Preliminary study on the use of polished otoliths in the age determination of Pacific sardine (*Sardinops sagax*) in British Columbia waters

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Abstract

Accurate age estimates are fundamental to understanding stock dynamics of fish. In this study we compare two methods for estimating the age of Pacific sardine (*Sardinops sagax*) using the otolith surface and polished otolith methods. In addition, first and second annuli could be better identified using mean annulus measurements to estimate the location of these annuli. Estimated ages ranged from 2 to 9 using the otolith surface method and from 2 to 10 years using the polished otolith method. However, individual fish ages estimated using the polished otolith method were generally 1 to 3 years older than corresponding otolith surface ages. Average percent error was much lower (3.3) for the polished otolith method than for the otolith surface method (5.3). Accurate ages are essential for determining growth trajectories, age compositions and maturation schedules. Identifying first and second annuli using mean annulus measurements to estimate the location of those annuli. Polishing the otolith surface enhanced those annuli, and greatly enhanced annuli near the edge in fish 5 years and older.

Introduction

The Pacific sardine (*Sardinops sagax*) supported the largest fishery in British Columbia from the 1920s to the mid-1940s. Catches during this period averaged 40,000t annually, then collapsed in 1947 as sardines disappeared from the British Columbia coast. The collapse of sardines off the west coast of North America was historically cited as an example of overfishing (Hilborn and Walters, 1992) rather than a result of distributional change, and sardines were not expected to return to Canadian waters (Murphy 1966; MacCall 1979). Sardines first reappeared in British Columbia waters in 1992 in commercial and research catches of Pacific hake (*Merluccius productus*) (Hargraves et al. 1994), and an experimental scientific fishery was opened in 1995. Research surveys targeting sardines were first conducted in 1997, capturing the fish in large numbers in surface waters. The abundance of sardines in British Columbia has continued to increase and has averaged 244kt since 2006 (Schweigert et al. 2009). This recent reappearance of sardines off British Columbia has been linked to changes in climate/ocean conditions (McFarlane et al. 2000; McFarlane and Beamish 2001).

Accurate age information is fundamental to stock assessment and management. Historically, the methods of estimating the age of Pacific sardine have changed from length-frequency analysis to annuli counts from either scales or otoliths (Butler et al. 1996). There is some evidence for the annual periodicity of age increments for sardine captured in California waters. Otoliths were used to validate daily growth in juvenile sardines (Butler, 1987), and Barnes and Foreman (1994) documented the periodicity of annuli in sardines up to age 3 using otoliths surface readings. Yaremko (1996) postulated that because sardines are a relatively short lived species, examination of the external surface of the otolith is a satisfactory technique for determining annual growth increments. However, she went on to point out that as the fish ages and otoliths grow, some deposition occurs across the entire otolith surface, and otolith thickness increases. Additionally, grooves and spiny protuberances on the ventral interior surface of the otolith become more pronounced. This can lead to difficulty in identifying annuli (particularly in older fish). Without using additional characteristics to help identify annuli; Butler et al. (1996) proposed two potential sources of ageing error: 1) variable periodicity for major growth increments, and 2) indistinct or ambiguous appearance of major growth increments. Butler et al., 1996 suggested these difficulties in interpreting otolith appearance was the greater source of error.

Larger (and presumably older) sardines migrate north into British Columbia waters during the summer feeding season (Beamish et al. 1999 and McFarlane and Beamish, 2001). No studies have examined the reliability of the otolith surface method for these larger fish in northern waters. Difficulties with identifying annuli in older fish has been a reoccurring problem in our studies. Given the importance of accurate ages for determining growth trajectories, age compositions and maturation schedules, we initiated a study to examine the reliability of the otolith surface method of age determination for sardines captured in British Columbia waters. This paper presents preliminary results of this study.

Methods

Sample collection

Surface-trawl surveys have been conducted annually (with the exception of 2007) off the west coast of Vancouver Island, Canada, to determine the abundance, distribution and biology of sardines. In all surveys a model 250/350 14 mid-water rope trawl (Cantrawl Pacific Ltd, Richmond, British Columbia) was used. Fork length (mm) and sex were recorded for all fish sampled. Sardine otoliths were collected from randomly selected samples of 50-75 fish from randomly selected sets during each survey. Samples were collected throughout the entire range of the survey. For this study otoliths were selected from fish from 7 sets. These otoliths were collected from fish which covered a range in size. In addition, samples of small fish were selected for 1st and 2nd annuli measurements from sets conducted from 1999 to 2005.

