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Abstract

Bocaccio (*Sebastes paucispinus*) are an important part of the commercial and recreational fisheries in California. Since 1983, this species has been repeatedly subjected to stock assessments. By the early 1990's, it was apparent that the species was in danger of being overfished, and regulatory measures were implemented to protect it. In spite of those efforts, the species was declared overfished in 2000, and catches in the fishery were heavily constrained. Age data were only marginally useful in early assessments because the species was deemed too difficult to age, and reliable criteria had not been developed. Since 1999, assessments have excluded age data due to inconsistencies with the length data. In this paper we describe how we finally developed age determination criteria for this species. The approach to development relied on using otoliths from Washington as well as a combination of other methods, based on fish length compositions and cross-sectioning of the otoliths, to obtain a better idea of how annuli were formed. After developing the criteria, a total of 850 otolith samples were double-read by two age readers. Age determination error analysis showed that there were generally good agreements between the two age readers. Estimated standard deviations of age determination errors increased for older fish, ranged from 0.270 for age 1 to 6.77 for age 36.

INTRODUCTION

Bocaccio (*Sebastes paucispinus*) have historically been a large component of California's commercial and recreational fisheries (Field, 2013; PacFIN, 2014; RecFIN, 2014). In 1983, landings of Bocaccio in the California commercial fishery peaked at 5,075 metric tons. By the late 1980's, concern over the possibility that the fishery was beginning to collapse, caused the Pacific Fishery Management Council to begin regulating the fishery (Bence and Hightower, 1990). In 2000, the stock was declared overfished and both commercial and recreational catches were dramatically reduced through the early 2000's (to a low of 13 metric tons in 2003). As the stock has recovered, due to both reduced harvest rates and more favorable environmental conditions (leading to improved recruitment), both the stock abundance and catches have increased.

Since the mid-1990's, stock assessments of the species have historically excluded age composition data, as this species has long been considered too difficult to age and no reliable age determination criteria could be developed (Ralston and Ianelli 1998, MacCall et al. 1999). Since reliable ages were not available, all stock assessments for this species have relied on length based approaches. The resulting assessment models have been considered robust, as the combination of very rapid growth and highly variable recruitment in this stock have allowed for the resolution of strong cohorts (and, subsequently, growth) within the modeling framework (Ralston and Iannelli 1998, MacCall 1999, Field 2013). Despite this, an ongoing research recommendation for this stock has been the development of age determination criteria for southern Bocaccio, the production age determination of existing age structures (current archives include over 60,000 age structures for this species), and the incorporation of such data into the assessment model.

Attempts at the Southwest Fisheries Science Center to develop age determination criteria for this species began in the early 1980’s and efforts have continued intermittently as new approaches became available. Initial efforts to age this species in the early 1980’s focused on using surface aging of whole otoliths. In 1986, the break and burn method was first tried. In 1988, daily aging of presumed 3-year old fish was attempted in collaboration with the California Department of Fish and Wildlife. Since then, thin sectioning and image processing techniques have been tried. In all cases, the efforts to develop reliable age determination criteria failed.

The principle reasons that age readers have found this species so difficult to age were 1) the presence of numerous marks that seemed to be real annuli but did not persist around the otolith, suggesting they were checks (false marks); 2) the inability to reliably identify the inner annuli; and 3) the lack of a clearly detectable pattern in the laying down of the annuli as the fish grew older. Another problem is that the morphology of the otoliths, even from fish of the same size, is highly variable: some otoliths may be very thick while others from the same size fish may be thin. Every effort to age this species resulted in a very low level of agreement both between and within age readers. Age differences of more than 5 years on fish presumed to be less than 25 years old were typical.

In 2005, an outside effort was made to validate ages and determine the maximum age of Bocaccio using lead-radium disequilibria and bomb carbon (Andrews et al., 2005). They found that measured levels of lead and radium were among the lowest in the literature, resulting in poor age resolution. Although this study arrived at a maximum age estimate of approximately 37 years, the age estimates originating from break and burn analysis were not based on established age determination criteria, and only a relatively small number of age estimates were made. A small number of age estimates have also been developed and validated for Bocaccio off of

Washington state based on bomb radiocarbon methods (Piner et al. 2006); that study also estimated a maximum age of at least 37 years. Bocaccio in Canada have been aged with break and burn methods, with maximum ages as old as 57 years, although the criteria used to develop these age estimates have not been described nor do those estimates seem to have been formally validated (Stanley et al. 2009). Growth and maturity patterns also appear to vary among the larger scale regions of the California Current, with Bocaccio in southern waters growing faster and maturing at smaller sizes than fish in northern waters (Field et al. 2009); this appears to correspond with greater difficulty in age determination of fish from the southern extent of the range.

In 2011, anecdotal information from age readers in Washington state indicated that age structures from Washington were not difficult to age, although they were not doing production age determinations of the species. Since our experience with other species of rockfish on the west coast had shown that some species are easier to age in the northern areas, we examined 33 otoliths collected from Washington. We found that the pattern of presumed annuli were, in fact, easier to identify than was typically observed in otoliths from California. As a result, we undertook yet another effort to develop a set of age determination criteria for this species.

