Abstract.—Age determination of sablefish (*Anoplopoma fimbria*) is typically done by counting growth zones on the burnt cross-section of the otolith. The break-and-burn method of age determination is difficult to apply to sablefish. Therefore, we applied a relatively new method of fish age validation, using the disequilibrium of Pb-210/Ra-226 in the otoliths. This method of validation complements previous methods which used oxytetracycline (OTC) marking to validate incremental growth in sablefish otoliths. The Pb-210/Ra-226 disequilibria generally confirmed the ageing criteria used to interpret the otolith's burnt cross-section.

Using Pb-210/Ra-226 disequilibria for sablefish, *Anoplopoma fimbria*, age validation

Craig R. Kastelle
Daniel K. Kimura
Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA
7600 Sand Point Way NE, Seattle, WA 98115-0070

Ahmad E. Nevissi
Donald R. Gunderson
School of Fisheries, WH-10, University of Washington
Seattle, WA 98195

Sablefish (*Anoplopoma fimbria*) is an important commercial species distributed continuously along the North Pacific Rim from California to northern Japan. On central California's continental shelf, the spawning of sablefish takes place from October to February at depths of over 823 m (Hunter et al., 1989). Both eggs and larvae have been collected at depths of over 400 m in April off British Columbia. After hatching, postlarval sablefish move into the surface waters where onshore or offshore transport may take place (Mason et al., 1983). Postlarvae have been found offshore, but as juveniles they are usually seen inshore (Bracken, 1983; Mason et al., 1983). The juveniles are believed to reside inshore for several years and then move to deeper offshore waters as they near maturity and join the spawning population (Bracken, 1983; Mason et al., 1983). Mature sablefish are typically caught at 700 m (Mason et al., 1983). Funk and Bracken[1] note that the growth of young fish is fast compared to very slow growth of mature fish. An abrupt slowing of growth coincides with the onset of sexual maturity. Mean fork length increases with depth and the length at 50% maturity for females is 60 cm (Hunter et al., 1989).

At the Alaska Fisheries Science Center (AFSC) sablefish ages are determined by counting growth zones (assumed annular) seen on the distal surface of the otolith, or more frequently in the burnt dorsal-ventral cross-section (break-and-burn method, Beamish et al., 1983). Even though ageing criteria have been established for sablefish by using the break-and-burn method (Beamish and Chilton, 1982; Beamish et al., 1983), variability between individual sablefish in the morphology of their otoliths and the appearance of growth zones makes this method difficult to apply. Between-reader variability in sablefish ages are far greater than for any other species routinely aged at the AFSC (Kimura and Lyons, 1991).

Sablefish age validation has been examined by using oxytetracycline (OTC) studies, mark and recapture of known age fish, tagging studies, length-frequency analysis of young fish, and daily growth zone counts in juvenile sablefish otoliths (Beamish

---

[1] Funk, F., and B. E. Bracken. 1983. Growth of sablefish in southeastern Alaska. AK Dept. Fish and Game Info. Leaflet No. 223, 40 p.

Manuscript accepted 27 October 1993
Fishery Bulletin 92:292-301 (1994)

and Chilton, 1982; Beamish et al., 1983). The age range considered by Beamish and Chilton (1982) was from 0 to 43 years, but their study had few individuals from the upper end of the age range. The OTC method can validate only incremental growth zones after injection, leaving interpretation of earlier growth in any one fish questionable. Ideally, the age range of OTC-injected fish spans all ages. Younger OTC-injected fish can then be used to infer that incremental growth zones seen prior to an OTC injection on older fish are annuli. With these limitations, the procedures used by Beamish and Chilton (1982) and Beamish et al. (1983) confirmed that ages counted on the burnt cross-section were accurate.

Our goal was to use radiometric dating techniques to validate the break-and-burn ageing criteria used at the AFSC for sablefish aged to approximately 35 years. This validation used the measured ratio of Pb-210/Ra-226 in the otoliths to provide estimates of total age, thereby complementing previous OTC validation work which only confirmed incremental growth.

In the first application of radiometric ageing to fishes, Bennett et al. (1982) used the ratio of Pb-210/Ra-226 to validate ages up to 80 years for splitnose rockfish (*Sebastes diploproa*). More recently, these radioisotopes were used by Campana et al. (1990) and Fenton et al. (1990, 1991) in age validation and longevity studies in a variety of fish species. Additional radioisotope pairs such as Th-228/Ra-228 have also been used to age fish (Smith et al., 1991).

The isotopes Ra-226 and Pb-210 are part of the naturally occurring decay chain of U-238:

$$\begin{gathered} U\text{-}238 \underset{4.5\times 10^{9}\,yr}{\dashrightarrow} Ra\text{-}226 \underset{1600\,yr}{\dashrightarrow} Rn\text{-}222 \underset{3.8\,d}{\dashrightarrow} Pb\text{-}210 \\ Pb\text{-}210 \underset{22.3\,yr}{\dashrightarrow} Po\text{-}210 \underset{138\,d}{\dashrightarrow} Pb\text{-}206 \end{gathered} \qquad (1)$$

where the dashed lines indicate short-lived intermediary nuclides that are not shown.

Both Ra-226 and Pb-210 are found naturally in seawater. Ra-226 is a calcium (Ca) analogue which accompanies Ca through the food chain and is deposited in fish tissue, particularly calcified structures, along with Ca (Swanson, 1985; Porntepkasemsan and Nevissi, 1990). The otoliths of teleosts consist of an acellular organic protein matrix mineralized with aragonite, a form of calcium carbonate in which the radioisotopes are deposited (Mugiya, 1977; Campana and Neilson, 1985). Pb-210 is also accumulated by the biota through the food chain (Shannon et al., 1970; Heyraund and Cherry, 1979). In fish, Pb-210 is preferentially deposited in the bone or liver[2] (Swanson, 1985). Its initial activity in the otolith must be measured (later as R^*) for the application of radiometric ageing.

