1997

Age and growth of the Pacific Grenadier (Family Macrouridae, Coryphaenoides acrolepis) with age estimate validation using an improved radiometric ageing technique

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AGE AND GROWTH OF THE PACIFIC GRENADIER

(FAMILY MACROURIDAE, *Coryphaenoides acrolepis*)

WITH AGE ESTIMATE VALIDATION USING AN IMPROVED RADIOMETRIC

AGEING TECHNIQUE

A Thesis

Presented to

The Faculty of Moss Landing Marine Laboratories

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Allen Hia Andrews

December, 1997

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ABSTRACT

AGE AND GROWTH OF THE PACIFIC GRENADIER (FAMILY MACROURIDAE, Coryphaenoides acrolepis) WITH AGE ESTIMATE VALIDATION USING AN IMPROVED RADIOMETRIC AGEING TECHNIQUE

by Allen Hia Andrews

Longevity estimates for the Pacific grenadier, *Coryphaenoides acrolepis*, range from 6 to greater than 60 years. Age estimates in this study using growth increments in otolith sections indicate the Pacific grenadier is long-lived. Prior to this study, traditional ageing of this fish was not validated. In this study, the radioactive disequilibria of ^{210}Pb and ^{226}Ra in otolith cores from Pacific grenadier were used to validate age estimates. Accuracy of radiometric ageing using ^{210}Pb:^{226}Ra disequilibria in otoliths was improved using ion-exchange chromatography and thermal ionization mass spectrometry (TIMS) to measure ^{226}Ra. Because TIMS counts atoms, the accuracy and precision of the technique is superior to α-spectrometric methods. This procedure was applied to otoliths from three other fish species. Results indicate the procedure reduced sample size and processing time, and increased accuracy. Radiometric ages for the Pacific grenadier agree with traditional age estimates and indicate the Pacific grenadier can live at least 56 years.

ACKNOWLEDGEMENTS

Head, South Carolina in 1991. Because of this symposium, I firmly committed to pursuing a thesis in age and growth of fishes.

Chapter 2, <u>Application of a new ion-exchange separation technique and isotope-dilution thermal ionization mass spectrometry to ^{226}Ra determination in otoliths for radiometric age determination of long-lived fishes,</u> was a collaborative effort that was made successful with the additional efforts of Jocelyn Nowicki, Craig Lundstrom, Zenon Palacz, and Erica Burton. Development of the new ion-exchange separation technique to measure radium using TIMS was facilitated by colleagues in the Department of Earth Sciences at University of California, Santa Cruz. Use of the thermal ionization mass spectrometer was an essential part of this research. Special thanks to Craig Lundstrom, Zenon Palacz, and Pete Holden for sample processing and teaching me how to use the instrument.

Fish specimens were procured from the following organizations; all of which have been very cooperative. In some cases, valuable ship-time was given.

• National Oceanic and Atmospheric Administration, Southwest Fisheries Science Center, National Marine Fisheries Service. Special thanks to John Butler.

• National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, National Marine Fisheries Service. Special thanks to Bob Lauth.

• Oregon Department of Fish and Wildlife. Special thanks to Mike Hosie.

• California Department of Fish and Game. Special thanks to Bob Lea and Bob Leos.

• University of California, San Diego/Scripps Institute of Oceanography.

PREFACE

This thesis presents results from using a novel technique used to determine ^{226}Ra activity in otolith material, with an application to the validation of Pacific grenadier age estimates using radiometric age determination. Chapter one is a comprehensive introduction which covers the history and application of radiometric age determination to many species and the age and growth of the Pacific grenadier, with documentation of the rapidly developing fishery. Chapter two details the research and development of the new technique used to determine ^{226}Ra activity in otolith material. Chapter three reveals new age estimates for the Pacific grenadier, compares them to existing estimates, and validates the new age estimates by applying the improved radiometric ageing technique. Chapter four is a comprehensive conclusion to chapters two and three that includes more details and further discussion indicating potential management strategies. Chapters two and three are prepared as complete scientific papers that will be submitted to the Canadian Journal of Fisheries and Aquatic Sciences, a refereed journal of our trade. These chapters are in the format required by this journal.

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CHAPTER 1

Introduction

The Pacific grenadier, *Coryphaenoides acrolepis*, is a member of the globally important cod fishes (order Gadiformes) in the grenadier family (family Macrouridae), also known as the rattails or whiptails. Gadiform fishes composed 17% (13,700,000 t) of the global marine landings in 1987, of which 0.4% (51,000 t) was grenadier (Cohen et al. 1990). The grenadier is a cosmopolitan family with the exception of the Arctic Ocean and basins with a shallow sill depth (Cohen et al. 1990). The family is the most diverse of the benthopelagic fishes and consists of an ever increasing 300+ species (Tomio Iwamoto, California Academy of Sciences, San Francisco, CA, personal communication). Depth distribution ranges from the upper continental slope to abyssal depths over 6000 m (Marshall 1979). Most members of this family occupy the benthopelagic waters of the continental slopes between 250 and 2000 m (Marshall 1979, Cohen et al. 1990).

The Pacific grenadier is a benthopelagic inhabitant of the continental slopes of the northern Pacific Ocean. Its range is circum-north Pacific Ocean from Baja California, Mexico to northern Japan and into the Bering Sea (Iwamoto and Stein 1974, Cohen et al. 1990). It is one of approximately 11 grenadier species found along the central northeastern Pacific Ocean margin (Hart 1973, Iwamoto and Stein 1974, Cohen et al. 1990). Its typical depth range is from 600 to 2500 m (Iwamoto and Stein 1974) with a population density maximum near 1500 m (Stein and Pearcy 1982, Matsui et al. 1990).

Historically, the Pacific grenadier fishery has been an incidental fishery where landing statistics were poorly documented. Because the Pacific grenadier has not been a targeted species, landings in most cases have been lumped into a poorly defined category. In northern Japan the grenadier catch consists of four species (*C. acrolepis, C. longifilis,*

C. cinereus, and *Albatrossia pectoralis*). These species are not differentiated from one

another and are pooled with a morid (*Laemonema longipes*). The landings for this group

show a rapid increase from an average of approximately 3500 t per year (1980-1989) to

an average of approximately 14,000 t per year (1990-1993), with a high of over 27,000 t

for 1991. Increased landings are attributed to a major decrease in the landings of walleye

pollock (*Theragra chalcogramma*), typically used in the surimi (processed white fish)

market, where grenadier are used as a secondary filler (Daiji Kitigawa, Touhoku National

Fisheries Institute, Hachinohe Aomori, Japan, personal communication). Russian catch

per unit effort (CPUE) for Kamchatka and the Kurils indicate the highest grenadier catch

success occurred in the Navarin region of the northern Bering Sea (Il'inskii 1991).

Significant increases in grenadier catch occurred in the early 1980's as CPUE for other

fishes decreased (i.e. sablefish (*Anoplopoma fimbria*)). Grenadier species composition is

not described in this study.

The Pacific grenadier fishery of the northeastern Pacific Ocean began as incidental

landings in the thornyhead rockfish, dover sole, and sablefish fisheries. In 1972, Eureka

Fisheries (based in Fields Landing south of Eureka, California) began marketing Pacific

grenadier, which has grown slowly through to the mid 1980's. In 1983, a single landing of

798 pounds at Eureka Fisheries processing plant in Coos Bay, Oregon was the beginning

of the Oregon fishery, but no additional landings were made until 1987. That year, a

Japanese fish buyer, Tomio Ao from Choshi, Japan began to develop an export thornyhead

rockfish (*Sebastolobus spp.*) fishery to Japan. Thornyhead soon increased in value from

approximately $0.25 per pound in an almost exclusively United States market to over

$1.00 per pound in an almost exclusively Japanese market. To increase catch, many northern California and Oregon fishermen increased maximum fishing depth from approximately 800 m to approximately 1500 m by investing in larger winches and more cable at a cost of $15,000 to $25,000 per vessel. As a consequence, incidental landings of Pacific grenadier began to increase (Mike Hosie, Oregon Department of Fish and Game, personal communication).

Until recently, fishermen regarded the Pacific grenadier as a trash fish because the fillets were small (~25% of the total fish weight) and had low market value ($0.08 to 0.16 per pound ex-vessel). In recent years the Pacific grenadier has been discovered to have desirable culinary attributes (Kremsdorf et al. 1979, Matsui et al. 1990) and has become a large commercial fishery in California and Oregon (Figure 1, Chapter 3). Landings for Monterey Bay, California have increased substantially from practically zero for 1992 to nearly 900 t (~2 million pounds) for 1996 (Figure 2, Chapter 3). Prior to 1995, greater than 95% of the landings were from trawlers targeting other fish species. For 1995 and 1995, greater than 90% of the landings were from set lines targeting the Pacific grenadier (Leos 1997). Because of these landings, the Pacific grenadier is no longer in the "other" category and has become the fifth largest fishery in Monterey Bay, California and thirteenth in the state of California at 1,130 t (~2.5 million pounds) for 1996 (Leos 1996, 1997).

To properly manage this rapidly developing fishery, the age structure and growth characteristics of the Pacific grenadier must be known along with other life history parameters. Some life history aspects are known (Stein and Pearcy 1982, Matsui et al.

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1990), but longevity estimates remain controversial. Recent and historical age and longevity estimates range from 6 to greater than 60 years (Kulikova 1957, Brothers et al. 1976, Mulcahey et al. 1979, Wilson 1982, Matsui et al. 1990).