Developing age determination criteria for a species is a required step before production aging can begin (Campana, 2001; Matta and Kimura, 2012). Development of age determination criteria assures that different age readers are all counting the same marks. It is important to note that age determination criteria are more than just a method (e.g. break and burn or thin sectioning). Properly developed criteria include how to identify the first annulus, how much summer growth should be present on the edge of the aging structure at different times of the year, what constitutes a check, and what the general pattern of growth is. The age determination criteria may specify that all otoliths are broken and burned, as well as specifying what axis of the aging structure is used for age determination. The criteria may also specify when an aging structure is not to be used, for example if the otolith is vateritic ("crystallized"). Ideally the criteria are based on some understanding of the biology of the fish as well as some form of validation of the accuracy of the ages. Direct validation methods, such as examination of known age fish from mark-recapture or aquarium studies and bomb-radio carbon analysis, are often prohibitive due to the availability of samples as well as time and monetary costs. However, several lines of indirect evidence (e.g. edge type analysis or following strong cohorts over time) can lend credibility as to the accuracy of the age determination criteria. After the criteria are developed, age readers must then be trained to use them. Cross reads (second, independent reads) between age readers and within age readers are performed to make sure the criteria are being consistently applied and to measure the precision between age readers.

In this paper, we describe the methods used to finally develop age determination criteria for Bocaccio. These criteria are currently being used to production age this species. When the next stock assessment is conducted in 2015, it is believed that there will be about 7,000 aged fish with about 20% of the otoliths having been read more than once for use in aging error analysis which will be needed for Bocaccio stock assessment models.

Materials and Methods

To develop the age determination criteria, we used several approaches: 1) side by side comparisons of otoliths from similar sized fish caught in Washington and California, 2) annual length composition data to determine fish likely to be 2 years old, 3) quarterly length composition data of small fish to identify the amount of summer growth appearing on the edge, 4) examination of serial sections from otoliths, and 5) detailed examination of otoliths from many fish of both sexes over a range of sizes. After the criteria had been developed, the primary ager (Pearson) aged approximately 300 otoliths using the criteria, and then reread them without reference to the first set of ages. This was done to confirm that the criteria could be consistently applied by the primary ager. The next step was to train a second age reader (Lefebvre) in the use of the criteria, conducting cross reads to verify that the criteria could be successfully communicated to other age readers to produce similar ages. After at least 1,000 fish had been aged in production mode, we analyzed between reader agreement.

General Age Determination Methods

For developing the age determination criteria we used dissecting microscopes with magnification capabilities of up to 80x. When examining the otoliths, we looked at whole otolith surface views, ¾ views, and sectioned views. The whole otolith surface was viewed by placing an otolith in a watch glass filled with water against a black background. The ¾ view is obtained by placing a cross-sectioned and burned otolith in clay at an angle such that both the surface and sectioned surface can be seen simultaneously (Fig. 1). This view allows surface features to be related to internal features. For much of our work we broke the otoliths by hand; however, for capturing images, we used a Buehler Isomet diamond saw to cut the otolith. The otoliths used for the ¾ and sectioned views were burned over an alcohol flame to enhance the annuli.

Both during and after development of the age determination criteria, we performed cross reads of the otoliths. Age readers were provided with year and month of capture, length, sex, and location of capture. The age readers were not able to refer to previous ages. If an ager was unhappy with the quality of the otolith, they were free to use the second otolith.

Comparison of Otoliths from Washington and California

In order to determine whether a consistent pattern of growth could be detected among otoliths and to attempt identify the first annulus, we compared otoliths from Bocaccio collected off the Washington and California coasts. For this comparison, we only had 33 otoliths from Washington state, none of which had been previously aged. We used 20 otoliths collected in Washington from fish ranging in size from 49cm (the smallest fish we had available) to 84cm (the largest fish we had available) and paired them with otoliths from similar sized fish collected in central California. The otoliths were from various months and years. The otoliths were cut in half across the dorso-ventral axis using a Buehler Isomet diamond saw. Each otolith was then burned over an alcohol lamp to enhance growth marks. The paired sets of otoliths were then observed under a dissection microscope at various magnifications. The whole otolith surface, ¾, and section views were observed for each pair. Images were taken from selected pairs of otoliths.

Annual Length Compositions

Since Bocaccio are known to grow very rapidly during the first few years of life (MacCall et al., 1999), it is often possible to identify weak and strong cohorts by examining their length compositions on an annual basis. Using this information, we knew that 1983 was a weak cohort while 1984 was a relatively strong cohort. We plotted the length compositions from the California commercial market samples for 1986 and 1987. From this we were able to determine the likely size of fish in the 1984 cohort for 2- and 3- year-old fish. Using this information, we examined the whole surface, ¾, and sectioned views of the otoliths from this group of fish, attempting to identify characteristics of the first and second presumed annuli.

Quarterly Length Compositions

To look at seasonal growth, we plotted the length compositions for the months of January-March and October-December from fish caught in 1986. We only used fish that were likely to be 2 year olds based on the previous analysis. The whole surface, ¾, and sections views were examined.

