1993

Age determination and confirmation from otoliths of the bank rockfish, *Sebastes rufus* (Scorpaenidae)

Diana L. Watters
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DOI: <https://doi.org/10.31979/etd.5kmw-3ns4>
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rockfish, *Sebastes rufus* (Scorpaenidae)**

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San Jose State University, 1993

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Ann Arbor, MI 48106

AGE DETERMINATION AND CONFIRMATION FROM OTOLITHS
OF THE BANK ROCKFISH, *SEBASTES RUFUS* (SCORPAENIDAE)

A Thesis

Presented to

The Faculty of Moss Landing Marine Laboratories
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by

Diana L. Watters

December, 1993

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ABSTRACT

AGE DETERMINATION AND CONFIRMATION FROM OTOLITHS OF THE BANK ROCKFISH, *SEBASTES RUFUS* (SCORPAENIDAE)

by Diana L. Watters

Methods for determining and confirming age for *Sebastes rufus* from otoliths were examined. The periodicity of growth zone formation and of calcium deposition in otoliths was studied to verify ages estimated from counts of otolith growth zones; results were inconclusive.

Pb-210/Ra-226 activity ratios were measured in otoliths to confirm ages estimated from counts of otolith growth zones. Radiometric ages agreed well with growth zone counts, giving substantial validity to them and suggesting longevity of 50 years or more *Sebastes rufus*.

Otolith length, width, area, perimeter and weight were evaluated as criteria for determining age. All parameters were highly correlated with growth zone counts, with otolith weight showing the most promise as an age predictor.

Von Bertalanfy growth parameters were $L_{\infty}=488$, $K=0.048$, $t_0=-8.372$ for 81 males and $L_{\infty}=594$, $K=0.039$, $t_0=-6.96$ for 86 females. *Sebastes rufus* are long-lived, slow growing and reach reproductive maturity at 10-15 years of age.

ACKNOWLEDGMENTS

This work is a result of research sponsored in part by NOAA, National Sea Grant College Program, Department of Commerce, under project numbers R/NP-1-20D and R/F-113, through the California Sea Grant College Program, and in part by the California State Resources Agency.

Otoliths were provided by Bill Lenarz, of the National Marine Fisheries Service, and Frank Henry, of the California Department of Fish and Game. Milton Love, UC Santa Barbara, also donated his collection of "otoliths from hell".

Special thanks to my committee members, Greg Cailliet, Kenneth Coale and Ralph Larson, for their guidance and support; Mary Yoklavich, "otolith queen", for invaluable guidance and encouragement; Ken Severin, who made the electron microprobe x-ray analysis portion of my study possible; Donna Kline, Kenneth Coale, Ken Bruland and Ross Williams who made the radiometric ageing portion of my study possible; Lucy Wold and Kate Hutchison, who understood the "working girl's dilemma" and were supportive every step of the way.

Finally, I thank the students and staff of Moss Landing Marine Laboratories for creating a truly unique learning environment which I was fortunate to experience.

TABLE OF CONTENTS

	Page
Acknowledgments	iv
List of Figures	vi
List of Tables	viii
Introduction	1
Rockfishes	1
Bank rockfish	1
Age Determination	2
Otoliths	3
Verification and Validation of Age Estimates	5
Present Study	7
Methods	9
Sample Collection	9
Opaque and Translucent Zone Formation	9
Microprobe Analysis	10
Radiometric Age Analysis	12
Traditional Otolith Ageing	14
Otolith Preparation	15
Pb-210 Analysis	16
Ra-226 Analysis	17
Pb-210/Ra-226 Age Determination	18
Otolith Morphometrics and Weight as Estimators of Age	19
Growth Curves	20
Results	20
Opaque and Translucent Zone Formation	20
Microprobe Analysis	21
Radiometric Age Analysis	21
Otolith Morphometrics and Weight as Estimators of Age	22
Growth Curves	23
Discussion	23
Opaque and Translucent Zone Formation	23
Microprobe Analysis	25
Radiometric Age Analysis	28
Otolith Morphometrics and Weight as Estimators of Age	32
Growth Curves	34
Summary and Recommendations	36
Literature Cited	38
Appendix A: Sample Preparation for Radiometric Analysis	58
Appendix B: Pb-210/Ra-226 Error Calculation	59

LIST OF FIGURES

Number		Page
1	<i>Sebastes rufus</i> otolith cross section relative to its position in the inner ear. Age was estimated from counts of growth zones in the dorsal region; opaque and translucent zone formation was examined along the dorso-proximal margin; electron microprobe x-ray analysis for Ca was conducted at points along the dorso-ventral axis	44
2	Number of opaque and translucent margins observed by month	45
3	Sample 5105: otolith section analyzed for Ca along the dorso-ventral axis with a wavelength dispersive electron microprobe at 25 micron and 6.25 micron intervals. Data from the 6.25 micron interval are smoothed with a 5 point running average. The specimen is a male, 270 mm TL, aged 7+ yrs. from growth zone counts	46
4	Sample 5104: otolith section analyzed for Ca along the dorso-ventral axis with a wavelength dispersive electron microprobe at 25 micron and 6.25 micron intervals. Data from the 6.25 micron interval are smoothed with a 5 point running average. The specimen is a female, 290 mm TL, aged 6+ yrs. from growth zone counts	47
5	Sample 5105: wavelength dispersive electron microprobe data for Ca analyzed at 25 micron intervals. Error bars represent ± 1 s.d. based on counting statistics	48
6	Sample 5104: wavelength dispersive electron microprobe data for Ca analyzed at 25 micron intervals. Error bars represent ± 1 s.d. based on counting statistics	49
7	Radiometric ageing results for otolith core samples representing 'young', 'medium' and 'old' fish. Points represent observed Pb-210/Ra-226 activity ratios plotted against mean growth zone counts for each sample. The curve represents expected Pb-210/Ra-226 activities over time. Error bars represent ± 1 s.d..	50

Number		Page
8	Age (growth zone counts) plotted against otolith length, width, perimeter, area and weight for male and female <i>Sebastes rufus</i>	51
9	Von Bertalanffy growth curve of male and female <i>Sebastes rufus</i> . Parameter estimates, followed by 95% confidence limits in parentheses, are shown in the inset	54

LIST OF TABLES

Number		Page
1	Summary of 'young', 'medium' and 'old' pooled otolith core samples from <i>Sebastes rufus</i> used for Pb-210 analysis	55
2	Results of radiometric ageing of 'young', 'medium' and 'old' otolith core samples from <i>Sebastes rufus</i> . Observed Pb-210/Ra-226 activity ratios (dpm/g), radiometric age (yrs.), average age (yrs.) from growth zone counts, and expected Pb-210/Ra-226 activity (dpm/g)	56
3	Equations describing the relationship between otolith parameters and age (growth zone counts) for male and female <i>Sebastes rufus</i> , and their corresponding coefficients of determination, F statistics and probabilities	57

Introduction

Rockfishes

Many species in the rockfish genus *Sebastes* are long-lived and slow growing, with some reaching confirmed ages in excess of 60 years (Beamish 1979, Archibald et al. 1981, Bennett et al. 1982, Beverton 1986, Beamish and McFarlane 1987, Campana et al. 1990, Leaman 1991). Over 60 species of this genus occur in the northeast Pacific (Gulf of Alaska to Baja California), and many are important to commercial and sport fisheries (Lea 1992). Rockfishes inhabit a wide variety of habitats including nearshore kelp beds, rocky bottom and offshore soft bottom. Some species are mid-water schoolers while others are benthic and solitary. All are predators and viviparous.