Fisheries management strategies rely heavily on accurate age determinations. Age is typically determined in fishes by performing one of several techniques, but most commonly the quantification of growth increments in calcified structures (Chilton and Beamish 1982, Beamish and McFarlane 1987). The annual periodicity of growth increments in these structures is often assumed. Until recently this assumption was rarely validated (Beamish and McFarlane 1983). As a consequence, underestimation of longevity may have had serious consequences to existing fisheries; for example the decline of the Pacific ocean perch (*Sebastes alutus*) of the northeastern Pacific Ocean and the orange roughy (*Hoplostethus atlanticus*) off New Zealand (Beamish 1979, Archibald et al. 1983, Mace et al. 1990, McFarlane and Beamish 1995, Smith et al. 1995) can be attributed to inaccurate age estimates and overfishing. The problem with typical growth-increment age validation techniques is that they have limited applicability to deepwater or long-lived fishes because of slow growth, fine growth increment structure, and barotrauma upon capture (MacDonald 1987, Mace et al. 1990, McFarlane and Beamish 1995). One technique that can be used to validate age estimates for these fishes is the radiometric ageing technique which uses the disequilibria of ^{210}Pb and ^{226}Ra in otoliths as a natural chronometer (Smith et al. 1991).

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Radiometric age determination

An essential requirement for utilizing ^{210}Pb:^{226}Ra disequilibria for age determination is the capability of measuring the activity of these radioisotopes with high precision and accuracy at very low levels, from femtogram (10^{-15} g) to attogram (10^{-18} g) quantities. The detection of ^{210}Pb uses the auto-deposition and alpha-spectrometric determination of its daughter proxy, ^{210}Po (Flynn 1968). Two techniques traditionally used to determine ^{226}Ra are the solid scintillation counting of its daughter proxy ^{222}Rn via radon emanation, and direct α-spectrometry of plated ^{226}Ra samples.

The radon emanation technique has been applied to otoliths, as part of the radiometric ageing technique, of six fish species: 1) *Sebastes diploproa* (Bennett et al. 1982); 2) *Sebastes mentella* (Campana et al. 1990); 3) *Anoplopoma fimbria* (Kastelle et al. 1994); 4) *Sebastes rufus* (Watters 1995); and 5) *Sebastolobus altivelis* and *S. alascanus* (Kline 1996). This technique uses the α-decay of ^{222}Rn as a proxy for ^{226}Ra determination. The first ichthyological application of this technique used whole otoliths for both ^{210}Pb and ^{226}Ra determination (Bennett et al. 1982). However, use of whole otoliths can be imprecise and requires assumptions of constant growth which is not always a robust assumption (Francis 1995). To circumvent the need for such assumptions, a method was developed to extract the oldest part of the otolith, the core, which typically represents the first few years of growth (Campana et al. 1990). Because cores are small (approximately 0.01 to 0.05 g), they must be pooled to attain measurable ^{210}Pb activity ($\sim$1 g). A sample size of approximately 1 g, however, is typically too small for the radon emanation technique (Bennett et al. 1982). Therefore, ^{226}Ra determination is usually

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performed on pooled whole otoliths with the necessary assumption that ^{226}Ra uptake is in constant proportion to the otolith mass growth-rate.

To determine ^{226}Ra, direct α-spectrometry has also been used as part of the radiometric ageing technique for six fish species: 1) *Hoplostethus atlanticus* (Fenton et al. 1991, Smith et al. 1995); 2) *Allocyttus verrucosus* (Stewart et al. 1995); 3) three species of the family Lutjanidae (Milton et al. 1995); and 4) *Macruronus novaezelandiea* (Fenton and Short 1995). In this technique ^{226}Ra was isolated by co-precipitation with a ^{133}Ba tracer where the α-decay of ^{226}Ra was measured directly using α-spectrometry (Fenton et al. 1990). Because this technique is more sensitive, a lower sample size is possible (~1 g). Therefore, this technique has been applied to otolith core samples, and whole otolith samples where cores were too small to be efficiently extracted.

Determining ^{226}Ra in otoliths using these techniques has worked well for radiometric ageing, but aspects of the technique made improvement desirable. Difficulties in developing relevant and verifiable mass growth-rate models and the uncertainty of the constant ^{226}Ra uptake assumption make the use of otolith cores preferable when possible. The large sample size typically required for radon emanation, however, make the use of cores improbable because hundreds to thousands of comparably aged fish would be necessary (>10 g; Campana et al. 1990, Watters 1995, Kline 1996). For small sample sizes typically associated with the use of otolith cores, direct α-spectrometry is currently the most applicable. Both techniques, however, are time consuming (3-5 weeks) and can

lead to large analytical uncertainties (range of ~4-150% from all previously cited

radiometric ageing studies).

Thermal ionization mass spectrometry

In recent studies, thermal ionization mass spectrometry (TIMS) was used to

measure very small quantities of ^{238}U and ^{232}Th series isotopes, including radium, in

volcanic rocks with high precision and accuracy, and low detection limits (Cohen and

O'Nions 1991, Volpe et al. 1991, Cohen et al. 1992, Chabaux et al. 1994). By using

isotope-dilution TIMS, the isotope of interest is measured directly by counting the ionized

atoms. Although few thermal ionization mass spectrometers exist and processing is

expensive, this technique has significant advantages over the existing radium determination

techniques: 1) detection limits are at least 1000 times lower (~1 fg or 3 million atoms); 2)

analytical precision at low concentrations is better than 1.5% (95% conf. level); 3)

measurement of radium is direct and therefore not dependent on activity or decay

products; and 4) TIMS processing time requires only 1-2 hours (Volpe et al. 1991). In

addition, lower detection limits would allow a smaller sample size and may make it

unnecessary to pool otoliths. Given these advantages, a technique was developed to

enable the application of TIMS to the measurement of ^{226}Ra in otoliths.

Previous applications of TIMS to the determination of ^{226}Ra in natural samples

involved volcanic rocks. The elemental composition of otoliths are, however, very

different from that of volcanic rocks and published methods had to be substantially

modified. Of concern for the otolith analyses are high concentrations of calcium and

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barium and an organic component called otolin. Each of these constituents must be reduced to very low levels, while conserving radium, to prevent ionization suppression of the radium signal and elevated background levels during TIMS analysis (Cohen and O'Nions, 1991).

The following two chapters present the findings of two concurrent studies in journal submission format. The goals of theses studies were to: 1) develop a new technique using TIMS to determine ^{226}Ra directly in otoliths, 2) independently estimate age and growth of the Pacific grenadier using traditional ageing techniques, and 3) apply radiometric age determination and the new separation technique with isotope-dilution TIMS to age validation of the Pacific grenadier.

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CHAPTER 2

Application of a new ion-exchange separation technique and isotope-dilution thermal ionization mass spectrometry to ^{226}Ra determination in otoliths for radiometric age determination of long-lived fishes.

Abstract

To improve the accuracy and precision of radiometric age determination using ^{210}Pb: ^{226}Ra disequilibria in otoliths of fishes, a technique was developed using isotope-dilution thermal ionization mass spectrometry (TIMS) to determine ^{226}Ra. Because TIMS measures radium directly by counting ionized atoms, the accuracy and precision of the technique is superior to conventional α-spectrometric methods. Due to their chemical similarities, radium must be separated from interfering quantities of calcium and barium in the otolith matrix. This was accomplished using a new ion-exchange separation procedure. This procedure was tested by applying it to otolith samples from three fish species in three separate radiometric ageing studies. The resultant separations and TIMS determinations indicate the procedure efficiently separates radium from calcium and barium. Measured ^{226}Ra activities for each species were comparable to previous radiometric ageing studies, with the exception of one sample. When results are compared to traditional ^{226}Ra determination techniques, radon emanation and α-spectrometry, the new separation technique with isotope-dilution TIMS has significant advantages. Samples over three times smaller than attempted in other studies were processed with increased accuracy and decreased processing time.

Introduction

Fisheries management strategies rely heavily on accurate age determinations. Age is typically determined in fishes by performing several techniques, but the most common is the quantification of growth increments in calcified structures (i.e. otoliths; Chilton and Beamish 1982, Beamish and McFarlane 1987). The annual periodicity of growth increments in these structures is often assumed. Until recently, however, this assumption was rarely validated (Beamish and McFarlane 1983). As a consequence, underestimated longevity may have had serious consequences to existing fisheries (Beamish 1979, Archibald et al. 1983, Mace et al. 1990, McFarlane and Beamish 1995, Smith et al. 1995). The problem with typical growth-increment age validation techniques is that they have limited applicability to deepwater or long-lived fishes because of slow growth, fine growth increment structure, and barotrauma upon capture (MacDonald 1987, Mace et al. 1990, McFarlane and Beamish 1995). One technique that can be used to validate age estimates for these fishes is a radiometric approach which exploits the disequilibria of ^{210}Pb and ^{226}Ra in otoliths as a natural chronometer (Smith et al. 1991).

An essential requirement for utilizing ^{210}Pb:^{226}Ra disequilibria for age determination is the capability of measuring the activity of these radioisotopes with high precision and accuracy at very low levels, from femtogram (10^{-15} g) to attogram (10^{-18} g) quantities. Because background in the ^{210}Pb-^{210}Bi beta-counting technique via lead sulfate coprecipitation is relatively high (6 to 12 counts per hour), this technique was not employed (Koide and Bruland 1975). Due to the relatively short half-life and low activity of ^{210}Pb, the detection of ^{210}Pb is typically accomplished through the autodeposition and

alpha-spectrometric determination of its daughter proxy, ^{210}Po (Flynn 1968). Differences in half-life for ^{210}Pb and ^{226}Ra lead to low ^{210}Pb:^{226}Ra atom ratios. Therefore direct mass determination methods are not feasible for ^{210}Pb at this time. Because of these constraints, the long counting times required, and the low backgrounds associated with α-spectrometry, autodeposition and α-spectrometric analysis of ^{210}Po was chosen to determine ^{210}Pb activity in this study. Two techniques traditionally used to determine ^{226}Ra are the solid scintillation counting of its daughter proxy ^{222}Rn via radon emanation, and direct α-spectrometry of plated ^{226}Ra samples.