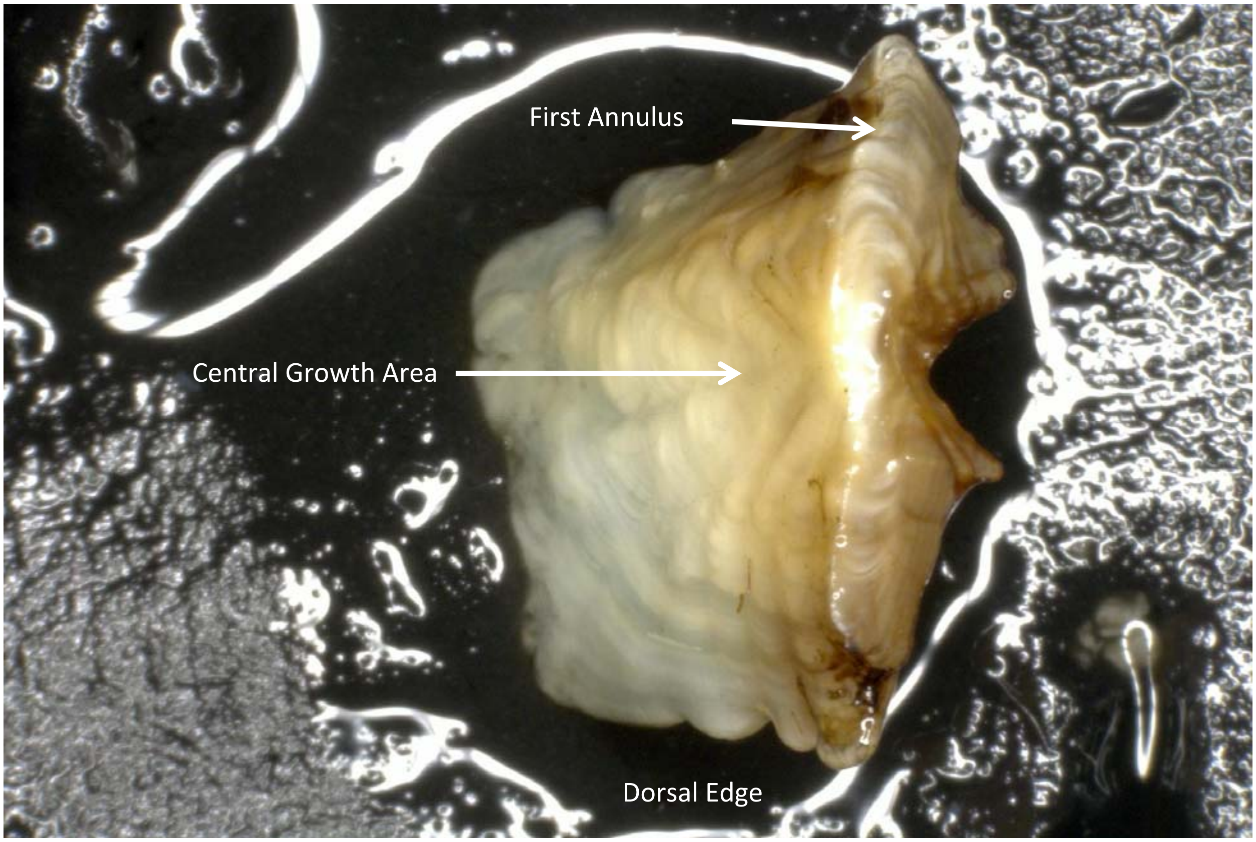

Figure 1. Photograph of a typical Bocaccio otolith using the ¾ view showing general structure, the large central growth zone, and the presumed first annulus.

Serial Sections

In an effort to understand the nature of check formation, we examined sections of otoliths from different parts of ten otoliths. The ten otoliths were selected from fish ranging in length from 45 to 60 cm forklength. Each whole otolith was first examined under water and otoliths were chosen which had marks which joined with other marks, suggesting that they were checks. The selected otoliths were then cut using a Dremel mototool equipped with a diamond saw blade. The cuts were made at the point where the marks joined, which was typically not through the nucleus. The otoliths were then burned and surface, 3/4, and cross-section views were examined.

Size Range Observations

In order to get a sense of the overall growth pattern and help identify checks, we arbitrarily selected 200 otoliths from fish caught in various years and from a broad size range for further examination. Over the span of several weeks, we examined the whole surface, ¾, and section views for each otolith numerous times, often placing otoliths from different sized fish side by side.

Testing the Criteria

After the primary age reader developed the age criteria and practiced using it, a second age reader was trained in the new methods. To test the age determination criteria we performed cross reads within and between age readers. Cross reads between age readers were performed on 200 otoliths. The otoliths were from both sexes and a range of sizes, years, and locations. The ager was provided with year and month of capture, length, sex, and location of capture. The ager did not have access to previous ages.

Age Determination error analysis

A total of 850 otoliths, collected from commercial fisheries and trawl surveys, were read independently by two age readers. Some otoliths were intentionally selected from larger fish (> 60cm) to ensure that there were sufficient sample sizes in the calculations of age determination errors for old fish. In order to more formally evaluate age determination error (defined as when the age estimate differs from that of the true age of the fish) within and among the two primary age readers, and because age determination error is a critical element to be accounted for when using age-structured stock assessment models, we used the method and model developed by Punt (2008) to derive aging error matrices that account for both aging bias and aging imprecision, as enabled by a recent R program by J. Thorson (Thorson[1]). Note that this program is not itself an age validation tool, as it requires making the assumption that at least one age reader is unbiased.

