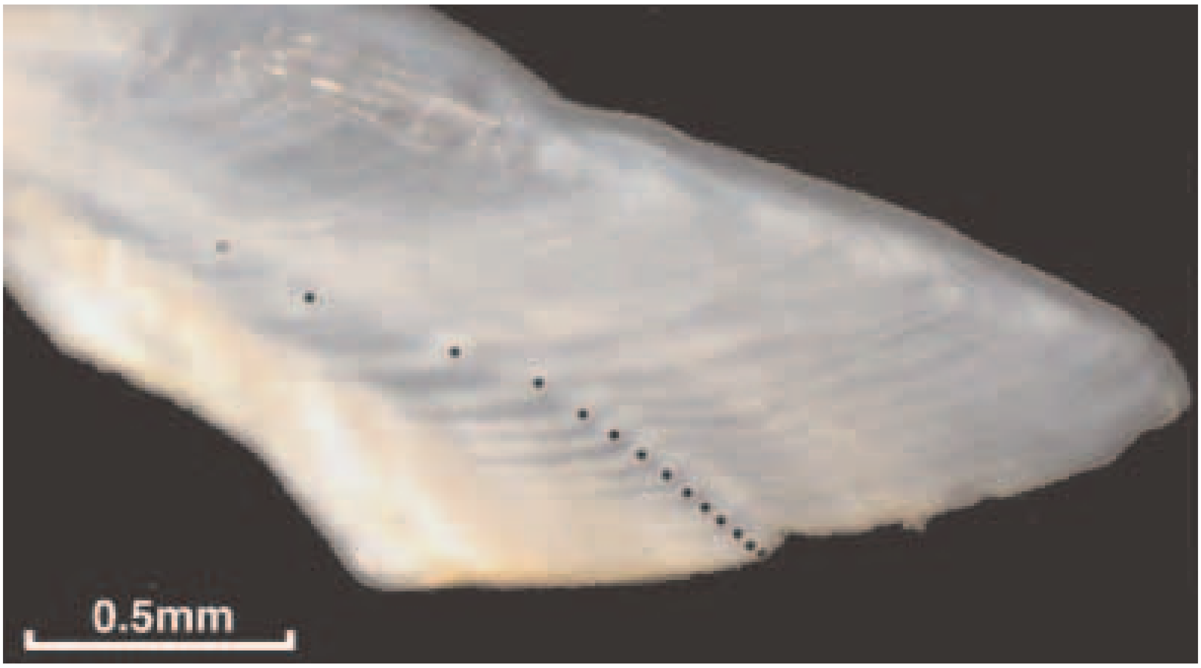

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