Sources of age determination errors for sablefish (*Anoplopoma fimbria*)*

Donald E. Pearson

Santa Cruz Laboratory
National Marine Fisheries Service
110 Shaffer Road
Santa Cruz, California 95060
E-mail address: Don.Pearson@Noaa.Gov

Franklin R. Shaw

Alaska Fisheries Science Center
National Marine Fisheries Service
7600 Sand Point Way NE
Seattle, Washington 98118

Abstract—This study was undertaken to resolve problems in age determination of sablefish (*Anoplopoma fimbria*). Aging of this species has been hampered by poor agreement (averaging less than 45%) among age readers and by differences in assigned ages of as much as 15 years.

Otoliths from fish that had been injected with oxytetracycline (OTC) and that had been at liberty for known durations were used to determine why age determinations were so difficult and to help determine the correct aging procedure. All fish were sampled from Oregon southwards, which represents the southern part of their range. The otoliths were examined with the aid of image processing.

Some fish showed little or no growth on the otolith after eight months at liberty, whereas otoliths from other fish grew substantially. Some fish lay down two prominent hyaline zones within a single year, one in the summer and one in the winter. We classified the otoliths by morphological type and found that certain types are more likely to lay down multiple hyaline zones and other types are likely to lay down little or no zones. This finding suggests that some improvement could be achieved by detailed knowledge of the growth characteristics of the different types.

This study suggests that it may not be possible to obtain reliable ages from sablefish otoliths. At the very least, more studies will be required to understand the growth of sablefish otoliths.

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Sablefish (*Anoplopoma fimbria*) are a valuable groundfish resource off the west coast of North America. The fishery in California, Oregon, and Washington is tightly regulated according to periodic stock assessments. Between 1990 and 1998 landings averaged more than 8000 metric tons per year and an average exvessel (retail) value of 12.5 million dollars per year (PFMC, 1999).

Sablefish are distributed in the northeastern Pacific Ocean from Baja California to the Bering Sea and southeast to northern Japan (Miller and Lea, 1972). Males and females are sexually mature between 55 and 67 cm, although there is considerable variation (Fujiwara and Hankin 1988a; Hunter et al., 1989). Off Washington, Oregon, and California, sablefish spawn from October through April and spawning peaks in January and February. Sablefish are oviparous, releasing eggs that float near the surface (Hunter et al., 1989). After hatching, larvae and juveniles inhabit surface waters offshore for several years after which they migrate inshore and settle to the bottom.

Sablefish are found on the continental slope and are commercially fished at depths from 200 to 1400 meters (Leet et al., 1992). Adult sablefish feed on fish, cephalopods, and crustaceans (Laidig et al., 1998). They reach a maximum length of 102 cm (Miller and Lea, 1972) and are believed to be a very long-lived species (possibly 100 years or more).

Many physical features have been used to age this species, including scales, finrays, thin-sectioned otoliths, and broken and burned otoliths, but all methods have resulted in less than 45% agreement among readers (Lai, 1985; Fujiwara and Hankin 1988b; Kimura and Lyons, 1991; Heifetz et al., 1999). The broken and burned otolith method (Chilton and Beamish, 1982) is the principal method used in aging of the species in both the United States and Canada. Typically, age readers agree on ages less than 50% of the time, and for fish older than 7 years, agreement drops to less than 15% (Kimura and Lyons, 1991).

There have been repeated efforts at validating sablefish ages and developing aging criteria. Beamish et al. (1983) successfully used oxytetracycline (OTC) marking to validate ages and repeated his experiment in 1995 when additional marked fish were recovered (MacFarlane and Beamish, 1995). Lai (1985) validated the use of otoliths for aging sablefish. Fujiwara and Hankin (1988b) examined otolith growth characteristics to help refine aging criteria. Heifetz et al. (1999) validated the currently accepted aging practices and examined sources of error in the aging of sablefish. Kastelle et al. (1994) used radiometric methods to generally validate the aging criteria currently used. Even with all of these studies that have validated age

* Contribution 119 from the Santa Cruz Laboratory, National Marine Fisheries Service, Santa Cruz, CA 95060.

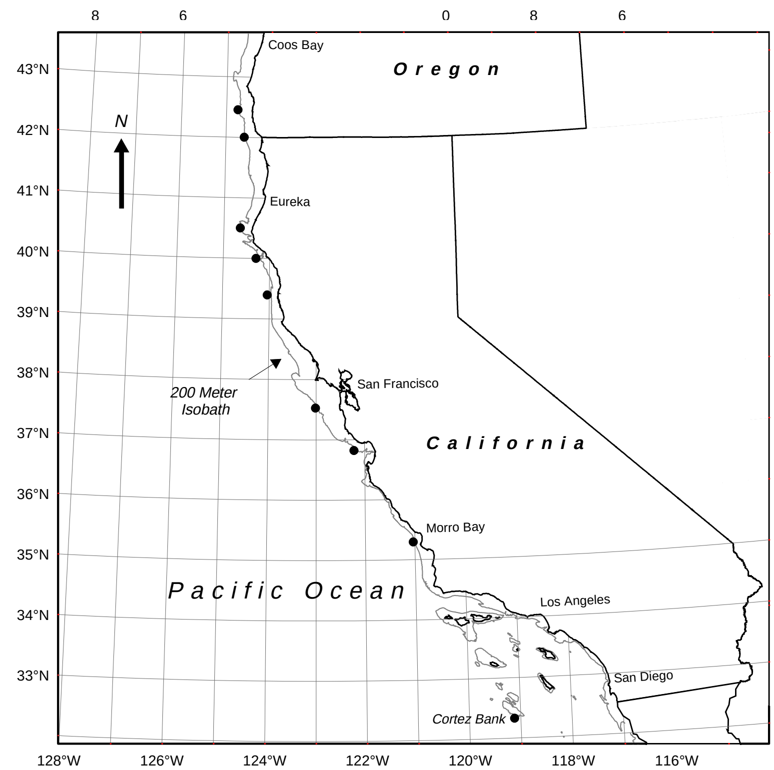

Figure 1

Map of California and southern Oregon showing the locations (black dots) of sablefish sampling and tagging in September and October of 1991.

determinations, independent age readings seldom are in agreement. This suggests that the methods used to validate the ages were insufficient to allow development of precise aging criteria. The lack of reliable age data has made stock assessments difficult and controversial (Crone et al., 1997), and in addition, accurate aging is needed to support ecological and habitat studies.

In September and October of 1991, a tagging and oxytetracycline (OTC) injection study was included as part of a fish trap survey of the abundance of sablefish in southern Oregon and California. The purpose of this study was to attempt, once more, to improve our ability to reliably age sablefish, thereby improving our ability to manage the species.