Results

California Versus Washington

The examination of otoliths from fish collected off Washington showed patterns of growth that helped identify the growth patterns in fish from California (Fig. 2). We were looking for a pattern of growth where the inner annuli are large, get progressively smaller, enter a transitional growth phase characterized by checks, and then, on older fish, become thin stacked lines which is typical of many species of rockfish that we had worked with. In the figure, the first six presumed annuli in Washington fish are shown, and the likely analogous marks on the California fish are indicated. This comparison suggested that Washington fish could help to identify the pattern of growth in California fish. In particular, the first presumed annulus was determined to be at the edge of the large central growth zone.

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[1] Thorson, J. 2014 Northwest Fisheries Science Center. Montlake, WA, Personal Commun.

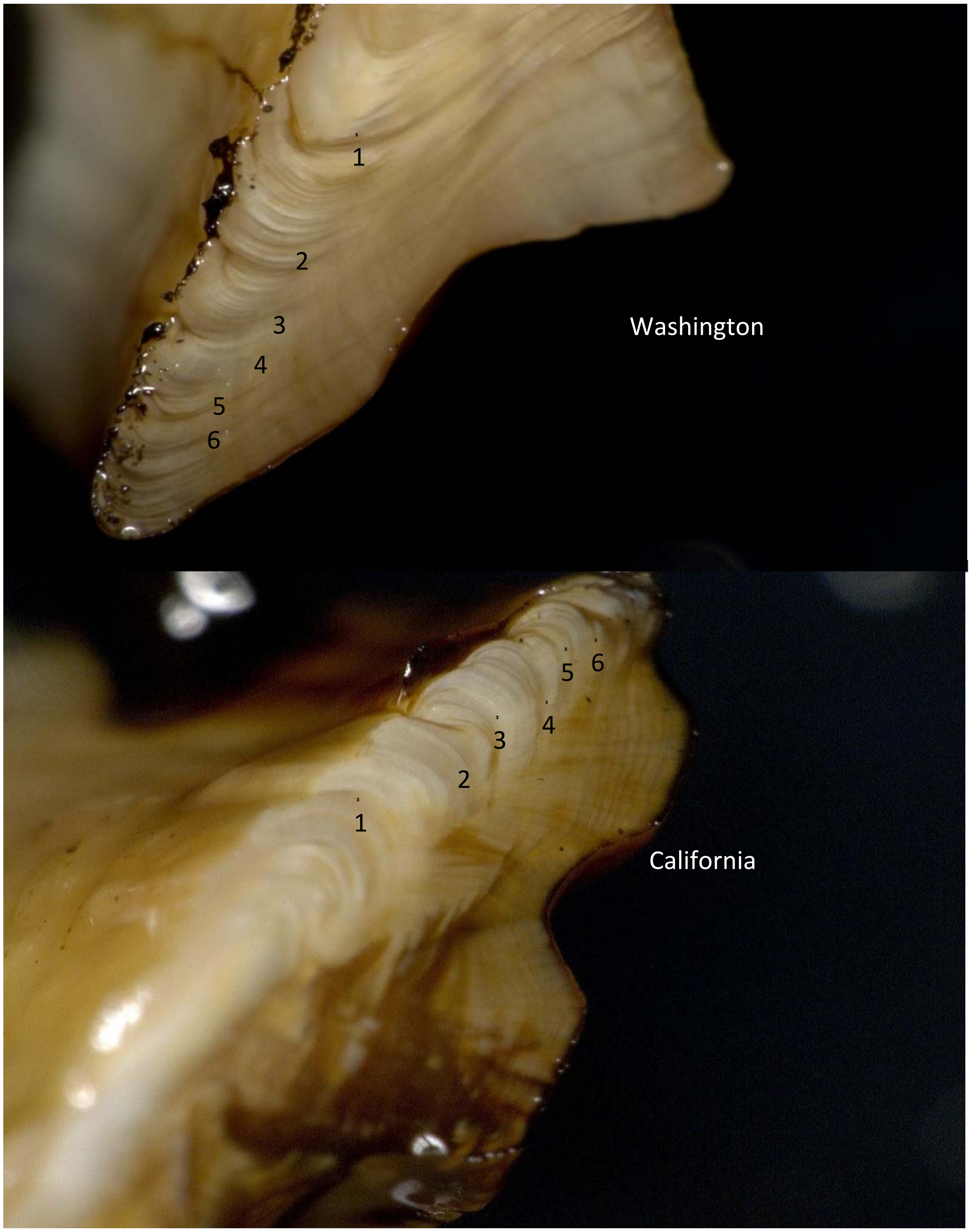

Figure 2. Side by side photographs of Bocaccio otoliths from California and Washington. Both otoliths were from 63cm fish.

Annual Length Compositions

The length compositions from 1986 and 1987 showed a strong mode at 37cm for 1986 and 43cm for 1987 suggesting that most of these fish were probably the 1984 cohort. We examined 75 fish with lengths less than 50cm, which had a high chance of including a large number of 1- and 2- year old fish (Fig. 3). From this examination, we were able to identify the probable first and second annuli and the characteristics associated with them. The key characteristic of the first annulus is that it is at the edge of a large irregular growth area (Fig. 4). This growth area is readily visible on most otoliths although the boundary is not always clear. The second probable annulus is often fairly distinct; however, there are exceptions as described in the section on size range viewing results.

