Developing Ageing Criteria for Shortraker Rockfish *(Sebastes borealis)*

By
Charles Hutchinson

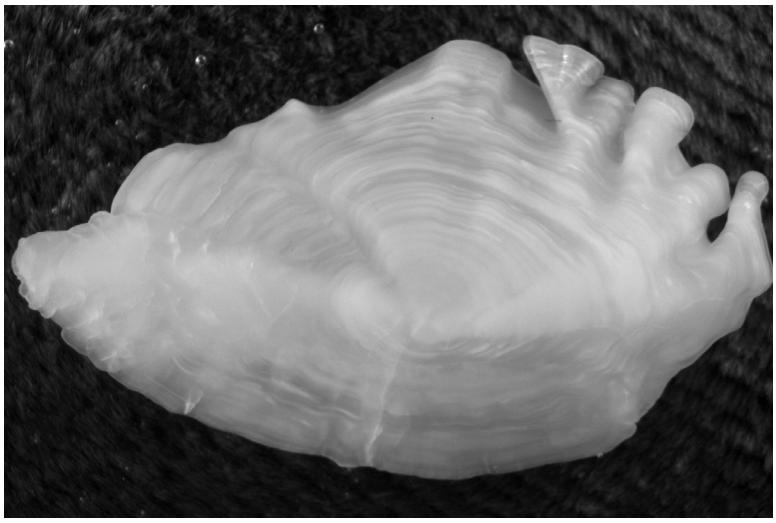

Figure 1. A shortraker rockfish otolith photographed at a magnification of approximately 5X.

The Alaska Fisheries Science Center's (AFSC) Age and Growth Program provides age data that contribute to our understanding of fish species with focus on age data for age-structured modeling of exploited fish populations. Determining accurate information on the ages of commercially important fish stocks is critical for accurate stock assessment modeling and effective fishery harvest and management strategies. Inaccurate fish age data can lead to over- or under-exploitation of fish resources.

Historically hard body parts such as scales, spines, and otoliths (ear bones) have been used to determine the ages of fishes. The most common structures used to age fish in the Age and Growth Program are otoliths (Fig. 1). Otoliths exhibit growth zones that chronicle the yearly growth cycle of fish, similar to the yearly growth rings seen in trees. One fast and one slow growth ring correspond to one yearly growth cycle, called an annulus. By examining growth zones in an organism, scientists can determine what effect the environment had on the growth of an organism and by enumerating the annuli can determine the organism's age. However, age reading is complicated because otoliths often contain many growth zones that may or may not be associated with true annuli.

Growth zones appear on the surface of an otolith and are read by placing the otolith in a water-filled Petri dish with a black felt background and examining the otoliths under a dissecting microscope with a light source (Fig. 2). In cases where no surface age can be determined, an otolith preparation technique is used to more clearly view the growth zones. The most common otolith preparation technique used by the Age and Growth Program is the break and burn method. In the break and burn method the otolith is snapped in half and a broken end toasted over an alcohol flame. The broken otolith is placed in modeling clay, and the toasted (burnt) end viewed through a dissecting microscope. A fiber optic light source reflects light off of the burnt half so that the growth zones appear as light (opaque) and dark (translucent) growth zones of fast and slow growth, which may or may not represent an annulus (Fig. 3).

Criteria are used to distinguish an annulus. An annulus is characterized by the ease with which it can be followed continuously along the axes of the burnt otolith from dorsal or ventral tip, the strength and uniformity of the dark and light growth zone,

Figure 2. The author examines a shortraker rockfish otolith using a dissecting microscope in the Age and Growth Laboratory.