When Ra-226 is deposited in otoliths, like Ca it remains immobile, and a disequilibrium is created between Ra-226 and all of its progeny. With time, the activity of shorter-lived daughter products like Pb-210 will increase. In the pair of radioisotopes used here, Ra-226 and Pb-210, the difference between their half-lives is great (Eq. 1). Therefore, after about 100 years in a closed system the activity of both Ra-226 and Pb-210 will become equal, establishing a so called "state of secular equilibrium" (Faure, 1986). A chronometer is started when Ra-226 is first deposited in the otolith, and the activity ratio Pb-210/Ra-226 is a function of the time elapsed since deposition.

Radiometric dating applied to fish otoliths relies on three basic assumptions (Faure, 1986; Smith et al., 1991):

1 The otoliths are closed with respect to the loss or gain of any radioisotopes in the decay chain.
2 The initial activity ratio of Pb-210/Ra-226 in the otoliths should be much smaller than one, ideally close to zero, and known or measured.
3 The specific activity [disintegrations per minute per gram (dpm/g)] of the radioisotopes in the material incorporated into the otoliths must be constant.

These assumptions will be considered in detail later in the "Discussion" section. But first, it is important to consider Assumption 3 because it explains why we did not use whole otoliths. Assumption 3 is the most problematic of the three assumptions when applied to whole otoliths because it also requires assuming a mass-growth rate for the otoliths (Smith et al., 1991). Campana et al. (1990) used otolith cores in their application of radiometric dating to fish. When using otolith cores and individual measurements of Ra-226 for each sample being radiometrically aged, Assumption 3 becomes unnecessary. However, if measurements of Ra-226 are averaged over the different samples being radiometrically aged, Assumption 3 requires that the different core samples have the same activity levels. Our study followed the procedures of Campana et al. (1990) and used otoliths cores. But unlike Campana et al. (1990), we used individual Ra-226 measurements for each sample being aged so that Assumption 3 was unnecessary.

---

[2] Noshkin, V. E., K. M. Wong, R. J. Eagle, T. A. Jokela, and J. A. Brunk. 1988. Radionuclide concentrations in fish and invertebrates from Bikini Atoll. Lawrence Livermore National Laboratory, Livermore, Ca., UCRL-53846, 53 p.

Materials and methods

Otolith collection

Sablefish were collected along the Eastern Pacific Continental Shelf during two research cruises by the AFSC. The first collection was made from the research vessel *Alaska* on 8 August 1986 and contained 61 fish captured at 135 m in Morro Bay, California. The second collection was made from the vessel *American Viking* between 23 September and 31 October 1986 and contained 423 fish captured at depths of 246–1426 m off the California coast between 32°23'N and 42°23'N. Both sagittal otoliths were removed at sea. The sacculus membranes were removed and otoliths were stored in 50% ethanol.

Ages were estimated by the first author (Reader 1) from one otolith per fish applying the break-and-burn method, and the other otolith was used in the radiometric study. The criteria used to count annuli for this study were similar to those typically used by experienced age readers at the AFSC and Tiburon Laboratories of the National Marine Fisheries Service (NMFS), and were the same used by Beamish and Chilton (1982) and Beamish et al. (1983). By using these criteria, an age range of up to ±5 years for older fish was often possible. The oldest age consistent with these criteria was often assigned as the most probable age. When a fish was aged as 1 year old the otolith surface was usually adequate and no break-and-burn was done. The otoliths were subsequently pooled into four age categories (1 year, 9–11 year, 14–23 year, and 24–34 year), on the basis of Reader-1 ages.

For comparison, a subsample of otoliths initially aged ≥14 years (n=186) was read by a second experienced sablefish age reader at the AFSC (Reader 2). Additionally, all fish initially aged ≥14 years (n=266) were read by an experienced sablefish age reader at the Pacific Biological Station (PBS), Canadian Department of Fisheries and Oceans (Reader 3).

In the radiometric dating procedures, we used that part of the otolith which was deposited during the first year of life (i.e. the first year core). For age category 1, whole otoliths representing the first 15 to 18 months of growth were used. These otoliths were those classified as age 1 by Reader 1 and were intact and unburnt. For other age categories, the first year cores were isolated by grinding away excess otolith material. The grinding was done with a Buehler metallurgical polishing machine equipped with Buehler wet and dry #600 or #900 paper. Otolith material representing the first year was readily identifiable in older fish from the distal surface of the ground otolith, and from the broken-and-burnt section, viewed with a dissecting microscope (25×) as a guide. Removal of material was done slowly, with frequent viewing of the otolith during the grinding process (Kastelle, 1991). The position of the first annulus on the otolith has been confirmed by several authors in age validation studies of young sablefish (Beamish and Chilton, 1982; Beamish et al., 1983; McFarlane and Beamish, 1983). Average measurements of the core dimensions were not used as an aid in the grinding process. Instead, grinding was completed when the contours of the first year were approximated. Small inaccuracies in the grinding were inconsequential because samples were pooled into four categories based on age ranges.