Methods

Capture, tagging, injection, and recovery

In September 1991, the fisheries research vessel *Alaska* was chartered by the National Marine Fisheries Service to conduct a trap survey from Coos Bay, Oregon, to Cortez Bank, California (Fig. 1). A total of nine sites were visited. At each site seven strings of ten traps were deployed in various depths between 250 and 1900 meters. The traps were retrieved after 24 hours, the catch was removed, and the traps reset for an additional 24 hours. All the sablefish were counted, otoliths were removed from the first 20 arbitrarily selected fish at each station, and the rest of the fish were tagged with blue spaghetti tags. Three of every four tagged fish were injected intraperitoneally with 30 mg of OTC per kilogram of fish (Beamish et al., 1983) and the fourth fish was used as a control. A complete description of the survey can be found in Parks and Shaw (1994).

A scientist visited the major commercial fishing ports in California and southern Oregon to make port samplers, commercial dealers, and fishermen aware of the importance of the study and to explain handling procedures in the study. A $50.00 reward was offered for the return of whole tagged fish.

When a tagged fish was returned, the port sampler measured it (fork length in mm), determined the sex, and removed the otoliths. The otoliths were cleaned and stored in painted glass vials (because the OTC mark was light labile) with a 50% ethanol solution.

Processing of the otoliths

Two pairs of otoliths were initially selected to develop the procedures to be used in the study. It was found that the OTC mark was very faint and upon heating (as required by conventional age determination methods), the mark disappeared. Accordingly, we developed a method to obtain images of the otoliths before and after heating, and to superimpose the two images of the same otolith; the first viewed under UV light and, the second, after heating, under white light.

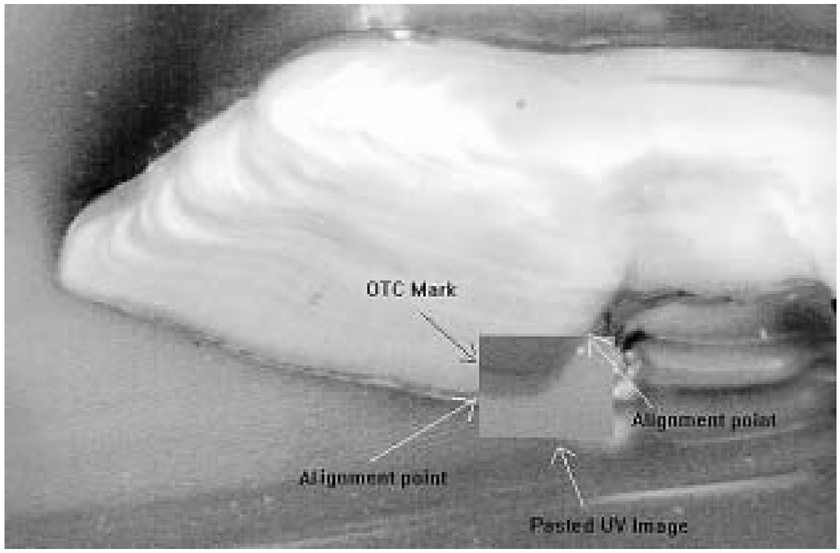

Figure 2

Composite image of a sablefish otolith. The otolith was first viewed under UV light and an image was captured. It was then baked and a second image was captured by using white light. Then a small rectangle from the UV image was electronically cut and pasted on the image of the baked otolith. The flourescent mark produced by the OTC appears as a dark line on the UV section. Points on the otolith used for correct positioning of the pasted section are shown.

The otoliths were embedded in epoxy casting resin. After the resin hardened, the blocks containing the otoliths were sliced in half across the dorsoventral axis with a diamond saw.

Images were captured in a two-stage process. The first stage used ultraviolet light to reveal the OTC mark, and the second stage used white light to reveal the growth marks used for age determination. In the first stage, the room was completely darkened and an image of the otolith, including the OTC mark, was captured by using a video camera capable of capturing images under low light conditions. We used an ultraviolet lamp which produced a strong beam of light at 365 angstroms. The otolith was viewed on a compound microscope using reflected light. The camera and image processing system were connected to a PC computer equipped with a frame grabber card. A version of NIH Image, a public domain image processing software (Scion Corporation, Frederick, MD), was used to process the images.

The embedded otolith was placed on the microscope and a drop of mineral oil was placed on the surface of the otolith. The limited amount of UV light available to the camera required the use of frame averaging. Usually 30 frames were sufficient to produce a sharp view of the otolith and the fluorescing mark. In some cases, the mark was too faint to allow an image to be captured. When there was sufficient fluorescence, two composite images were captured, one at 4× and one at 40×.

In the second stage, the same embedded otolith was placed in a small toaster oven at 270°C and heated for 20 to 25 minutes until it had turned dark brown. This baking process enhanced the growth rings for visual analysis and approximated what age readers see using the break and burn method; however, the latter process results in darker hyaline zones than those obtained with this method. After cooling, the otolith was viewed under white light. A second set of images was then captured. A section of each UV image was then electronically cut and pasted onto the image captured under visible light. With some experimentation it was found that the pasted sections could be aligned exactly over the visible light images, creating a final composite image as shown in Figure 2.

Initial examination of the otoliths

Initially, all OTC-marked otoliths were examined with knowledge of the year and season of release, but without any other information about the fish. Composite UV and white light images were obtained as previously described. The age reader determined the following: whether or not the OTC mark was visible; whether the OTC mark was in a hyaline or opaque zone; the number of annual hyaline zones visible beyond the OTC mark (and whether or not the edge was included in the count); edge type (hyaline, narrow opaque, wide opaque, or unidentifiable), and the shape of the otolith. In some cases the OTC mark could not be identified or the mark was too faint to be captured as a composite image; these specimens were excluded from subsequent analyses.

