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Reevaluation of the interpretation of annuli from otoliths of a long-lived fish, *Anoplopoma fimbria*

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Abstract

Sablefish (*Anoplopoma fimbria*) were recaptured up to 20 years after being injected with oxytetracycline (OTC). After 20 years the OTC mark is clear in most otoliths. Our examination of these otoliths confirmed that sablefish are long-lived and grow slowly after maturity. Annuli that formed beyond the OTC mark could be difficult to interpret in broken and burnt sections. However, polished thin sections improved the clarity of annuli. Despite improved preparations, annuli in some sections were difficult to distinguish, resulting in annuli counts after the OTC mark that did not correspond exactly to the years at liberty of the recaptured fish.

Keywords: Sablefish; Otolith sections; Oxytetracycline; Annuli; Mark/recapture

1. Introduction

In 1982 a new method of estimating the age of sablefish (*Anoplopoma fimbria*) was used to show that this fish was considerably older than previously thought (Beamish and Chilton, 1982). Estimated ages ranged up to 45 years in this earlier study. More recently we have estimated ages of sablefish greater than 100 years (maximum 114 years). Using oxytetracycline (OTC) injections, Beamish et al. (1983) and McFarlane and Beamish (1995) were able to confirm that as sablefish grow older, otolith growth is reduced and occurs primarily in thickness on the ventral surface. This growth on the ventral surface was not detectable on otoliths of older fish by viewing the surface of the otolith. We noted in these previous studies that despite confirming that the narrow growth zones were formed annually, there was some difficulty in identifying annuli, which would reduce precision. A major difficulty with these interpretation problems in the management of sablefish was the inability to be confident that the strength of year classes could be identified accurately.

In this study we examined otoliths from sablefish that were at liberty between 13 and 20 years after being tagged and injected with OTC. Of particular interest was a detailed examination of annual growth zones that formed after the OTC mark was deposited. As the exact number of years of growth was known and the fish had been at liberty long enough to experience a diversity of growing conditions, we expected that the complexity of annulus formation characteristic of the slow growth phase would be found in the otolith growth after the OTC mark.

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Our initial examination of these otoliths quickly indicated that our previous method of estimating ages from burnt otolith sections (Beamish and Chilton, 1982) was not adequate. Careful preparation using polished surfaces of thin sections of the otolith greatly improved the interpretation. In this report, we describe the difficulties of interpreting the very narrow annual growth rings that form in the otoliths of this long-lived fish.

2. Methods

The methods of capturing, tagging, and injecting sablefish have been described previously (McFarlane and Beamish, 1987, 1995). Sablefish do not have a swim bladder and can be readily captured in traps, kept alive at sea, and tagged and released. Tagged sablefish are routinely recaptured in the commercial fishery, and the whole fish is returned for processing. The fishery for sablefish is managed using quotas allocated to individual fishermen, who work cooperatively with biologists and managers. As a consequence of this cooperative approach, the reporting and retrieval rate of recaptured, tagged fish is believed to be high.

Only fish that had been at liberty for 13 years or longer were examined. Sagittal otolith pairs from all injected and recaptured fish were recovered and stored in 50% glycerin in a darkened container. Prior to storage, the otoliths were wiped clean of all tissue. Some otoliths were stored for up to 5 years before being examined. One otolith from each pair of a selected number of fish was processed using the method described previously by McFarlane and Beamish (1995). The otolith was broken dorso-ventrally through the nucleus, and the broken surface was examined using a Leitz Laborlux 12 compound microscope equipped with an ultraviolet light. The position of the OTC mark was noted, and the distance from the distal edge of the mark to the edge of the otolith section was measured. This procedure was required because burning destroys the mark. The section was then burnt and treated with cedar wood oil to enhance the contrast between growth zones (Beamish and Chilton, 1982); the age was then determined. The position of the OTC mark was identified using the previous measurements, and the number of annuli formed after the marking was counted. The annulus was defined as the translucent zone or the zone of slower growth that appeared as a dark zone under reflected light. All other otoliths in this study were mounted in a fiberglass resin treated with a black pigment and sectioned according to the procedures described in Beamish (1979). Sections were approximately 1.0 mm thick. The thin sections were examined using the Leitz Laborlux 12 compound microscope with an ultraviolet source. Annual growth zones were detected using transmitted light to view the section while the ultraviolet light source remained on. Growth zones were also studied using phase contrast and regular transmitted light sources. Oil applied to the surface sometimes improved the clarity, as did polishing the otolith section (in the epoxy section) using very fine silicon-carbide polishing paper (grades 320, 600, 1200, 4000). Two readers assessed the ages and annual growth, but both readers knew that the numbers of annuli that formed after the OTC mark ranged from 13 to 20. Measurements of the widths of annual zones were made using an eyepiece micrometer on the thin polished sections only.