Otolith processing and annuli counts

Otoliths were extracted from sardines during onboard sampling, or were extracted in the laboratory from sardines that were frozen at sea, and processed at the Pacific

Biological Station, Nanaimo, British Columbia. After otoliths were extracted, they were rinsed in water to remove tissue and stored dry for subsequent ageing.

For surface ageing, otoliths were placed under a film of water in a shallow container and observed under a dissecting microscope on 25X and 50X power with a 10X eye piece, using reflected light. Against a dark background, opaque zones appeared light, translucent zones appeared dark. Annuli were defined as the area consisting of one opaque zone (summer growth) and one translucent zone (winter growth).

For polishing, each otolith was mounted sulcus side down on a microscope slide using a thermal setting resin (Crystalbond ™). Using fine sandpaper (ranging from 600 to 8000 grit), the otoliths were wet sanded to remove surface overburden and enhance underlying annuli (presumably on the outer edge). Polished otoliths were then aged as described above.

In larger (and presumably older) fish, it was sometimes difficult to identify the annuli (from checks) that would correspond to the first year or two of life. In these instances, the mean distance from the center of the focus to the interface between the translucent and opaque zone for the 1st and 2nd annuli measurement from young sardines in which these rings were clear were used to help identify the annuli.

Criteria for annuli determination

- ✓ An annulus is defined as the interface between an inner translucent growth increment and a distal opaque growth increment (Collins and Spratt, 1969).
- ✓ The translucent ring must be continuous along the entire structure. If the translucent ring merges with another or fails to go around the entire otolith, it is considered a false annulus, also called a check (Collins and Spratt, 1969).
- ✓ A surface-relief view of the otolith reveals a ridge for opaque depositions.
- ✓ The outermost translucent ring is not counted as an annulus unless an opaque zone can be seen distally from it (Yaremko, 1996)
- ✓ Some parts of the otolith may have stronger formations than other areas. Age interpretations were based upon the examination of all parts of the otolith. Often the best ridging was observed on the antirostrum part of the otolith.
- ✓ A January 1st birth date was used to avoid straddling the summer growth season.

Data analysis

Caillet and Goldman (2004) suggested that precision within a given study should be evaluated using an average percent error index. Both readings conducted in this study were done by a single reader so the precision between readings was evaluated using the average percent error index of Beamish and Fournier (1981). A student's t-test was used to evaluate the differences in the frequency of assigned ages between the otolith surface and polished otolith surface methods.

Results

In this study, 173 Pacific sardines were examined in the comparison of otolith surface and polished otolith methods for age determination. These fish were collected from research survey sets off the south west coast of Vancouver Island in 2003 (91 fish) and 2008 (82 fish), and ranged in length from 173 mm to 286 mm. In addition 252 small fish were sampled and the position of the 1st and 2nd annuli measured.

An annulus was defined as the interface between an inner translucent growth increment and the successive outer growth increment (Fitch, 1951; Yarmenko 1996). Although numerous checks were present, using the criteria presented above, the first and second annuli were usually easily identified in smaller, younger fish. In older fish, as deposition occurs across the entire otolith surface, and otolith thickness increases, identification of annuli near the focus became increasingly problematic as it may be more difficult to distinguish checks. In addition, annuli near the edge of otoliths from older fish are close together, and may be faint due to increased deposition on the exterior surface of the entire otolith, as well as increased deposition on the ventral interior surface near the edges. Polishing the exterior surface of the otolith removed the surface deposition over the entire otolith surface and annuli (particularly near the edge) became clearer and easier to identify.

As it was sometimes difficult to identify the 1st and 2nd annuli we used the measurement criterion of Chilton and Beamish (1982) to locate the annuli (fig.1). The mean first annulus diameter was 0.92mm +/- 0.12mm and the mean second annulus diameter was 1.21mm +/- 0.10 (fig. 2). There was little overlap between the measured diameter and the two measures were significantly different (t-test, $p<0.05$).