Bank Rockfish

The bank rockfish, *Sebastes rufus*, is one of 59 species of this genus that occur off the coast of California. Miller and Lea (1972) reported the species' range from central Baja California north to the Mad River, California, at water depths of 31 to 247 meters. In recent years it has appeared in Oregon commercial landings (Neil T. Richmond, Oregon Department of Game and Fish, pers. comm.), and has been caught at depths up to 400 meters off California (California Department of Fish and Game (CDFG), unpublished commercial landing data). It occurs over mud and sand

bottom in schools, and is often caught with the dark blotched rockfish, *S. crameri*.

Since the early 1970's, with the expansion of commercial trawling and gill netting into deeper waters, bank rockfish have become an increasingly important component of the California commercial rockfish fishery. From 1981 through 1987 this species ranked among the top five rockfish species landed in central California (Morro Bay to San Francisco Bay), averaging 1,075,120 pounds annually (CDFG, unpublished commercial landing data).

Little published information exists for this species. First described by Eigenmann and Eigenmann (1890), it is considered to be closely related to the widow rockfish (*S. entomelas*) and speckled rockfish (*S. ovalis*), and was once synonymized with the latter by Phillips (1957). Love et al. (1990) and Wyllie Echeverria (1987) included *S. rufus* in their studies of rockfish life histories. Both authors reported difficulties in ageing the species, with only Wyllie Echeverria (1987) presenting limited age-specific information.

Age Determination

The importance of accurate age determination to the proper management of a population is well known. Basic demographic information such as growth rate, natural mortality, age-specific fecundity, age at first maturity, and longevity can be determined from a population's age struc-

ture, and used in models to predict acceptable harvest yields. Ages can be determined through size frequency analysis of species that are fast growing and short lived, or by studying growth in captivity or in the field. The most common method of age determination involves the time-consuming practice of ageing individuals by counting growth zones deposited in calcified structures such as scales, cleithra, spines, vertebral centra, fin rays and otoliths.

For a given species, one or more of these structures reveal an optical pattern of alternating opaque and translucent zones of growth, which are assumed to form annually. Interpreting these zones, however, can be difficult and subjective, especially for older fish (Chilton and Beamish 1982, Boehlert and Yoklavich 1984). This can be due to irregularities in growth, the faintness of growth zones, or the presence of sub-annual growth patterns (Panella 1971), leaving which zones to count as annuli up to the subjective interpretation of the reader (Williams and Bedford 1974).

Otoliths

Otoliths have been used to age rockfishes for many years (Six and Horton 1977, Kimura et al. 1979, Chilton and Beamish 1982). Three pairs of otoliths exist in the inner ear of fishes; the largest of these, the saggita, is most commonly used in ageing studies. They are composed of aragonite (calcium carbonate) which is accreted in an organic matrix composed of a unique protein called otolin (Degens

et al. 1969). Although bone tissue in most teleosts is acellular (lacking enclosed osteocytes), and calcium metabolism and the mechanism for mineralization is poorly understood (Moss 1963, 1961, Pannella 1980), there is little evidence that resorption commonly occurs in otoliths (Simkiss 1974, Campana and Neilson 1985). Thus, they may represent a permanent record of fish growth over time.

Opaque and translucent zones are visible on the distal surface of whole otoliths, and can be used to age young fish. With time, however, the axis of growth often changes and the otolith thickens, as more material is deposited proximally (Panella 1980). It is necessary to view a dorsal-ventral cross section of the otolith through the nucleus to reveal additional growth zones. Zones can be elucidated by grinding thin sections or by breaking the otolith in half and burning the broken surface over an alcohol flame (Christensen 1964). The latter technique enhances contrast by burning the protein layer in the translucent zone. When compared, maximum ages determined from thin sections or the break and burn method are greater than those determined from the surface of whole otoliths (Beamish 1979, Archibald et al. 1981, Chilton and Beamish 1982, Campana et al. 1990), and it is now standard procedure to use these methods in rockfish ageing studies. The decision about which zones to count as annuli, however, is still left up to the subjective interpretation of the otolith reader.

Verification and Validation of Age Estimates

The subjectivity and associated errors involved in determining age from calcified structures has created a need for more objective methods. Recently, the importance of verification (measuring precision), and, more importantly, validation (measuring accuracy) of ageing methods has been emphasized (Brothers 1983, Cailliet et al. 1986, Cailliet 1990). Both require the use of an independent, objective method of determining age which should, ideally, verify or validate all ages. Beamish and McFarlane (1983) found that although 65% of the papers that they reviewed had mentioned or attempted validation of ageing methods, less than 3% had successfully done so.

Invalid age estimates can lead to errors in predicting sustainable yield levels based on population models. The collapse of the Pacific ocean perch, *Sebastes alutus*, fishery is an example. Based on ages determined from the surface of whole otoliths, this species was thought to be short lived and fast growing. Ages determined from otolith cross sections revealed that this species lived twice as long and had a much lower natural mortality rate than previously thought (Beamish 1979, Archibald et al. 1981).

Several methods have been used to verify or validate rockfish age determinations. Mark-recapture studies have been used for species that are less susceptible to barotrauma.

ma (injury due to the expansion of gases with decrease in pressure). Leaman and Nagtegaal (1987) tagged yellowtail rockfish, *Sebastes flavidus*, and injected them with oxytetracycline, which leaves a fluorescent mark in bone. Upon recapture, they found that the number of opaque and translucent zones formed beyond this mark in otoliths agreed with the number of years the fish had been at liberty. Using this method, they were able to validate ages determined with the break and burn method for up to 35 years in this species.

Analysis of the timing of opaque and translucent zone formation along the otolith margin has been used as supporting evidence for their annual formation, but the success of this method has been limited to young fish. Using this method, ages have been verified for the widow rockfish, *Sebastes entomelas*, up to 10 years (Lenarz 1987), for the Atlantic redfish, *Sebastes marinus* to 7 years (Mayo et al. 1981), and for *S. constellatus*, *S. elongatus*, *S. rosenblatti*, *S. saxicola* and *S. semicinctus* to about 6 years (Love 1990).

Electron microprobe X-ray analysis provides an objective tool for measuring the elemental composition of opaque and translucent zones and detecting variations in elements deposited over time. The analysis is unique in that the distribution and concentration of elements can be measured in an intact specimen; thus, the nature of opaque and

translucent zones with respect to calcium and other elements can be measured directly. Coupling this information with seasonal changes in variables such as temperature, food availability, or reproduction, the periodicity of deposition can be determined and age confirmed. This tool has been used to verify ages estimated from elasmobranch centra (Jones and Geen 1977, Cailliet and Radtke 1987) and from cleithra of northern pike *Esox lucius* (Casselman 1987). Casselman (1987) concluded that the translucent zone is more heavily mineralized than the opaque zone, and that a pattern of calcium concentration, analogous to growth patterns, could be used to verify age interpretations.