3. Results

As reported previously (McFarlane and Beamish, 1995) 21 989 sablefish were tagged and injected with OTC from 1977 to 1981. As of 31 December 1997, 1618 (Table 1) or 7.4% of all fish released were recaptured. Otoliths were recovered from 26 of the 32 fish that were at liberty for 13 years or more but 5 of the 26 fish had otoliths in which no mark was visible. Thus 21 pairs of otoliths were available for study (Table 2). The total ages ranged from 18 to 65 years and the average yearly increase in fork length was 3.1 mm with a range from 0.8 to 10.0 mm. There was no relationship between yearly growth rate and years at liberty ($p>0.05$) or between total age and yearly growth rate ($p>0.05$). One fish apparently had negative growth. Unfortunately it is not possible to be certain that all lengths at either release or recapture were recorded correctly, thus there is always some doubt whether negative growth is real.

The annual growth zones that formed in the otolith after the OTC mark was deposited were quite narrow

Table 1
Recaptured sablefish tagged and injected with OTC between 1977 and 1981

Release year	Number released	Number recaptured (years at liberty)													
		0–4	5–9	10	11	12	13	14	15	16	17	18	19	20	Total
1977	5279	162	57	3	7	2	1	0	1	2	0	1	0	2	238
1978	9881	695	74	8	11	5	2	2	2	3	0	0	0	0	732
1981	6829	531	79	16	12	4	6	7	3	0	0	0	0	0	648
Total	21989	1388	210	27	30	11	9	9	6	5	0	1	0	2	1618

and in some cases close to the level of differentiation with the preparations used (Figs. 1 and 2). Annual growth (measured on the thin polished sections) in the otolith thickness for the two fishes that were recaptured after 20 years at liberty was approximately 0.01 mm with a range 0.035–0.009 mm. In all otoliths there was a gradual decrease in the width of the annual growth zones. After about age 8–10 years, the annual growth zone width decreased from approximately 0.05 to 0.03 mm in about 5 years. After about age 15 years, the annual growth zones ranged from 0.03 to 0.01 mm, with most zones being 0.01 mm wide or less. In about one half of the samples, the annual growth zones were distinguishable and counts corresponded to the number of years at liberty. The first few annuli (3–5 years) are difficult to identify by defining objective criteria similar to the criteria used for years of slower otolith growth. It is possible, however, to use measures of annual otolith growth to assist in identifying the annulus. It is also useful to use the otolith surface and a broken and burnt section to identify the annuli in these faster growing zones.

Table 2
Total age and average annual growth during the period of release and recapture

Years at liberty	Total age	Length at tagging (mm)	Average yearly growth after tagging (mm)
20	58	470	1.4
20	61	530	1.9
18	51	540	8.8
16	48	510	0
16	51	690	1.6
16	49	610	2.5
16	33	620	−0.8
15	38	710	1.5
15	47	490	0.9
15	31	610	2.4
15	38	713	4.9
15	65	610	6.0
14	18	520	10.0
14	47	632	1.3
14	39	715	4.6
14	49	700	4.9
14	61	656	3.1
13	31	610	2.8
13	24	743	1.2
13	28	530	7.2
13	43	749	2.9

In the thin sections in which the annual growth zones were readily identifiable, the number of annuli that formed after the OTC mark was similar to the number of years at liberty (Table 3). However, we were not unbiased in our determinations, as we knew that there would always be 13–20 annuli. It was important to recognize the width of growth zones that formed after the OTC mark. This helped to interpret the annual growth zones prior to tagging according to their similarity in width and appearance.