All otolith cores were cleaned with an ultrasonic cleaner in distilled and deionized water for a minimum of 30 seconds. Any soft tissue remaining on the otolith after collection was visibly broken down by the ultrasonic cleaner. The goal of the cleaning was to remove any contamination from soft tissue or grinding paper. After cleaning, the otoliths were stored again in a fresh 50% ethanol solution prior to analysis for radioisotopes.

Approximately one gram of material was necessary for radioisotope activity to be measurable above background levels, which meant that 83 to 141 otoliths were used for each age category. To increase the weight of category 1 (1 year olds), an additional unburnt half of the aged otolith from some specimens was included with the intact otoliths (83 specimens total: 83 whole otoliths plus 65 half otoliths from some of the same fish).

Activity measurements

The methods employed in the chemical separation and counting techniques for Ra-226 and Pb-210 were similar to those used by Bennett et al. (1982) and are detailed in Kastelle (1991).

In general, the activity of Pb-210 in the otoliths was determined by counting the alpha decays of Po-210 (the granddaughter-proxy of Pb-210 with which it is in secular equilibrium, Eq. 1) by using a yield tracer of Po-209. Reagent blanks, with and without yield tracers, were processed with each age category as follows: category 1, one blank without yield tracer; categories 2 and 3 processed simultaneously, one blank with a yield tracer and one without; category 4, one blank with a yield tracer and one without. The counting time for each sample or reagent blank was approximately three weeks.

The activity of Ra-226 was determined by counting alpha decays of Rn-222 (the daughter-proxy of Ra-226 with which it grows into secular equilibrium, Eq. 1) in a Lucas cell (Lucas, 1957). The samples were stored in a Rn-222 de-emanation flask for a minimum of three weeks prior to counting. During storage, Rn-222 reached secular equilibrium with Ra-226. Reagent blanks were processed with each sample. Lucas cell (Rn-222) counting times were 4,000 minutes for each sample or reagent blank. Lucas cell and electronic backgrounds (counting time 1,000 minutes) were measured multiple times before and after any sample or reagent blank. The counting efficiency (i.e. the percentage of decays that was detected) of the de-emanation technique, Lucas cells, and associated electronics was determined to be 53.75% with the use of a Ra-226 standard solution supplied by the U.S. Environmental Protection Agency. Activities of Ra-226 and associated errors were calculated by using the methods of Sarmiento et al. (1976). Details concerning incorporation of blank and background measurements into the activity calculation for Po-210 and Ra-226 are given in Kastelle (1991). All sources of error were propagated through the calculations to estimate errors (standard deviation or SD) for the Ra-226 and Pb-210 measurements.

Data analysis

ANOVA with contrasts was used to test whether break-and-burn otolith ages from the different age readers were significantly different within each age category. Statistical analyses of activity measurements from samples, backgrounds, and reagent blanks were conducted by using Z, t, and a likelihood ratio χ^2 test as described below. To test if the Ra-226 (or Pb-210) activity from each age category was statistically different, the likelihood ratio test was employed. Assuming X_i is distributed as $N(\mu_i,\sigma_i^2)$, where the σ_i^2 are known, the likelihood ratio χ^2 test for $H_o:\mu_1=\ldots=\mu_n$, is

$$\hat{\chi}^2=\sum(X_i-\hat{\mu})^2/\sigma_i^2$$

which is distributed as χ^2_{n-1}. Here X_i is the measured Ra-226 (or Pb-210) activity for age category i,

$$\hat{\mu}=\sum(X_i/\sigma_i^2)/\sum(1/\sigma_i^2),$$

and σ_i^2 is the variance for age category i. If σ_i^2's are underestimated, then the $\hat{\chi}^2$ would be inflated. Z tests were carried out between the reagent blanks plus background, and background alone, for Po-209 and Po-210 measurements; a t-test was performed between the mean Rn-222 reagent blank plus background and the background alone.

Two sets of estimated Pb-210/Ra-226 ratios were calculated: one by using the mean Ra-226 activity from the four categories and the second by using the Ra-226 activity measured for each category. The delta method was used to determine the variance of the ratios (Seber, 1982).

The measured ratio of Pb-210/Ra-226 in the otoliths can be used to predict a radiometric age from the curve:

$$\frac{A_2}{A_1}=1-e^{-\lambda_2 t}+R^{*}e^{-\lambda_2 t}, \qquad (2)$$

where A_1 is the activity for Ra-226, A_2 and λ_2 are the activity and decay constant respectively (λ=ln (2)/half-life, for Pb-210, t is time (i.e. age), and R^* is the initial ratio of Pb-210/Ra-226 (Fig. 1, see also Kastelle [1991]). The initial ratio was estimated by solving Equation 2 for R^* and applying the activity ratio from age category 1 (R^*=-0.034). The negative value for R^* is due to measurement error. Therefore, we assume $R^* = 0$ in Figure 1. The actual fish ages were predicted from the radiometric ages by subtracting the time between collection and analysis (4.5 yr) from the radiometric ages in Figure 1. Alternatively, it is possible to calculate the radiometric ages by correcting the Pb-210 activity estimates to the time of otolith collection. These adjusted radiometric ages were then compared with ages read from the burnt otolith cross-section by the three readers. For each of the readers, a linear regression line was fit through the origin and compared with the 45° line of agreement by using a t-test.