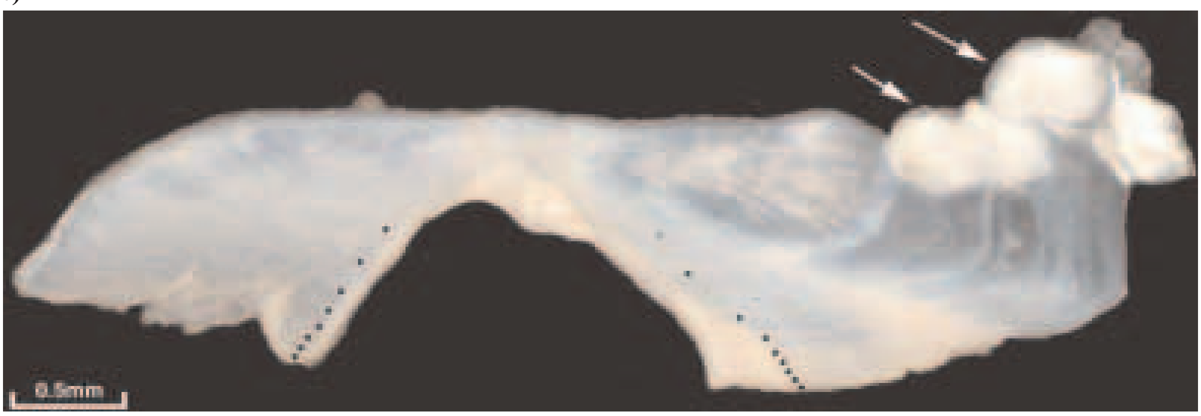
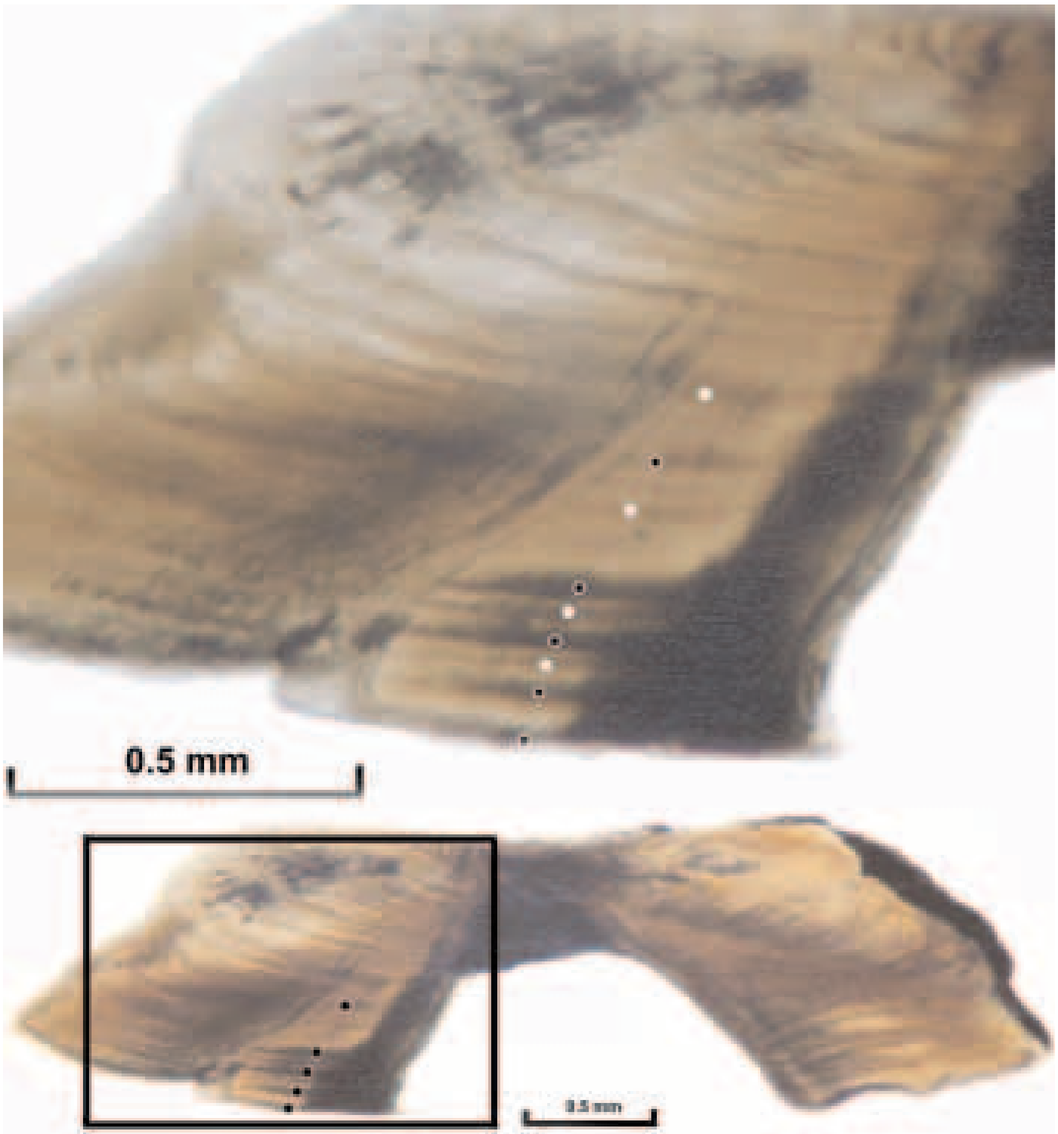