Objectives

Beginning in 2000, the Age and Growth Program began investigating new methodologies for ageing shortraker rockfish. The methodology known as thin sectioning proved better than the break and burn method at eliminating burning artifacts and glassy areas in the reading surface of the otolith. Using the thin section method, three different strategies were examined based on growth patterns seen on the otoliths, including determination of a "transition age," an age where the fish's somatic growth slows. Ages using the different strategies were compared to radiometric ages to determine which strategy was most accurate. The use of radiometrics was also used to validate the transition age in shortraker rockfish. This article outlines the development of our new otolith preparation technique and ageing criteria using the thin section method and the testing and validation of the ageing criteria using radiometrics.

and the expected spacing between annuli in reference to the earlier growth pattern and the overall pattern (Fig. 3). A set of guidelines such as the characteristic of an annulus, placement of the first year, and edge growth viewed on an otolith determine the ageing criteria for each species aged in the Age and Growth Program. The ageing criteria should generate ages close to the fish's true age. In order to check on the accuracy of the ageing criteria, ages are validated by reading the ages of fish whose ages are already known. This is accomplished by marking or tagging live fish at birth (or later) and recapturing those fish in later years. If no known-age fish are available, then the ageing criteria are supported using other independent data such as cohort length frequency.

An important task of the Age and Growth Program is to carry out research on ageing methodologies in order to develop ageing criteria for commercially important species that are difficult to age. Shortraker rockfish together with other rockfish species make up a commercially important species group in the Alaska groundfish fishery. Historical efforts at ageing shortraker rockfish have aged the species to 120 years, however, age readers with the Age and Growth Program have not been able to age this species because of the difficulty in interpreting the growth patterns due to the appearances of artifacts and "glassy areas" (Fig. 4) on the otolith surface. Thus, no ageing criteria have been set for shortraker rockfish. As a result, with no ages generated for the species by the Program, AFSC stock assessment scientists have been unable to establish an age structure model for shortraker rockfish.

Methods

Thin Sectioning

A mass production thin section method used at the Central Ageing Facility in Queenscliff, Victoria, Australia, was adapted to produce shortraker otolith thin sections. Thin sectioning equipment includes a high speed Tyslide™ saw that produces thin sections approximately 0.4 mm thick. We modified the technique by reducing the thickness of the thin sections to approximately 0.2 mm using a Hillquist™ grinding wheel.

Otoliths were embedded in blocks made with a polyester resin (i.e., Artificial Water™). Blocks were formed in a silicone mold that held four individual block molds measuring 7 × 6.5 × 1.2 cm. A bottom layer of resin was applied 40 minutes prior to placement of the otoliths in order to allow the semi-cured resin to be scored. This process facilitated aligning the otoliths and prevented them from losing alignment by sinking into the resin. Otoliths were lightly marked with a pencil transversely through the middle of the first-year annulus and arranged on the scored line so that pencil marks lined up. Along with the otolith, a label was placed at the top end of the resin block. Another layer of resin was applied to

encase the otoliths in resin and allowed to cure for 2 days. Each block contained two rows of otoliths, with up to eight shortraker specimens fitting into one block. Three to five thin sections were cut from each specimen to ensure that a thin section contained a cross section of the first year. The thin section was then mounted on a glass slide using a UV adhesive, Loctite 349™, as the mounting medium. The adhesive was cured by exposure to UV light for 20 minutes. The thin sections were reduced in thickness by grinding the mounted slide on the grinding wheel to approximately 0.2 mm, which appeared to provide optimal viewing of growth zones.

Age Determination Strategies

In the case of shortraker rockfish, many fine marks punctuated by occasional major marks are visible on the thin section of an otolith. Thus, the question for the age reader is which marks represent an annulus? We developed three age determination strategies: Strategy 1, 2, and 3. Strategy 1 was based on counting major marks--growth zones that can be split into finer growth zones. Strategy 2 was based on counting fine marks. Strategy 3 was based on the combination of Strategies 1 and 2 and included a "transition age," an age where the fish's somatic growth slows due to the production of reproductive material as the fish matures. Change is represented in the otolith when annular marks change from broad growth zones or major marks to narrow growth zones or fine marks. If only the major marks are annular, then only the major marks should be counted (Strategy 1) and the age reader obtains a young age. If all of the fine marks are annular, then all of the fine marks should be counted (Strategy 2) and the age reader obtains a much greater age. For short-lived species such as Pacific cod, only major marks need to be counted. However, for long-lived species such as shortraker rockfish, counting only major marks is not the correct strategy, because once growth slows fine marks represent annular zones. The correct strategy has often been found to count major marks up to a "transition age," and then count fine marks past this point (Strategy 3). Pacific ocean perch and sablefish are long-lived species that have been successfully aged using Strategy 3.