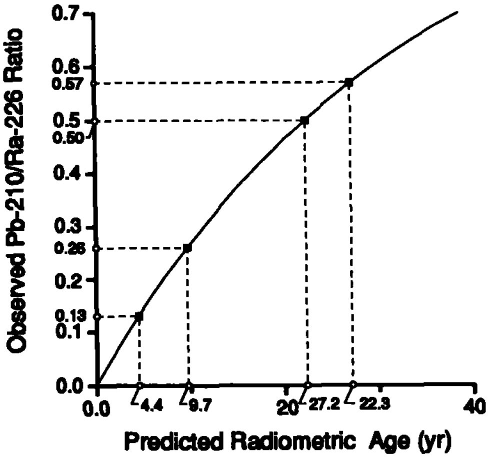

Figure 1

Predicted radiometric age of samples from observed activity ratios and curve of expected activity ratio (Eq. 2). Predicted radiometric age includes the 4.5 years between collection and analysis.

Results

ANOVA with contrasts showed the average age generated by the three age readers within each age category were all pair-wise significantly different ($P<0.001$). Reader 3 estimated the youngest ages, followed by Reader 1, and Reader 2 produced the highest ages (Table 1). Age categories were based on ages from Reader 1; therefore, the age ranges in each category for Readers 2 and 3 were greater.

The reagent blanks plus background for Po-210 and Po-209 did not contain significant activity above the background alone ($P>0.05$). The background activity for Po-209 and Po-210 ranged up to $6.413\times10^{-4} \pm 1.472\times10^{-4}$ cpm (counts per minute) and $5.600\times10^{-4} \pm 9.75\times10^{-5}$, respectively (Table 2). Therefore, the unadjusted sample data (including background counts) were reduced by the appropriate background only; no adjustment was made for the reagent blank. Specific activity of Pb-210 at the time of separation from Ra-226 ranged from 0.037 ±0.007 dpm/g in category 1 to 0.265 ±0.041 dpm/g in category 4 (Table 3). The Pb-210 activity levels were significantly different among the four age categories ($P<0.001$).

The average background count ($n=66$) for the Rn-222 system was $8.500\times10^{-3} \pm 5.27\times10^{-4}$ cpm. The mean reagent blank plus background for Rn-222 was not significantly greater than the background alone ($P=0.077$). Mean Ra-226 activity ($n=4$) was 0.414 ±0.050 dpm/g (Table 3). Ra-226 values from the four age categories were significantly different ($P<0.001$). Therefore, the Ra-226 measurements were specific to each age category.

The adjusted radiometric ages (from category specific Ra-226 measurements) for age categories 1 to 4 respectively were –0.09, 5.21, 17.83, and 22.66 years. Although the adjusted radiometric ages were consistently younger than the burnt cross-section ages from Reader 1, there was general agreement between the two methods (Fig. 2). A *t*-test between the slope of a line fit through the origin vs. the 45° line did not show a significant difference for any of the age readers ($P>0.05$).

Discussion

Principal findings

The principal result of this study is that the radiometric ages generally confirmed the burnt otolith ageing criteria that are used to age sablefish by U.S. and Canadian age readers. A factor which facilitated this confirmation is that sablefish otoliths apparently accumulate higher levels of radioisotopes than do other fish species that have been previously studied. We found the specific activities of Pb-210 and Ra-226 in sablefish otoliths (Table 3) to be a full order of magnitude greater than values reported in other species (Bennett et al., 1982; Campana et al., 1990; Fenton et al., 1991). These large differences in activity levels may be explained by biological and environmental considerations. Radium-226 is incor-

Table 1

For each age category of sablefish, *Anoplopoma fimbria*, comparison of average estimated age, number of samples read, age range by each age reader, and radiometric age are shown. The time between collection and analysis (4.5 yr) was subtracted from the radiometric age at the time of analysis to make the ages comparable.

Age category	First Reader: average age (yr), *n* read, range (yr)	Second Reader: average age (yr), *n* read, range (yr)	Canada (PBS): average age (yr), *n* read, range (yr)	Radiometric age: average age (yr), *n* pooled, range (yr)[1]
1	1, n=83, (1)	—	—	–0.09, n=83 (–0.89, 0.74)
2	9.79, n=130, (9, 11)	—	—	5.21, n=130 (3.40, 7.12)
3	18.91, n=141, (14, 23)	22.07, n=101, (14, 42)	15.58, n=139, (8, 27)	17.83, n=141 (13.05, 23.44)
4	28.55, n=127, (24, 34)	31.01, n=85, (17, 49)	23.75, n=127 (11, 48)	22.66, n=127 (16.50, 30.30)

[1] Based on measured Pb-210/Ra-226 ratio and error with individual Ra-226 measurements and Eq. 2.

Table 2

Comparison of background (Bk) and blank (BL) activity reported in the literature for tracer spikes, Po-210 in equilibrium with Pb-210, and Ra-226. NR = not reported

		Tracer spike (Po-209 or Po-208)	Po-210	Ra-226
This study	Bk (cpm)	$1.867 \times 10^{-4} \pm 5.63 \times 10^{-5}$ to $6.413 \times 10^{-4} \pm 1.472 \times 10^{-4}$	$2.353 \times 10^{-4} \pm 6.53 \times 10^{-5}$ to $5.600 \times 10^{-4} \pm 9.75 \times 10^{-5}$	$8.500 \times 10^{-3} \pm 5.27 \times 10^{-4}$
	Bl (dpm)	Not significant	Not significant	Not significant
Bennett et al. (1982)	Bk (cpm)	NR	6.94×10^{-4}	1.4×10^{-2} to 2.8×10^{-2}
	Bl (dpm)	NR	Not measurable above Bk	3.9×10^{-2}
Campana et al. (1990)	Bk (cpm)	NR	6.94×10^{-4}	NR
	Bl (dpm)	NR	5×10^{-4}	NR
Fenton et al. (1990)	Bk (cpm)	NR[1]	NR[1]	
	Bl (dpm)	NR	$1.03 \times 10^{-2} \pm 2.7 \times 10^{-3}$	$1.25 \times 10^{-2} \pm 4.3 \times 10^{-3}$
Fenton et al. (1991)	Bk (cpm)	6.94×10^{-4}	6.94×10^{-4}	1.4×10^{-3} to 3.5×10^{-3}
	Bl (dpm)	NR	$1.03 \times 10^{-2} \pm 2.7 \times 10^{-3}$	$2.55 \times 10^{-2} \pm 2.3 \times 10^{-3}$

[1] Not reported specifically for this radioisotope, but this author suggested a lower value here than reported by Bennett et al. (1982).