Figure 5. Transverse sections of *S. commerson* otoliths showing annuli (black dots) and secondary opaque zones (white dots); a) complete section from a 1204 mm FL male showing orientation, 16 annuli either side of the sulcus, the first radial striae zone (black triangles), the bump at the end of this zone (white arrow), b) ventral portion of section from a 1189 mm FL female showing 12 annuli and the secondary opaque zone at the completion of the radial striae, c) complete section from 1246 mm FL female showing 8 annuli on both dorsal and ventral portions, indicating abnormal outgrowths (white arrows).

d)



e)

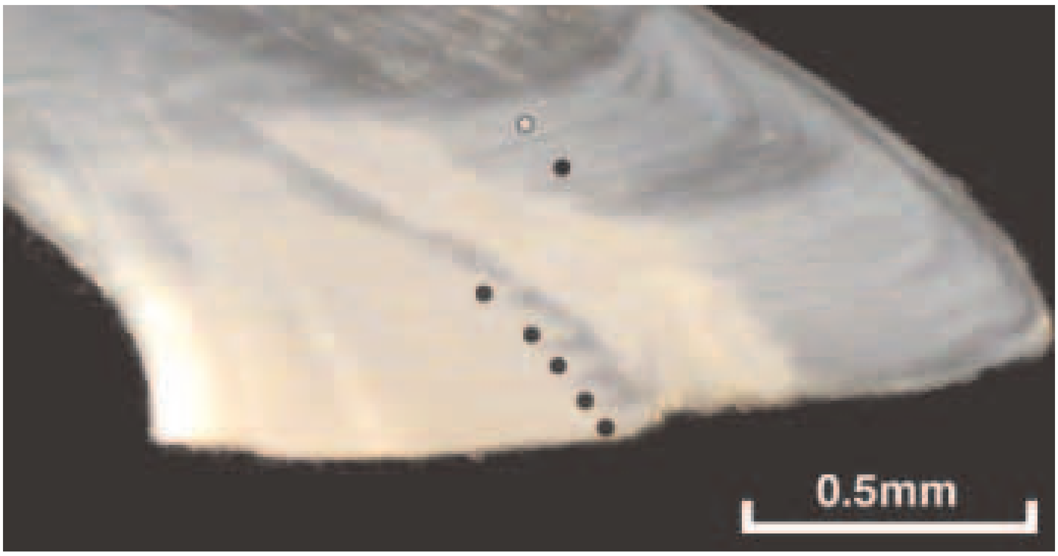


Figure 5. Transverse sections of *S. commerson* otoliths showing annuli (black dots) and secondary opaque zones (white dots); d) ventral portion of section from a 1070 mm FL female showing 4 clear annuli and clear secondary opaque zones, e) ventral portion of section from a 1175 mm FL female with clear secondary opaque zone, first annulus and 5 broad annuli.

a)