The radon emanation technique has been applied to otoliths, as part of the radiometric ageing technique, of six fish species: 1) *Sebastes diploproa* (Bennett et al. 1982); 2) *Sebastes mentella* (Campana et al. 1990); 3) *Anoplopoma fimbria* (Kastelle et al. 1994); 4) *Sebastes rufus* (Watters 1995); and 5) *Sebastolobus altivelis* and *S. alascanus* (Kline 1996). This technique uses the α-decay of ^{222}Rn as a proxy for ^{226}Ra determination. The first ichthyological application of this technique used whole otoliths for both ^{210}Pb and ^{226}Ra determination (Bennett et al. 1982). However, use of whole otoliths can be imprecise and requires assumptions of constant growth which are not always robust (Francis 1995). To circumvent the need for such assumptions, a method was developed to extract the oldest part of the otolith, the core, which typically represents the first few years of growth (Campana et al. 1990). Because cores are small (approximately 0.01 to 0.05 g), they must be pooled to attain measurable activity. A sample size of approximately 1 g, however, is typically too small for the radon emanation technique (Bennett et al. 1982). Therefore, ^{226}Ra determination is usually performed on

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pooled whole otoliths with the necessary assumption that ^{226}Ra uptake is in constant proportion to the otolith mass growth-rate.

To determine ^{226}Ra, direct α-spectrometry has also been used as part of the radiometric ageing technique for six fish species: 1) *Hoplostethus atlanticus* (Fenton et al. 1991, Smith et al. 1995); 2) *Allocyttus verrucosus* (Stewart et al. 1995); 3) three species of the family Lutjanidae (Milton et al. 1995); and 4) *Macruronus novaezelandiea* (Fenton and Short 1995). In this technique ^{226}Ra is isolated by co-precipitating with barium, using a ^{133}Ba yield tracer followed by α-spectrometric determination of the ^{226}Ra on calibrated detectors (Fenton et al. 1990). Because this technique is more sensitive, a lower sample size (~1 g) is possible. Therefore, this technique has been applied to otolith core samples, and whole otolith samples where cores were too small to be efficiently extracted.

Determining ^{226}Ra in otoliths using these techniques has worked well for radiometric ageing, but aspects of the technique made improvement desirable. Difficulties in developing relevant and verifiable mass growth-rate models and the uncertainty of the constant ^{226}Ra uptake assumption make the use of otolith cores preferable when possible. The large sample size typically required for radon emanation, however, make the use of cores improbable because hundreds to thousands of comparably aged fish would be necessary (>10 g; Campana et al. 1990, Watters 1995, Kline 1996). Yield determinations using ^{133}Ba, a β-technique, introduce additional errors, and direct α-counting of ^{226}Ra often leads to detector contamination and high backgrounds. For the small sample sizes typically associated with the use of otolith cores, direct α-spectrometry is currently the most applicable. Both techniques, however, are time consuming (3-5 weeks) and can lead

to large analytical uncertainties (range of ~4-150% from all previously cited radiometric ageing studies).

In recent studies, thermal ionization mass spectrometry (TIMS) has been used to measure very small quantities of ^{238}U and ^{232}Th series isotopes, including radium, in volcanic rocks. High precision and accuracy, and low detection limits have been reported for this technique (Cohen and O'Nions 1991, Volpe et al. 1991, Cohen et al. 1992, Chabaux et al. 1994). By using isotope-dilution TIMS, the isotope of interest is measured directly by counting the ionized atoms. Although few thermal ionization mass spectrometers exist and processing is sometimes expensive, this technique has significant advantages over the existing radium determination techniques: 1) detection limits are at least 1000 times lower (~1 fg or 3 million atoms); 2) analytical precision at low concentrations is better than 1.5% (95% conf. level); 3) measurement of radium is direct and therefore not dependent on decay; and 4) TIMS processing time requires only 1-2 hours (Volpe et al. 1991). In addition, lower detection limits would allow a smaller sample size and may make it unnecessary to pool otoliths. Given these advantages, a technique was developed to enable the application of TIMS to the measurement of ^{226}Ra in otoliths.

Previous applications of TIMS to the determination of ^{226}Ra in natural samples has involved volcanic rocks. The elemental composition of otoliths are, however, very different from that of volcanic rocks and published methods had to be substantially modified. Of concern in the analysis of otoliths are high concentrations of calcium and barium and an organic component called otolin. Each of these constituents must be

reduced to very low levels, while conserving radium, to prevent ionization suppression of the radium signal and elevated background levels during TIMS analysis (Cohen and O'Nions, 1991).

This paper describes a new technique using TIMS to determine ^{226}Ra directly in otoliths which was adapted from a recently developed ion-exchange separation technique for geological samples (Chabaux et al. 1994). To test the application of this new technique, we have determined ^{226}Ra in otoliths of three fish species using isotope-dilution TIMS. In some otolith samples, ^{210}Pb was also measured for the purpose of radiometric age determination, the results of which are only briefly discussed here. The focus of this paper is the determination of ^{226}Ra in otoliths using a new ion-exchange technique and isotope-dilution TIMS, with a comparison to the traditional techniques, where the associated analytical features of merit are discussed.

Materials and Methods

The activity of ^{226}Ra was determined in sagittal otoliths from three fish species was performed. The species analyzed were shortspine thornyhead (*Sebastolobus alascanus*) collected from Monterey Bay, California, Atlantic tarpon (*Megalops atlanticus*) from inshore waters of Florida, and yelloweye rockfish (*Sebastes ruberrimus*) from off southeastern Alaska. Whole *S. alascanus* otoliths were selected and pooled to attain samples of approximately 1 g. The purpose of these samples was for comparison with similar whole otolith samples processed using radon emanation (Kline 1996) and direct α-spectrometry (John Butler, Southwest Fisheries Science Center, P.O. Box 271, La Jolla, CA 92038-0271, personal communication). Whole young-of-the-year *M. atlanticus* otoliths were pooled based on collection site and date to determine if exogenous ^{210}Pb would present a problem in ageing adults. Because age is less questionable for young-of-the-year, determining radiometric age can be used to determine if exogenous ^{210}Pb is incorporated. Otolith cores (first 4 years growth) were extracted using procedures given elsewhere (Andrews et al. 1997, Chapter 3) and were pooled into age-groups for *S. ruberrimus* to attain approximately 0.5 g core samples for the purpose of determining radiometric age. In addition, one whole otolith sample was processed for ^{226}Ra determination to compare ^{226}Ra activity with the core samples.

Due to the extremely low levels of ^{226}Ra and ^{210}Pb, trace-metal precautions were exercised during sample processing (Linn 1988, Fabry and Delaney 1989, Watters 1995). All acids used were double distilled (GFS chemicals®) and dilutions were made using Millipore® filtered Milli-Q (MQ) water (18 MΩ cm^{-1}). Because ^{210}Pb determination was

performed prior to ^{226}Ra analysis in radiometric age determination, the procedures used are briefly described here.

Otolith cleaning and dissolution

Otolith samples were cleaned, dried, and weighed prior to dissolution. Rough cleaning began with hydrating the otoliths in de-ionized water for 5 minutes. Samples were agitated and rinsed three times with each of the following: 1) a mixture of de-ionized water and Micro® laboratory cleaner, 2) de-ionized water, and 3) MQ water. Fine cleaning was performed by sequentially stepping between agitation in four cleaning solutions with a Branson 2200 sonicator and a triple rinse with MQ water. The four cleaning solutions with agitation times were: 1) MQ water (10 min), 2) 0.15N HNO_3 (1 min), 3) basic 1:1 mixture of 30% H_2O_2:0.4N NaOH (10 min), 4) MQ water (10 min), and 5) 0.001N HNO_3 (3 times at 1 min). After the final rinse, samples were dried for at least 24 hr in an oven at 80°C, cooled in a desiccator, and weighed to ±0.0001 g. Samples were dried to a constant weight.

Dried and weighed samples were placed in acid cleaned 100 mL Teflon® PFA griffin beakers. While on a hot plate at 80°C, 8N HNO_3 was added to the otoliths in 1 mL aliquots until dissolved. The dissolved sample was dried, re-dissolved with 1 mL of 8N HNO_3, and dried again. This was repeated 5 times. Prior to drying the fifth dissolution completely, 1 mL of 6N HCl was added to form an aqua regia solution. This solution was dried, re-dissolved with 1 mL 6N HCl, and dried again. Dissolution in 1 mL of 6N HCl was repeated 5 times which left the final sample in the desired chloride form. Drying

temperatures were kept low in order to minimize volatilization potential of polonium. The repeated drying and dissolution enhanced the oxidation of otolin, a potential interferant.

Determination of ^{210}Pb activity

To determine ^{210}Pb activity in the otolith samples, the α-decay of ^{210}Po (t½ = 138.4 d) was used as a daughter proxy for ^{210}Pb. To ensure that ^{210}Po:^{210}Pb was in secular equilibrium and that all of the ^{210}Po was due to ingrowth from ^{210}Pb, all samples were greater than 2 years old. Samples prepared for ^{210}Po analysis were spiked with a yield tracer, ^{208}Po, calibrated against NBS and geological standards (Williams 1988). The amount added was estimated to be 5 times the activity of ^{210}Po in the otolith sample to reduce error in the ^{210}Pb activity determination. This amount was added to the sample once the otoliths were first put into solution and before the repeated dissolutions previously discussed.