Table 3

For each age category of sablefish, *Anoplopoma fimbria*, number of specimens, average estimated age and estimated age range (from Reader 1), specific activity (dpm/gram) of Ra-226 and Pb-210, and the ratios Pb-210/Ra-226 and the ratios Pb-210/mean Ra-226, at the time of analysis 4.5 years after collection are shown. Errors are ±1 SD and are rounded up in the last significant digit for presentation.

Age category	Number pooled	Average age (yr)	Age range (yr)	Ra-226 (dpm/g)	Pb-210 (dpm/g)	Pb-210 Ra-226[1]	Pb-210 Ra-226[2]
1	83	1	1	0.288 ± 0.012	0.037 ± 0.007	0.128 ± 0.023	0.089 ± 0.019
2	130	9.79	9–11	0.517 ± 0.021	0.135 ± 0.022	0.260 ± 0.043	0.325 ± 0.065
3	141	18.91	14–23	0.386 ± 0.017	0.193 ± 0.030	0.500 ± 0.080	0.467 ± 0.092
4	127	28.55	24–34	0.465 ± 0.019	0.265 ± 0.041	0.570 ± 0.091	0.640 ± 0.126

[1] Activity ratios calculated with Ra-226 measured from each group.

[2] Activity ratios calculated with mean Ra-226: 0.414 ± 0.050.

porated into the biota or food chain through a variety of sources, such as water during the osmoregulation process, from food (Porntepkasemsan and Nevissi, 1990) such as phytoplankton (Shannon and Cherry, 1971) and zooplankton (Evans et al., 1938), and from contact with sediments (Swanson, 1985). The geographic area the species inhabits, its vertical distribution in the water column, and the efficiency of transfer of Ra-226 through its food chain could play a role in the uptake of Ra-226 (Cochran,

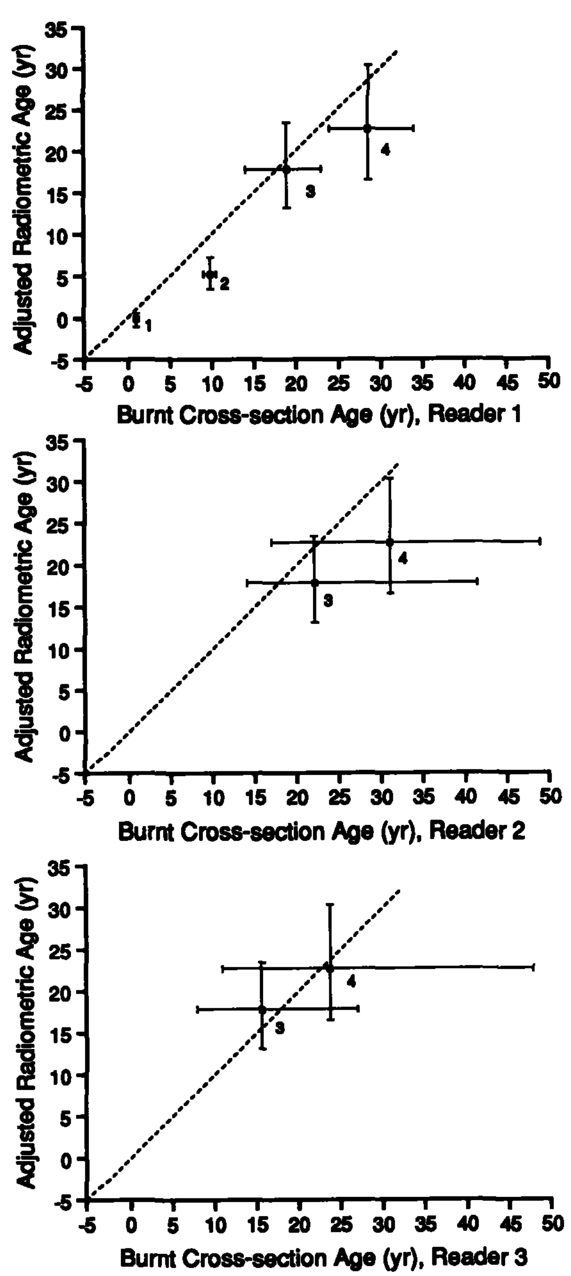

Figure 2

Comparison of burnt otolith cross-section ages (from Readers 1, 2, and 3) with adjusted radiometric ages (4.5 years were subtracted for the time between capture and analysis). Range in adjusted radiometric ages is 1 standard deviation, and the range in burnt otolith cross-section ages are the maximum and minimum in each age category (see Table 1). Ranges of burnt cross-section ages for Readers 2 and 3 are wider than for Reader 1 because age categories were defined by Reader 1 ages.

1982; Swanson, 1985; Fenton et al., 1990). Additionally, feeding rate, metabolic rate, and calcium deposition rate could all affect the specific activities found in otoliths. Sablefish are one of the fastest-growing epipelagic juvenile fishes (Shenker and Olla, 1986; Kendall and Matarese, 1987). Therefore, the higher Ra-226 and Pb-210 activity levels seen in sablefish otolith cores could be related to rapid uptake of Ra-226.