What is special (and difficult) about shortraker rockfish is that many specimens will display occasional major marks past the transition age. The problem for the age reader is to determine the location of the transition age when the otolith contains several major marks that are candidates for the transition age. To perform this task we used radiometric ages to help determine the probable location of the transition age.

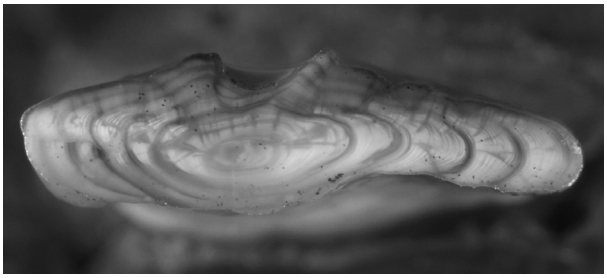

Figure 3. A break and burn rockfish otolith showing the dark (slow growth) zones and thicker (fast growth) zones.

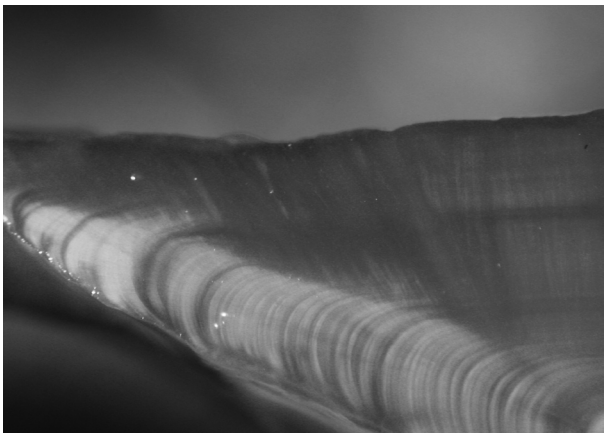

Figure 4. A shortraker rockfish otolith containing an example of the "glassy effect" (dark area) where the annuli are blurred and indistinquishable.

Ageing Radiometrically-aged Otoliths

The ratio of radioisotopes of lead and radium (^{210}Pb and ^{226}Ra) found in otoliths can be used to determine the ages of long-lived fish. Radiometric ageing has been used to validate several ageing criteria for long-lived species such as sablefish, Pacific ocean perch, northern rockfish, and yelloweye rockfish. Radiometric ageing does not generate ages for individual fish, but rather for a group of fish. The use of radiometrics in ageing is to verify or substantiate the ageing criteria.

An earlier study conducted in the Age and Growth Program on a group of shortraker rockfish used length as age proxy to determine shortraker rockfish longevity. In that study Craig Kastelle, a research scientist with the program, used radiometrics to age several length categories of shortraker rockfish (Table 1). In order to help establish the ageing criteria for shortraker rockfish, the remaining oto-

Table 1. Strategy 3 ages based on assumed transition ages are shown. Estimated ages for the length groups for a given transition age are derived by adding on the Strategy 2 incremental increases to the transition age starting with next larger length group after the transition age. Sum of squares (SS) residuals are listed at the bottom of the table.