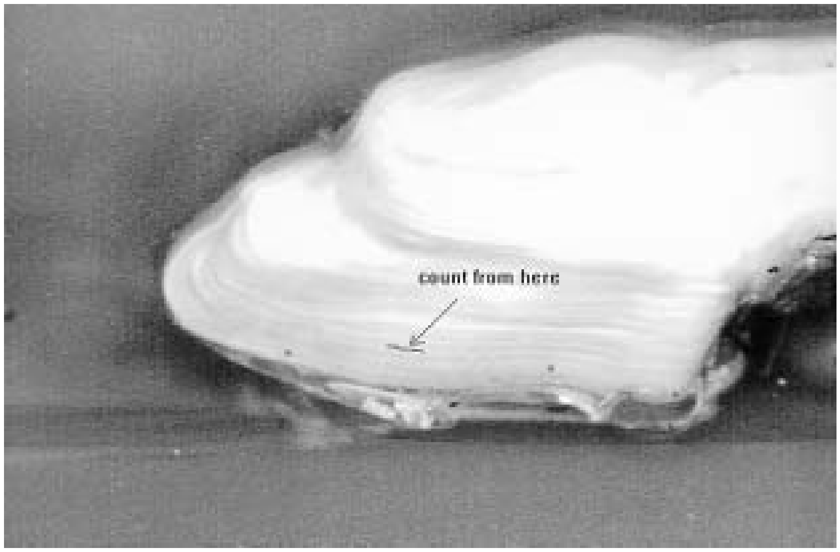

Figure 3

Example of an image of a baked sablefish otolith which has been annotated with a mark. The image is an example of one of the images provided to three researchers in order to obtain cross-reading comparisons.

Following standard age determination procedures (Chilton and Beamish, 1982), if a hyaline zone was not visible on the edge between January and March, then the edge was counted. If a mark was not visible on the edge between April and May and there was a wide opaque zone, then the edge was counted as a mark. If a mark was visible on the edge and the month was after May, the edge was not counted. This procedure is used to properly assign the fish to an annual cohort. Because the reader was not given the month of recapture, the ages were adjusted based on the count of hyaline zones, the month of recapture, and whether the edge had been counted. This adjustment provided a corrected reader count of annual marks. The corrected count was compared to the number of annual marks that would have been present if marks were laid down annually.

Previous experience suggested that there are different patterns of sablefish otolith growth. We attempted to classify and characterize these different types of growth patterns based on morphology of the otoliths as seen in cross section. After the otoliths had been examined, we developed a standard classification scheme of morphological classes and types which could be used to classify the most commonly observed morphological types. The otoliths were re-examined and reclassified to see if difficulties and discrepancies in aging were associated with morphological type. It was hoped that this process could be used to refine the aging criteria and improve precision.

Because sample size was small, we used a Fisher exact test (Agresti, 1990) to test for independence of morphological type versus tendency to over-estimate, correctly estimate, or under-estimate the number of annual marks. The columns in the test indicated whether the fish had been over-aged, correctly aged, or under-aged. The rows in the test were the four morphological types identified in this study.

Examination of the otoliths by the age readers

To determine how age readers would count the marks on the otoliths, we selected a subsample of 25 otoliths to be aged at four West Coast fisheries laboratories. The otolith selection was based on having good quality images and otoliths. The images of the baked otoliths (not the composite images) were annotated with a mark (Fig. 3). The mark was placed in a location which could be readily located on the actual otolith by the readers—on the zone just inside of the OTC mark. Readers were given the following: a set of printed images, an electronic file of the images for viewing on a computer screen, the embedded otolith, the month of capture, the size and sex of the fish from which the otolith came, and a set of instructions for examining the otoliths. Readers were not told where the mark on the image was placed in relation to where the OTC mark was in order to reduce bias from readers who may have known when the fish were injected and recaptured. Readers were asked to provide the following: the number of annual marks visible outside the mark on the image, whether the edge was counted, how confident they were of their readings, and any comments they might have.

Three readers participated in this analysis, two of whom had extensive, long-term experience in aging sablefish. The readings and age determination criteria (including edge count criteria) were compared to each other and to the time known to have passed between OTC marking and recapture.

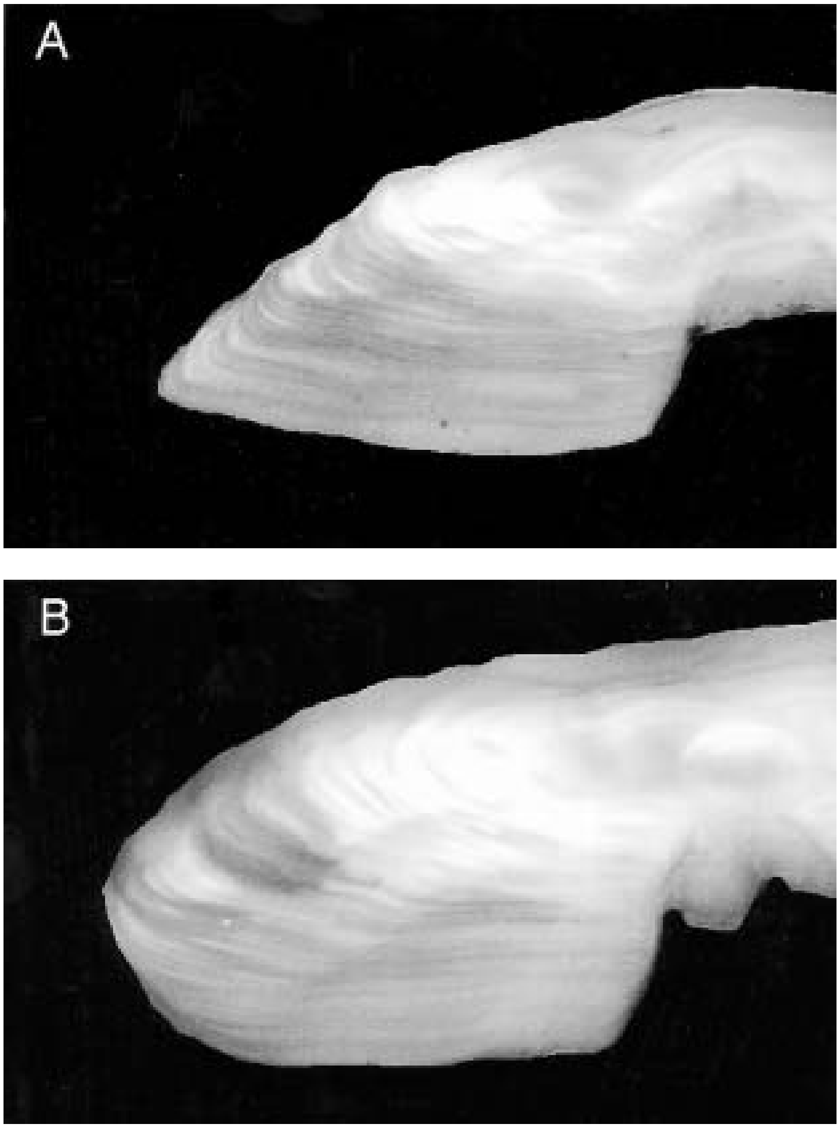

Figure 4

Images of four otolith morphological types. (**A**) Otolith is a wide type, (**B**) otolith is a wide, wedge subtype. (**C**) otolith is a thick type, and (**D**) otolith is a thick, wedge subtype.