Assumptions for radiometric dating

We discussed three assumptions which must be satisfied for the radioisotope ageing of sablefish to be valid. Assumption 1, that otolith cores are closed with respect to loss or gain of any radioisotopes in the decay chain, has not been tested. Considering the decay chain containing Ra-226 (Eq. 1), Rn-222 is a source of concern. For Ra-226 to decay to Pb-210, it must first become Rn-222 (half-life=3.82 days) which is a noble gas. It is conceivable that Rn-222 could migrate in the otolith. A loss of Rn-222 would lead to underestimation of the true age. However, the calcium carbonate crystalline structure of the otolith probably prevents radioisotopes from migrating.[3]

Welden (1984) measured Pb-210 activity in the calcified cartilage of vertebral centrum from sharks, applying a procedure similar to the dating of sediments. The calcified cartilage allowed the Pb-210 to migrate in the vertebra so the assumption of a closed system did not hold true. Previous research by Goreau and Goreau (1960), Moore et al. (1973), Dodge and Thomson (1974), and Veeh and Burnett (1982) confirmed that calcium carbonate in coral acted as a chemically closed system. In Rn-222 diffusion experiments, Moore et al. (1973) could not detect migrations in coral.

For our study, Assumption 2 required that in the core of otoliths, initial ratios of the two radioisotopes be measured or known, and ideally be near zero. More Ra-226 than Pb-210 may be encountered by fish since Ra-226 is not in equilibrium with Pb-210 in seawater (Bacon et al., 1976). The environmental residence time of Ra-226 in seawater has been reported to be as high as 950 years (Szabo, 1967) and as low as 0.7 to 5.5 years (Shannon and Cherry, 1971). The environmental residence time of Pb-210 in surface waters has been reported to be as high

---

[3] At the "CSIRO, International workshop on otolith chemistry" 2–6 March 1992, in Hobart, Tasmania, Australia the possibility of Rn-222 migration was discussed with some enthusiasm. G. Fenton suggested that the otolith is relatively impermeable, and that Rn-222 migration is not a major problem.

as 3.5 years (Shannon et al., 1970) and as low as 1.4 years (Bacon et al., 1976). Pb-210 is incorporated into particulate matter whereby it is removed from the water column and deposited into sediments. To correct for Pb-210 incorporation, we measured the initial ratio, R^*, with young fish (1 yr olds). By analyzing very young splitnose rockfish, Bennett et al. (1982) found that the initial ratio of Pb-210/Ra-226 was between 0.1 and 0.2. Similar values for the initial ratio were also found in redfish (*Sebastes mentella*) (Campana et al., 1990), orange roughy (*Hoplostethus atlanticus*) (Fenton et al., 1991), and blue grenadier (*Macruronus novaezelandiae*) (Fenton et al., 1990). These results are comparable with the $R^*=0$ we used. The initial ratio cannot be estimated from older age categories because their true ages are uncertain. Therefore, $R^*=0$ for older age categories was assumed.

Assumption 3 states that the specific activity of Ra-226 incorporated into the otolith core be constant over the time span that the otoliths are receiving the radioisotopes. Assumption 3 is not required if only the otolith core is used and individual Ra-226 measurements are made for each age category. In sablefish the core of 1–year-old fish was appropriate because it is large, and there is a strong possibility that fish migration prior to maturity might cause a change in Ra-226 uptake. The likelihood ratio χ^2 test suggested that Ra-226 measurements differed among age categories. Therefore, ratios calculated by using individual Ra-226 measurements were preferred over those calculated with the Ra-226 mean. Campana et al. (1990), using cores composed of the first 5 years of growth, and a mean of 5 whole otolith samples (13 g per sample), found no significant difference in Ra-226 activity between the cores and whole otoliths. This suggested a constant rate of Ra-226 uptake. The differences we found between the Ra-226 activity measured in the four age categories were considerable (Table 3). Because the factors controlling Ra-226 uptake are complex and not well understood, the observed differences may well be real.

The use of whole otoliths requires modeling the mass growth rate of the otolith over the life of the fish and making assumptions concerning the uptake of Ra-226 and Pb-210 each year. Bennett et al. (1982) modeled the otolith's mass growth rate and assumed that the uptake of Ra-226 over the life span of the fish was proportional to otolith size (i.e. they assumed a constant rate of uptake). In whole otoliths from blue grenadier, the rate of uptake of Ra-226 changed over the life of the fish (Fenton et al., 1990). Therefore, the assumption of a constant rate of uptake of Ra-226 was violated. In whole otoliths from orange roughy, the Ra-226 specific activity increased with age (Fenton et al., 1991). Therefore, Fenton et al. (1991) used two Ra-226 averages: one for young age categories and a second for old age categories.

The conservative approach is to use the otolith core and individual category Ra-226 measurements making Assumption 3 unnecessary. Also, by using cores the closed system considered under Assumptions 1 and 2 is reduced. The behavior of otolith material deposited later in a fish's life need not be considered. The different approaches (otolith core, whole otolith, larger multi-gram samples, or any averaging of different age ranges) have trade-offs which should be evaluated in light of a species' biology.

Sources of error in ageing methodologies

Differences between the three sets of burnt cross-section ages are explainable. First, storage of broken-and-burnt otoliths in ethanol between readings may cause a fading of growth patterns. Second, variations in application of the otolith interpretation criteria of Beamish and Chilton (1982) and Beamish et al. (1983) could also lead to differences.