Bennett et al. (1982) first used the naturally occurring radionuclides Pb-210 and Ra-226 to radiometrically date otolith samples which had been aged from cross sections. This study confirmed ages to 80 years for the splitnose rockfish, *Sebastes diploproa*. Since then, several authors have attempted to use this technique to validate ages for elasmobranchs (Welden et al. 1987), for the Atlantic redfish, *Sebastes mentella* (Campana et al. 1990), the blue grenadier, *Macruronus novaezelandiae* and orange roughy, *Hoplostethus atlanticus* (Fenton et al. 1990, 1991) and for sablefish, *Anoplopoma fimbria* (Kastelle 1991).

Present Study

Of the aforementioned age verification and validation methods, mark-recapture and captive rearing of bank rockfish

are not feasible because the depth at which this species occurs makes it susceptible to barotrauma and unlikely to survive being brought to the surface.

This study examines objective methods for testing the validity of ages determined from otolith cross sections and for determining age from otoliths for *Sebastes rufus*. Age verification is attempted through examination of opaque and translucent zone formation and microprobe analysis; radiometric dating is used as a validation method, as part of a collaborative effort with Kline (in prep.). In addition, otolith morphometrics and weight are evaluated as objective predictors of age, and growth curves are presented for the species.

The questions addressed in this study were:

1. Do opaque and translucent growth zones form annually? In which season does each form?
2. Does a pattern in any element exist across growth increments which can be used to independently assess age?
3. Do ages determined from otolith cross sections agree with ages determined radiometrically?
4. Can otolith morphometrics and/or weight be used to predict age as determined from growth zones?
5. Can the von Bertalanffy growth function be fit to *Sebastes rufus* age at length data?

Methods

Sample Collection

Sebastes rufus otoliths and accompanying data, collected at ports from Morro Bay to Eureka, were provided by the California Department of Fish and Game and the National Marine Fisheries Service, from a cooperative sampling program of commercial groundfish landings. Landings are subsampled by randomly selecting 50 pound "clusters" from each market category. Species, total length (TL), sex, port of landing, and the Fish and Game block number and depth at which the catch occurred were recorded. Sagittal otoliths were removed, cleaned with water and stored dry in coin envelopes. Beginning in 1984, extrudable larvae from females was also noted. Additional otoliths were provided by Dr. Milton Love, of the University of California, Santa Barbara. These were collected from Santa Barbara area fish markets and were used to supplement the sample size used to assess opaque and translucent zone formation.

Opaque and Translucent Zone Formation

Otolith sections from fishes caught throughout the year were examined along the dorso-proximal margin (Figure 1) to determine if opaque and translucent zones are formed during different seasons. Because otolith margin type (opaque or translucent) was difficult to discern from fishes larger than 310 mm TL, only otoliths from fishes equal to or less

than this size were examined.

Otoliths were cast in fiberglass resin (Evercoat Casting resin) and mounted on wood chucks. A 0.6 mm section was cut dorso-ventrally through the nucleus with a Buehler Isomet low speed diamond blade saw. Sections were then mounted on slides and ground on one side with 600 grit paper and water on a Buehler Ecomet grinder to ca. 0.3 mm thickness or when zones were clearly discernible. They were then immersed in water on a black background and examined along the dorso-proximal margin using a dissecting microscope at 25x-50x and fiber optic reflected light. The date of capture was unknown during readings to prevent bias. Margins were determined to be opaque or translucent when two independent readings by the author agreed.

Microprobe Analysis

Otoliths from small fishes were also used in this analysis because otolith growth zones are broader during the first years of growth. This makes them easier to interpret visually, allows for fewer analysis points, and simplifies the comparison between growth zones and analysis data.

Otoliths were cast in fiberglass resin (Evercoat Casting Resin) and sectioned as above. Microprobe analysis requires a flat and highly polished sample surface to prevent X-ray resorption and analytical error caused by topography. Therefore, sections were mounted on petrographic slides with Super Glue and polished in two steps: 1) by hand

on glass with a slurry of water and 12 micron diameter AlO_2 disks (WCA3 Microgrit, Micro Abrasives Corp.); and 2) on a polishing wheel with a glass interface, silk (Buehler selected silk polishing cloth) and a slurry of water and 0.05 micron Buehler Gamma Alumina Micro-polish. A thin layer of carbon (250-300 Angstroms) was applied with a Polaron E5600 carbon sputter coater to increase electron conductivity and to prevent specimen charging.

A Cameca SX 50 microprobe was used for elemental analysis. The instrument focuses a beam of electrons on the sample surface, causing the elements present to emit X-rays with characteristic energy and wavelength. The wavelength and intensity (counts) of X-rays is measured by wavelength dispersive detectors, resulting in an atomic weight percent value for each element detected. Elements with atomic weights of 10 (sodium) or less cannot be reliably measured because their x-ray energies are too low for detection. The system was calibrated for calcium with a calcite standard (USNM 136321, Jarosewich and MacIntyre 1983); intrinsic variation in calibration counts was 1% for calcium. Operating voltage was 15 kev.

A qualitative analysis using a 50 micron beam diameter and 30 nA beam current was performed to detect elements present. For elements with atomic weights greater than 10, calcium was the only element present in sufficient amount to be reliably measured, based on this analysis.

Approximately 20 preliminary scans across several sections were performed to determine the best axis for analysis, and the optimal beam current, beam size and count time to use. The dorso-ventral otolith axis was chosen for analysis due to the greater width and visibility of growth zones in this area.

Due to time and cost constraints, only two sections were analyzed quantitatively for calcium composition. Densities of this element were measured at equally spaced points along the entire dorso-ventral axis (Figure 1) of each section under two operating conditions: 1) at 25 micron intervals, using a 20 micron beam diameter, 10 nA beam current and 10 second count rate; and 2) At 6.25 micron intervals, using a 5 micron beam diameter, 4 nA beam current and 10 second count rate.

Radiometric Age Analysis

Radiometric ageing of bank rockfish otoliths was conducted in collaboration with Kline (in prep.), and served as a precursor for her further work on two species of thorny-head, *Sebastolobus altivelus* and *Sebastolobus alascanus*.

The radiometric dating method used in this study is based on the incorporation of the naturally occurring radio-nuclide Ra-226 (half life 1622 yrs.) into otolith material (Porntepkasemsan and Nevissi 1990) and its subsequent decay to daughter product Pb-210 (half life 22.3 yrs.), as part of

the U-238 decay series:

Ra-226 (1622 yrs.) ---> Rn-222 (3.8 days) ---> Po-218 (3.1 min.) --->
Pb-214 (26.8 min.) ---> Bi-214 (19.7 min.) ---> Po-214 (< 1 sec.) --->
Pb-210 (22.3 yrs.) ---> Bi-210 (5 days) ---> Po-210 (138.4 days) --->
Pb-206 (stable)

Because of the short half lives of the intermediary radionuclides involved in the series, Ra-226 can be thought of as decaying directly to Pb-210.