To isolate the polonium isotopes for the purpose of α-spectrometry, the isotopes were autodeposited onto a silver planchet. Spiked samples were redissolved in approximately 50 mL of 0.5N HCl on a hot plate covered with a watch-glass. In previous studies, ascorbic acid was added to inhibit autodeposition of divalent iron (Kline 1996). Because ascorbic acid is an organic that can act as an interferant in the TIMS analysis and because iron content in otoliths is low (Dannevig 1956), it was not used in this study. Samples were completely dissolved and the temperature was elevated to 90-100°C before plating. The ^{210}Po and ^{208}Po-tracer were autodeposited at this temperature (higher than suggested because of inaccurate temperature monitoring, 80°C) onto a purified silver

planchet (99.999%) held in a rotating teflon holder over a 4 hour period (Flynn 1968). Planchets were counted using both silicon surface barrier detectors and ion implant detectors in Tennelec TC256 α-spectrometers interfaced with a multi-channel analyzer and an eight channel digital multi-plexer with Nucleus® software on an IBM-PC. The activity of ^{210}Po was quantified by its integrated peak area relative to that of the ^{208}Po yield-tracer, which when decay corrected, was quantitatively equivalent to ^{210}Pb activity. Radium blanks included these steps to account for any potential radium contamination. The sample remaining after polonium autodeposition was taken to dryness and saved for ^{226}Ra analysis.

The separation of radium from calcium and barium is essential for obtaining good ionization efficiency during TIMS. This was achieved by applying a three column, ion-exchange separation procedure. Elution characteristics for each column separation were analyzed using calcium and barium standards and flame atomic absorption spectrophotometry (AA) to optimize the sample collection intervals. In the first two ion-exchange columns, most of the calcium was separated from barium and radium. Because barium co-elutes with radium, and not calcium, barium was used as a proxy for radium in determining radium elution characteristics. The eluate from the resultant collection interval for the barium and radium fraction was used in the third ion-exchange column. Distribution coefficients provided by the manufacturer were used to estimate the acid strength necessary to achieve the greatest separation between radium, barium, and any remaining calcium in the third cation exchange column. Based on these findings, an optimized ion-exchange separation procedure was developed (Table 1).

Radium separation

The radium separation procedure consisted of two cation-exchange columns. The first column type was a 10 mL chromatography column with a 20 mL reservoir (Bio-Rad Laboratories, Econo-Pac 10 Column). The conditioned column contained 10 mL of Bio-Rad AG® 50W-X8 cation exchange resin and had an aspect ratio of 3.9. The second column type was a custom 150 µL microcolumn made of TFE heat-shrink tubing (6.35 mm ID, Penntube Plastics) shrunk over a hand machined stainless steel die. Die dimensions were 6.35 mm (0.25") in diameter by 57 mm (2.25") in length for the reservoir which tapers down to 2.38 mm (0.094") in diameter by 35 mm (1.38") in length for the column volume. Enough tubing was used to create an 800 µL reservoir and a constricted tip with minimal dead volume. A frit made of porous polyethylene (2 mm thickness with 10 µm pore size) was cut and squeezed tightly down the column and into the tip. The conditioned microcolumn contained 150 µL of Sr® resin and had and aspect ratio of 13.1.

To determine ^{226}Ra using isotope-dilution TIMS, the dissolved otolith sample (prior to repeated dissolutions and plating) should be spiked gravimetrically with a ^{228}Ra yield-tracer. The ^{228}Ra solution was prepared by separating ^{228}Ra from its parent, ^{232}Th. The atomic ratio of ^{232}Th to ^{230}Th in the solution is greater than 1.6 million, producing a ^{228}Ra to ^{226}Ra ratio in the yield tracer of 0.2855 as of June 1, 1995. The ^{228}Ra spike solution was calibrated extensively against NBS and geological standards. The amount of ^{228}Ra added to each sample was estimated to attain a ^{226}Ra:^{228}Ra atom ratio close to one. The spiked sample was dried and examined to determine the next step in the separation procedure. If the residue was not white, the sample was redissolved with 1 mL of 8N

HNO$_3$ and dried. This was repeated until a white residue was obtained. Before the final drying was complete, an aqua regia transition to 6N HCl was created by adding 1 mL of 6N HCl. This solution was taken to dryness. Dissolution with 6N HCl and drying was repeated three times. All of these steps were repeated until the residue was as white as possible.

Calcium removal: two column separation

A rinsed slurry of Bio-Rad AG® 50W-X8 cation exchange resin and MQ water was added into an acid cleaned 10 mL chromatography column with a 20 mL reservoir (Bio-Rad Laboratories, Econo-Pac 10 column) to achieve 13 mL of settled resin. The settled resin was cleaned and conditioned by passing 100 mL of MQ water followed by 5 mL 2.5N HCl, 5 mL 4.0N HCl, and 40 mL 6.0N HCl through the column.

The sample was re-dissolved in 5 mL of 6N HCl over mild heat (50-60°C) covered with a watch glass. The 5 mL sample was cooled, loaded onto the column, allowed to settle into the resin and the beaker was rinsed with an additional 5 mL of 6N HCl. Using the sample pipette tip, the acid rinse was introduced by allowing 1 mL to settle into the resin before adding the remaining 4 mL. Once the acid rinse settled into the resin, two 1 mL aliquots of 6N HCl were added to begin washing the column. Each of these aliquots were allowed to settle into the resin prior to adding 28 mL of 6N HCl column wash. The column wash was allowed to settle into the resin and the collected eluant containing the bulk of the calcium was discarded (40 mL). An acid cleaned 100 mL Teflon® PFA griffin beaker was placed under the column to collect the radium fraction. The radium fraction

was collected in the next 80 mL of 6N HCl added to the column in portions that kept the reservoir full to maximize the flow rate (~1.5 mL min^{-1}). Once the 80 mL sample fraction was collected, the beaker was placed on a hot plate at 90-100°C and taken to dryness. The sample was never boiled and the heat was reduced when a crystalline residue began to form. While samples were drying, the same columns were cleaned and conditioned, as in the first preparation, for a second pass and the separation was repeated.

Barium removal: third column separation

The acid cleaned microcolumn was prepared for sample processing by adding a slurry of MQ water and 50-100 μm Sr® resin (EiChroM Industries). For best results, the column was filled with MQ water first, then the resin was added to the water and allowed to settle into the column to just below the taper of the reservoir. Cleaning and conditioning were performed by passing 1000 μL of MQ water followed by 800 μL of 1.1N HNO$_3$ through the column.

To prepare a sample for introduction to the third column, the sample was dissolved with 100 μL of 8N HNO$_3$. Droplets were swirled in the beaker bottom, gathered together, and dried at 90-100°C. If the dried sample spot was not white, the sample was treated with an aqua regia solution of 10-30 μL of 8N HNO$_3$ and 10-30 μL of 6N HCl, dried at 90-100°C, and re-dissolved in 10-30 μL of 8N HNO$_3$. The last two steps were repeated until the sample was as white as possible.

The sample spot was re-dissolved with 50 μL of 1.1N HNO$_3$ over mild heat (60-70°C), cooled, and added to the microcolumn with a 100 μL pipette. The sample was

allowed to settle into the resin and then a beaker rinse of 50 μL 1.1N HNO$_3$ was added to the column. A column wash of 150 μL of 1.1N HNO$_3$ was added and allowed to settle into the resin. The eluant (250 μL) was discarded. The final sample fraction was collected in an acid cleaned 3 mL Teflon® PFA sample vial. Radium elution was performed by adding 450 μL of 1.1N HNO$_3$ to the column, while the barium remained on the column. The collected sample was then placed on a hot plate at 90-100°C and dried. If the spot was not clear and very small (0.5 mm), an aqua regia solution of 1 drop of 8N HNO$_3$ and 1 drop of 6N HCl was added and dried at 90-100°C. These steps were repeated until the sample would not lighten or shrink any further. The sample spot was then re-dissolved two times in 1 drop of 6N HCl and dried into the chloride form. Microcolumns were cleaned by passing 2000 μL of MQ water and were stored upright in a vial of MQ water.

Thermal ionization mass spectrometry

To utilize TIMS, the final sample from the separation procedure must be loaded onto a metallic filament. The design was a single filament assembly made of 4 pass zone-refined 99.999% rhenium (0.020" wide and 0.001" thick; H. Cross Co.). Prior to loading the sample, the filament was out-gassed in a diffusion pump vacuum chamber at 3.5 amps for 30 min (~1700°C) once the vacuum was less than 10^{-6}torr. Filaments were allowed to cool in the pumped down chamber before removal. To minimize organic interference during analysis, sample loading began immediately after out-gassing. Two μL of tantalum activator (1% Ta solution, Birck 1986) solution were evaporated on to the filament to

enhance ionization (Cohen and O'Nions 1991). Samples in the teflon vials were transferred to the filament in two successive 1 μL additions of 1N HCl. The tantalum activator and the sample were dried as they were loaded by passing 0.8 amps through the filament. Once loaded, an additional 20 s period of increased amperage (1.5 amps) was applied to further secure the sample to the filament and drive of any remaining acid.