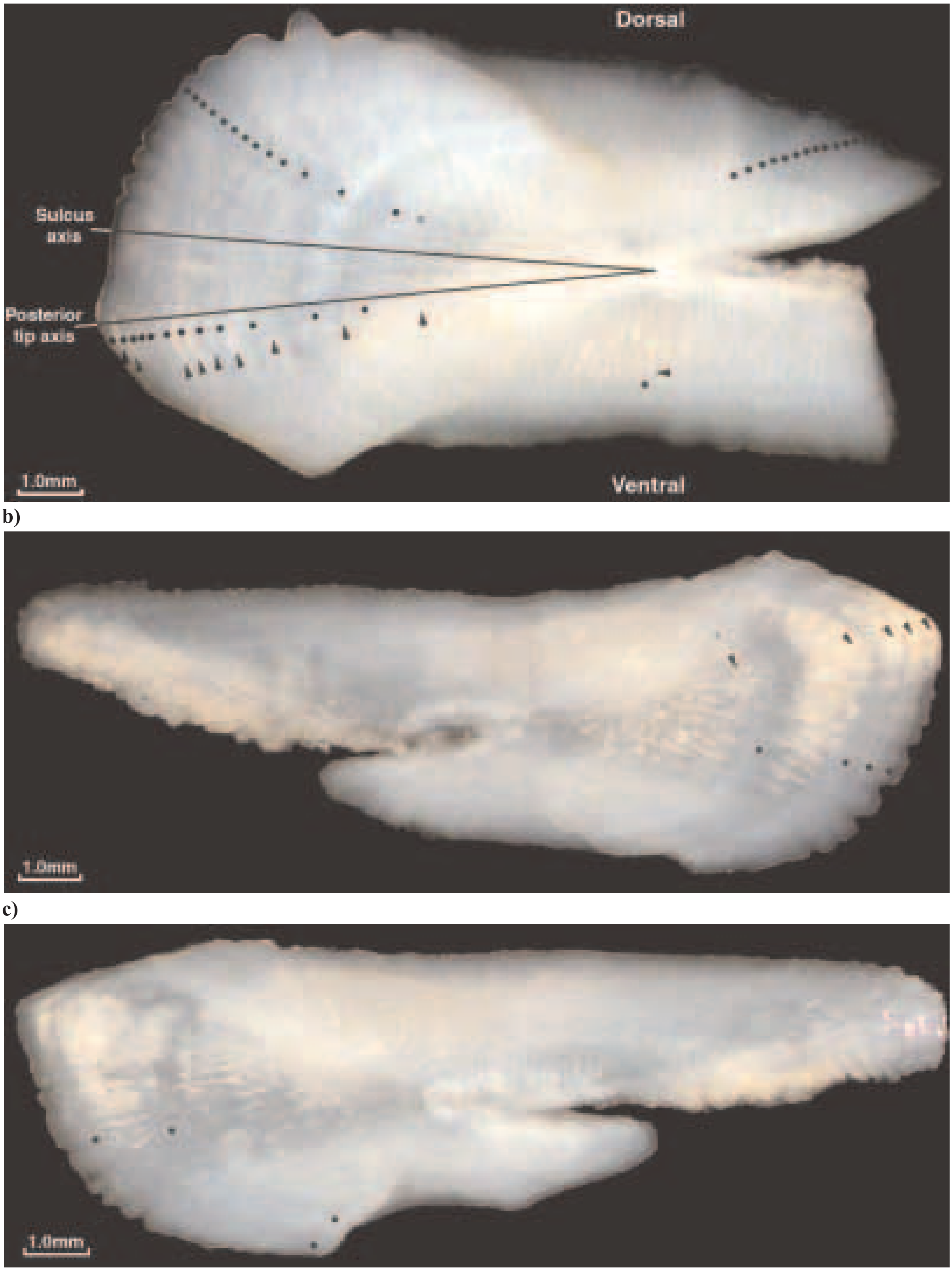


Figure 6. Distal views of whole adult *S. commerson* sagittal otoliths showing annuli (black dots) and radial striae (triangles); a) sagittae from a 1510 mm FL female showing 12 clear annuli along the posterior margin and antirostrum, a secondary opaque zone (white dot) and the axes along which most readings were taken, b) sagittae from a 1053 mm FL male showing 5 clear translucent zones with radial striae and 4 annuli, c) sagittae from a 1034 mm FL female showing 2 faint annuli between zones of radial striae.

a)