Ra-226 exists in seawater, is water soluble, and acts as bio-geochemical analog for calcium (Porntepkasemsan and Nevissi 1990, Smith et al. 1992). It takes about 120 years for the activity of Pb-210 to reach secular equilibrium with its parent Ra-226 (a daughter/parent activity ratio of 1), thus the pair is suitable for ageing long-lived fishes such as *Sebastes*. Age is determined based on the ratio of Pb-210 to Ra-226 activities.

Bennett et al. (1982) and Fenton et al. (1990) made the following assumptions when radiometrically ageing whole otoliths: 1) the uptake of Ra-226 is constant over time; 2) Ra-226 is incorporated into a closed system (there is no loss or exchange of parent or daughter products with tissue, fluids or seawater); and 3) uptake of Pb-210 from outside is minimal.

The first assumption must be made when whole otoliths are used because Pb-210 activity will differ appreciably

with newly accreted material, and the mass growth of the otolith must be included in the analysis to compensate for this (Bennett et al. 1982, Fenton et al. 1990). Campana et al. (1990) were the first to avoid some of the potential errors associated with this assumption by using core material representing the first five years of growth for their analysis. In the present study otolith cores were also used. The second assumption is supported by the fact that otoliths are acellular, housed within a membrane in the auditory bullae, and that unlike other fish bone, osteolysis in otoliths is not known to occur commonly (Simkiss 1974, Campana and Neilson 1985). The third assumption can be addressed by measuring activity ratios from otoliths of very young fish.

The extremely low activity of Ra-226 present in otoliths (<1.0 dpm/g) requires the elimination of any sources of contamination during collection, processing and analysis. To avoid potential sources of error, trace metal clean techniques were used to prepare otoliths for analysis, after Fabry and Delaney (1989) and Linn (1988) (see Appendix A).

Traditional Otolith Ageing

Otoliths from fishes caught in 1989 and 1987 were aged to test the validity of break and burn and section age determination methods, which are both commonly used in present day rockfish ageing studies. In most cases, growth

zone counts were made from the left otolith of each pair, and the right otolith was used for radiometric ageing. Otoliths from 1989 were aged using the less time-consuming break and burn method as described by Christensen (1964) and Chilton and Beamish (1982). Each broken and burned otolith half was placed in modeling clay with the burnt surface facing upward and the surface was brushed with mineral oil to enhance the contrast of growth zones. Otoliths from 1987 were sectioned and ground as mentioned above, and immersed in water on a black background to enhance the contrast of growth zones. For both methods, growth increments were interpreted and counted in the dorsal region (Figure 1) under a dissecting microscope using reflected light at 25x to 50x magnification. For each specimen, counts were made independently by the author up to three times if the first two did not agree; if none of the three counts agreed, the middle one was used. Crystallized or otherwise unreadable otoliths were not used.

Otolith Preparation

To minimize the error associated with whole otoliths (Bennett et al. 1982), a sectioned core encompassing only the first five years of growth was used for Pb-210 analysis, after Campana et al. (1990). Core dimensions were determined by averaging the length (mm), width (mm) and thickness (mm) of the first five years of growth in twenty three otoliths ($\bar{x}$ =7.0 s.d.= 0.8, $\bar{x}$ =3.7 s.d.=0.3, $\bar{x}$ =0.8 s.d.=0.07,

respectively). To remove cores, otoliths were cast in small ice cube trays with LR White histological mounting medium and attached distal side up to a cardboard price tag with more LR White. Cores were cut from the center of each otolith with a Buehler Isomet diamond blade saw; dimensions were marked on the tag to aid in cutting. The mounting medium was then removed from each core with a razor blade, and the sulcus was ground on 600 grit paper to the required thickness.

A minimum of one gram (ca. 20 cores) of material was required for Pb-210 analysis. Therefore, it was necessary to pool cores into 'young', 'medium', and 'old' age categories as determined from growth zone counts in order to adequately measure Pb-210 activity.

Samples were then subjected to a series of cleaning steps to remove surface contamination (see Appendix A) and were kept dust free in acid washed containers at all times. After cleaning, they were oven dried at 30°C for at least 12 hours and weighed to the nearest milligram.

Pb-210 Analysis

Pb-210 activity was measured via its alpha-emitting daughter product Po-210 (half life 138.4 days), using an alpha spectrometer calibrated by Kline (in prep.). Each sample was dissolved in 8N HNO₃ acid and spiked with 0.25 ml Po-208 (0.4 dpm) as a yield tracer for Po-210 (Appendix A). The isotopes in each sample solution were then plated onto a

silver planchet at 80°C using a stirrer/hot plate for a minimum of four hours (Flynn 1968), as modified by Kline (in prep.).

Alpha decays of the Po-208 spike and Po-210 were counted for three weeks. Po-210 activities were determined from alpha spectra, and corrected for tailing, spike (Po-208 activity at mid-point of counting), background counts due to the detector (Kline in prep.) and preparation and reagent blanks which were cored, cleaned and plated in the same manner as above. They were also decay corrected to the time of plating and to the time of capture of specimens in the sample to produce Pb-210 activity.

Ra-226 Analysis

Ra-226 was measured through its daughter proxy Rn-222 (half life 3.825 days) using a Rn-222 gas emanation stripping technique followed by solid scintillation with a Lucas cell and photomultiplier tube. Kline (in prep.) details the calibration and background determination for this instrument. Whole otoliths were used for this analysis for two reasons: 1) the amount of Ra-226 decay in whole otoliths was considered negligible due to its long half life (1,622 yrs.); and 2) a minimum sample size of at least 10 grams was required for adequate counts above background.

Two samples consisting of otolith pairs of similar weight (0.13-0.18 g per otolith) from females caught in 1989 were analyzed. The two samples weighed 10.957 g (n=76) and

11.047 g (n=72), respectively. They were cleaned of surface contamination and dissolved (Appendix A), and then placed in gas wash bottles in which radon gas was allowed to accumulate for a minimum of 30 days, in order to reach secular equilibrium with Ra-226 and to allow for adequate counts above instrument background.

Rn-222 gas was stripped from the sample solution using a He carrier and an activated carbon column at dry ice/isopropanol temperatures, before being transferred to a Lucas cell. The Lucas cell was placed on a photomultiplier tube where alpha decay scintillations were counted for a minimum of four days.

Pb-210/Ra-226 Age Determination

Sample ages were determined from the decay disequilibrium between the activity of Pb-210 in each sample and the expected Ra-226 activity of each sample as described above, using the relationship:

$$A_t = A_o(1 - e^{-\lambda t})$$

solved for t:

$$t = -\ln(1 - A_t/A_o) / \lambda$$

where A_t = activity of Pb-210 at time t, A_o = activity of parent Ra-226, t = time elapsed since the incorporation of Ra-226, and λ = the decay constant for Pb-210 ($\ln 2 / 22.26$ yrs.). Errors were calculated based on standard counting statistics, which are presented in Appendix B.

Sample activity ratios were then plotted against the

average age determined from otolith cross sections and the expected activity ratio curve for comparison.