Radium analysis was performed on a Vacuum Generators 54-30 Thermal Ionization Mass Spectrometer, equipped with an energy filter and an ion-counting daly-detector. The dark current on the detector was 20 cpm and dead time was 22 ns. The accuracy and linearity of this detector at signals up to 1 million cps has been verified by repeated measurements of NBS standard U-010. This TIMS radium analysis procedure is similar to the procedure outlined in Cohen and O'Nions (1991).

Prepared filament samples were placed in the TIMS sample turret, pumped down overnight, and analyzed the following day. First a programmed warm-up sequence raised the filament to 2.4 amps (1150°C) over 15 min. After raising the filament manually to approximate operating temperatures (1197 to 1220°C), the residual ^{138}Ba signal was used to focus the beam for counting the much weaker ^{226}Ra and ^{228}Ra signals. The ^{138}Ba signal typically varied between 20,000 and 3 million counts per second (cps) at this point. Once focused, unspiked samples (samples containing no added ^{228}Ra) were monitored to determine if measurable natural ^{228}Ra existed in the otoliths. No measurable natural ^{228}Ra was recovered and, therefore, no adjustment was necessary.

Spiked samples were analyzed with a programmed sequence of readings at atomic mass unit (amu) 226 and 228 with background readings at amu 225.5. First the scanning

of the amu range 224.5 to 228.5 was performed to ensure that organic interference had been minimized. When the background from amu 224.5 to 228.5 appeared uniform, and the ^{226}Ra signal was as high as could be attained by focusing procedures (30 to >100 cps), analysis was begun. On spiked samples, where ^{226}Ra:^{228}Ra was near unity, analysis was performed by peak-hopping between amu 226 and amu 228 after taking a 20 s baseline measurement at amu 225.5. A measurement cycle consisted of a 5 s integration on amu 228 , a 10 second integration on amu 226, and a 5 s integration on amu 228. Twenty ratios were taken per block and an analysis generally consisted of 5 blocks (or until the desired precision was attained). Data were obtained in the filament temperature range of 1180-1300°C. At higher temperatures, calcium ionization interfered with the radium ionization. The ^{226}Ra:^{228}Ra atom ratio was calculated as the mean (±SE) of all the readings taken in the analysis with outlying values statistically eliminated by the analysis routine.

Mass fractionation during analysis can not be accounted for, but is believed to be much less (~1% per amu) than our normally obtained precision (~1% ± 2 SE). This assumption is strongly supported by the absence of a systematic decrease in measured atomic ^{226}Ra:^{228}Ra ratios on extended runs.

Because the TIMS detected atomic ^{226}Ra:^{228}Ra ratio was equal to the unknown atomic ratio of the sample, the known number of atoms in the ^{228}Ra spike can be used to determine the unknown number of ^{226}Ra atoms. Hence, ^{226}Ra activity is determined by multiplying the number of ^{226}Ra atoms by the ^{226}Ra decay constant.

Results

Radium separation

    Based on AA determinations, the separation of calcium and barium (used as a proxy for radium) for the first pass on the AG® 50W-X8 ion-exchange column was good, but the elements co-eluted to a small extent (Figure 1). To conserve any sample in the co-elution, the selected sample collection interval began in the tail of the calcium elution at 40 mL and ended at 120 mL. Less than 10% of the total calcium was found in the selected collection interval.

    The second pass on the AG® 50W-X8 ion-exchange column better separated the calcium and barium (radium) fractions which did not co-elute to a measurable extent (Figure 2). The selected sample collection interval was the same as in the first column pass. A calculated one percent of the total calcium may have remained in the sample fraction. Recovery of barium, and presumably radium, through the first two ion-exchange columns was greater than 90%.

    An acid strength of 1.1N HNO_3 achieved the greatest separation between calcium, barium, and radium on the Sr® resin microcolumn. Based on the distribution coefficients of the Sr® resin, a radium sample collection interval of 250 to 700 µL was selected between the calcium and barium elution intervals (Figure 3). A small sample spot (<0.5 mm) remained after drying and was used for radium determination on the TIMS.

    To optimize the elemental separations and radium recovery, the collection intervals for each column separation were shifted slightly from the first trial run (Table 1). The initial collection interval for the first two columns was from 35 to 120 mL. Occasional

high calcium in the TIMS analysis was attributed to this collection interval. Therefore, a shift of the collection interval away from the calcium peak to 40 to 120 mL was adopted for each column pass (Figures 1 & 2). The amount of crystalline residue visible at the end of each column pass was reduced as a result of this shift. The trial collection interval for the third column was from 250 to 700 μL. This collection on this interval resulted in the high ^{226}Ra counts using TIMS (100-130 counts per second for 20 min). Samples analyzed after this shift improved because they have significantly lower calcium, a stronger radium signal, and lower analytical uncertainty (<1%).

Thermal ionization mass spectrometry

Seventeen samples consisting of 15 otolith samples from three fish species and two spiked ^{228}Ra yield-tracer blanks were processed for radium determination using isotope-dilution TIMS. Three of the 15 otolith samples, one from each species, were not spiked with ^{228}Ra to verify that natural quantities of ^{228}Ra were insignificant relative to potential spike quantities. Results indicate that naturally occurring ^{228}Ra for each species is at or below TIMS background. Spiked blanks indicated the ion-exchange separation procedures do not contribute measurable ^{226}Ra to samples. The twelve spiked samples produced ^{226}Ra activities ranging from 0.0251 ± 0.0001 dpm g^{-1} to 1.63 ± 0.02 dpm g^{-1} (Table 2).

During sample analysis, the signal strength of calcium, barium, and radium were observed qualitatively. The ^{226}Ra signal was usually strong (as high as 500 cps) and had low or no background interference from organics (usually <1 cps). The residual calcium

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and barium signals were much stronger than the radium signals (approximately 20,000 and 3 million cps, respectively). Samples with the highest analytical uncertainties typically had the highest residual calcium and barium signals.

Technique comparison

Determination of ^{226}Ra using the ion-exchange separation procedure and isotope-dilution TIMS in otoliths from three fish species revealed a wide range of activity levels (Table 2). This was expected due to habitat and geographical variation among the species. Whole and cored otolith samples from *S. alascanus* and *S. ruberrimus* contained the lowest ^{226}Ra activities and have comparable levels. The whole young-of-the-year otoliths from *M. atlanticus* contained ^{226}Ra activities that ranged from approximately 10 to 100 times the activity of the two deep-water species. The ^{226}Ra activities calculated from the TIMS results for each fish species were part of separate radiometric ageing studies which are discussed elsewhere.

To allow direct comparison of these results with existing studies, some reported ^{226}Ra uncertainties were recalculated as a percent of the reported ^{226}Ra activity. These comparisons are reported here with other features that are an improvement relative to existing techniques. The ^{226}Ra activities for *S. alascanus* are unique because they were compared with ^{226}Ra results from radon emanation and direct α-spectrometry for the same species. The TIMS ^{226}Ra activities for this species are the lowest, with radon emanation and direct α-spectrometry ranging higher (Table 3). Each determination was performed on similar, but independent samples. Because the *S. ruberrimus* samples are otolith-core

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samples, the sample size and ^{226}Ra measurements were compared to results from two

recent radiometric ageing studies where otolith cores were used (Table 4). Sample size,

analytical uncertainty, and processing time were significantly reduced relative to these

studies.

Discussion

The primary objective of this study was to separate radium from calcium and barium in otoliths, and to determine ^{226}Ra using isotope-dilution TIMS. The initial approach was to use a technique developed for volcanic rocks where barium is the primary suppressant to the detection of radium using TIMS (Volpe et al. 1991). Attempts to use this technique resulted in samples too high in calcium and barium to measure radium. Because otoliths are high in calcium (38% by weight; Dannevig 1956, Macpherson and Manriquez 1977), in addition to barium (~5-10 ppm; Edmonds et al. 1991), we applied a recently developed ion-exchange separation technique (Chabaux et al. 1994). With minor modifications, calcium and barium were successfully reduced and measurable radium was recovered.

In the developmental stages of the ion-exchange separation procedure there were problems with organic contamination. Samples analyzed using TIMS had an elevated and noisy background (high counts at atomic mass 225.5) which we attributed to organic interference from otolin (0.2-10% in otoliths by weight; Degens et al. 1969, Radtke 1984). This observation led to the rigorous sample dissolution and oxidation procedures reported here. As a result of this procedure, the product of the ion-exchange separation procedures had very little visible organic residue. However, occasionally there appeared to be a small amount of organic residue in the final sample, which we attributed to resin from the first two ion-exchange columns. When this residue is present, it should not be loaded onto the TIMS filament because it raises the organic background. Instead it should be brought up in acid and passed through the Sr® resin column a second time. As a result of the

dissolution and oxidation procedures, the observed organic interference on the thermal ionization mass spectrometer was reduced to very low levels (<1 cps).

The difference between the ^{226}Ra levels determined for *S. alascanus* otoliths using radon emanation and direct α-spectrometry prompted a follow up ^{226}Ra determination using the new TIMS technique (Table 3). The ^{226}Ra activities determined using TIMS are lower than the results for each determination, but more similar to Kline (1996). Because some samples were taken from a location further north off the Farallon Islands, California, variation may be from differing environmental concentrations of radium. Variation of radium in the natural environment is especially evident in the following study of juvenile *M. atlanticus*.

Determination of ^{226}Ra and ^{210}Pb for young-of-the-year *M. atlanticus* samples was part of a feasibility study to determine if radiometric age determination would be applicable to adult otolith cores. The order of magnitude range of ^{226}Ra activity among the samples (0.199 ± 0.001 to 1.63 ± 0.02 dpm g^{-1}) may be attributed to the variable water chemistry of the inshore waters where the juveniles were collected (Fanning et al. 1982, Crabtree et al. 1995, Moore 1996). However, this variability was not a detrimental factor for this technique because ^{226}Ra and ^{210}Pb were determined from the same sample.