In about one half of the material, the presence of checks within the narrow (older) and wider (younger) otolith growth zones resulted in difficulties in identifying both annuli and annual growth zones. Interpretation was most difficult in the parts of the otolith where annual growth was larger (younger ages or older parts of the otolith — not the recently formed parts). However, in a few otoliths, the number of zones formed after the OTC mark exceeded by 10–20% the actual number of years at liberty. We interpret this to indicate that on occasion a check will form at a location in the annual growth zone that creates the appearance of being an annulus. In such cases, the two halves of the annual growth zone it divides appear about the same width as adjacent annual growth zones and the structure of the check cannot be separated from the annulus.

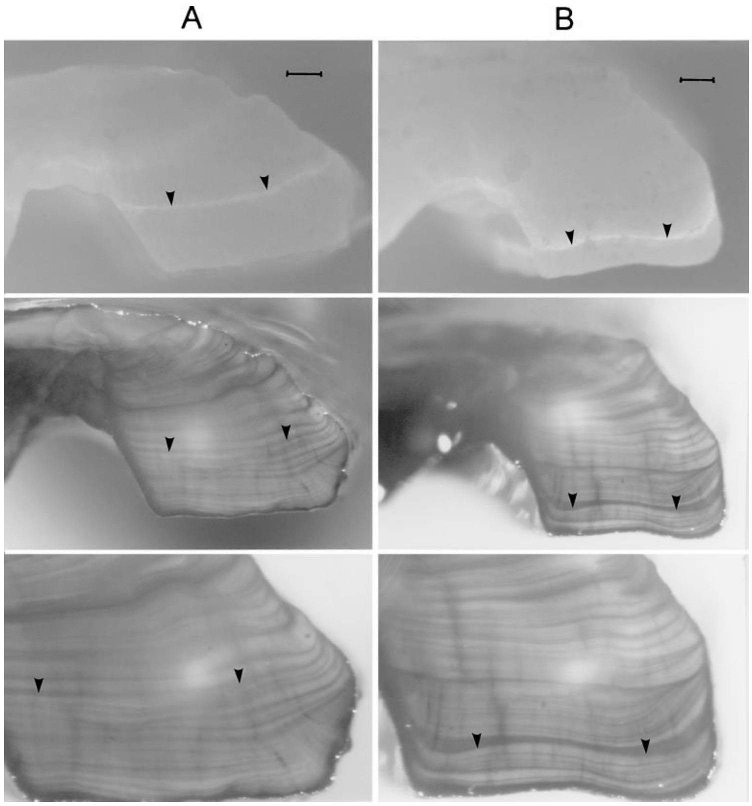

Fig. 1. Broken otolith sections showing OTC mark (arrows). The sections in the upper panels were viewed with a compound microscope using reflected ultraviolet light. The same sections in the middle and lower panels were burnt and viewed with a dissecting microscope using reflected white light. The position of the OTC mark is indicated with an arrow. Scale bar=0.2 mm. (A) Fish was tagged in November 1981 and recaptured in August 1995. The OTC mark (arrows) was located on the fourth annulus. The total age was estimated at 18 years. (B) Fish was tagged in May 1978 and recaptured in June 1993, aged as 65 years. The otolith was difficult to age because of an irregular pattern. The annual zones were narrow and difficult to discern. The OTC mark (arrows) is located on approximately the 50th annulus. The annual zones were so narrow that they could not be differentiated, even at 100× magnification.

We show two broken and burnt otolith sections (Fig. 1A and B). The OTC mark is clear in both otoliths, however, the amount of growth after the OTC mark is greater in section A than in section B. The burnt surface shows distinct zones in Fig. 1A that, if counted from the figure, underestimate the number of years at liberty. This is particularly evident in Fig. 1B. When viewed using reflected light and high magnification, it was possible to identify more annuli (as indicated by dots in Fig. 1A and B), but the tendency was to underestimate the actual age (years at liberty) after tagging. There were two major difficulties with this type of preparation. One was the narrow width of annual growth zones and the second was the difficulty in interpreting the ventral edge using the burning technique. It is apparent from the photographs that detail can be lost in the areas of slow growth. In areas of rapid otolith growth, near the nucleus, it is possible to see the checks that may be contributing to the difficulty in identifying annuli in the slower growth areas of otoliths. In general, the broken and burnt sections provided less detail in the slower growing areas of the otolith. Although the contrast achieved by burning appeared to differentiate annual zones, the pattern that resulted did not make it possible to distinguish some adjacent annual growth zones less than 0.01 mm in width. Thus this method was most useful when a thin section could be used as a reference. Although the production of both a thin section and a broken and burnt section was time-