Otolith Morphometrics and Weight as Estimators of Age

To test whether otolith morphometrics and/or weight could be used as objective predictors of age as determined from growth zones, 86 female and 81 male otolith pairs from fishes ranging from 231 mm to 545 mm TL were examined. Age was estimated for 85 specimens by examining sections, and for 82 specimens using the break-and-burn technique. In all cases, age was estimated from the left otolith of each pair, as described previously. The right otolith of each pair was weighed to the nearest milligram, and its length (anterior-posterior), width (dorso-ventral), area (distal side) and perimeter were measured, using an Olympus image analyzer and Cue II software (Olympus Corp.). The program was calibrated to one millimeter.

Measurements of each variable (weight, length, width, area and perimeter) were plotted against growth zone counts for each sex. Regression lines were fit to the data using the program Systat. Non-linear functions were fit to the data with the program Table Curve (Jandel Scientific). The best fit was then chosen based on the highest correlation coefficient. For each regression, residuals were plotted as a function of their corresponding value to examine them for heteroscedasticity.

Growth Curves

Fish total length and age estimate data from the otolith morphometrics/weight analysis were also used to estimate von Bertalanffy growth parameters with the program FISHPARM (Prager et al. 1987):

$$L(t) = L_{\infty} (1 - \exp^{-K(t - t_0)})$$

where $L(t)$ = total length at time t , L_{∞} = asymptotic length, K = growth constant at which L is reached, and $t = t_0$ when $L = 0$.

Results

Opaque and Translucent Zone Formation

The margins (dorso-proximal) of 139 otolith sections from all months were examined and classified as opaque or translucent. Otoliths from four months (February, March, July and September) were excluded from further analysis due to their small sample size (< 5/month). Classification of opaque and translucent zones along the otolith margin was difficult and subjective, despite the fact that otoliths from fishes less than 310 mm TL were used. Opaque and translucent zones were not always continuous along the margin within the area examined, and slight adjustments in lighting or angle made interpretation confusing. Further, because translucent zones are much narrower than opaque zones, their formation on the otolith margin was much more

difficult to see.

Based on the frequency of occurrence of opaque and translucent zones by month, a seasonal pattern of formation was not apparent (Figure 2). Neither opaque nor translucent margins were found exclusively during any time of the year; rather, both were observed in every month that at least five specimens were available. Translucent edges were observed more frequently than opaque edges only in December, and equaled the number of opaque edges in January.

Microprobe Analysis

Quantitative analyses of calcium along the entire dorso-ventral axis of the two samples under two operating conditions revealed no apparent pattern in density which could be used to match the opaque and translucent zones used to age the fish (Figures 3 and 4). For sample 5104, 870 points were analyzed at 6.25 micron intervals and 216 points at 25 micron intervals. Sample 5105 was analyzed at 744 points with 6.25 micron intervals and 186 points at 25 micron intervals. Calcium profiles obtained at these two intervals for both samples were similar, indicating the consistency of the results and the suitability of both intervals (Figures 3 and 4). However, counting error overlapped between adjacent points, making them indistinguishable from each other, except in the region of the nucleus, where calcium density was lower (Figures 3,4,5,6).

Radiometric Age Analysis

Age was estimated from growth zone counts for 274 otoliths from 1987 and 1989, using thin section and break-and-burn techniques, respectively. Of these, 134 were cored and pooled into two sets of 'young', 'medium' and 'old' age category samples (Table 1). Individual age estimates within these six samples ranged from 9 to 53 years.

Po-210 activities ranged from 0.0185 dpm/g for the 'young' sample from 1989, to 0.0515 dpm/g for the 'old' sample from 1989 (Table 2). The 'medium' 1989 sample could not be used due to insufficient counts above background in the Po-210 region.

Rn-222 activities for the two samples were very similar (0.0734 and 0.0700 dpm/g), and were averaged to calculate Pb-210/Ra-226 activity ratios.

For the five samples used, Pb-210/Ra-226 activity ratios agreed well with the ratios expected for the average ages of the samples as determined from growth zone counts (Figure 7, Table 2). The closest agreement was between the oldest sample (mean traditional age of 39.65) which included a core from an individual aged traditionally at 53.

Otolith Morphometrics and Weight as Estimators of Age

Otolith weight, length, width, area and perimeter were compared with growth zone counts ranging from 7 to 53 for males and 6 to 50 for females (Figure 8). Males ranged in

length from 254 to 492 mm TL, and females ranged from 231 to 545 mm TL.

For males, the relationship between age and all otolith parameters was linear (Figure 8, Table 3). Female otolith parameters also showed linear relationships with age, with the exception of otolith length, which was best described as curvilinear (Figure 8, Table 3). For both sexes, all five otolith parameters showed highly significant relationships with age, with otolith weight having the highest correlation coefficients (Table 3). Heteroscedacity was not detected for any of the variables analyzed with regression.

Growth Curves

The von Bertalanfy growth curve fit total length at age data reasonably well (Figure 9). Parameters of this equation, followed by 95% confidence limits were: L_{∞} =488 (± 46.2), $K=0.0482$ (± 0.019), $t_0=-8.372$ (± 4.91) for males, L_{∞} =594 (± 89.5), $K=0.0391$ (± 0.018), $t_0=-6.96$ (± 4.97) for females, and L_{∞} =557 (± 56.5), $K=0.041$ (± 0.014), $t_0=-7.61$ (± 3.85) for the sexes combined.

Discussion

Opaque and Translucent Zone Formation

The most detailed study of opaque and translucent zone formation in otoliths of *Sebastes* was conducted by Mayo et al. (1981), for the redbfish, *Sebastes marinus*. They examined the margins of 922 otoliths from the same year-class. The width of translucent zones allowed for eight descriptive

categories to be used to classify the width and type of zone forming, with only 15% of otoliths being unclassifiable.

Based on examination of the otolith margin alone, evidence for the annual formation of opaque and translucent zones used to age *Sebastes rufus* was inconclusive. Although the smallest specimens available were used for this analysis to reduce error, classification was still difficult and subjective, and did not result in a distinct pattern of one opaque and one translucent zone being formed within a year. This can be attributed to the fact that the translucent zone in bank rockfish otoliths is very narrow, and, therefore, more difficult to see as it forms even in small otoliths. In addition, the small sample size available included specimens collected during different years and from ports from Santa Barbara in the south to Fort Bragg in the north. This may have clouded results due to variation in the timing of opaque and translucent zone formation among individuals during different years and at different latitudes.

Although it is possible that the translucent zone is formed during a very short period of the year (December) as indicated, translucent zones were, nevertheless, still observed in all months from which data were available.

As stated previously, the success of this method has been limited to young fish. For this reason, its application as an age verification method is very limited for long-

lived fishes such as *Sebastes*. For example, the study by Mayo et al. (1981) was only successful to an age of seven years, considerably less than the confirmed longevity of 75 years for this species (Campana et al. 1990).

Microprobe Analysis

Electron microprobe analysis of otoliths was used to objectively detect patterns in elemental composition across opaque and translucent zones used for ageing. This assumes that differences between these two zones exist and can be detected. Qualitative evidence for this assumption has been provided by several studies, particularly with regard to calcium.