Even with the high specific activity (compared with other species) found in sablefish otoliths, the generally low activity levels of Po-210 and Ra-226 found in otoliths makes it important to carefully evaluate reagent blanks and background activities. Therefore, activity levels for reagent blank and background measurements reported in four previous studies (Bennett et al., 1982; Campana et al., 1990; and Fenton et al., 1990, 1991) were compared with those found here (Table 2). Considering the magnitude of errors, the Po-210 background we found was similar to other reported values (Table 2). The literature showed a much greater range of activities measured in the reagent blanks. Like Bennett et al. (1982), we found a nonsignificant activity level in the Po-210 reagent blank. The Po-210 reagent blank of 0.0103 ±0.0027 dpm reported by Fenton et al. (1990, 1991) seems high compared with the other findings. Also, we were the only study to report a nonsignificant Ra-226 reagent blank activity. Except for the Ra-226 reagent blank result, our background and reagent blank measurements for both Ra-226 and Pb-210 are in the same range as those reported in previous applications of radiometric ageing to fish.

The Ra-226 (or equivalent Rn-222) activity levels were low and difficult to measure. The signal to background ratio can be increased by using a greater sample weight. Fenton et al. (1991) argued that measuring Ra-226 by using barium co-precipitation with alpha spectroscopy (Sill, 1987) could also produce a better signal to background ratio. However, our measurements of Ra-226 background were similar to those of Fenton et al. (1991).

The low activity of Ra-226 and Pb-210 in the otoliths also requires that care be taken to avoid contamination. The otoliths were stored in 50% ethanol at sea. The grade of the ethanol was not ultra-pure which could introduce contaminating radioisotopes. Ethanol may also leach out some of the radioisotopes during storage. Some previous fish age-validation studies using Pb-210/Ra-226 (Fenton et al., 1990) stored the otoliths dry with desiccated adhering tissues. The dry tissue proved very difficult to remove and introduced Po-210 contamination. We relied on ultrasonic cleaning to break up any soft hydrated tissue after which the otoliths were rinsed thoroughly. This appeared to remove any organic material on the surface of the otolith. Contamination by Po-210 in the adherent tissues would increase the estimated radiometric age. In our study, the results from age category 1 indicate that this was not the case. Also, the grinding paper used could also introduce contamination.

Conclusions

The goal of this study was to validate the break-and-burn otolith ageing criteria used for sablefish at the AFSC. Although usually lower, the radiometric ages were within two standard deviations of the break-and-burn ages (Table 1; Fig. 2). If a range of possible ages occurred in the break-and-burn method, the older age was usually chosen. Since radiometric ages were consistently lower than burnt cross-section ages (from Reader 1), this method was probably not the best way to interpret the broken-and-burnt otolith. Possible migration of Rn-222 from the otolith, as discussed earlier, could also explain the difference.

Nevertheless, the break-and-burn method of age determination for sablefish has been validated by this study for fish up to 34 years old. This is the maximum age regularly seen in commercial or research catches. We have shown that the break-and-burn ageing criteria applied to sablefish otoliths produces, on average, ages similar to radiometric ages.

Acknowledgments

We thank age readers from the Pacific Biological Station, Canadian Department of Fisheries and Oceans, and the Alaska Fisheries Science Center, NMFS, for providing sablefish ages. We thank the Scientific Editor and three anonymous reviewers for comments which led to significant improvements in the paper.

Literature cited

Bacon, M. P., D. W. Spencer, and P. G. Brewer.
1976. ^{210}Pb/^{226}Ra and ^{210}Po/^{210}Pb disequilibria in seawater and suspended particulate matter. Earth Planet. Sci. Lett. 32:277–296.

Beamish, R. J., and D. E. Chilton.
1982. Preliminary evaluation of a method to determine the age of sablefish (*Anoplopoma fimbria*). Can. J. Fish. Aquat. Sci. 39:277–287.

Beamish, R. J., G. A. McFarlane, and D. E. Chilton.
1983. Use of oxytetracycline and other methods to validate a method of age determination for sablefish. *In* Proceedings of the second Lowell Wakefield fisheries symposium, Anchorage, AK, p. 95–116. Alaska Sea Grant Report 83-8.

Bennett, J. T., G. W. Boehlert, and K. K. Turekian.
1982. Confirmation of longevity in (*Sebastes diploproa*) (Pisces: Scorpaenidae) from ^{210}Pb/^{226}Ra measurements in otoliths. Mar. Bio. 71:209–215.

Bracken, B. E.
1983. Sablefish migration in the Gulf of Alaska based on tag recoveries. *In* Proceedings of the second Lowell Wakefield fisheries symposium, Anchorage, AK, p. 185–190. Alaska Sea Grant Report 83–8.

Campana, S. E., and J. D. Neilson.
1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42:1014–1032.

Campana, S. E., K. C. T. Zwanenburg, and J. N. Smith.
1990. ^{210}Pb/^{226}Ra determination of longevity in redfish. Can. J. Fish. Aquat. Sci. 47:163–165.

Cochran, J. K.
1982. The oceanic chemistry of the U- and Th-series nuclides. *In* M. Ivanovich and R. S. Harmon (eds.), Uranium series disequilibrium: applications to environmental problems, p. 384–430. Oxford Univ. Press, NY.

Dodge, R. E., and J. Thomson.
1974. The natural radiochemical and growth records in contemporary hermatypic corals from the Atlantic and Caribbean. Earth Planet. Sci. Lett. 23:313–322.