		Strategy 1	Strategy 2		Strategy 3 transition age with estimated ages for each length group						
Ave length cm	Radio-metric age	Average age	Average age	Incremental increase in Strategy 2 ages	8.5	13.7	22.6	23.2	25.3	26.1	36.0
40.0	22.4	8.5	65.0	0.0	8.5	8.5	8.5	8.5	8.5	8.5	8.5
43.0	36.6	13.7	51.2	-13.8	-5.3	13.7	13.7	13.7	13.7	13.7	13.7
61.0	30.8	22.6	63.1	11.9	6.6	25.6	22.6	22.6	22.6	22.6	22.6
76.0	51.3	23.2	59.1	-4.0	2.6	21.6	18.6	23.2	23.2	23.2	23.2
81.7	57.3	25.3	58.8	-0.3	2.3	21.3	18.3	22.9	25.3	25.3	25.3
83.7	56.2	26.1	79.6	20.8	23.1	42.1	39.1	43.7	46.1	26.1	26.1
97.0	92.6	36	117.0	37.4	60.5	79.5	76.5	81.1	83.5	63.5	36.0
SS residuals					10,080	3,308	3,942	3,060	2,796	4,364	6,721

liths from that radiometric study were thin sectioned. The thin sections from each of these length categories were then conventionally aged (i.e., counting growth zones) using Strategy 1 and Strategy 2 and the ages grouped into the same length categories of the radiometric study. The ages for each given length category were averaged. The averaged thin section age was compared to the average radiometric age for the corresponding length category. Strategy 1 gave ages that were too young, and Strategy 2 gave ages that were too old, suggesting that Strategy 3 would be the best strategy.

In order to compare radiometric ages with the Strategy 3 ageing criteria we posited that the transition age occurred at one of the Strategy 1 ages for the length category groupings. For example, for the 43-cm length grouping, we found these specimens to have an average of 13.7 major marks by using Strategy 1 and an average of 51.2 minor marks by using Strategy 2. For the next length category 61 cm, Strategy 2 gave an average fine mark count of 63.1. For Strategy 3 if we posited a transition age of 13.7 years, the estimated average age for the 61-cm category fish was 13.7+(63.1-51.2) = 25.6 years. The value 63.1-51.2 = 11.9 shows up in Table 1 under the category "incremental increase in Strategy 2 ages," and 25.6 shows up under the Strategy 3 ages with a posited 13.7 years transition age for the 43-cm length category. In this manner we completed Table 1. Negative incremental ages, which were used in the calculations, were the result of averaging the ages from a group of fish and reader counting errors.

The sum of squared residuals was calculated between the radiometric and Strategy 3 ages for each of the posited transition ages (Table 1). A parabola was fit to the sum of squares that were obtained with the posited transition age as the independent variable. The lowest point on the parabola was solved using calculus to obtain an estimate of the "true" transition age. This estimate of transition age was approximately 23 years.

The thin sections from the radiometric study were then re-aged assuming a transition age of around 23 years. After ageing the thin sections, the average observed transition age for individual otoliths was 20.7 years, which was close to the statistical transition age of around 23 years using the radiometric data. Strategy 3 is the combination of Strategies 1 and 2 where we combined fine growth marks into annular bands, major marks, up to a transition age of around 20 years, and then all growth zones counted after that age (Fig. 5) as shown in Figure 6.

Testing the 20-year Transition Age

A new set of otoliths was used in testing Strategy 3 assuming a transition age of around 20 years. These otoliths were collected during the 2000 Gulf of Alaska summer survey conducted by the AFSC's Resource Assessment and Conservation Engineering (RACE) Division. The otoliths were thin sectioned and aged using Strategy 3.

In this radiometric analysis, we used otolith material laid down in the first 3 years of the fish's life.

Previous radiometric studies have shown that young fish or otoliths modified to represent young fish (i.e., "cored" otoliths where all material beyond the third year has been removed), are less likely to violate assumptions associated with radiometric ageing. Because approximately one gram of otolith material is needed to run the analysis, the otoliths needed to be grouped into age-category samples. Three samples were chosen based on age availability, experimental design, and time concerns. Two samples conventionally aged 20 ±2 years were termed SR1 and SR3. SR1 and SR3 samples were selected so that their conventional ages were the transition age of 20 years. The third sample (SR2), conventionally aged 30 ± 2, was selected because it was 10 years past the transition age of 20 years.