To determine if age determination difficulties were related to sex, size, area of capture, depth of capture, or otolith morphological type; Fisher exact tests were performed. In each test, the variables were compared to whether the fish had been correctly aged, over aged, or under aged.

Results

Recoveries

A total of 2575 fish were tagged at the nine sites, and 368 tagged fish were recaptured. Of the recaptured fish, 284 had been injected with OTC. Of the 284 injected fish, usable otoliths were recovered from 191 fish; for the remaining fish, otoliths either were not recovered or were too badly damaged during removal to be used.

Otolith morphological types

After examination of all the otoliths, “wide” and “thick” morphological types were identified, and each type had a “wedge” subtype (Fig. 4). Each otolith in the study was then classified according to this scheme.

The wide type (Fig. 4A) is characterized by new growth that steadily increases cross sectional width along the dorsal and ventral surfaces. In the wedge subtype (Fig. 4B), initial growth increases the width, but the most

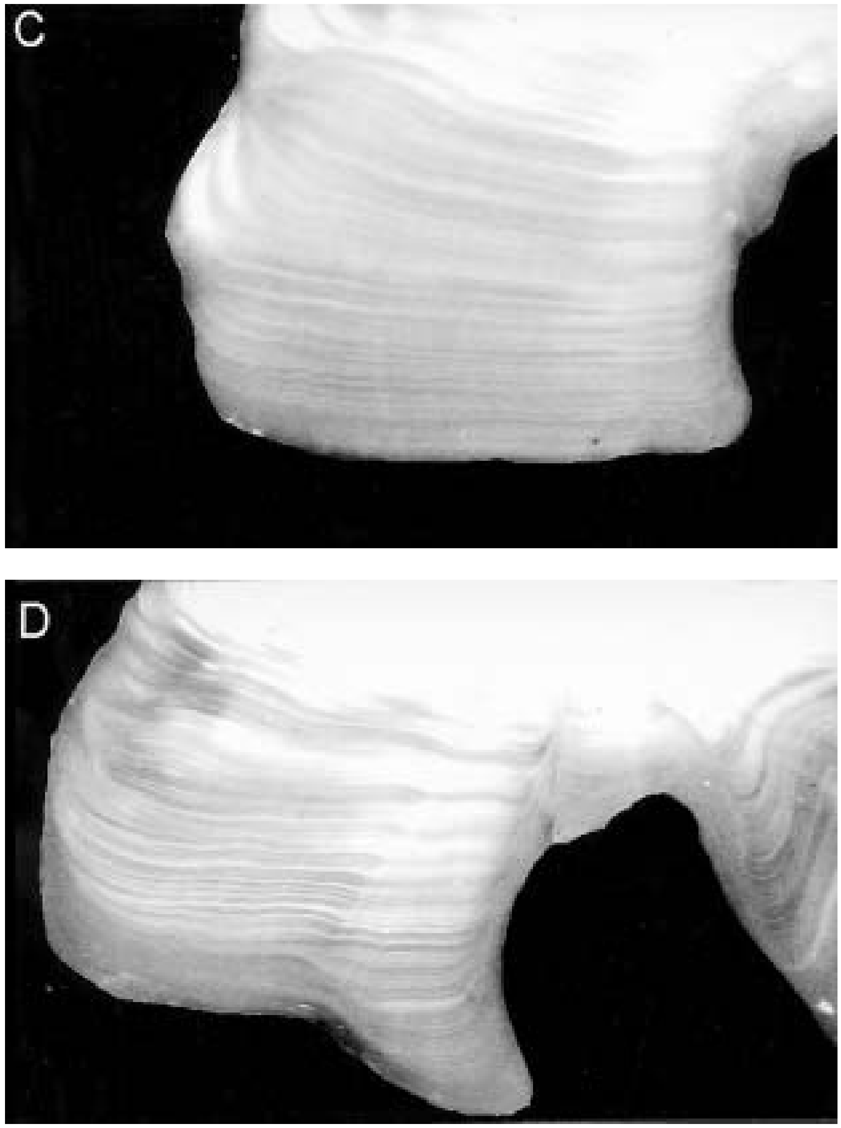

Figure 4 (continued)

recent growth is concentrated on the medial or lateral surface at the sulcus, decreasing towards the dorsal and ventral surfaces, resulting in a wedgelike appearance.

The thick type (Fig. 4C) is characterized by new growth that increases the thickness of the otolith without increasing the cross sectional width, causing the annulii to appear closely spaced on the lateral surfaces. In the wedge subtype (Fig. 4D), the most recent growth is concentrated at the sulcus and narrows towards the dorsal and ventral surfaces, forming a wedge shape.

It should be noted that these types and subtypes are not always clearly defined. It should also be noted that classification to the subtype is based on the most recent one or more hyaline zones. A wedge subtype is formed when a single hyaline zone widens near the sulcus and comes to a point at the outer edge.

Of the 191 otoliths examined, 63 (33.0%) were classified as "wide" types, 76 (39.7%) were classified as "wide, wedge subtypes," 32 (16.8%) were classified as "thick" types, 5 (2.6%) were classified as "thick, wedge subtypes,' and 15 (7.9%), could not be classified by this scheme.

Position of the OTC mark

There was no detectable OTC mark in 22 of 191 otoliths. The absence of marks appeared to be a random event, occurring in otoliths from several different recovery years and equally likely to be found among different sexes, otolith types, different depths, and locations.

Of the 169 otoliths with detectable marks, the OTC mark was found in a hyaline zone in 129 otoliths (76.3%), in an opaque zone in 36 otoliths (21.3%), and could not be reli-

Table 1

Frequency of otoliths with an OTC mark appearing on the edge versus those with the marks inside the edge. All fish were injected between September and October of 1991.

Year	Month	Mark on edge	Mark not on edge
1991	Oct	2	1
	Nov	1	3
	Dec	4	2
1992	Jan	2	4
	Feb	1	6
	Mar	7	4
	Apr	3	1
	May	7	26
	Jun	2	
	Jul	1	7
	Aug	1	4
	Sep	1	2
	Oct	5	
	Nov	3	
	Dec		

Table 3

Number of visible hyaline zones occurring after an OTC mark on otoliths from fish recaptured in 1992. This is shown by three-month interval to show the progression of development of the hyaline zones. All fish were injected in September and October of 1991.