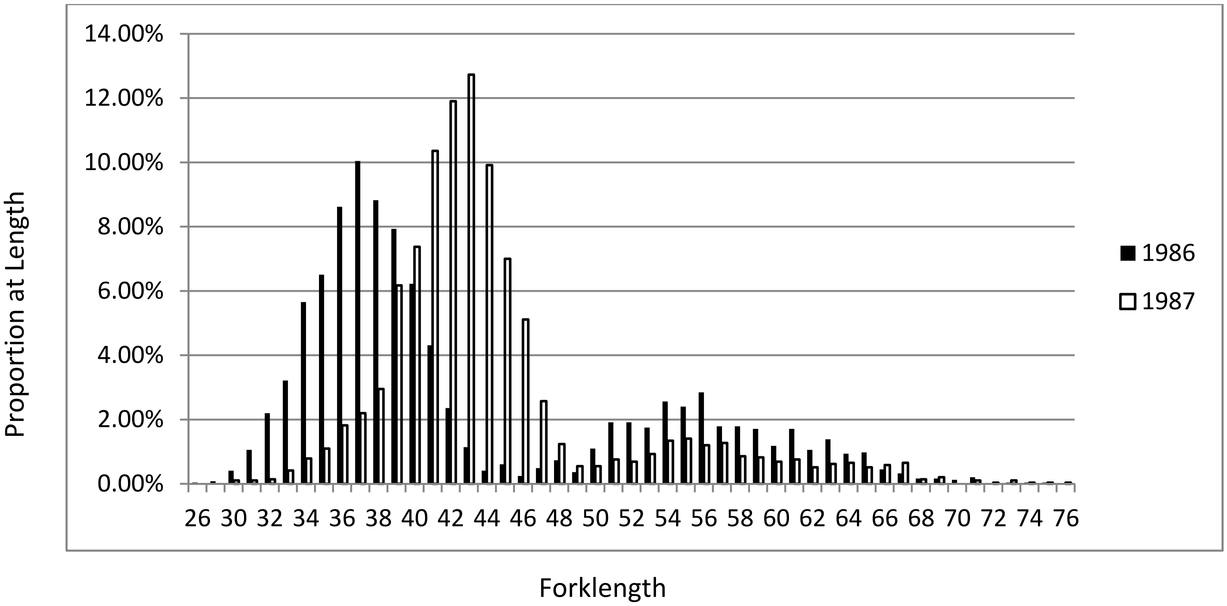

Figure 3. Annual length compositions for Bocaccio from California's commercial fisheries in 1986 and 1987. A strong mode is visible at 37 cm in 1986, and 43 cm in 1987. These modes are believed to be the 1984 cohort.

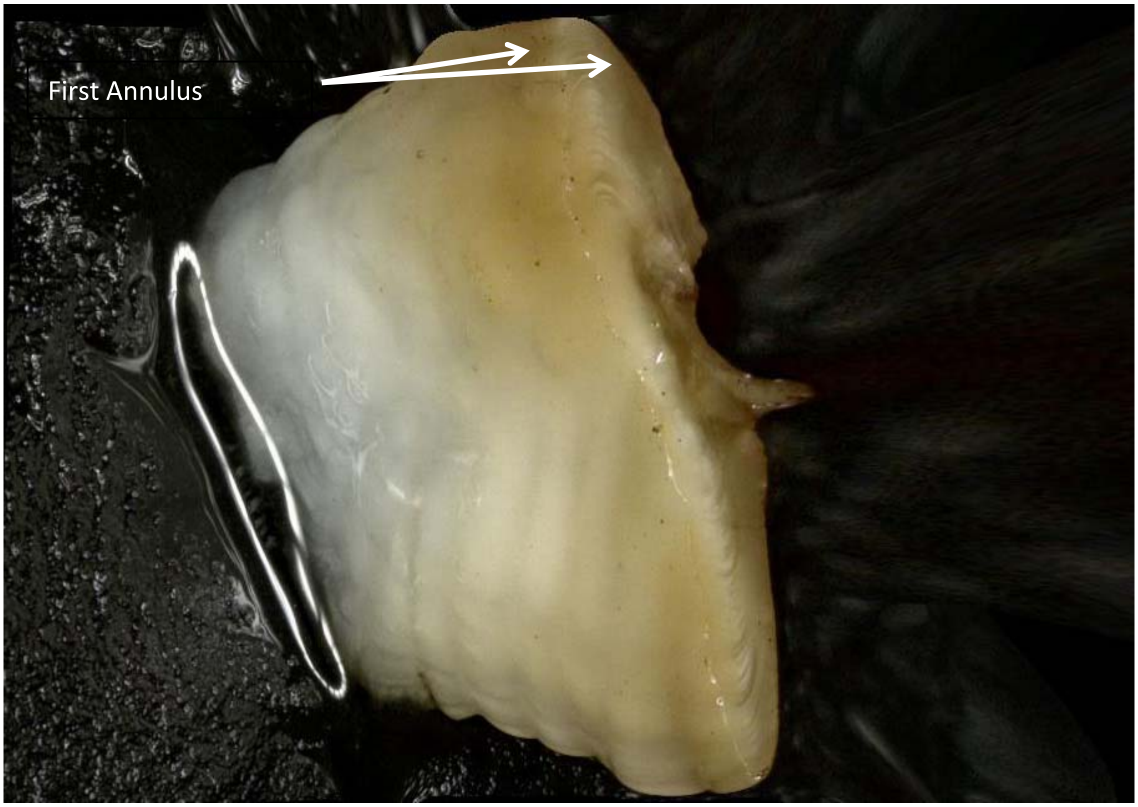

Figure 4. Photograph of an otolith from a one year old fish showing the presumed first annulus.