But confusion exists in the literature as to whether the opaque or translucent zone has higher calcium content. Casselman (1987) concluded that the translucent zone in cleithra of the pike, *Esox lucius* contained higher calcium concentrations, based on electron microprobe analysis. In this study, line scans for calcium were overlaid on photographs of the analyzed section with the same dimensions for comparison. Peaks in calcium concentration appeared to match up with translucent zones, but were very similar to data from the opaque zone, and were not distinguished statistically. Irie (1960) also concluded that the translucent zone (in this case formed in summer and autumn) had a higher concentration of calcium and lower protein content based on scanning electron microscope observations of CaCO_3 crystals.

Likewise, Dannevig (1956) concluded that the opaque zone contains less calcium based on burning and decalcination of the otolith cross section.

On the other hand, Panella (1980) stated that for temperate fishes, the ratio of calcium carbonate to otolin in the translucent zone is much less than in the opaque zone. Christensen (1964) interpreted burned zones in otolith cross sections as organic membranes, after Dannevig (1956), but described their location as being in between "transparent" and opaque zones.

Even at 6 micron intervals, calcium data did not demonstrate detectable patterns of increasing and decreasing concentration in this study. Counting error, though low, made adjacent points indistinct from each other. The beam size and spacing of analysis points (even at 6 micron intervals) may have been inadequate for analyzing the narrow translucent zones observed in this species, but this is unlikely, since translucent zones were roughly 50-100 microns in width. Because otoliths are composed primarily of CaCO_3 , it may be unrealistic to expect significant fluctuations in calcium density across otolith growth zones.

One obvious feature that was present in all samples examined was the lower densities of calcium measured in the region of the nucleus than in subsequent zones of growth. This represents embryonic and possibly larval growth during the first months of life. Rockfishes are viviparous, giving

birth to larvae which lead a pelagic existence for several months before settling into juvenile or adult habitat within their first year. Lower calcium levels in the nucleus may be due to differences in water temperature, diet, and/or metabolism during this period. However, specific information about *Sebastes rufus* life history during this period is lacking.

Dannevig (1956) also concluded that this region contains less calcium by comparing the amount of CaO in small otoliths with large ones. Lower levels of calcium and phosphorus were detected from microprobe analysis of the core in elasmobranch centra by Jones and Geen (1977) and Cailliet and Radtke (1987); the latter authors interpreted these lower levels as occurring during embryonic growth.

Although the data were collected objectively by the electron microprobe, the relationship between calcium distribution and opaque/translucent patterns was still left to human subjective interpretation. Unless data from elemental profiles can be interpreted objectively (with Fourier analysis (Rosenberg et al. 1980), for example) electron microprobe analysis can only be considered as a descriptive tool for defining opaque and translucent zones.

The structural and chemical composition of opaque and translucent zones in otoliths and the factors which influence their formation has just begun to be addressed. Environmental factors such as temperature, water chemistry, and

food availability, and physiological factors such as growth, life history stage and plasma and endolymph chemistry have singularly and in combination been hypothesized as affecting otolith formation and chemistry (Mugiya 1984, 1987, Kalish 1989, 1991, Radtke and Targett 1984, Sadovy and Severin 1992). In his work on bearded rock cod, *Pseudophycis barbatus*, Kalish (1991) concluded that changes in otolith chemistry were due primarily to physiological factors, with environmental factors playing an indirect role. Similar conclusions were made by Sadovy and Severin (1992), who examined the role of water temperature in Sr/Ca levels in otoliths. Further work in this area will aid in clarifying the nature of opaque and translucent zones and our interpretation of them.

Radiometric Age Analysis

Radiometric ages agreed well with samples aged from otolith cross sections, giving substantial validity to ages determined from them. Results indicate that the zones used in ageing *Sebastes rufus* from otolith cross sections in this study are deposited annually, and that it is appropriate to use a cross sectional method (thin section or break-and-burn) when determining age. Further, results suggest longevity of 50 years or more for *Sebastes rufus* since the oldest individual used in the analysis was aged to be 53.

As stated previously, three principal assumptions must be made when applying this technique to whole otoliths. The

potential error associated with the constant uptake assumption was largely avoided in the present study by only using core material, after Campana et al. (1990). Although microprobe analyses of two otolith sections detected lower densities of calcium in the region of the nucleus, this is a small area relative to the first five years of growth used for analysis. Further, on an atomic weight percent basis, the difference in densities between the nucleus and subsequent growth zones was only ca. 1% and is considered negligible for the purposes of this assumption.

The closed system assumption could not be addressed in this study; however, it is supported by the fact that otoliths are acellular, housed within a membrane in the auditory bullae, and that resorption in otoliths does not commonly occur (Simkiss 1974, Campana and Neilson 1985). Welden et al. (1987) found this assumption to be invalid in the cellular cartilage of shark vertebral centra, where Pb-210 was apparently able to migrate. A source of concern is the possible diffusion of daughter product Rn-222, a noble gas, out of otolith material. This would result in lower Pb-210 activity and underestimation of age.

The third assumption, that Pb-210 from outside sources is minimal, can be addressed by examining Pb-210 activity in young fish. At $t=0$, the activity of Pb-210 should ideally be $=0$. Bennett et al. (1982) measured Pb-210 activity of 0.0009 ± 0.002 in whole otoliths of 1-2 year old fish, while

Campana et al. (1990) reported 0.0077 ± 0.001 in a core sample averaging 8.4 years of age. In the present study, the youngest sample of cores, based on the average growth zone counts ($\bar{x}=13.24$), had a Pb-210 activity of 0.0198 ± 0.0022 . None of these three levels resulted in significant deviations from the expected Pb-210/Ra-226 activity ratio curve.

Detection limits of the alpha decays of Po-210 necessitated the pooling of otoliths into age categories based on growth zone counts for Pb-210 analysis. Results may have been more precise if more age categories had been analyzed, and if samples had been composed of otoliths of a single age or narrower age ranges. Time constraints prevented this; it would have taken much longer to make enough individual age estimates to obtain the minimum one gram (ca. 20 otoliths) needed for each sample, particularly for older age categories which have fewer individuals.

Using smaller otolith cores instead of the first five years of growth also may have improved results, but could possibly introduce more age estimate error, since more cores would have to be pooled to obtain the required material.

The 0.0717 ± 0.0011 dpm/g activity for Ra-226 measured in this study was higher than that reported for two other species of *Sebastes* and for two species of thornyhead, *Sebastolobus*, but much lower than the 0.414 ± 0.050 dpm/g reported by Kastelle (1991) for sablefish. Bennett et al. (1982) reported 0.043 ± 0.004 dpm/g for *Sebastes diploproa*,

while Campana et al. (1990) measured 0.033 ± 0.002 dpm/g for *Sebastes mentella*. Kline (in prep.) reported Ra-226 levels of 0.045 ± 0.0018 dpm/g and 0.043 ± 0.0053 dpm/g for *Sebastes altivelus* and *Sebastes alascanus*, respectively.