	No. of hyaline zones		
Interval	0	1	2
Jan–Mar	12	8	1
Apr–Jun	5	14	4
Jul–Sep	6	2	
Oct–Dec	3	1	

ably determined in four otoliths (2.4%) because the marks were between a hyaline and opaque zone. Of the 36 otoliths with the mark in an opaque zone, the mark occurred just after a hyaline zone in four otoliths. In 24 of the 36 otoliths with the mark in an opaque zone, the mark was on the edge where it can be difficult to determine whether it is opaque or hyaline. In no case did the reader indicate that the mark was in a hyaline zone at the edge and thus the edge appeared to be opaque in most cases.

The OTC mark occurred on the otolith edge in 30 of the otoliths recaptured prior to 1993 (up to 16 months after injection). Examination of the monthly distribution of otoliths with marks on the edge (Table 1) indicated that some fish exhibited little or no otolith growth for substantial lengths of time.

Otoliths from fish recaptured in 1992 with marks on the edge (i.e. showing little growth) were examined and classified by morphological type (Table 2). This examination indicated that the thick type is more likely to have little growth because 32% of the otoliths with marks on the edge were the thick type, yet they made up only 17% of the otoliths in the study. Conversely, only 18% of the otoliths with the mark on the edge were of the wide type; however, they made up 33% of the otoliths in the study. This trend was not statistically significant, however, because the *P*-value was 0.106.

Number of visible hyaline zones

The number of prominent hyaline zones after the OTC mark for fish recaptured in 1992 at three-month intervals is shown in Table 3. This distribution shows the otoliths that had no detectable growth but also shows that a hyaline zone forms in many fish during the winter. It also shows that in some fish, a summer hyaline zone is formed; however, the sample size for October–December was small and this is a period when a summer hyaline zone would be expected to be fully visible.

The number of visible and prominent hyaline zones after the OTC mark for fish recaptured after 1992 (Table 4), compared with the number of zones which should have been counted, showed that if a reader had counted each of the prominent hyaline zones as an annulus, the count would have overestimated the age of the fish. An example of an otolith with a larger number of prominent hyaline zones than expected is shown in Figure 5. It should be noted that a reader would not necessarily have counted each of the

Table 2

Number of otoliths in 1992 with OTC marks on the edge by otolith morphological type. Also shown is the overall percentage of the morphological types in the present study. All fish were injected in September and October 1991.

	Otolith type							
	Wide		Wide, wedge		Thick		Thick, wedge	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
1992 otoliths	4	18	10	45	7	32	1	5
Otoliths in this study	63	33	76	40	32	17	5	3

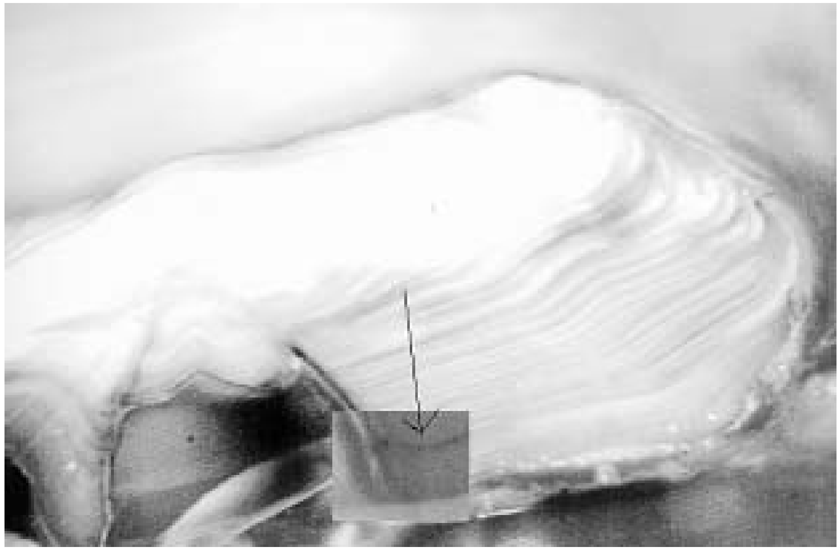

Figure 5

Image of a sablefish otolith having more prominent hyaline zones than should have been present. The fish was caught after eight months at liberty. A single hyaline zone should have formed; however, there is a zone on the edge and one midway between the dark OTC mark.

Table 4

Counts of the number of prominent hyaline zones versus the number of annual hyaline zones that should have been present after an OTC mark. These counts are for fish recaptured more than 15 months after initial capture. Agreement between counts and number of expected annual hyaline zones is shown in bold.

		No. of prominent hyaline zones										
Year	Expected number	0	1	2	3	4	5	6	7	8	9	10
1993	2	3	1	**7**	6	4	3					
1994	3	2		1	**5**	2	1	3	1			
1995	4	1				**2**		5	1	2	1	
1996	5					1	**1**		1	2		
1997	6				1			**1**	1		2	1

Table 5

Percent and number (in parentheses) of sablefish otoliths with more hyaline zones than were expected, with the expected number of hyaline zones (correct count), and with fewer hyaline zones than were expected for each otolith type.

Otolith type	More zones	Expected number of zones	Fewer zones
Thick	10.3% (3)	41.4% (12)	48.3% (14)
Thick, wedge	0 (0)	40.0% (2)	60.0% (3)
Wide	39.3% (22)	48.2% (27)	12.5% (7)
Wide, wedge	35.2% (25)	45.1% (32)	19.7% (14)

prominent hyaline zones as an annulus (they might have considered them to be checks). In many of these otoliths, there were less prominent zones that were not counted and which were interpreted as checks.

Thick type otoliths and thick, wedge subtype otoliths tend to have fewer visible hyaline zones than expected (Table 5). In contrast, wide type and the wide, wedge subtype otoliths are more likely to have more hyaline zones than expected. The Fisher exact test yielded a significant P-value of 0.001.

Blind comparisons of reader counts

A comparison of the counts of annual hyaline zones for each reader to the expected number of annual hyaline zones

Table 6

Comparison of number of annual hyaline zones counted by reader 1 versus the expected number of annual hyaline zones that should have been counted. Agreement with the expected counts are shown in bold.

	Reader 1 count						
Expected count	1	2	3	4	5	6	7
1	**2**	7	2	1			
2		**2**	4	1	1		
3		1			1		
4				**2**			
5			1				

Table 7

Comparison of number of annual hyaline zones counted by reader 2 versus the expected number of annual hyaline zones that should have been counted. Agreement with the expected counts are shown in bold.

	Reader 2 count						
Expected count	1	2	3	4	5	6	7
1		5	2	3	1	1	
2			3	2	2		1
3			**1**	1			
4						1	1
5					**1**		

Table 8

Comparison of number of annual hyaline zones counted by reader 3 versus the expected number of annual hyaline zones which should have been counted. Agreement with the expected counts are shown in bold.