Quarterly Length Composition Examination

Plots of length compositions from January-March versus October-December of 1986 showed a clear mode at 34cm in the first three months of the year which had increased to 40cm in the last three months of the year (Fig. 5). This indicated the amount of growth likely to have occurred for the presumed 2-year-old fish from the 1984 cohort. We selected 50 fish from the first quarter in the 30cm size class and 50 from the 40cm size class from the fourth quarter and examined them. Summer growth (opaque) was clearly defined in the fourth quarter while only winter growth is visible on the edge in the first quarter (Fig. 6). This analysis provided additional confirmation that we were correctly identifying the first two annuli.

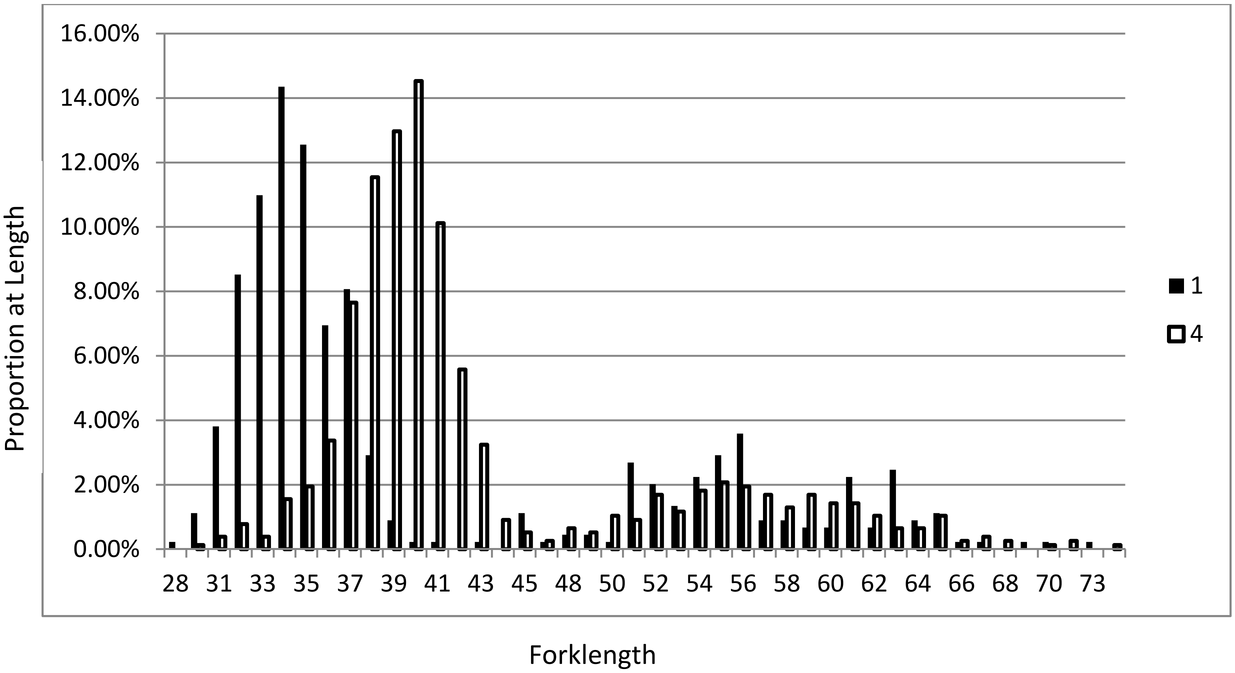

Figure 5. Quarterly length compositions from Bocaccio rockfish caught in 1986. In quarter 1 (January-March), a strong mode is visible at 34 cm, and in quarter 4 (October-December), a strong mode is visible at 40 cm, suggesting that presumed 2-year old fish grew approximately 10 cm during that time.

Figure 6. Photographs of otoliths from two young fish captured in January and October showing the amount of summer growth on the edge.

Serial Sections

One of the key observations made by examination of the serial sections was that during the early years of life, two real marks can merge into a single mark. This occurs because new growth at the margin can overlay the surface of the previous mark, particularly on or near the nucleus where the dorso-ventral axis is at its narrowest (Fig. 7). While Bocaccio have numerous checks, the phenomena of some presumed annuli merging with other apparent annuli can now be explained.