Evans, R. D., A. F. Kip, and E. G. Moberg.
1938. The radium and radon content of Pacific Ocean water, life, and sediments. Am. J. Sci. 36:241–259.

Faure, G.
1986. Principles of isotope geology. John Wiley and Sons, New York, NY, 589 p.

Fenton, G. E., D. A. Ritz, and S. A. Short.
1990. ^{210}Pb/^{226}Ra disequilibria in otoliths of blue grenadier, *Macruronus novaezelandiae*: problems associated with radiometric ageing. Aust. J. Mar. Freshwater Res. 41:467–473.

Fenton, G. E., S. A. Short., and D. A. Ritz.
1991. Age determination of orange roughy, *Hoplostethus atlanticus* (Pisces: Trachichthyidae)

using ^{210}Pb:^{226}Ra disequilibria. Mar. Bio. 109:197–202.

Goreau, T. F., and N. I. Goreau.
1960. The physiology of skeleton formation in corals. IV: On isotopic equilibrium exchanges of calcium between corallum and environment in living and dead reef-building corals. Biol. Bull. Mar. Biol. Lab., Woods Hole 119:416–427.

Heyraund, M., and R. D. Cherry.
1979. Polonium-210 and lead-210 in marine food chains. Mar. Biol. 52:227–236.

Hunter, J. R., B. J. Macewicz, and C. A. Kimbrell.
1989. Fecundity and other aspects of the reproduction of sablefish (*Anoplopoma fimbria*) in central California waters. CalCOFI Rep. 30:61–72.

Kastelle, C. R.
1991. Radioisotope age validation and an estimate of natural mortality from the gonad to somatic weight index for sablefish (*Anoplopoma fimbria*). M.S. thesis, Univ. Washington, School of Fisheries, 87 p.

Kendall, A. W., and A. C. Matarese.
1987. Biology of eggs, larvae, and epipelagic juveniles of sablefish (*Anoplopoma fimbria*) in relation to their potential use in management. Mar. Fish. Rev. 49(1):1–13.

Kimura, D. K., and J. J. Lyons.
1991. Between-reader bias and variability in the age-determination process. Fish. Bull. 89:53–60.

Lucas, H. F.
1957. Improved low-level alpha scintillation counter for radon. Rev. Sci. Instruments 28(9): 680–683.

McFarlane, G. A., and R. J. Beamish.
1983. Preliminary observations on the juvenile biology of sablefish (*Anoplopoma fimbria*) in waters off the west coast of Canada. *In* Proceedings of the second Lowell Wakefield fisheries symposium, Anchorage, AK, p. 119–135. Alaska Sea Grant Report 83-8.

Mason, J. C., R. J. Beamish, and G. A. McFarlane.
1983. Sexual maturity, fecundity, spawning, and early life history of sablefish (*Anoplopoma fimbria*) off the Pacific coast of Canada. Can. J. Fish. Aquat. Sci. 40:2126–2134.

Moore, W. S., S. Krishnaswami, and S. G. Bhat.
1973. Radiometric determinations of coral growth rates. Bull. Mar. Sci. 23(2):157–176.

Mugiya, Y.
1977. Effect of acetazolamide on the otolith growth of goldfish. Bull. Jpn. Soc. Sci. Fish. 43(9):1053–1058.

Porntepkasemsan, B., and A. E. Nevissi.
1990. Mechanism of radium-226 transfer from sediments and water to marine fishes. Geochem. J. 24:223–228.

Sarmiento, J. L., D. E. Hammond, and W. S. Broecker.
1976. The calculation of the statistical counting error for ^{222}Rn scintillation counting. Earth Planet. Sci. Lett. 32: 351–356.

Seber, G. A. F.
1982. The estimation of animal abundance and related parameters. Macmillan, New York, NY, 654 p.

Shannon, L. V., and R. D. Cherry.
1971. Radium-226 in marine phytoplankton. Earth Planet. Sci. Lett. 11:339–343.

Shannon, L. V., R. D. Cherry, and M. J. Orren.
1970. Polonium-210 and lead-210 in the marine environment. Geochim. Cosmochim. Acta 34:701–711.

Shenker, J. M., and B. L. Olla.
1986. Laboratory feeding and growth of juvenile sablefish, *Anoplopoma fimbria*. Can. J. Fish. Aquat. Sci. 43:930–937.

Sill, C. W.
1987. Determination of Radium-226 in ores, nuclear wastes and environmental samples by high-resolution alpha spectrometry. Nucl. Chem. Waste Management 7:239–256.

Smith, J. M., R. Nelson, and S. E. Campana.
1991. The use of Pb-210/Ra-226 and Th-228/Ra-228 dis-equilibria in the ageing of otoliths of marine fish. *In* P. J. Kershaw and D. S. Woodhead (eds.), Radionuclides in the study of marine processes, p 350–359. Elsevier Applied Science, England.

Swanson, S. M.
1985. Food-chain transfer of U-series radionuclides in a northern Saskatchewan aquatic system. Health Phys. 49(5):747–770.

Szabo, B. J.
1967. Radium content in plankton and sea water in the Bahamas. Geochim. Cosmochim. Acta 31:1321–1331.

Veeh, H. H., and W. C. Burnett.
1982. Carbonate and phosphate sediments. *In* M.Ivanovich and R. S. Harmon (eds.), Uranium series disequilibrium: applications to environmental problems, p. 459–480. Oxford Univ. Press, NY.

Welden, B. A.
1984. Radiometric verification of age determination in elasmobranch fishes. M.S. thesis, Calif. State Univ., Hayward, Moss Landing Marine Labs, 108 p.