	Reader 3 count						
Expected count	1	2	3	4	5	6	7
1	**10**	2					
2	2	**1**	3	1	1		
3	1	1					
4	2						
5	1						

after the OTC mark are shown in Tables 6, 7, and 8. In these tables, it is assumed that the readers should not have counted the zone in which the OTC mark occurred because that mark is presumed to have formed in the summer of 1991. Readers 1 and 2 tended to overestimate, whereas reader 3 (the least experienced age reader) had generally good agreement. Reader 1 agreed with the expected count 24% of the time, reader 2 agreed with the expected count 4% of the time, and reader 3 agreed with the expected count 44% of the time. The result for reader 3 is deceptive, however, because that reader did not follow accepted methods of when to count the edge.

Reader 1 and reader 2 agreed on whether to count the edge of the otolith in 24 of 25 otoliths (Table 9). Reader 3 agreed with reader 1 on whether to count the edge in 16 of 25 otoliths and 17 of 25 otoliths with reader 2. Had reader 3 followed accepted practice, agreement with the expected count would have been much less.

Efforts to determine what factors (depth of capture, location of capture, sex, size of the fish, and otolith morphological type) resulted in a miscount of the true number of annual marks were inconclusive. We first corrected the count for the fact that all readers counted the mark in which the OTC mark had occurred by subtracting one from their counts, and we then eliminated the readings from reader 3 because of his lack of experience and anomalous age determination criteria. Then we examined the relationship of how many otoliths had been over-aged, correctly aged, and under-aged to the above factors. Depth of capture was divided into two groups: less than 600 m and 600 or more m. Location was divided into two groups: north and south of latitude 39 north. Sizes were divided into two groups: <55 cm FL and ≥55 cm FL. And finally, we tested each of the four otolith morphological types.

We used Fisher exact tests to determine the probability that differences were due to chance alone. There were no detectable differences from the null hypothesis for depth, sex, or location of capture (Table 10); however, there was some evidence that fish length and otolith morphological type might be related to miscounting. Small fish showed a slightly greater tendency to be over counted (more rings than should have been present) than larger fish (P=0.150). Otolith morphological type showed some departure from randomness; thick types appeared to be more likely to be undercounted (fewer rings than should have been present) and wide types were more likely to be over counted (P=0.066).

Discussion

Position of mark

There was no visible mark on 22 of the 191 otoliths (11.5%). Beamish et al. (1983) reported that 14 of 129 OTC-injected fish (10.9%) had no detectable mark. They attributed this to improper handling of the fish after recapture. The similarity in the number of otoliths failing to show the OTC mark between their study and our study suggests that some portion of the population may not absorb sufficient OTC to produce a visible mark.

The finding that most of the OTC marks were in a hyaline zone is important. This indicates that many of the sablefish in our study laid down a prominent hyaline zone in the summer. Age readers who conventionally assume that an annual mark is laid down only in the winter

Table 9

Blind reading results of 25 sablefish otoliths by 3 readers. All fish had been captured and injected with OTC in September and October of 1991. The counts they provided are the number of annual marks outside of the OTC mark. "Expected count" indicates how many winter hyaline zones should have been present. The columns labeled "Edge" refer to whether or not the edge was included in the age reader's counts.

			Reader 1		Reader 2		Reader 3	
Fish ID no.	Recapture date	Expected count	Count	Edge	Count	Edge	Count	Edge
10375	4 May 92	1	2	Y	2	Y	1	N
10030	14 May 92	1	1	Y	2	Y	1	N
10267	17 May 92	1	2	Y	3	Y	1	N
10408	17 May 92	1	2	Y	2	Y	2	Y
10417	18 May 92	1	2	Y	3	Y	1	N
10630	25 May 92	1	4	Y	4	Y	1	N
12148	26 May 92	1	3	Y	4	Y	1	N
12176	26 May 92	1	2	Y	5	Y	1	N
12431	26 May 92	1	2	Y	2	Y	2	Y
10568	29 Jul 92	1	1	N	2	N	1	N
11121	1 Oct 92	1	2	N	6	N	1	N
11117	16 Oct 92	1	3	N	4	N	1	N
10400	12 Jan 93	2	3	Y	5	Y	3	Y
10370	14 Jan 93	2	4	Y	4	Y	3	Y
10870	15 Feb 93	2	2	Y	7	Y	5	Y
10246	15 Apr 93	2	3	N	3	Y	3	Y
11735	16 May 93	2	3	Y	3	Y	2	Y
11586	18 May 93	2	2	Y	4	Y	4	Y
11106	3 Aug 93	2	3	N	3	N	1	N
10617	2 Dec 93	2	5	N	5	N	1	N
10580	23 May 94	3	2	Y	4	Y	2	Y
10714	9 Dec 94	3	5	N	3	N	1	N
11516	3 Aug 95	4	4	N	7	N	1	N
11524	16 Dec 95	4	4	N	6	N	1	N
11761	25 Apr 96	5	3	Y	5	Y	3	N

Table 10

Comparison of the number of fish under counted, correctly counted, and over counted by two experienced age readers versus depth of capture, location (north or south of 39 degrees latitude), sex, fork length, and otolith morphological type. The *P*-value from the Fisher exact test is shown, indicating the level of significance.

	Under counted	Correctly counted	Over counted	*P*
Depth				
<600 meters	7	14	15	0.987
>600 meters	3	6	5	
Location				
South	4	8	10	0.606
North	3	12	7	
Sex				
Male	3	2	5	0.381
Female	7	18	15	
Length				
<55 cm	4	14	15	0.150
≥55 cm	6	6	5	
Otolith type				
Thick	4	2	2	0.066
Thick, wedge	1	1	0	
Wide	2	5	11	
Wide, wedge	3	12	7	