Differences in Ra-226 activity between species may be due to differences in sources of Ra-226 and/or metabolism. Porntepkasemsan and Nevissi (1990) examined these possible mechanisms of uptake of Ra-226 by marine fishes, measuring Ra-226 activities in several benthic fish species. They noted that Ra-226 levels five times higher were reported for salmon, which are pelagic, by Jenkins (1969). Based on this comparison, they concluded that Ra-226 is most likely taken up by fishes through water and food, not the sediments from which it originates, and that the higher Ra-226 activities found in salmon were probably due to their high metabolic rate.

Since Bennett et al. (1982) first measured Pb-210/Ra-226 ratios in *Sebastes diploproa*, subsequent works have improved on the method while applying it to other species. Fenton et al. (1990) pointed out the need for stringent control over sources of contamination and error, due to the low level activities being measured. The results of this (Fenton et al. 1990) study were inconclusive, however, because whole otoliths were used and the constant Ra-226 uptake assumption was violated for blue grenadier.

Campana et al. (1990) eliminated the error associated

with the constant uptake assumption by only using core material representing the first five years of growth for *Sebastes mentella*, but reported no other details regarding other sources of potential error. Kastelle (1991) used cores representing only the first year of growth in his radiometric aging study of sablefish, *Anoplopoma fimbria*. Cores were washed in distilled and deionized water in an ultrasonic cleaner, and acid washed glassware was used. The present study and that of Kline (in prep.) add to the list of improvements made with regard to minimizing sources of error, by using only core material for analysis and by using trace metal clean techniques for preparing samples.

The radiometric dating technique shows promise for other species, particularly deep water ones, for which more traditional age validation methods are not feasible. The method is currently limited by low activities of Ra-226 and Pb-210, and will greatly improve once detection limits are lowered and individual otoliths can be analyzed. Future research is needed to fully validate the assumptions of the method.

Otolith Morphometrics and Weight as Estimators of Age

Although all of the otolith parameters examined showed highly significant relationships with age from growth zone counts, otolith weight appears to be the most promising predictor, for three reasons. The first is that otolith weight showed the strongest relationship for both sexes.

Secondly, it accounts for the thickening process which occurs with age; this change in the axis of growth is not reflected in the other parameters. Third, otolith weight is relatively easy to obtain and doesn't require the more time-consuming use of an image analyzer or ocular micrometer.

Otoliths used in this analysis were not dried to a constant weight prior to weighing them, and this may have affected results. However, all otoliths were stored dry in paper coin envelopes, and it is assumed that any moisture retained by the specimens used was proportionately the same for all sizes.

Otolith weight is an objective measurement which can be easily and quickly obtained, and could possibly replace time-consuming, subjective counts of growth zones in determining age, once the initial relationship between otolith size and age has been determined. This would provide a much more rapid and efficient process for making the hundreds of individual age estimates which are needed in population ageing studies. However, further evaluation of the regression model is needed before it can be considered a substitute for estimating age from growth zone counts. The precision with which the model agrees with growth zone counts for individual specimens should be tested to determine its appropriateness as an age predictor.

Boehlert (1984) first suggested the use of otolith morphometrics and weight as objective criteria for determin-

ing age. This study examined the relationship between growth zone counts and otolith weight, length, width, and thickness for *Sebastes diploproa* and *Sebastes pinniger*. When evaluated individually, otolith weight was found to have the strongest linear relationship with growth zone counts. Boehlert (1984) used these otolith variables in combination to develop multiple regression models for estimating age. The appropriateness of these models is questionable, however, since the variables are not truly independent of each other (Zar 1984).

Although otoliths are useful ageing tools, it is important to remember that their function is that of hearing and hydrostatic organs, and their shape and size are species specific (Popper 1978, Wyllie Echeverria 1987, Gauldie 1988). For bank rockfish, as in other rockfishes, otolith size at age reflects the sexual dimorphism in body size. For this reason, any otolith parameter used in ageing should be modeled separately for the sexes.

Growth Curves

The maximum total length predicted by the von Bertalanffy equation for both sexes combined ($L_{\infty}=557$ mm) exceeds the maximum length for *Sebastes rufus* reported by Miller and Lea (1972) (510 mm), but this is not surprising, since many specimens (particularly females) in the collection exceeded this length. One of two type specimens of this species measured 540 mm (Eigenmann and Eigenmann, 1890).

As with other members of the genus *Sebastes*, maximum length for females exceeds that of males. Female growth appears to slow considerably at about 475 mm TL (ca. 30 years of age), while male growth slows at about 400 mm TL (ca. 28 years of age).

The rate at which bank rockfish reach maximum total length, or k as predicted by the model (0.041), is slightly lower than that reported for other rockfishes. Love et al. (1990) summarized k values reported in the literature for 42 northeast Pacific rockfish species. He warned that comparisons should be made with caution, since some of the species included had been aged using whole otoliths, which would tend to underestimate age and increase k values. Values for k ranged from 0.048 for the rougheye rockfish, *Sebastes aleutianus* (Nelson and Quinn 1987) to 0.789 for the Puget Sound rockfish, *Sebastes emphaeus* (Moulton 1977), with most ranging between 0.1 and 0.3. Love (1990) noted a trend in which species that are the largest and longest-lived have the lowest k values, while the smallest and shortest-lived species exhibit the highest k values. However, for most rockfish species, which fall between these extremes in size and longevity, a trend in k values was not apparent.

Love (1990) and Wyllie Echeverria (1987) reported lengths at first, 50% and 100% maturity for bank rockfish which were very similar. For males, both authors estimated

TL at 50% maturity at 310 mm. Based on the results of the present study, this corresponds to age of approximately 15 years (Figure 9). Wyllie Echeverria (1987), who estimated age from whole otoliths, reported an age of three for males of this length. For females, length at 50% maturity was estimated as 360 mm TL (Love 1990) and 340 mm TL (Wyllie Echeverria 1987). This corresponds to approximately 17 years based on the present study; Wyllie Echeverria (1987) estimated an age of three years for this length.

Summary and Recommendations

Based on the results of the radiometric ageing portion of this study, the parameters of the von Bertalanffy equation, and comparisons with length at maturity reported by Love et al. (1990) and Wyllie Echeverria (1987), bank rockfish appear to have a long lifespan, are slow-growing, and reach reproductive maturity at a late age. Although natural mortality was not estimated in this study, the confirmed longevity of individuals aged here suggests that it is low. Population characteristics such as these make rockfishes, in general, susceptible to overfishing, and should be considered and monitored for management purposes (Leaman 1991).

Future ageing studies of this species should utilize the thin section or break and burn methods of age estimation, possibly for all age categories, given the large discrepancy in size at age for young fishes between this study and Wyllie Echeverria (1987). Otolith weight should

be evaluated further as an objective criterion for determining age.

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Figure 1. *Sebastes rufus* otolith cross section relative to its position in the inner ear. Age was estimated from counts of growth zones in the dorsal region. Opaque and translucent zone formation was examined along the dorso-proximal margin. Electron microprobe x-ray analysis for Ca was conducted at points along the dorso-ventral axis.

Figure 2. Number of opaque and translucent margins observed by month.

Figure 3. Sample 5105: otolith section analyzed for calcium along the dorso-ventral axis with a wavelength dispersive electron microprobe at 25 micron and 6.25 micron intervals. Data from the 6.25 micron interval are smoothed with a 5 point running average. The specimen is a male, 270 mm TL, aged 7+ from growth zone counts.