The initial step in the radiometric work for SR1, SR2 and SR3 was to core each otolith. This was accomplishing by mechanically removing the otolith material beyond age 3 years using a Buehler™ low speed cutting saw and grinding wheel. The cored otolith size was determined by taking measurements of the first 3 years on whole and thin-sectioned otoliths from samples processed for radiometric analysis. Growth zones were distinguishable in the early years in shortraker rockfish, especially when the top layer was ground off on the distal side during the coring process.

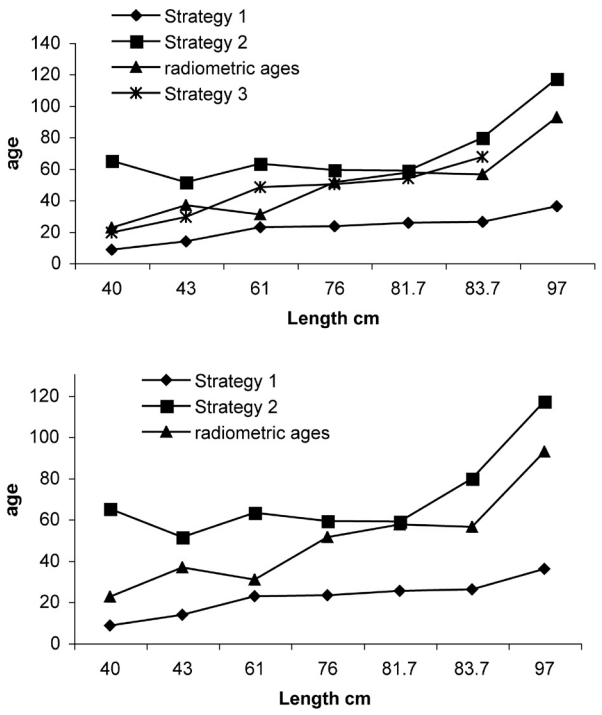

Figure 5. Shortraker rockfish ages (by length group) determined using four different ageing methods. Strategy 3 with transition age around 20 years is attained using the combination of Strategy 1 and Strategy 2.

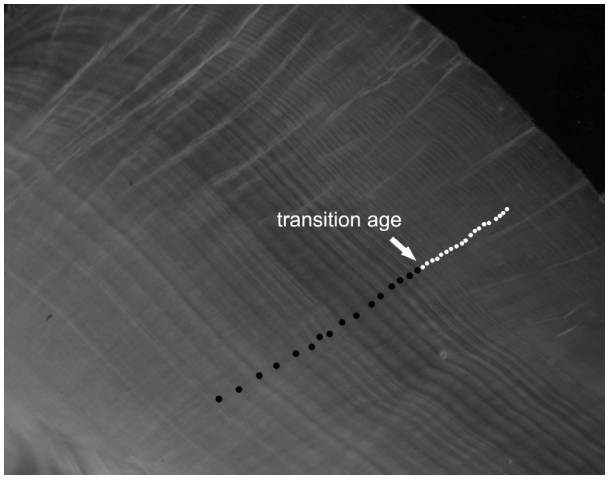

Figure 6. A thin section aged with Strategy 3 and a transition age of 22 years. Dark dots indicate major or banded zones and light dots indicate closely spaced zones.

Cores were rinsed with distilled water, blotted dry, and weighed to the nearest milligram. Cores were then placed in a clean, acid-washed vial containing a 60% ethanol solution. The procedure was continued until the vial contained enough cores to total around 1 gram of material. The average weight for cores representing the first 3 years of life was 43 mg. The radiometric work was again performed by Craig Kastelle.