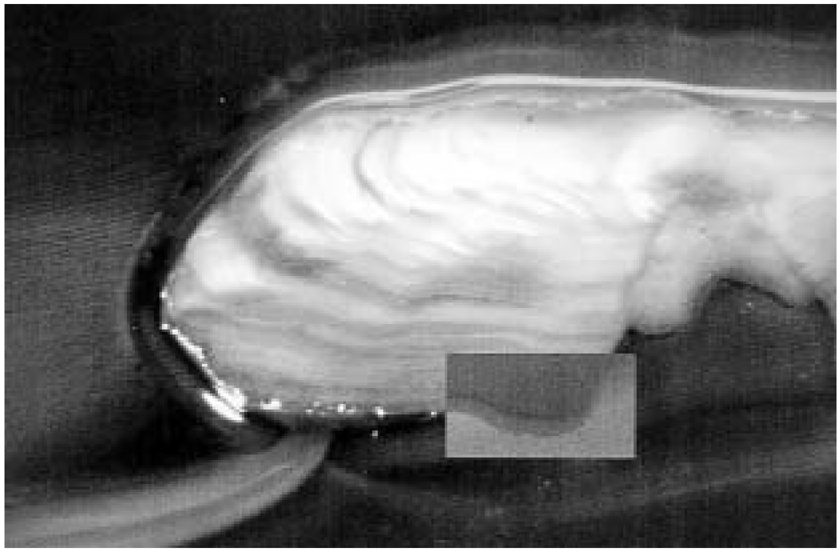

Figure 6

Image of a baked sablefish otolith with an electronically pasted section taken from an image captured under UV light. The dark OTC mark is clearly located within a hyaline zone, and the hyaline zone persists through the entire otolith. The fish was injected with OTC on 5 October 1991.

would probably mis-age these fish. Because the age readers who examined the otoliths without knowledge of the recapture information were not informed that the point they were counting from was just inside the summer mark, it was interesting to note that all three of them counted the hyaline zone in which the OTC mark had occurred as an annual hyaline zone in all cases. In other words, the summer hyaline zone did not appear to be a check to the readers. The readers indicated that the manner of preparation of the otoliths (embedded and baked) was not the manner in which they were accustomed to view otoliths and may have influenced their results. The fact that the hyaline zones were not as dark with the baking method as opposed to the burning method may have influenced the readers age estimates; however, some otolith burns can be quite light and experienced readers recognize the various levels of burning, particularly when cross reading otoliths from other age readers. Readers sometimes use multiple sections and are free to manipulate the otolith to improve viewing, which was not possible in the present study. Beamish et al. (1983) indicated that when readers knew how many marks to look for, they were able to identify false annual marks (checks). According to their study, a check is not persistent throughout the otolith. In Figure 6, the hyaline zone in which the OTC mark appeared clearly persists throughout the otolith. If the hyaline zone which contained the OTC mark began to be laid down in the winter, then there would be very little time for the formation of a wide opaque zone to form after injection in the fall. Because the age readers counted the hyaline zone in which the OTC mark occurred, they clearly assumed that it was not a check. If the age readers had known that the hyaline zone (in which the OTC mark occurred) had formed in the summer, then they presumably would not have counted it. It is therefore of interest to see the effect on agreement between reader counts minus the hyaline zone where the OTC mark occurred and the actual number of hyaline zones that should have been present. When we adjusted the reader counts by subtracting one year from their original counts and compared their adjusted counts to the expected number of annual marks (Table 11), agreement for readers 1 and 2 improved, whereas it decreased for reader 3 (the least experienced reader).

Also of importance is the fact that on some otoliths, even after eight months at liberty, no growth had occurred, as evidenced by the fact that the OTC mark was on the edge. For example, otoliths from two fish, recaptured after eight months at liberty showed marked differences in otolith growth (Fig. 7). On otolith A there was no detectable growth with the OTC mark on the edge, whereas on otolith B there was substantial growth. The OTC marks on both otoliths were very prominent. These otoliths came from similar fish; that is, otolith A came from a 597-mm female fish caught in 680 meters of water at 40°52′ latitude, and otolith B came from a 610-mm female fish caught in 480 meters of water at 41°54′ latitude. This provides strong evidence that otolith growth, and presumably fish growth, varies greatly among individual sablefish. Beamish et al. (1983) reported that the OTC mark was on or near the edge in 28 otoliths (18.1%) of 154 fish which had been at liberty for two to three years. In a similar time interval, we found that 34 of 126 (27.0%) had the OTC marks on or near the edge. Both the finding of a summer hyaline zone and the differences in growth of the otolith among individual fish

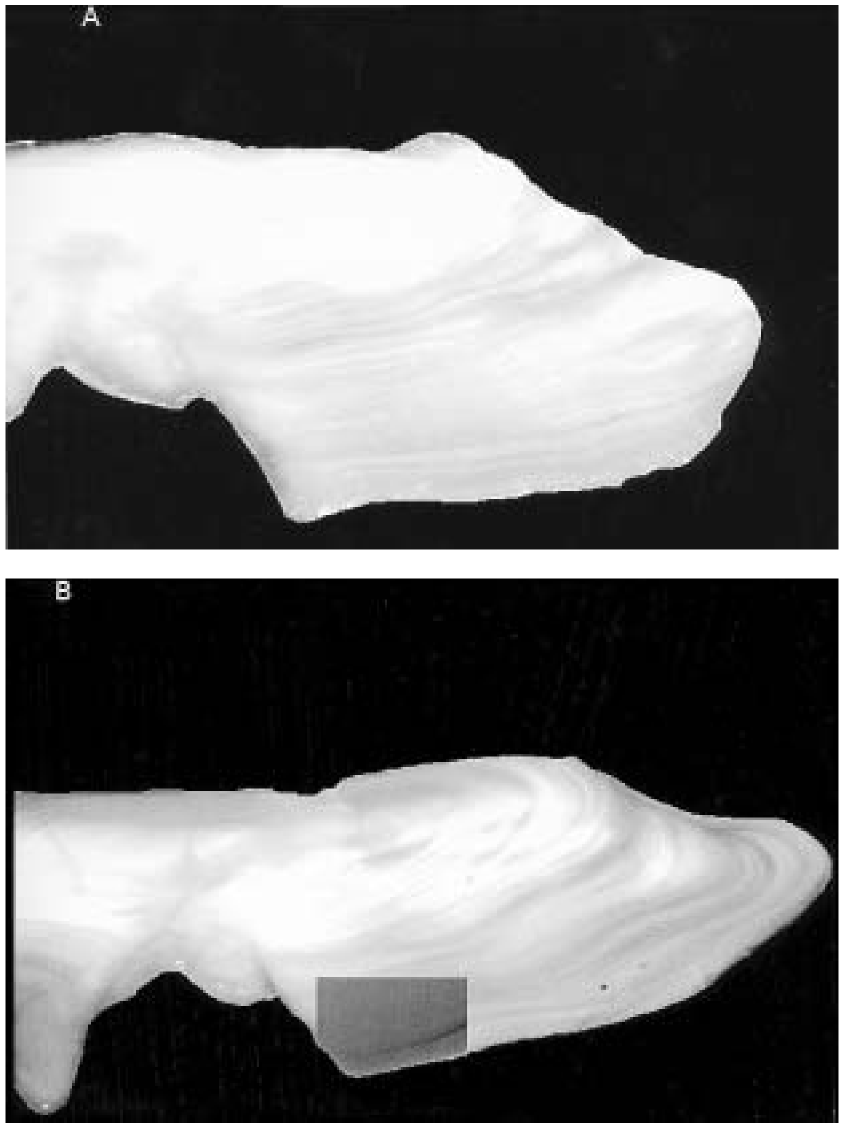

Figure 7

Images of otoliths from two sablefish showing differences in otolith growth rate. Both fish were injected with OTC in early October of 1991 and were recaptured in May of 1992. (**A**) Otolith was from a 597-mm female caught in 680 meters of water at 40°52′ latitude. (**B**) Otolith was from a 610-mm female fish caught in 480 meters of water at 41°52′ latitude. The OTC mark in A was on the edge, whereas the position of the OTC mark in B is shown on the insert.

are important factors in developing reliable and consistent age determination criteria.