Figure 4. Sample 5104: otolith section analyzed for calcium along the dorso-ventral axis with a wavelength dispersive electron microprobe at 25 micron and 6.25 micron intervals. Data from the 6.25 micron interval are smoothed with a 5 point running average. The specimen is a female, 290 mm TL, aged 6+ from growth zone counts.

25 micron interval

6.25 micron interval

Figure 5. Sample 5105: wavelength dispersive electron microprobe data for calcium analyzed at 25 micron intervals. Error bars represent ± 1 s.d. based on counting statistics.

Figure 6. Sample 5104: wavelength dispersive electron microprobe data for calcium analyzed at 25 micron intervals. Error bars represent ± 1 s.d. based on counting statistics.

Figure 7. Radiometric ageing results for otolith core samples representing 'young', 'medium' and 'old' fish. Points represent observed Pb-210/Ra-226 activity ratios plotted against mean growth zone counts for each sample. The curve represents expected Pb-210/Ra-226 activities over time. Error bars represent ± 1 s.d..

Figure 8. Age (growth zone counts) plotted against otolith length (mm), width (mm), perimeter (mm), area (mm²) and weight (g), for male and female *Sebastes rufus*, with best fit line through the data.

Males

Females

Figure 8 (continued). Age (growth zone counts) plotted against otolith length, (mm), width (mm), perimeter (mm), area (mm²) and weight (g), for male and female *Sebastes rufus*, with best fit line through the data.

Males

Females

Figure 8 (continued). Age (growth zone counts) plotted against otolith length, (mm), width (mm), perimeter (mm), area (mm²) and weight (g), for male and female *Sebastes rufus*, with best fit line through the data.

Males

Females

Figure 9. Von Bertalanffy growth curve of male and female *Sebastes rufus*. Parameter estimates, followed by 95% confidence limits in parentheses, are shown in the inset.

Table 1. Summary of 'Young', 'Medium' and 'Old' Pooled Otolith Core Samples from *Sebastes rufus* used for Pb-210 Analyses.

Otolith Sample	n	wt. (g)	Growth Zone Counts	Mean Counts	S.D.
<u>'Young'</u>					
1987	21	0.819	9 - 16	13.24	1.64
1989	21	1.130	10 - 15	13.57	1.29
<u>'Medium'</u>					
1987	24	1.719	20 - 27	23.42	1.98
1989	24	1.263	20 - 29	23.96	2.81
<u>'Old'</u>					
1987	21	1.323	30 - 43	33.14	3.49
1989	23	1.847	35 - 53	39.65	4.69

Table 2. Results of Radiometric Ageing of 'Young', 'Medium' and 'Old' Samples from Growth Zone Counts. Observed Po-210 Activities, Pb-210/Ra-226 Activity Ratios, Radiometric Ages, Mean Ages from Growth Zone Counts, and Expected Pb-210/Ra-226 Activity Ratios. Activities reported in decays/minute/gram.

Otolith Sample	Po-210 Activity	S.D.	Pb-210/ Ra-226	S.D.	Radio- metric Age	Mean Growth Zone Counts	Expected Pb-210/ Ra-226 Ratio
'Young'							
1987	0.0198	0.0022	0.2765	0.0305	10.4	13.2	0.3374
1989	0.0185	0.0028	0.2582	0.0390	9.6	13.6	0.3441
'Medium'							
1987	0.0462	0.0030	0.6438	0.0428	33.2	23.4	0.5171
1989	not used - counts in Po-210 region too low						
'Old'							
1987	0.0490	0.0034	0.6841	0.0483	37.1	33.1	0.6430
1989	0.0515	0.0049	0.7179	0.0696	40.7	39.7	0.7084

Table 3. Equations Describing the Relationship between Otolith Parameters and Age (growth zone counts) for Male and Female *Sebastes rufus*, and their Corresponding Coefficients of Determination, F statistics and Probabilities.

<u>Otolith</u> <u>Parameter</u>	<u>Equation</u>	<u>r²</u>	<u>F</u>	<u>P</u>
<u>Males (n=81) F_{0.05(1), 1, 79}=3.96</u>				
Weight	a= -2.47+188.29(wt)	0.913	885.0	<<0.0005
Length	a= -40.46+4.78(l)	0.838	408.1	<<0.0005
Width	a= -41.51+10.23(w)	0.786	290.0	<<0.0005
Area	a=-11.967+0.603(ar)	0.859	482.4	<<0.0005
Perimeter	a= -23.88+1.37(p)	0.812	340.3	<<0.0005
<u>Females (n=86) F_{0.05(1), 1, 84}=3.96</u>				
Weight	a= 1.09+139.48(wt)	0.909	841.7	<<0.0005
Length	a= 1.83+0.007(l ³)	0.904	788.7	<<0.0005
Width	a=-30.09+7.90(w)	0.851	479.5	<<0.0005
Area	a= -6.76+0.46(a)	0.899	745.4	<<0.0005
Perimeter	a=-18.21+1.14(p)	0.901	765.7	<<0.0005

Appendix A

Sample Cleaning Procedure

Rough cleaning:

Place pooled sample cores into plastic test tube.

1. Agitate/rinse in deionized water and Micro three times.
2. Rinse in deionized water three times.
3. Rinse in milli-q water three times.

Place in oven for 12 hours at 30°C to dry. Weigh sample.

Fine Cleaning:

Transfer to acid-cleaned beaker.

1. Soak 10 minutes in milli-q water in sonicator.
2. Soak 1 minute in 0.15N HNO_3 in sonicator.
3. Soak 10 minutes in 30% H_2O_2 /NaOH (pH 10) in sonicator.
4. Rinse with milli-q water three times.
5. Soak 1 minute in 0.15N HNO_3 in sonicator.
6. Soak 5 minutes in milli-q water in sonicator.
7. Rinse three times for 30 seconds with 0.001N HNO_3 in sonicator.
8. Rinse two times with milli-q water.

Dry at least 12 hours at 30°C in oven. Weigh sample.

Sample Dissolution

Add .8N HNO_3 until sample is almost completely dissolved (place in sonicator). Drop HNO_3 until dissolution is complete and record total volume added. Add milli-q water until final solution is 0.5 N and record volume added.

Appendix B

Error Calculation for Pb-210/Ra-226 Activity

Standard deviation for Pb-210/Ra-226 activity was calculated as follows:

$$\text{s.d. Pb-210/Ra-226} = \sqrt{\left(\frac{Pb_{sd}}{Pb}\right)^2 + \frac{Ra_{sd}}{Ra} \times \frac{Pb}{Ra}}$$

Where:

Pb = Pb-210 activity (dpm)

Ra = Ra-226 activity (dpm)

$$Pb_{sd} = \sqrt{\left(\frac{\sqrt{210_{cnts}}}{210_{cnts}}\right)^2 + \left(\frac{\sqrt{208_{cnts}}}{208_{cnts}}\right)^2} \times Pb-210 \text{ activity}$$

210_{cnts} = Po-210 counts

208_{cnts} = Po-208 counts