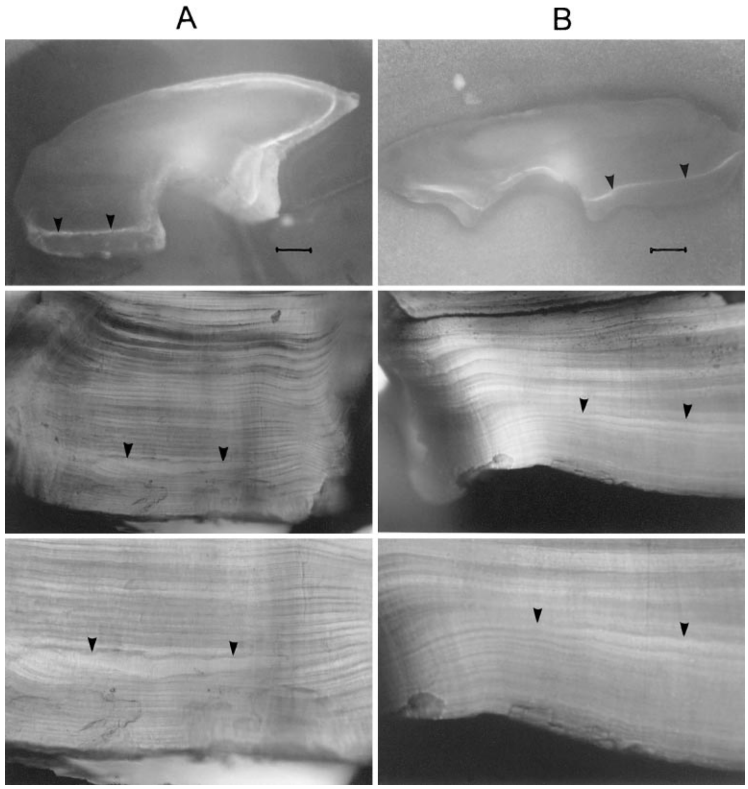

Fig. 2. Thin, polished, sections, with a drop of oil showing the OTC mark in both upper panels. The position of the OTC mark is indicated with an arrow. Scale bar=0.3 mm. The amount of growth after the OTC mark represent 15 years in Fig. 2A and 13 years in Fig. 2B, and is an indication of slow growth of the otolith. The middle and lower panels show the detail of the annual growth zones in the vicinity of the OTC mark to the ventral edge. (A) Fish was tagged in September 1977 and recaptured in November 1992. The total age was estimated at 47 years. There are very clear annual growth zones prior to the formation of the OTC mark. There was some difficulty in interpreting annuli around the OTC mark, but the narrow zones at the ventral edge were distinguishable. (B) Fish was tagged in November 1981 and recaptured in May 1994. The fish had a total age of 24 years and was at liberty for 13 years, but we identified 15 annuli and possibly up to 17. This is an example of the interpretation difficulties even though the preparation is suitable.

consuming, it was considered to be essential for interpreting the annuli in the otoliths of these old, slow growing fish.

Thin sections of otoliths (Fig. 2A and B) also had distinct OTC marks. The extent of the marks confirms that growth is restricted to the ventral surface, except in Fig. 2A, where there has been some growth on the dorsal surface. The position of the OTC mark within the particular annual growth zone could be distinguished by alternating between ultraviolet light and transmitted light. Thus there was no difficulty in studying the detail of the annual growth zones before and after the OTC mark. The detail of annual increment formation was clearer in the polished sections than in the burnt sections. Differentiation of the adjacent annual growth zones remained a problem with the narrowest zones (<0.01 mm) even with high magnification (Fig. 2A and B). Despite these interpretation difficulties, it was always possible to identify annual growth zones, particularly near the ventral edge. If checks formed within an annual growth zone, it was often possible to separate checks from annuli by viewing the particular growth zone in several areas of the section and noting whether the check was still present. However, there were some sections in which checks could not be separated from annuli, resulting in an overestimate of years at liberty. Separation may be possible if the microstructure is studied in more detail.