For example, a 226 mm male sardine (fig. 3) was aged 4+ using the otolith surface method, and 6+ using the polished otolith method. In this case, the 1st annulus was not identified correctly, as it did not meet all the criteria used by the reader. In addition, the 4th annulus was missed and initially called a check. Once the otolith was polished the two annuli could be identified and the 1st annulus confirmed using annulus measurements.

A 242 mm, male sardine aged 5+ using the otolith surface method was aged 6+ using the polished otolith method (fig. 4). In this case, the 5th annulus near the edge was not detected until the otolith was polished; however the 1st and 2nd annuli were easily identified in both the otolith surface and polished otolith.

The most extreme example of undetected annuli in our study was a 265 mm male sardine (fig. 5) which was aged 7+ using the otolith surface technique and 10+ using the polished otolith technique. All 3 undetected annuli were near the edge and were not visible on the otolith surface. Only after the edge was polished to reveal the otolith growth on the ventral interior portion of the edge were these annuli detected.

Reader agreement

Reader agreement between first and second readings of the otolith surface was not very precise (Table 1). Total agreement was 52% and agreement within +/- 1 year was 92%, similar to that presented by Butler et al. (1996). However, most fish aged by Butler et al. (1996) were less than 5 yrs. The index of average percent error (APE) (Beamish and Fournier, 1981; Chang 1982) was 5.3 identifying an acceptable level of precision. Reader agreement between first and second readings of the polished otolith surface improved dramatically (Table 1). Total agreement was 81% and agreement within +/- 1 year was 98%. The index of average percent error was 3.3, indicating a high level of precision.

Ages determined by using the annulus measurements in conjunction with the polished otolith surface were generally 1 to 3 years older, even in fish as young as 3 or 4 years (fig. 6). The age composition for ages determined using the polished otolith surface has a mode of 6 years a maximum age of 10 whereas the age composition for ages determined using the otolith surface had a mode of 5 yrs and a maximum age of 9 years (fig. 7). Older fish (>5+) comprised 57% of the polished otolith sample vs. 42% using the otolith surface. There was a significant difference between the ages estimated using the otolith surface and polished otolith surface methods (t-test; $p<0.05$).

Discussion

This study used standard age determination methodologies using otolith surface readings and polished otolith readings to estimate the ages of Pacific sardines. In general, the otolith surface readings produced ages 1 year younger than polished otolith readings, however some fish were aged 2 and 3 years younger. Although ageing inaccuracies of one year might not be critical in long-lived species, they have serious stock assessment implications for shorter-lived species such as sardine. This is likely true because shorter-lived species typically have only 3 to 5 year classes which dominate the fishery.

Butler et al. (1987) validated only growth rings in juvenile sardine and Barnes and Foreman (1994) validated annuli up to age 3. However Butler et al. (1996) and Yaremko (1996) pointed out that difficulties associated with otolith growth made annuli identification in older fish difficult. In particular, we found that ring (annuli) clarity near the focus (first and second annuli) was sometimes compromised and that rings (annuli) close to the edge were close together, faint and difficult to identify. This was due to deposition (in older fish) along the entire surface of the otolith and increased thickness of the otolith on the ventral interior surface near the edge in older fish. Identification of the first and second annuli was improved by using mean annulus measurements to estimate the location of those annuli, and polishing the otolith surface enhanced those annuli, and greatly enhanced annuli near the edge in fish 5 years and older.

We suggest that using these techniques when ageing Pacific sardine will improve the accuracy of the age estimates. Although a systematic under-aging of sardine of 1 year and a small percentage of under-ageing by 2 and 3 years does not appear, at first glance to be problematic – we believe that it could have serious consequences for understanding sardine dynamics and for stock assessment. For example, underestimates of age would overestimate mortality rates. If managers used a F=M strategy to estimate fishing yields, then overfishing would result (Tyler et al. 1989). If recruitment were estimated from catch-at-age analysis, erroneous age composition and overestimates of mortality would lead to overestimates of recruitment (Tyler et al. 1989). The use of annulus measurements and polished otoliths will also help us to assess the relative migration of older fish found in this stock.