Data Analysis

The ratio of ^{210}Pb and ^{226}Ra the otoliths can be used to predict a radiometric age by using the following equation:

$$\frac{A_2}{A_1} = 1 - \exp(-\lambda_2 t) + R^* \exp(-\lambda_2 t).$$

Here A_1 is the activity for ^{226}Ra, A_2 and λ_2 (λ_2=ln (2)/half-life in years) are the activity and decay constant respectively for ^{210}Pb, t is time (age in years), and R* is the initial ratio of ^{210}Pb/^{226}Ra. The initial ratio is based on the amount of ^{210}Pb and ^{226}Ra found in juvenile fish and has been estimated to be close to 0.0. In this study two R* values (R* = 0.0, an assumed value, and R* = 0.0636, from a similar spe-

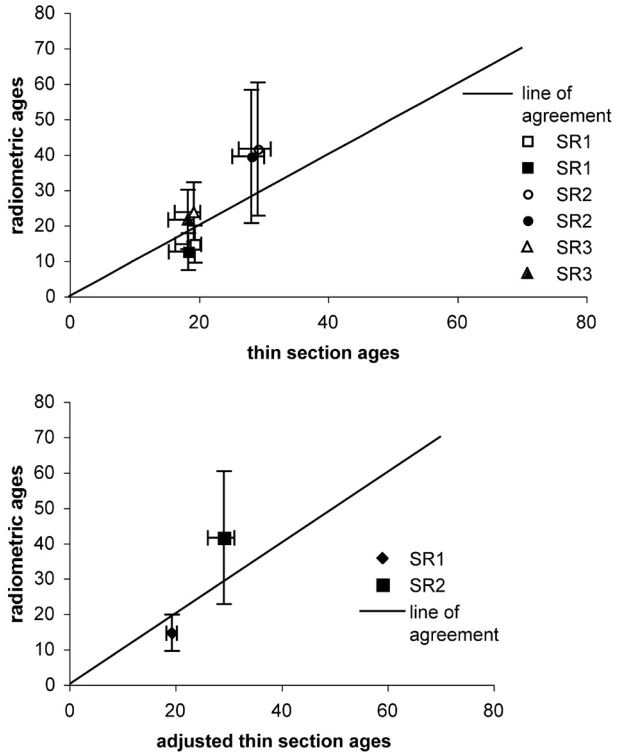

Figure 7. Radiometric ages for SR1, SR2, and SR3 for initial ratios of 0.0 (shown in clear symbols) and 0.0636 (shown in solid symbols) plotted against thin sections ages. The error bars on the x-axis represent the thin section age range in the samples. The y-axis error bars represent two standard errors for the radiometric ages. Point estimators for each sample on the x-axis are offset (by 1 year) for a clearer view of error bars.

cies rougheye rockfish, were used in the radiometric age determination of shortraker rockfish.

Sources of errors associated with the radiometric ageing come from blank and background measurements, chemical procedures, gravimetric measurements of otolith core weight, and errors produced from the counting statistics involved in measuring the decay activity of the radionuclides. Errors were propagated through all calculations to estimate errors in radiometric age.

Comparing Radiometric Ages to Thin Section Ages

For a more accurate comparison between thin section ages and radiometric ages, the thin section ages from the samples SR1, SR2 and SR3 were adjusted to take into account accumulation of radium over the 3-year time period of core growth. This adjustment reduced the nominal conventional ages of the samples from 20 years to 18.9 years for SR1, 30 years to 28.7 years for SR2, and 20 years to 18.8 years for SR3. Table 2 shows radiometric ages corresponding to the two initial activity ratios 0.0636 (value for rougheye rockfish) and 0.0 (assumed value) and their corresponding thin section ages (assuming a transition age at around 20 years). The point estimates for SR1, for both initial ratios, suggests that thin section ages were older than radiometric ages. Point estimates for SR2 and SR3, for both initial ratios, suggests that the thin section ages were younger than radiometric ages. The 95% confidence interval for the mean radiometric age range with initial ratio 0.0636 includes the mean thin section age for SR2 and SR3, but not SR1. The 95% confidence interval for mean radiometric age range with initial ratio 0.0 includes the mean thin section age for all three samples (Fig. 7).