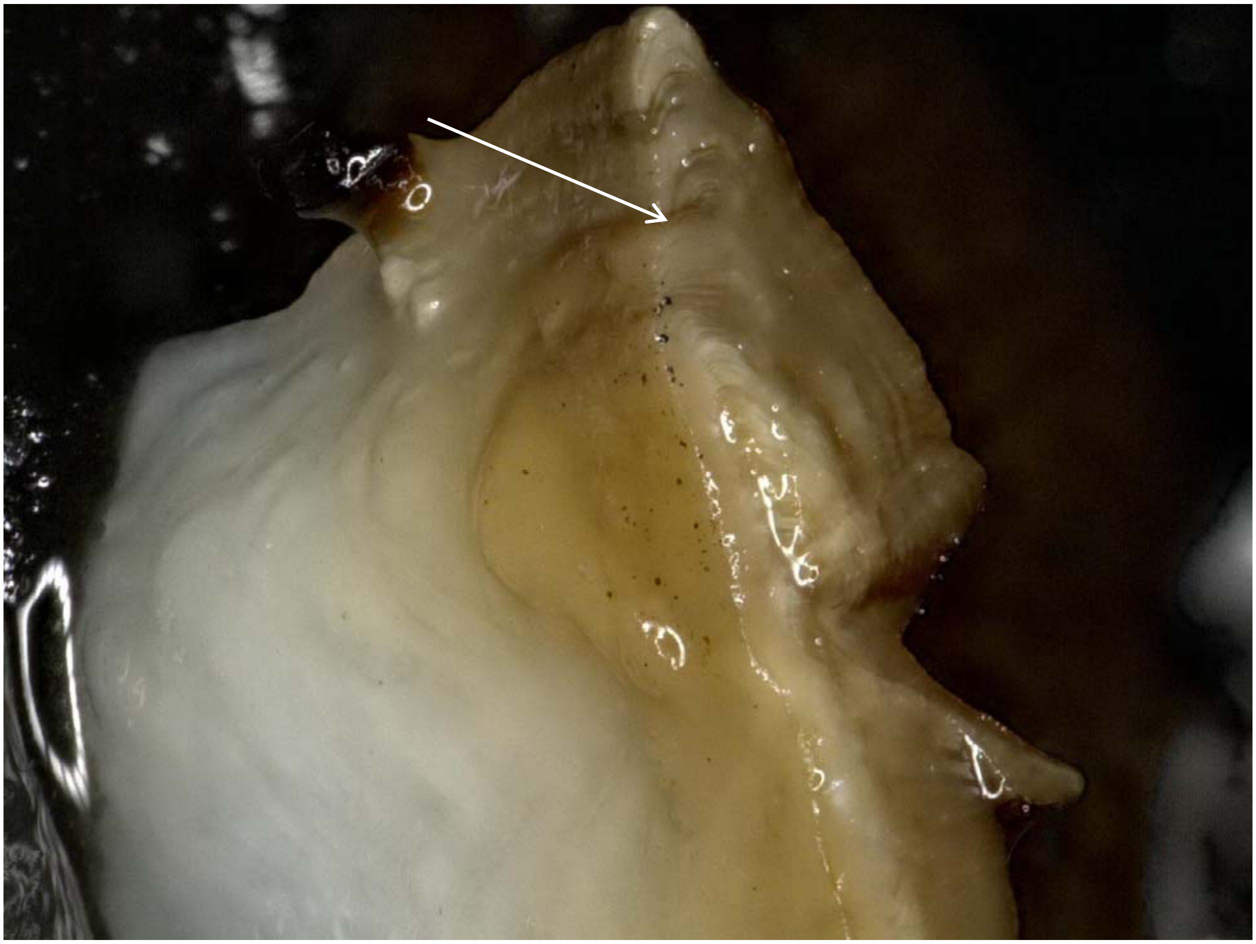

Figure 7. Photograph of an otolith showing the later growth wrapping around to the surface and hiding the surface expression of an inner annulus.

Size Range Observations

After many hours of careful examination of otoliths of various sizes, we began to see a pattern in the way growth is laid down on the otolith. Growth in the first year is very rapid. Between year 2 and 5, growth is still fast; however, it does slow down and annuli become narrower. Between about age 6 and 15, the growth goes into the transitional phase which is characterized by many checks and a great deal of variability in size of the annuli. After about 15 years, the growth slows down and often overlaps the outer surface and sometimes even folds in on itself (Fig. 8).

Figure 8. Photograph of an otolith from 20 year old fish showing the convoluted growth pattern that can occur in the later years of some fish.

Final Age Determination Criteria for Bocaccio

Based on the above observations, we developed the following age determination criteria for Bocaccio:

1. All otoliths must be broken and burned.
2. The 3/4 view must be used in conjunction with the section view.
3. The ventral side of the otolith is the only side of the section which should be read. If this is not possible, the otolith should not be aged.
4. The pattern of growth shows rapid growth for the first 4 or 5 annuli, a transition zone between rapid and slower growth occurring between about the fifth and fifteenth annuli, and slow growth following the transition zone.
5. Winter growth typically is visible on the edge in January-March and summer growth progresses after that.
6. It is common for two or more annuli to join at the surface. This can be identified by the “3/4” view used in conjunction with the section view.
7. Only otoliths with very good breaks and burns can be used for age determination.
8. A high percentage of cross reads is essential.

With these criteria established, the primary ager aged 200 fish knowing month of capture but not year. The ager was able to obtain 42% agreement to the year on fish younger than 5 years old, and 68% agreement to within one year.

The next step was to train another ager how to use the criteria. The second ager had extensive experience with two species of rockfish and one species of flatfish. After showing the ager the criteria, both age readers examined 25 otoliths using a double headed scope to clarify the criteria. The second ager was then given 100 otoliths to age. The primary ager then cross read the 100 otoliths. Differences were resolved by both age readers simultaneously viewing the otoliths using a double headed microscope to reach a consensus. This was repeated for another 100 otoliths, by which time there was reasonably good agreement between age readers.