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Tables

Table 1: Percent agreement and average percent error (APE) for reader 1 and reader 2 using surface otolith readings and polished otolith surface readings.

Method	Precision between reader 1 and reader 2	Number of fish	Percent agreement	APE
Surface age	No difference	90	52%	
"	+/- 1	69	92%	5.3
"	+/- 2	14	100%	
Polished surface	No difference	158	81%	
"	+/- 1	14	98%	3.3
"	+/- 2	1	100%	

Figure captions

Figure 1. Schematic representation of the otolith surface for measurements of first and second annuli. Measurements are made from the centre of the focus to the interface between the translucent and opaque zone. (Figure modified from Yaremko, 1996).

Figure 2. Scatterplot of distances (mm) from the focus to the first annulus (triangles) and second annulus (circles) for Pacific sardine.

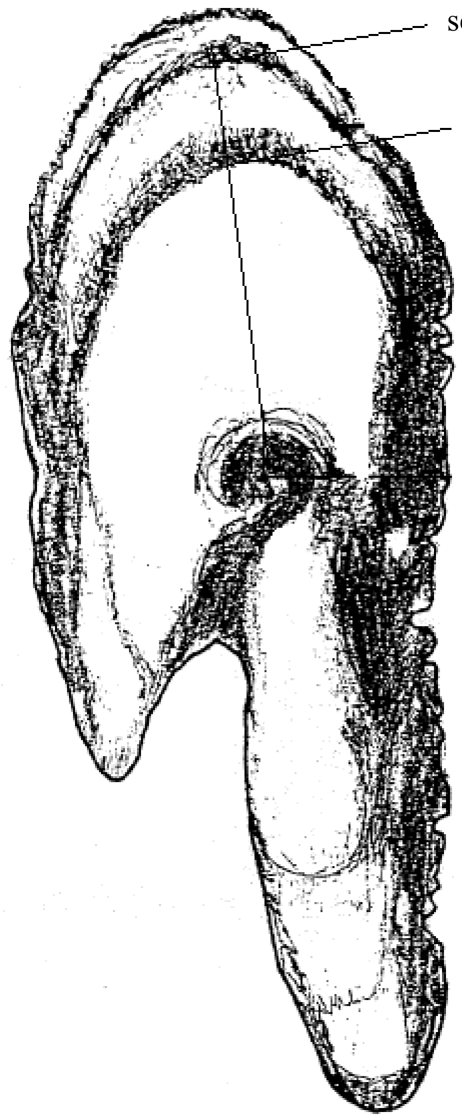
Figure 3. Otolith age estimates for a 226 mm male Pacific sardine showing (a) four distinct annuli using the otolith surface method to age the fish (b) six annuli using the polished otolith surface. X denotes the position of annuli which could not be identified using the surface method. Note: the first annulus was easily identified and confirmed on the polished surface using the annular measurement. (Scale bar=1mm)

Figure 4. Otolith age estimates for a 242 mm male Pacific sardine showing (a) five distinct annuli using the otolith surface method to age the fish (b) six annuli using the polished otolith surface. X denotes the position of annuli which could not be identified using the surface method. (Scale bar=1mm)

Figure 5. Otolith age estimates for a 265 mm male Pacific sardine showing (a) seven distinct annuli using the otolith surface method to age the fish (b) ten annuli using the polished otolith surface. X denotes the position of annuli which could not be identified using the surface method. (Scale bar=1mm)

Figure 6. The relationship between age estimates using otolith surface method and polished otolith method for Pacific sardine. The number in brackets indicates number of fish at that point.

Figure 7. Age composition for 173 Pacific sardine using the otolith surface method (open bars) and the polished otolith surface (solid bars).