To determine if exogenous ^{210}Pb would be a factor in radiometric age determination of adult *M. atlanticus*, age was calculated for the young-of-the-year samples using the measured ^{210}Pb and ^{226}Ra activities from each sample. If exogenous ^{210}Pb is accumulated in significant quantities, then the calculated radiometric age would be greater than the known age of these young-of-the-year juveniles (<1 yr). Measurements

of ^{210}Pb in these samples were very low (0.0559 dpm g^{-1}) and resulted in radiometric ages that were close to 1 yr. Therefore we concluded that exogenous ^{210}Pb is not a factor in juvenile otoliths of *M. atlanticus*. A follow up study to validate the estimated longevity of *M. atlanticus* using the new ion-exchange separation technique and isotope-dilution TIMS was successful (Andrews et al. 1997a).

The cored *S. ruberrimus* samples demonstrate several important aspects of this new technique (Table 4). First, the cored samples were analyzed for both ^{210}Pb and ^{226}Ra. This means it is not necessary to assume that radium activity among the cores in the sample is equal and constant. Second, the sample sizes required can be very small and have low ^{226}Ra activity. This demonstrates the capability of the new technique to detect ^{226}Ra in smaller samples than attempted in other studies with high accuracy and precision at low levels.

Because ^{226}Ra activities observed for *S. alascanus* and *M. atlanticus* were inconsistent among the samples, variation of ^{226}Ra uptake among the individuals in the sample must be factored in for future radiometric ageing studies. To increase the accuracy of radiometric age determination using otolith cores, measurement of ^{226}Ra must be performed on each pooled core sample and the assumption of constant ^{226}Ra uptake must be avoided. This assumption has been avoided in several studies by measuring ^{210}Pb and ^{226}Ra activities in each sample (Kastelle et al. 1994, Fenton and Short 1995). Variation of ^{226}Ra activity within the pooled sample has no effect on age determination because average ^{210}Pb:^{226}Ra disequilibria yields average age. Because of the analytical advantages

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inherent in the new technique, this assumption becomes unnecessary and radiometric age determination becomes more accurate.

In a companion study, this technique was sucessfully applied to the radiometric age determination of the Pacific grenadier, *Coryphaenoides acrolepis*. This study serves as important demonstration of the effectiveness and utility of the new technique in radiometric age determination (Andrews et al. 1997b; Chapter 3, Figure 7).

Some of the advances and advantages of the new technique are demonstrated by comparing the results of this study with two recent radiometric ageing studies (Table 4). By comparing the isotope-dilution TIMS results with the radon emanation results for *Anoplopoma fimbria* (Kastelle et al. 1994), the new technique measured ^{226}Ra activity that is about ten times lower in samples more than three times smaller with lower analytical uncertainty. A comparison with the α-spectrometry results for *Macruromus novaezelandiae* (Fenton and Short 1995) indicate ^{226}Ra activities are comparable, but sample size is about three times smaller with greatly reduced analytical uncertainty. Processing time for this technique was significantly reduced from 3.5-6 weeks, for the other techniques, to 7-10 days.

Because this technique has improved the accuracy and processing time of ^{226}Ra determination, the most significant source of error and analytical time in radiometric age determination is now associated with ^{210}Pb determination via α-spectrometry. In general, higher sample age and higher ^{226}Ra activity result in higher ^{210}Pb activity and consequently, lower analytical uncertainty. For samples with low ^{210}Pb activity, improvements in detection limits and reagent and background levels are the next steps to reduce error in

^{210}Pb determination. The time required, however, to acquire adequate counts in α-spectrometry for ^{210}Pb determination on young fish is still about 3 weeks for the low levels typically encountered. But for fish that are old (decades), count times can be lower and accuracy can increase because the activity of ^{210}Pb increases.

In the context of other radiometric ageing studies, the new ion-exchange separation technique with isotope-dilution TIMS provides a valuable contribution to the developing school of radiometric age determination. The ^{226}Ra activities determined for the otoliths of the three species studied are generally in the range of values reported by all of the previously cited fish ageing studies (0.004 ± 0.006 to 0.517 ± 0.021 dpm g^{-1}), with the exception of one *M. atlanticus* sample (1.63 ± 0.02 dpm g^{-1}). This is the highest ^{226}Ra activity yet reported in the otoliths of fish by more than a factor of three. Preliminary radiometric age determinations made for *S. ruberrimus*, and the successful application of the new technique to the Pacific grenadier (Andrews et al. 1997b, Chapter 3) and Atlantic tarpon (Andrews et al. 1997a), indicate the new ion-exchange separation procedure coupled with isotope-dilution TIMS is an effective technique for determining ^{226}Ra in otoliths for the purpose of radiometric age determination.

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Table captions

Table 1. Overview of column separation procedures. Radium and barium were first separated from calcium by passing the sample through the AG® 50W-X8 column twice. The radium and barium fraction from each pass was taken to dryness before the next column pass. Sample introduction on the second column pass was reduced from 5 mL to 1 mL because the dried sample is reduced and to narrow the elution peak for better separation. Radium was separated from barium and residual calcium by passing the redissolved sample through the Sr® resin column. The final radium fraction was taken to dryness and processed using TIMS.

Table 2. Summary of sample types for each species. Number of otoliths used corresponds to the number of fish in each sample because one otolith of each pair was sectioned for traditional ageing. Total sample weight and measured ^{226}Ra activity with % error (1 SE) are given for each sample.

Table 3. Comparison of results for *S. alascanus* otoliths using each technique. Otolith samples were similar, but from different fish samples. The lowest analytical uncertainty, for a sample size much lower than radon emanation and comparable to α-spectrometry, was the result of isotope-dilution TIMS.

Table 4. A comparison of the isotope-dilution TIMS results for *S. ruberrimus* cores with

two studies utilizing otolith cores and one of the other techniques. Because of the

advantages of TIMS, sample size is lower for samples with low activity, and analytical

uncertainty and processing time is reduced.

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Table 1. Cation-exchange column separation procedures.

	Calcium removal	Volume (mL)		Barium removal	Volume (µL)
	Reagent	First pass	Second pass	Reagent	
Ion-exchange resin	AG® 50W-X8 (Bio-Rad)	13[a]	13[a]	Sr® resin (EiChroM Ind.)	150[a]
Clean and condition	MQ water 2.5-6.0N HCl	100 50	100 50	MQ water 1.1N HNO$_3$	1000 800
Introduction	sample[b]	5	1	sample[d]	50
Beaker rinse	6.0N HCl	5	1	1.1N HNO$_3$	50
Wash	6.0N HCl	30	38	1.1N HNO$_3$	150
Elution	6.0N HCl	80[c]	80[c]	1.1N HNO$_3$	450[e]

[a] Rinsed resin added in MQ water.

[b] Sample dissolved in 6.0N HCl.

[c] Radium and barium fraction.

[d] Sample dissolved in 1.1N HNO$_3$.

[e] Radium fraction.

Table 2. Summary of sample characteristics for each species.

Species	Sample type (whole or core)	Number of otoliths	Sample weight (g)	^{226}Ra (dpm g^{-1})	^{226}Ra % error
Sebastolobus	whole	7	1.0150	0.0302	0.672
alascanus	whole	8	1.2607	0.0317	0.980
Megalops	whole[a]	35	0.5068	0.199	0.731
atlanticus	whole[a]	26	0.525	0.302	0.338
	whole[a]	28	0.564	1.63	1.22
Sebastes	whole	1	0.8819	0.0259	2.00
ruberrimus	core	10	0.319	0.0251	0.430
	core	20[b]	0.592	0.0279	2.33
	core	23	0.587	0.0292	1.00
	core	14	0.471	0.0294	0.719
	core	10	0.303	0.0301	0.768
	core	19	0.561	0.0327	1.82

[a] Young-of-the-year.

[b] Approximate number.

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Table 3. Comparison of results for the determination of ^{226}Ra activity in otoliths from *S. alascanus*.

	Technique	Sample size range (g)	^{226}Ra activity range (dpm g^{-1})	Analytical uncertainty (%)
This study	TIMS	1.0150-1.2607	0.0302-0.0317	0.672-0.980
Kline (1996)	Radon emanation	15.631-16.588	0.0387-0.0504	3.4-5.2
Butler[a] (unpublished data)	Alpha spectrometry	1.23-1.65[b]	0.0495-0.0697	5.8-6.6

[a] John Butler, Southwest Fisheries Science Center, P.O. Box 271, La Jolla, CA 92038-0271, personal communication.

[b] Before cleaning.

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Table 4. Comparison of technique results for otolith-core ageing studies.

	Technique	Sample size range (g)	^{226}Ra activity range (dpm g^{-1})	Analytical uncertainty (%)	Processing time
This study	TIMS	0.303-0.592	0.0251-0.0327	0.430-2.33	7-10 days
Kastelle et al. (1994)	Radon emanation	0.9227-1.3748	0.288-0.517	~4	5-6 wks[a]
Fenton & Short (1995)	Alpha spectrometry	approx. 1[b]	0.0179-0.0290	~12-21	3.5-4 wks[c]

[a] Craig Kastelle, Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, 7600 Sand Point Way NE, Seattle, WA 90915-0070, personal communication.

[b] Specific sample weights not reported.

[c] Gwen Fenton, Department of Zoology, University of Tasmania, Hobart, Tasmania, 700.0, Australia, personal communication.