The importance of using the same age determination criteria among readers cannot be overestimated. In the blind comparison, the readers were asked whether they had included the edge in their count of annual zones. With standard age determination methods, if no hyaline material is visible on the edge up to about May, then the edge is counted. This procedure is based on the assumption that a zone is in the process of forming but is not yet clearly visible. On the other hand, if hyaline material is observed on the edge after May, it is not counted because it is assumed to be either a check or the beginning of the next winter's hyaline zone. Reader 1 and reader 2 (the two most experienced age readers) agreed on whether to count the edge 96% of the time, indicating that they were using the same criteria. Reader 3, however, agreed with reader 1 only 64% of the time and with reader 2 only 68% of the time which suggests that reader 3 was using different edge-interpretation criteria.

Table 11

Percent agreement between number of hyaline zones counted by three age readers and the number which should have been present. Also shown is the effect of removing the count of a hyaline zone which formed in the summer and which should not have been counted as an annual mark.

Reader 1		Reader 2		Reader 3	
Original	Corrected	Original	Corrected	Original	Corrected
24%	44%	4%	36%	44%	20%

Effect of ages on stock assessments

Crone et al. (1997) noted that one of the problems with stock assessments of sablefish is that the size at 50% sexual maturity is between 55 and 67 cm (age 5–7) and that there is considerable variability in the these estimates. Further, they noted that there has been difficulty in determining age-specific selectivity because of problems with the ages used in previous assessments. Crone et al. (1997) further noted that there is a considerable discrepancy in ages among the age determination laboratories on the west coast. Finally, the model used to perform stock assessments has estimated that in order to obtain a good fit with the data, the actual level of aging error should be higher than has been reported. The lack of reliable age data has been used to criticize stock assessments.

Age and length at sexual maturity has been found to vary substantially by depth (Fujiwara and Hankin, 1988a). Fujiwara and Hankin found that both males and females had a length of 550 mm for the length at 50% sexual maturity in shallow water (<600 meters). In depths greater than 600 m, the size at 50% sexual maturity was 450 mm for males and 500 mm for females. To determine age, they used sectioned otoliths and methods that may not have been directly comparable to the methods used in other studies or the methods used in the present study; nonetheless, they found that both males and females matured at a younger age in deeper water. Saunders et al. (1997) also reported differences in length at maturity related to depth and location of capture. Methot[1] found that ontogenetic movement into deeper water for spawning was more closely related to age than size. If sexual maturity is more closely related to age than length as suggested by Methot, then unreliable ages may explain the variable maturity schedule for sablefish. In our study, fish were captured over a 900 nmi range at depths from 200 to more than 1000 m. If depth is related to growth of sablefish, then it is possible that the different morphometric types of otoliths observed in our study may also be a function of depth. If depth is responsible for the morphological types, it also suggests that reliability of ages may be a function of the depth at which the sablefish are found. Further, if depth influences growth, a fish which changes its depth over time, may exhibit different patterns of growth throughout its life which would further complicate the problem of determining reliable ages.

Potential sources of error in this study

This study used sablefish caught in the southern part of the sablefish range. Many species show latitudinal variation in growth (June and Reintjes, 1959; White and Chittenden, 1977; Leggett and Carscadden, 1978; Shepherd and Grimes, 1983; Pearson and Hightower, 1991). It is possible that the results of this study do not apply to the northern portion of their range.

Another potential source of error in our study is the effect of tagging on the growth of the sablefish. MacFarlane and Beamish (1990) found that tagged sablefish grew slower than untagged fish. If this is true, then the results of this study are much more difficult to interpret. MacFarlane and Beamish did not use OTC and as a result they based their ages on conventional aging methods. If they had injected the fish, it would have been interesting to note whether the ages for the fish in their study would have been interpreted differently. If fish do grow differently after tagging, many age, growth, and validation studies will need to be re-evaluated.

Conclusion

Obtaining accurate ages, with reasonable precision, for sablefish is very difficult. Previous aging studies of sablefish have obtained results similar to ours, even when the readers knew how many annual marks should have been present (Beamish et al. 1983; MacFarlane and Beamish, 1995). We found that some fish lay down two marks a year and others may not lay down any. We also found that certain morphological types of otoliths may be indicative of slow growing fish and others may be indicative of rapidly growing fish (assuming otolith growth relates to fish growth).

The fact that agreement among readers or with the correct age consistently ranges between 30% and 45% suggests that this imprecision may be inherent in sablefish aging. A substantial fraction of the population may not be able to be reliably aged: some otoliths do not appear to grow and others grow very rapidly, laying down prominent summer hyaline zones that even experienced age readers cannot differentiate from winter hyaline zones.

[1] Methot, R. D. 1995. Geographic patterns in growth and maturity of female sablefish off the U.S. west coast. Unpubl. manuscript, 39 p. NOAA, NMFS, Northwest Fisheries Science Center, Seattle, WA.

We believe the wide type and wide, wedge subtypes are often over-aged, and the thick type and thick, wedge subtypes are occasionally under-aged and further propose that readers be made aware that a hyaline zone typically forms in the winter, but that it is not uncommon for a second mark to form in the summer.

Another, less desirable approach, would be for age readers to record the morphological type of otolith as a routine part of aging. Users of the data could then incorporate this information into their studies by using a correction factor for fish likely to be under-aged and for fish likely to be over-aged. This factor could be in the form of an aging error matrix as suggested by Heifetz et al. (1999). This approach may not be practical until more data are available on the true effect on ages for the morphological types described in this study, including how many years would need to be added or subtracted for each type. Finally, a more complete description of the morphological types would be needed to assist the age readers.

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