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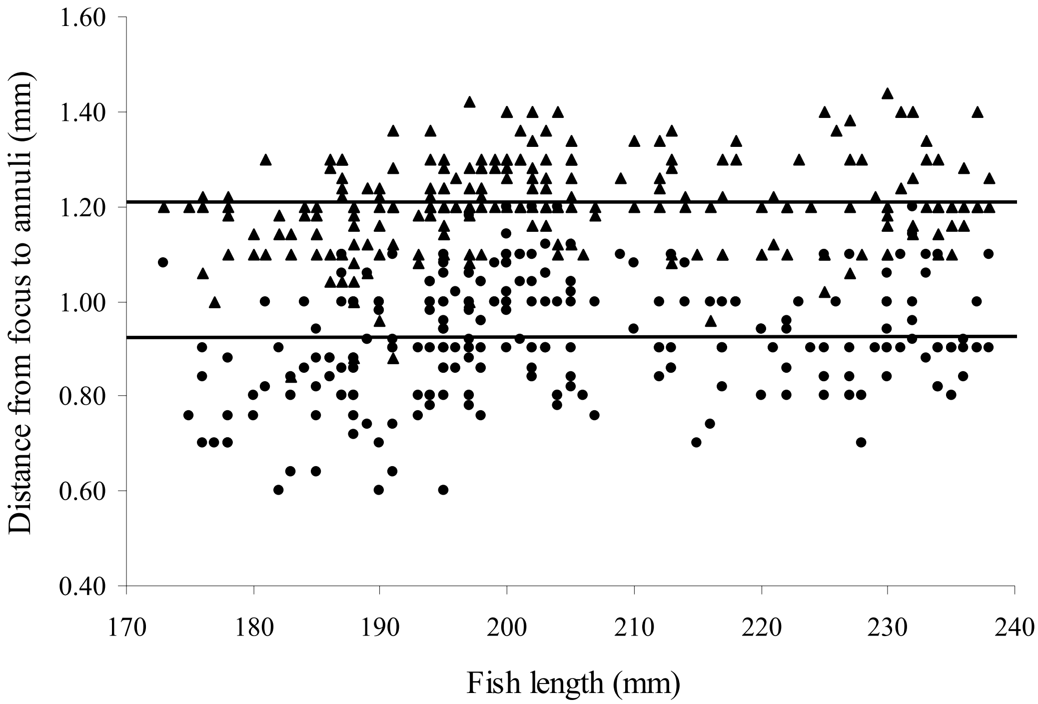

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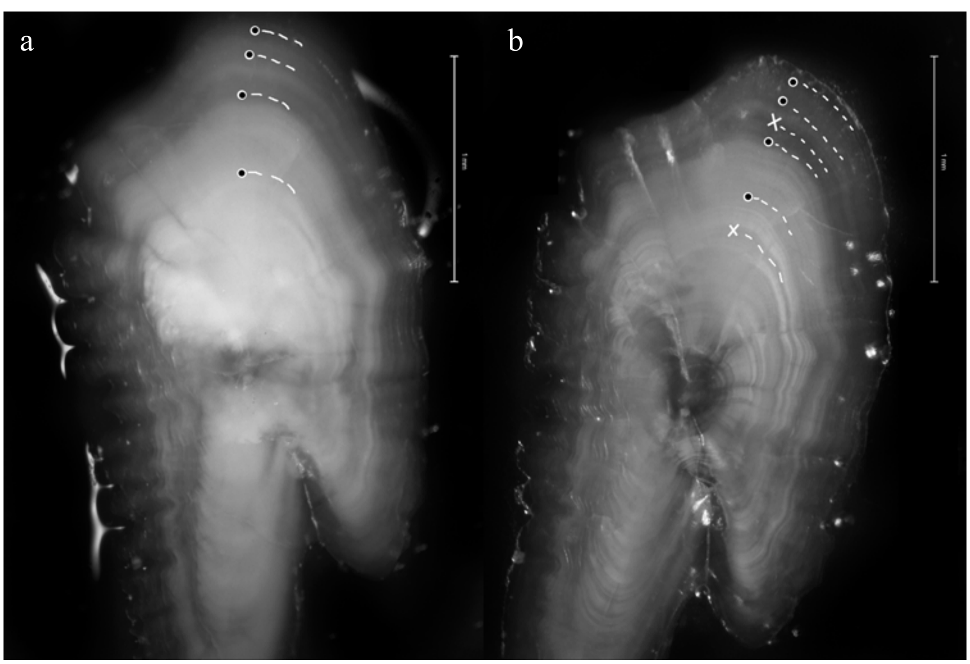