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Figure captions

Figure 1. Elution characteristics of the first pass using 6.0N HCl on the AG® 50-X8 cation exchange column for calcium (O) and barium (■). In these experiments barium was used as a stable proxy for radium. Calcium eluted strongly in the first 40 mL and then tailed to approach zero at 60 mL. The beginning of the barium elution is within the calcium tail and, therefore, the collection interval for the radium and barium fraction is from 40 to 120 mL.

Figure 2. Elution characteristics of the second pass using 6.0N HCl on the AG® 50-X8 cation exchange column for calcium (O) and barium (■). To more clearly evaluate the separation, calcium and barium elutions were performed separately, but plotted together. The collection interval for the radium and barium fraction was from 40 to 120 mL

Figure 3. Elution characteristics of the third column pass using 1.1N HNO_3 on the Sr® resin microcolumn for calcium (O) and barium (■). Based on the distribution coefficients provided by EiChroM Ind., radium eluted between calcium and barium. The optimal collection interval which minimizes calcium and barium was determined to be 250-700 μL. The small peaks in between the calcium and barium elution intervals were attributed to low level contamination. Radium in this interval was determined using TIMS.

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Figure axes: Percent of total recovered (%) vs Elution volume (mL)

Legend:
—○— % of total Ca
—■— % of total Ba

Radium co-elutes with barium

Collection interval

Radium collection interval

% of total Ca
% of total Ba

Elution volume (μL)

Percent of total recovered (%)

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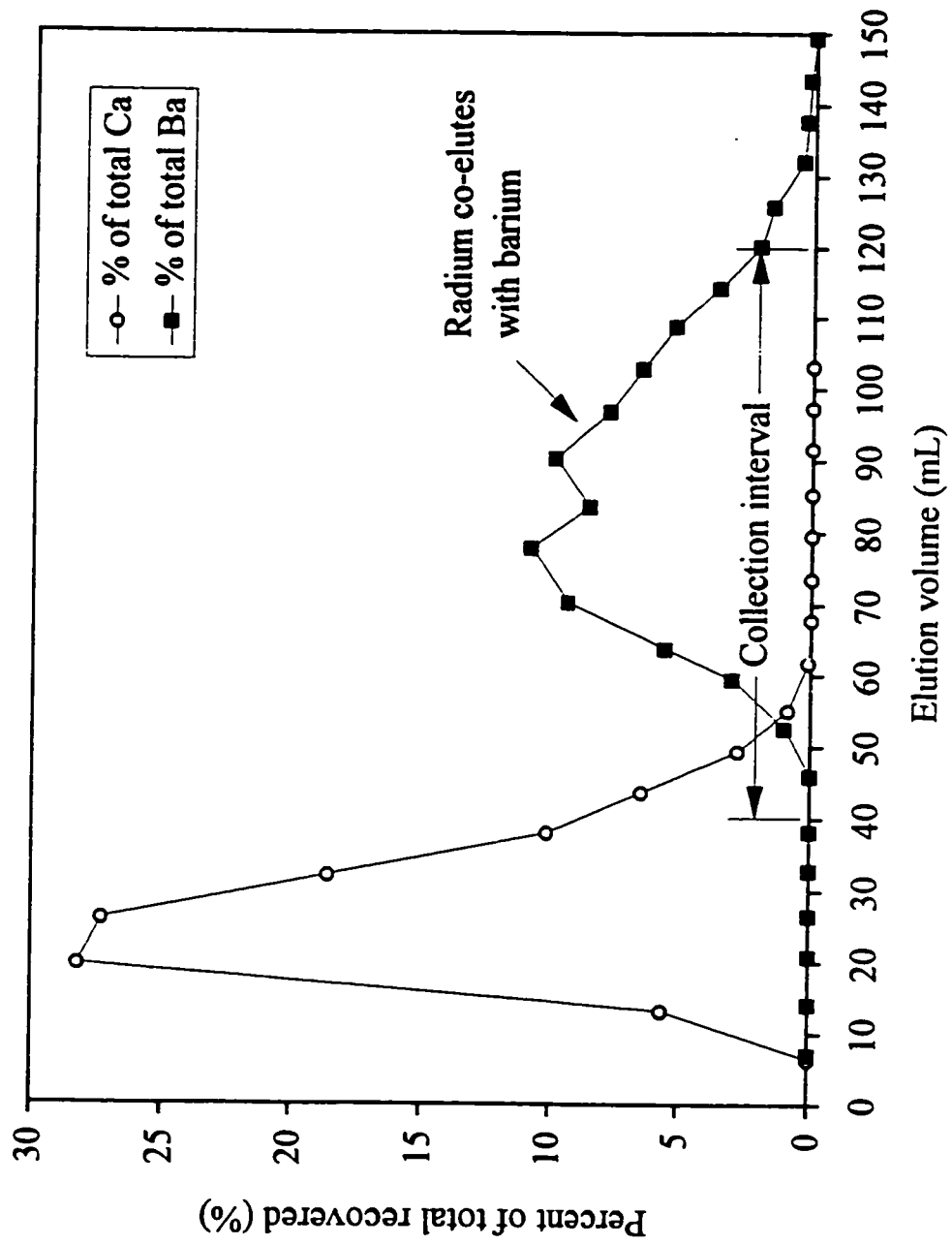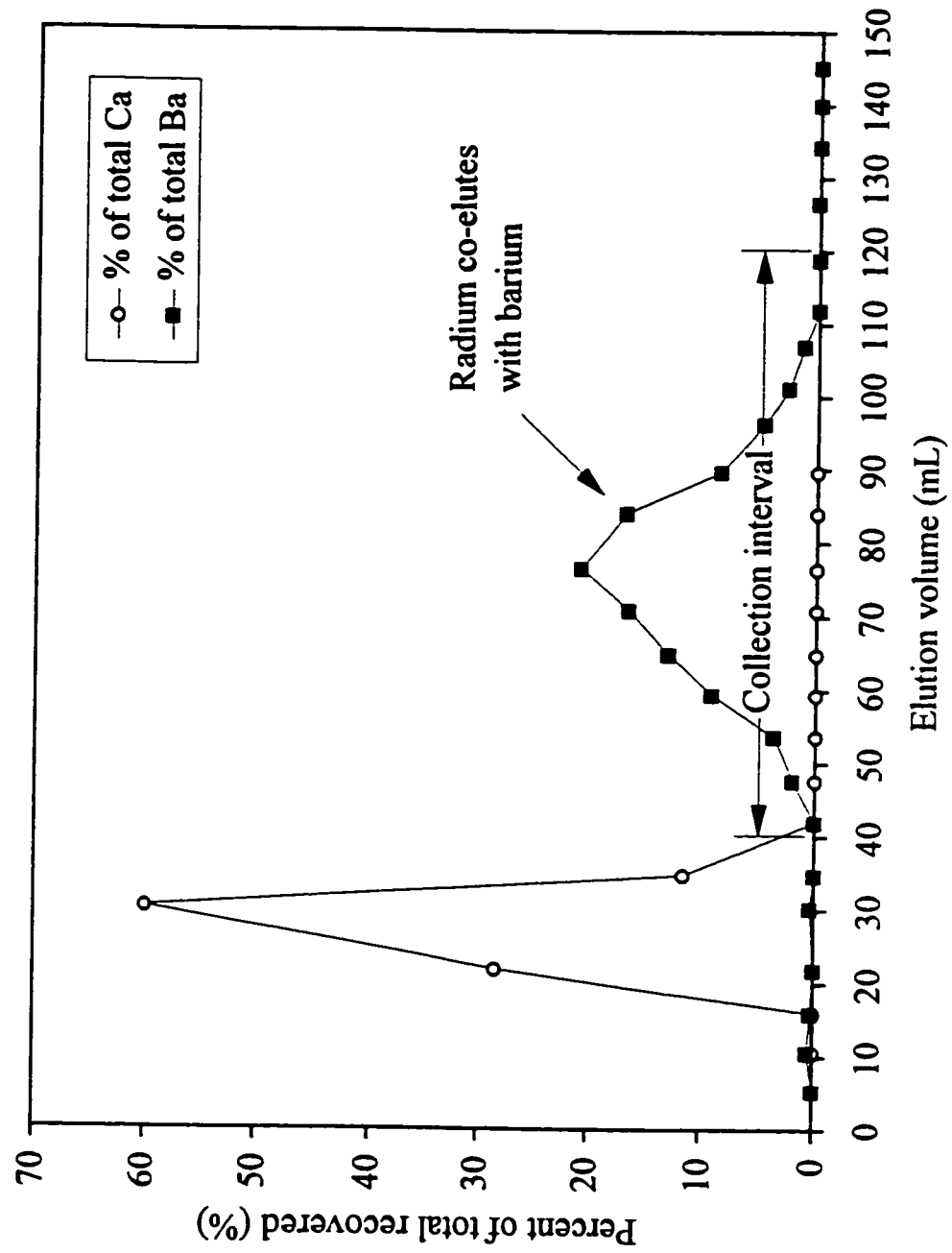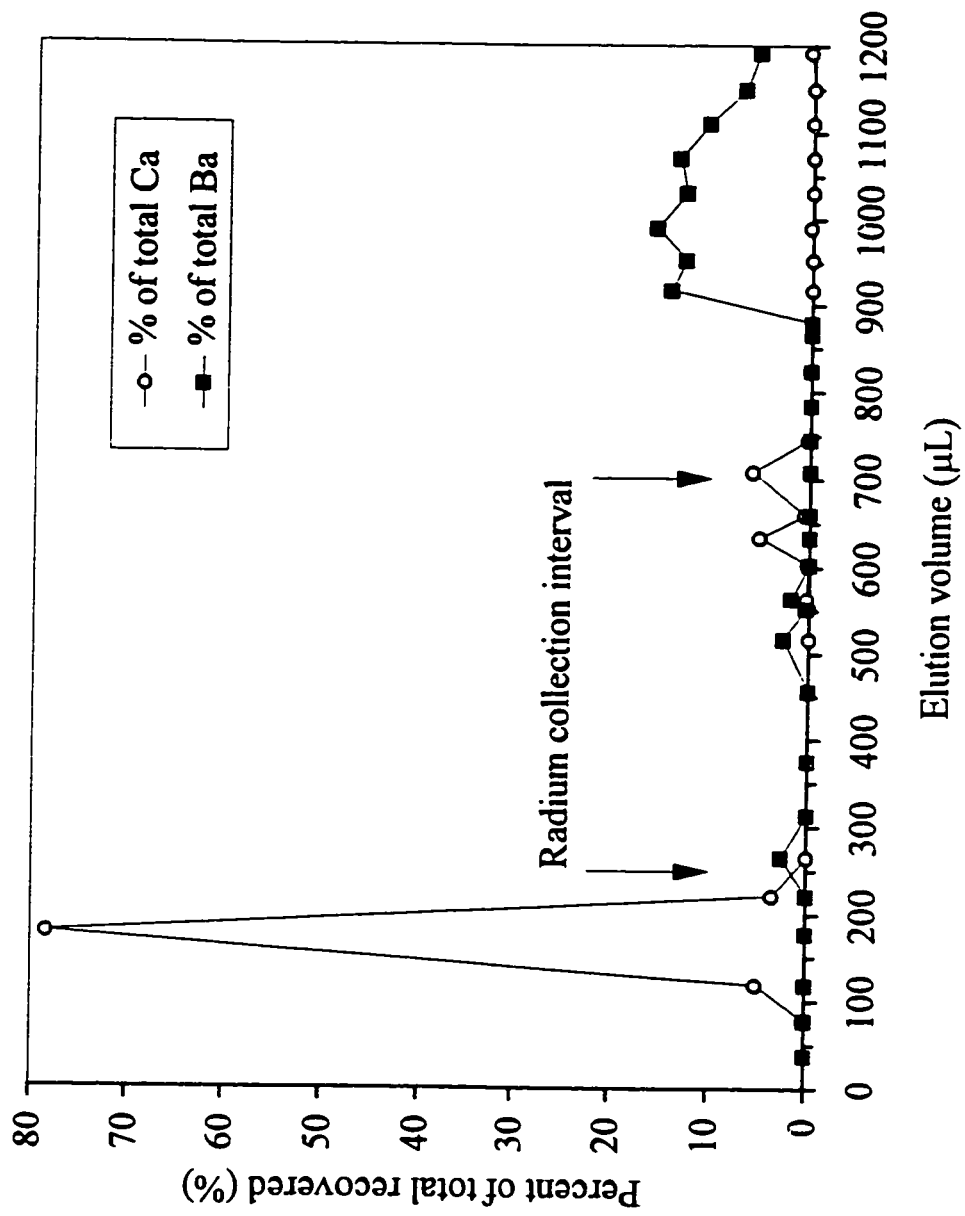
CHAPTER 3