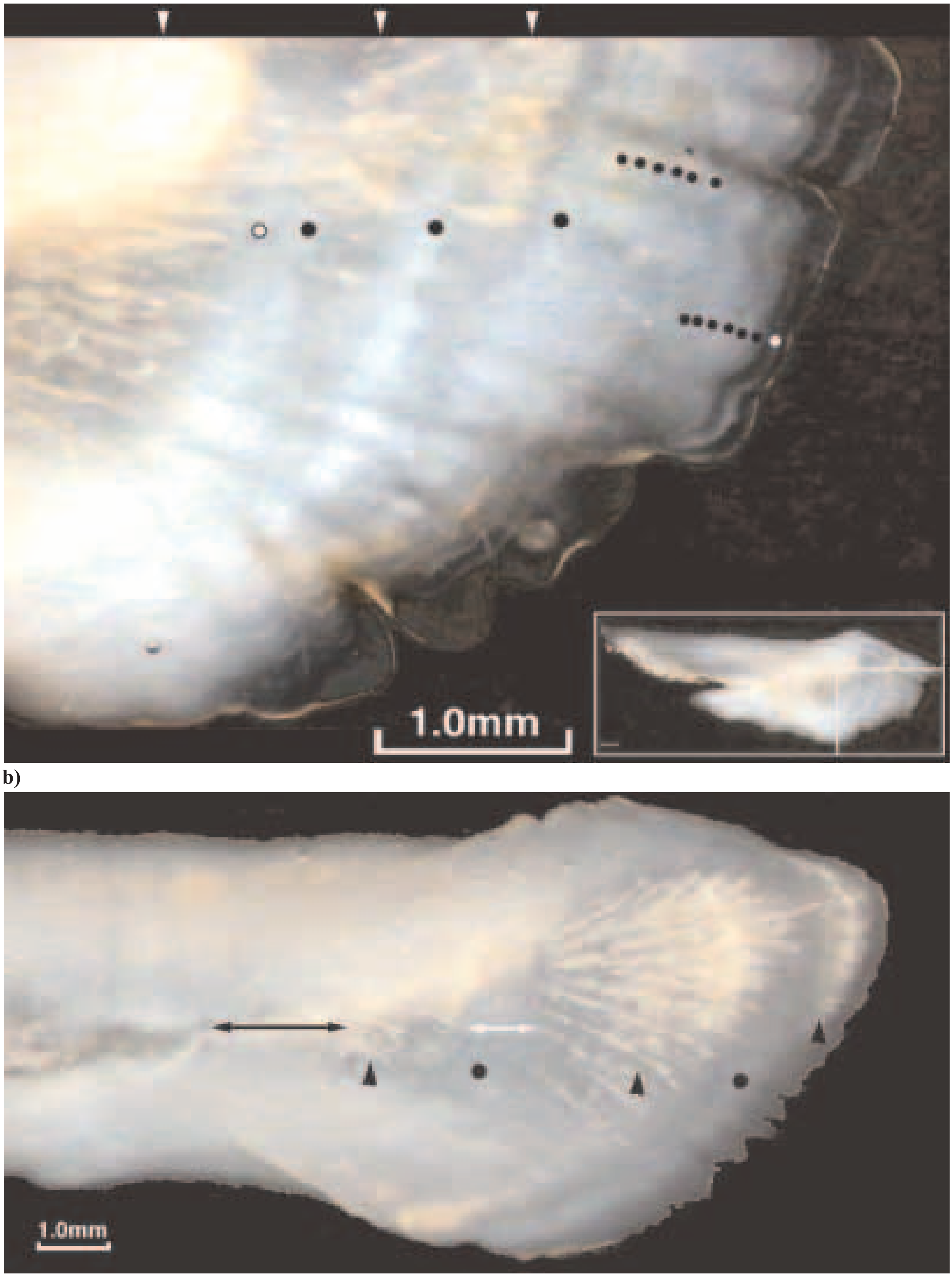


Figure 7. Distal view of posterior sections of *S. commerson* sagittal otoliths showing annuli (black dots), zones of radial striae (black triangles); a) from a 1280 mm FL male showing the uneven deposition of 15 annuli along the posterior margin, three zones of radial striae, and a clear secondary opaque zone (white circle) at the end of the first zone of radial striae, b) from a 1036 mm FL female showing three zones of radial striae (the first is quite small), a broad opaque zone at the first annulus (white arrows) and a clear second annulus. Note the broad translucent zone without radial striae near the core (black arrows).