Growth Analysis

Another piece of shortraker rockfish life history data included in our study that supports the theory of a transition age of around 20 years is the estimated age at 50% sexual maturity of shortraker rockfish. Otoliths were selected from female shortraker rockfish from the RACE 2000 summer cruises (N = 90) and 1993 specimens from the earlier radiometric study (N = 60) and were examined to determine von Bertalanffy growth parameters. Ages used in the von Bertalanffy equations were generated using Strategy 3.

Female shortraker growth parameters and age at 50% sexual maturity were determined from the von Bertalanffy growth equation:

$$L_t = L_\infty (1 - \exp(-k(t - t_0))) .$$

Here L_t is equal to length at age t, L_∞ is theoretical length at age infinity, t_0 is theoretical age at length zero and k is the growth rate. The von Bertalanffy curve was solved for t to estimate age at 50% sexual maturity using

$$t_{50} = t_0 - (1/k) \ln\left(1 - \frac{L_{50}}{L_\infty}\right) .$$

Table 2. Comparison of average ages for SR1, SR2, and SR3 samples from the thin section and radiometric ageing methods. The radiometric ages used ^{226}Ra activity from individual samples and include estimates of standard errors (SE).

Initial ratio	Samples	Radiometric age	± 1 SE	± 2 SE	Adjusted thin section age
0.0	SR1	14.6	2.6	5.2	18.9
0.0636	SR1	12.5	2.6	5.2	18.9
0.0	SR2	41.5	9.4	18.8	28.7
0.0636	SR2	39.4	9.4	18.8	28.7
0.0	SR3	23.7	4.2	8.4	18.8
0.0636	SR3	21.6	4.2	8.4	18.8

Table 3. Von Bertalanffy growth parameters and age at 50% sexual maturity for female shortraker rockfish. All samples were aged using the thin section method and Strategy 3.

Samples	Sex	N	Linf cm	K	To	50% Sexual Maturity	95 % CI
1993	F	60	83.2	0.042	4.68	23.22	± 4.4
2000	F	90	69.1	0.043	-2.89	21.55	± 4.12
Combined	F	150	84.6	0.03	-3.62	21.42	± 3.6

Here, t_{50} is the estimated age at 50% sexual maturity using the length at 50% sexual maturity (L_{50}), previously estimated to be 44.9 cm by scientist Suzanne McDermott, with the Center's Stock Assessment and Multispecies Assessment Program.

Nonlinear least squares was used to estimate von Bertalanffy growth parameters and to test for differences in growth parameters between samples, and the covariance matrix of parameter estimates and the delta method were used to construct 95% confidence intervals for age at 50% female sexual maturity.

Growth parameters and age at 50% sexual maturity were determined for each sample and also for samples combined (Table 3), indicating that t_{50} is approximately 20 years. Figure 8 shows the von Bertalanffy growth curves produced for separate and combined age samples.

Results

Results from the first radiometric study led us to dismiss Strategy 1 and Strategy 2 ageing as reasonable otolith ageing strategies for shortraker rockfish. A statistical comparison between radiometric ages and Strategy 3 ages suggest a predicted transition age of around 23 years.

Results from the second radiometric study supported our hypothesis of a transition age of around 20 years. The sample of older fish, SR2, strengthened the ageing criteria by extending ages past the transition age and confirmed that all growth zones should be counted after the transition age. This study suggests that the initial R* = 0.0 produced better agreement than the R* = 0.0636. The use of an initial R* = 0.0 can be rationalized because previous rockfish radiometric studies have found initial ratios were close to 0.0. Initial ratio estimates for shortraker rockfish could not be made because there were not enough young shortraker rockfish to perform such a study.