In the final step, we began production age determination with about 25% of otoliths being read more than once. For fish where there were substantial differences, we reexamined the otolith to make sure that we were using the same criteria.

Age determination error analysis showed that there were generally good agreements of ages by two age readers, especially for younger fish (Fig. 9). For older fish, there existed some differences between two age readers, as fish aged by Reader 1 seemed to be older than those aged by Reader 2. As expected, age determination errors increased for older fish. Standard deviations (SD) increased from 0.27 for age 0 fish to 6.77 for fish of 36 years old (Table 1).

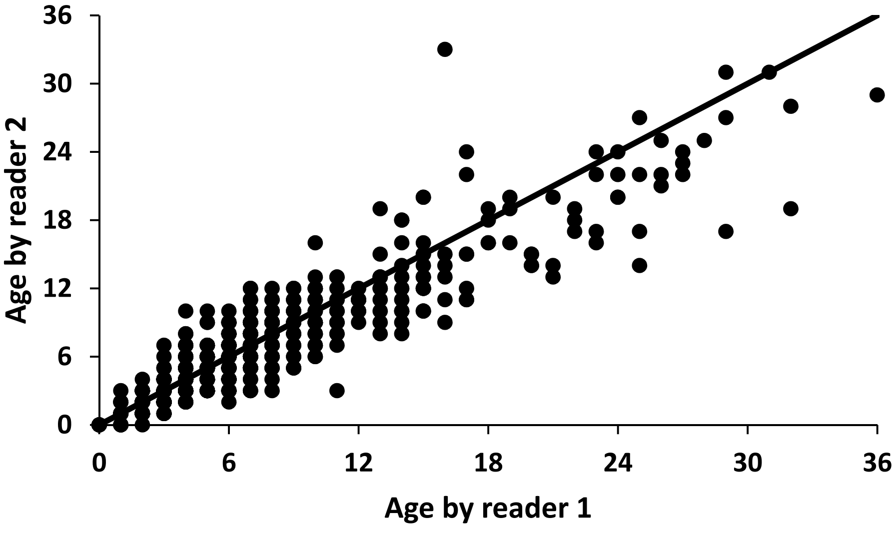

Figure 9. Plot of cross read age data by two age readers (reader 1 is Pearson, reader 2 is Lefebvre). Line represents identical ages by two age readers.

Table 1. Summary statistics of aging error analysis (CV = coefficient of variance; SD = standard deviation). Expected ages are 0.5 year older than observed ages, assuming most fish were caught in the middle-of-year (summer).

Observed age	CV	SD	Expected age
0	0.2702	0.2702	0.50
1	0.2702	0.2702	1.51
2	0.2104	0.4208	2.51
3	0.1910	0.5731	3.52
4	0.1818	0.7272	4.52
5	0.1767	0.8833	5.53
6	0.1735	1.0411	6.53
7	0.1716	1.2009	7.54
8	0.1703	1.3626	8.54
9	0.1696	1.5263	9.55
10	0.1692	1.6919	10.55
11	0.1690	1.8594	11.56
12	0.1691	2.0290	12.56
13	0.1693	2.2007	13.57
14	0.1696	2.3744	14.57
15	0.1700	2.5502	15.58
16	0.1705	2.7281	16.58
17	0.1711	2.9081	17.59
18	0.1717	3.0903	18.59
19	0.1724	3.2747	19.60
20	0.1731	3.4612	20.60
21	0.1738	3.6501	21.61
22	0.1746	3.8412	22.61
23	0.1754	4.0346	23.62
24	0.1763	4.2303	24.62
25	0.1771	4.4283	25.63
26	0.1780	4.6288	26.63
27	0.1789	4.8316	27.64
28	0.1799	5.0369	28.64
29	0.1808	5.2446	29.65
30	0.1818	5.4549	30.65
31	0.1828	5.6676	31.66
32	0.1838	5.8829	32.66
33	0.1849	6.1008	33.67
34	0.1859	6.3214	34.67
35	0.1870	6.5445	35.68
36	0.1881	6.7704	36.68

Discussion

After more than 25 years of intermittent efforts to develop age determination criteria, we have finally determined a set of criteria that can be used to age Bocaccio. Since this species remains difficult to age, high levels of within and among reader agreement should not be expected. It is anticipated that with practice, the level of agreement between and within age readers will improve. Unfortunately, otoliths collected in southern California have proven more difficult to age than those from central and northern California, so cross reader agreement rates will probably never be high for this area. Exploration of the environmental covariates that may drive the regional differences in age determination difficulties for Bocaccio as well as closely related species (such as Chilipepper rockfish [*Sebastes goodei*]) and age agreement rates is ongoing. Notwithstanding, the criteria developed in this research give us the ability to begin production age determinations with the expectation that the age estimates together with the associated aging error matrices will provide useful data for upcoming stock assessments.

While the age determination criteria established in this study have increased precision between age readers, the accuracy of the ages was limited to validation by indirect methods. As noted earlier, both Andrews et al. (2005) and Piner et al. (2006) estimated the maximum age for Bocaccio would be about 37 years. After production age determinations of more than 7,000 fish, our maximum age for the species is 36 years, indicating that the results of previous age validation efforts and the age estimates developed using these age determination criteria are consistent.

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