Table 3
Estimated number of annuli after the OTC mark using thin sections

Years at liberty	Annuli after OTC mark	Average
20	20, 20	20
18	19	19
16	15, 16, 18, 16	16.3
15	15, 15, 15, 15, 17	15.4
14	14, 13, 15, 11, 14	13.4
13	13, 15, 13, 13	13.5

In Fig. 2B, the growth zones that formed after the OTC mark are distinguishable in the photograph, particularly towards the edge. In this figure, the number of annuli after the OTC mark was equal to the years at liberty. In Fig. 2A, separation of the annuli before and after the mark was more difficult because of check formation. In this section, the number of annuli after the OTC mark were less clear as checks could not be identified.

4. Discussion

It is rather remarkable that we are still recapturing sablefish 20 years after they were tagged, injected with OTC, and released back into the northeast Pacific Ocean. The recapture of the fish reported in this study and the recovery of their otoliths provided clear evidence of both slow growth in fish length and the general longevity of the species. There was a general agreement between the number of annual growth zones identified after the OTC mark was deposited and the years at liberty, indicating that the narrow zones are annuli. However, the study also identified an important problem with the general interpretation of total age. In some otoliths the annual growth zones (and the annuli) are narrow and difficult to identify without high magnification and careful preparation of thin sections. The difficult problem is the presence of checks within the narrow and wider annual growth zones. Such checks are not found in all otoliths, but they are sufficiently common that they represent a problem in the interpretation of annuli and for routine age determination. Checks are more readily identified in the first 10 yearly growth zones as the annuli are wider, translucent structures compared to the checks. However, as the fish growth slows and otolith growth is reduced and restricted almost exclusively to the ventral surface of the otolith, the checks appear similar to annuli. We can make this statement because the otolith growth after the OTC mark produces a pattern of growth within a known number of years. Knowing the true age helped to categorize growth patterns. However, despite knowledge of the expected age after the OTC mark, some otoliths' patterns of check formation resulted in overestimates of age. It is important to emphasize that such overestimates do not mean that the fish were not long-lived and slow growing. It indicates the difficulties of estimating the age accurately. It is clear that detailed studies of otolith development are required.

A difficulty in age determination of older fish is the accurate separation of the narrow annual growth zones. Enhancement of growth patterns by staining or burning helps distinguish annuli, but consistently accurate age determination requires techniques that allow otolith structure to be studied. Material that is difficult to interpret because of the preparation simply cannot be aged reliably.

The polished thin sections used in our study were preferred for detailed study. Broken and burnt sections were useful but were difficult to examine using higher magnifications. In OTC studies, burning eliminates the OTC mark, requiring procedures to document the position of the mark and eliminating direct comparisons during the age estimating process. The technique of polishing the sections provided clearer preparations than previously observed (McFarlane and Beamish, 1995).

This study provided evidence that the method of aging sablefish is confounded by check development. If age is to be determined accurately, more detailed studies of otolith development are required. This problem is not new (Beamish and Chilton, 1982) and is another reminder that estimating the age of sablefish in particular and long-lived fishes in general is not a simple or unequivocal task. We believe this pattern of otolith growth is a feature of growth for all long-lived species (for example Power, 1978; Beamish, 1979; Leaman and Nagtegaal, 1987; MacLellan and Fargo, 1995).

It is more important to emphasize the preparation and interpretation of otoliths than to process large numbers. For sablefish, we think that periodic OTC injections and tagging continue to be useful and we think that a combination of breaking and burning and thin sectioning is necessary to estimate age accurately.

It may be necessary to rely on growth patterns in some parts of the otolith to estimate total age for some otoliths. For example, if 10–15 years of growth can be estimated accurately either through OTC injections or the differentiation of well formed annuli, it should be possible using computer-assisted annual-growth-increment matching techniques to match the growth pattern with known and validated growth patterns. The ages determined in this way may not be exact, but for older fish, the calculated age may provide a general indication of age composition and relative year-class strength. It is apparent that after 20 years of learning how to age sablefish, there is still more work to do.

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