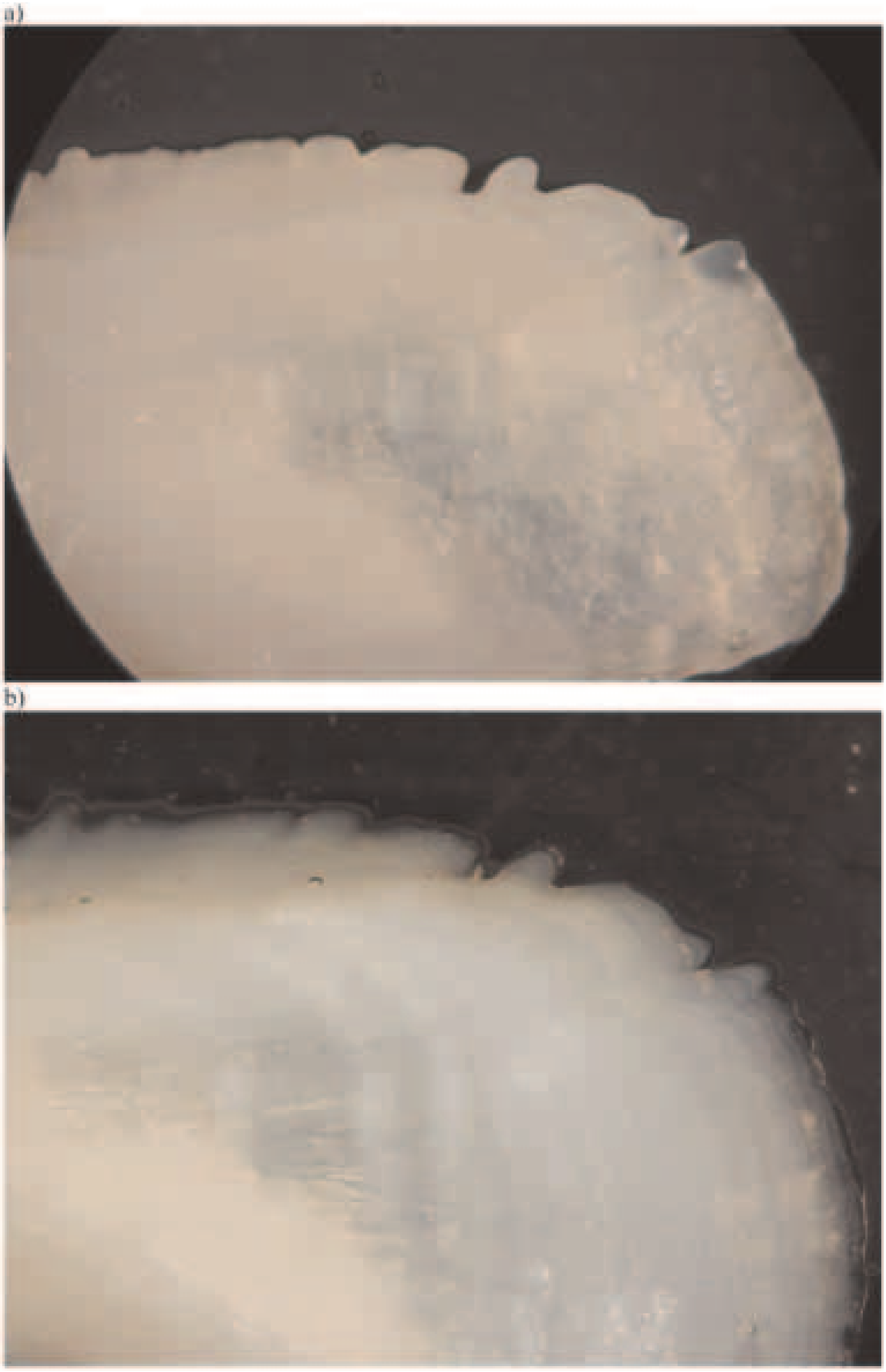


Figure 8. Distal view of posterior section of *S. commerson* sagittal otolith from a female of 1720 mm FL showing a) translucent appearance on the day of removal and b) clear annuli structure 3 days after removal.

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Not all have been listed here, a complete list is available online at http://www.wa.gov.au/westfish

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