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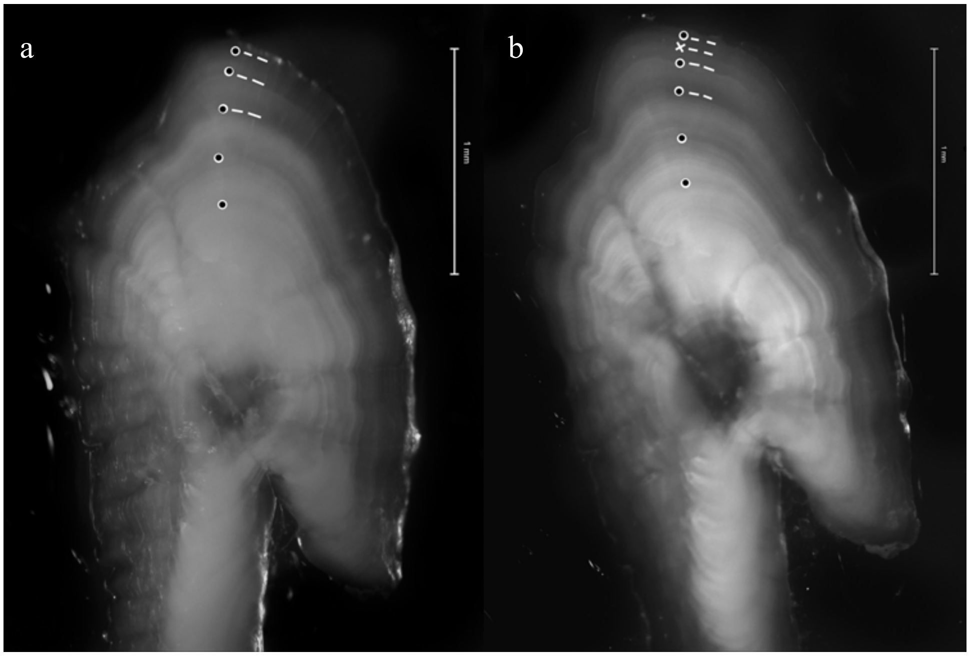

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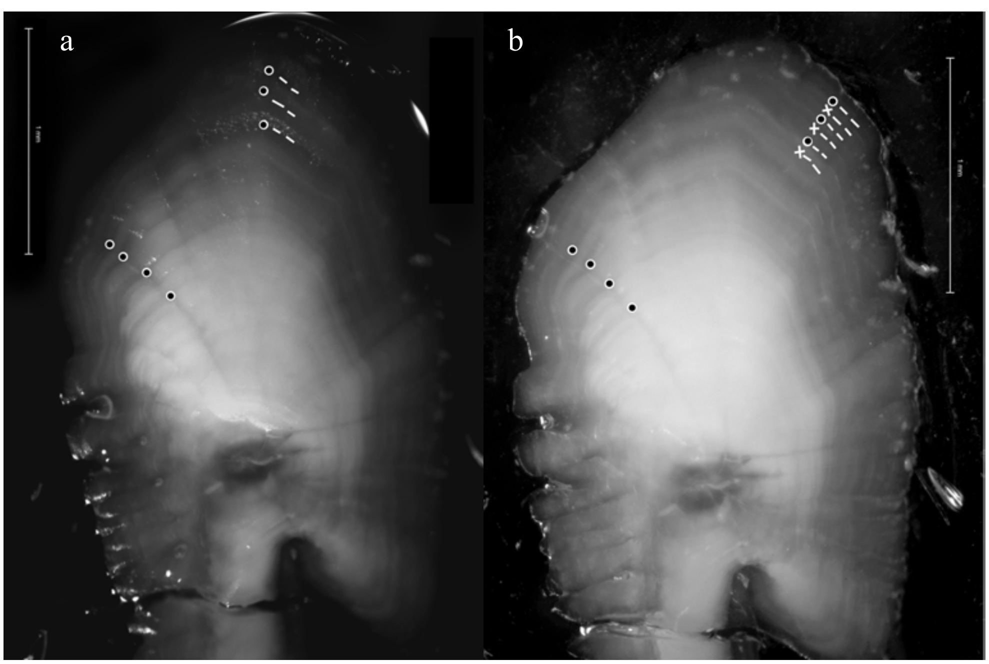