Under Strategy 3 ageing, the transition age in the otolith growth pattern signals a shift in otolith growth indicated by reduced thickness of annular marks. The statistical comparison between radiometric and thin section ages indicated that a transition zone was present around the age of 20 years. This was useful because a clear demarcation between fast growth and slow growth as represented in otolith growth patterns was lacking in many specimens. This result provides a rationale for using

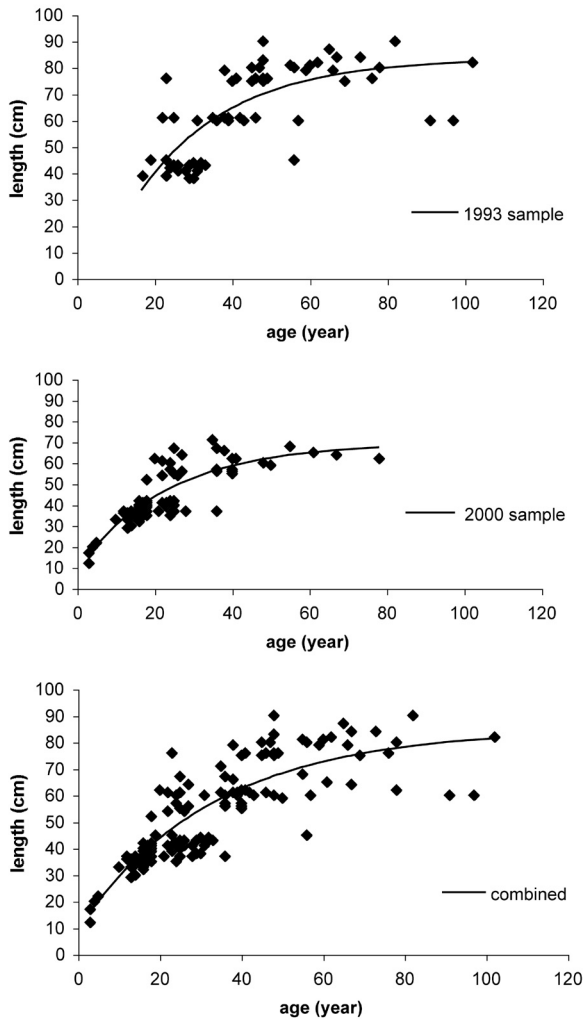

Figure 8. Von Bertalanffy growth curves of 1993, 2000, and combined samples.

a 20-year transition age when the "true" transition age was not apparent. Although specimens could be found that exhibited clear transition zones ages between 10 and 30 years (Fig. 6), most transition zones ages were located at around 20 years.

Confidence intervals for radiometric ages can encompass a wide range. Although there was fair agreement between radiometric and thin section ages using the new criteria, caution should be observed. The radiometric analysis that tested the transition age was age-based, but only three samples and a limited age range were used. More testing is needed to examine the accuracy of the ageing criteria over a wider age range and to examine the possibility of under ageing. One limitation of radiometric ageing is that a gram of material is required, and this pooling of material yields average rather than individual ages. As fish increase in age, their lengths tend to overlap and length becomes less correlated with age. This may mean that the pooled fish may represent a broad age range.

The age at 50% sexual maturity of female shortraker rockfish estimated using 1993 and 2000 samples ranged from 21.4 to 23.2 years. (Table 3). Sexual maturity in fish is associated with a reduction in somatic growth and an increase in gonad growth. The reduction in somatic growth is related to narrower spacing between translucent zones on the otolith pattern when viewed under reflected light. Although somewhat circular, the use of age data—the age at 50% sexual maturity—is consistent with the estimated transition age of 20 years as would be expected in theory.

The thin section ages generated by ageing Strategy 3 were validated by radiometric ages with a transition age of around 20 years. This strategy will be the starting point for setting ageing criteria for shortraker rockfish. Ideally the criteria would dictate that multiple age readers could age the same fish and produce similar ages. It is hoped that age readers can develop suitable ageing criteria that will produce ages usable for stock assessment purposes.

This article is based on the author's masters thesis "Using radioisotopes in the age determination of shortraker rockfish (Sebastes borealis)" presented 29 October 2004 at the School of Aquatic and Fisheries Sciences, University of Washington. Copies of the complete thesis are available at the University of Washington Fisheries-Oceanography Library and the AFSC's National Marine Mammal Laboratory Library .