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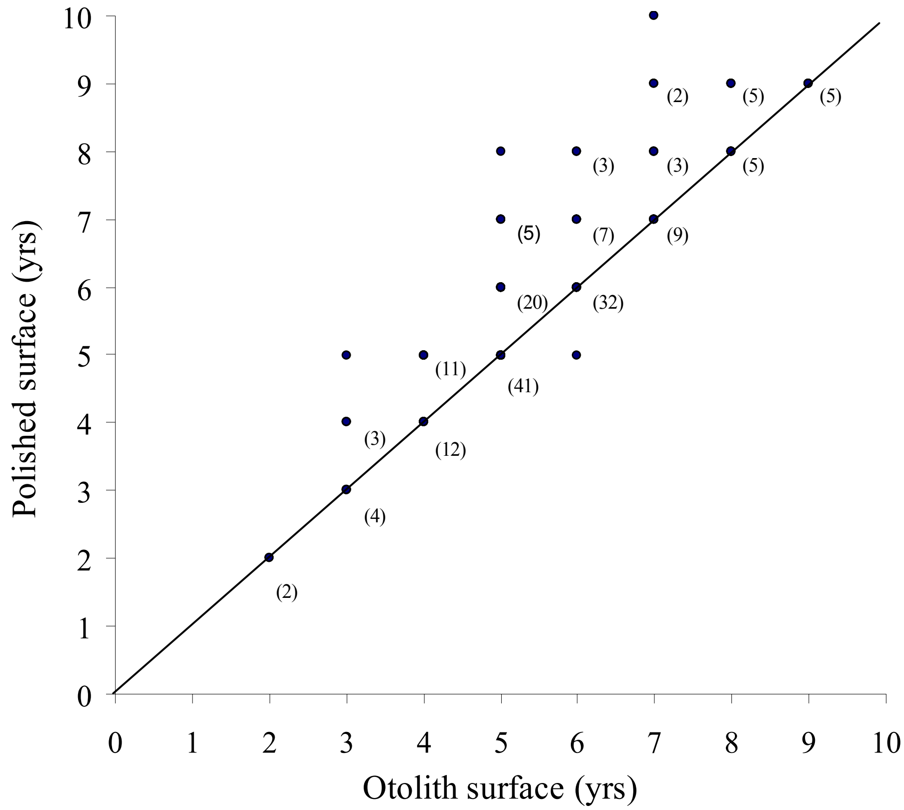

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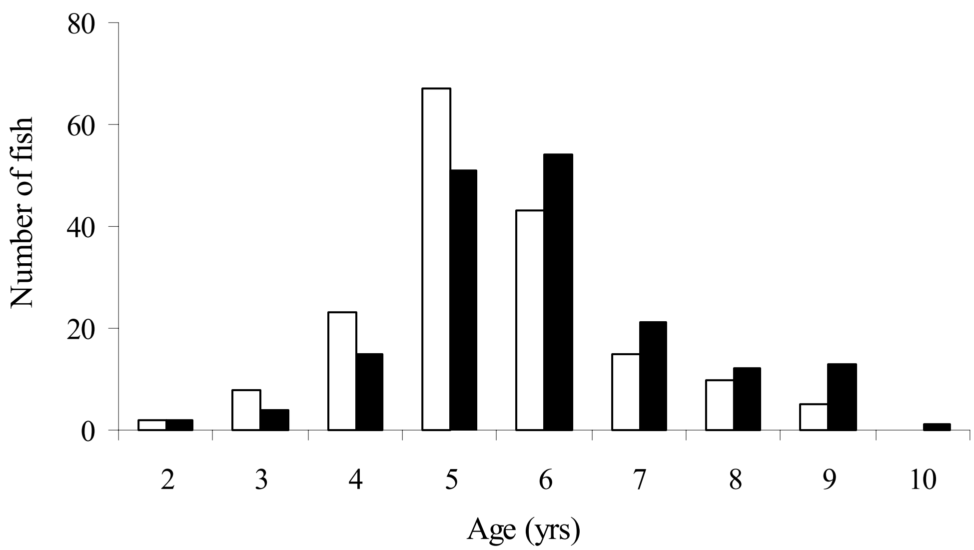

McFarlane et al (2009) Figure 7.