Age and growth of the Pacific grenadier (Family Macrouridae, *Coryphaenoides* *acrolepis*) with age estimate validation using an improved radiometric ageing technique

Abstract

Current and historic longevity estimates for the Pacific grenadier range from 6 to greater than 60 years. Age estimates in this study using the quantification of growth increments in thin otolith (ear-bone) sections indicate the Pacific grenadier is a long-lived fish that may approach 75 years. To validate this trend, age was determined using the radioactive disequilibria of ^{210}Pb and ^{226}Ra in otolith cores from adult Pacific grenadier. Radiometric ages closely agree with age estimates from the quantification of growth increments. This confirms the annual periodicity of these increments. Radiometric results indicate the Pacific grenadier can live at least 56 years and growth increments indicate longevity may be greater than 73 years. Because the Pacific grenadier is long-lived and matures late in life, it may be vulnerable to heavy fishing pressure. Therefore, conservation measures need to be taken immediately to sustain this rapidly developing fishery.

Introduction

The Pacific grenadier, *Coryphaenoides acrolepis* (family Macrouridae), is a benthopelagic, deep-water fish species which inhabits the continental slopes of the northern Pacific Ocean. Its range is circum-north Pacific from Baja California, Mexico to northern Japan and into the Bering Sea (Iwamoto and Stein 1974, Cohen et al. 1990). Its typical depth range is from 600 to 2500 m (Okamura 1970, Iwamoto and Stein 1974) with a population density maximum near 1500 m (Stein and Pearcy 1982, Matsui et al. 1990).

Until recently, fishermen regarded the Pacific grenadier as a trash fish because the fillets were small (~25% of the total fish weight) and had low market value ($0.08 to 0.16 per pound ex-vessel). In recent years, the Pacific grenadier has been discovered to have desirable culinary attributes (Kremsdorf et al. 1979, Matsui et al. 1990) and has become a large commercial fishery in California and Oregon (Figure 1). Landings for Monterey Bay, California have increased substantially from practically zero for 1992 to nearly 900 t (~2 million pounds) for 1996 (Figure 2). Prior to 1995, greater than 95% of the landings were from trawlers. For 1995 and 1996 greater than 90% of the landings were from set lines targeting the Pacific grenadier (Leos 1997). Because of these landings, the Pacific grenadier is no longer in the "other" category and has become the fifth largest fishery in Monterey Bay, California and thirteenth in the state of California at 1,130 t (~2.5 million pounds) for 1996 (Leos 1996 and 1997).

To properly manage this rapidly developing fishery, the age structure and growth characteristics of the Pacific grenadier must be known along with other life history parameters. Some life history aspects are known (Stein and Pearcy 1982, Matsui et al.

1990), but longevity estimates remain controversial. Recent and historic age and longevity estimates range from 6 to greater than 60 years (Kulikova 1957, Brothers et al. 1976, Mulcahey et al. 1979, Wilson 1982, Matsui et al. 1990).

Fisheries management strategies rely heavily on accurate age determinations. Age is typically determined in fishes by performing one of several techniques, but most commonly the quantification of growth increments in calcified structures (Chilton and Beamish 1982, Beamish and McFarlane 1987). The annual periodicity of growth increments in these structures is often assumed. Until recently this assumption was rarely validated (Beamish and McFarlane 1983). As a consequence, underestimation of longevity may have had serious consequences to existing fisheries; for example the decline of the Pacific ocean perch (*Sebastes alutus*) of the northeastern Pacific Ocean and the orange roughy (*Hoplostethus atlanticus*) off New Zealand (Beamish 1979, Archibald et al. 1983, Mace et al. 1990, McFarlane and Beamish 1995, Smith et al. 1995) may be attributed to age underestimation and over fishing. The problem with typical growth-increment age validation techniques is that they have limited applicability to deepwater or long-lived fishes (Macdonald 1987, Mace et al. 1990, McFarlane and Beamish 1995). One technique that can be used to validate age estimates for these fishes is the radiometric ageing technique which uses the disequilibria of ^{210}Pb and ^{226}Ra in otoliths as a natural chronometer (Smith et al. 1991). This technique has been successfully applied to 12 fish species (Bennett et al. 1982, Campana et al. 1990, Fenton et al. 1991, Kastelle et al. 1994, Fenton and Short 1995, Milton et al. 1995, Smith et al. 1995, Stewart et al. 1995, Watters 1995, Kline 1996).

In this study, the age and growth of the Pacific grenadier is estimated using a traditional ageing technique and validated using a novel radiometric ageing technique. Improvements made to the radiometric ageing technique in a concurrent study have been utilized here (Andrews et al . 1997, Chapter 2).

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Materials and Methods

Lengths were recorded for, and sagittal otoliths were extracted from, fish collected at various locations. Specimens ranged in size as much as was available from sampling. Fish collected east of Santa Cruz, California in November 1992 were processed fresh; others were collected during slope surveys conducted by Alaska Department of Fish and Game in October of 1992 and December 1993, shipped frozen, and then processed. Some otoliths were previously extracted and made available by the Oregon Department of Fish and Wildlife. As otoliths were extracted, total length (TL), pre-anal fin length (PAF), snout to dorsal fin (SD), and head length (HL) were recorded to the nearest millimeter. PAF was defined as snout to the posterior insertion of the anal fin. SD was defined as snout to the posterior insertion of the dorsal fin. HL was defined as snout to the anterior most tip of the operculum. The condition of the tail was carefully examined and specimens with intact tails were noted for a partial length analysis. Physical data for most otoliths was collected for comparison with fish lengths and age. Otolith weight was measured to the nearest milligram and length, width, and height were measured to the nearest 0.1 mm. Weight of right and left otoliths were compared using a t test to determine if significant differences occurred between sides.

To ensure a selection of clean otoliths for radiometric analysis, each otolith was carefully cleaned and stored. Extracted otoliths were initially stored in de-ionized water and ethanol (50%) buffered with marble chips. Further cleaning of adhering tissues was made later with the same solution in the lab. Cleaned otoliths were stored dry in acid cleaned glass vials.

Before otoliths were selected for traditional ageing, limitations of sample size in the radiometric age determination process were considered. Based on the findings of Campana et al. (1990), radiometric analysis of age-groups, consisting of pooled otolith cores, was selected. Because otolith cores are typically the first few years of life and can be very small, dimensions and weight of otoliths from juvenile fish (estimated age 5 yr) were examined. The pooled sample size of an age-group was typically limited to approximately 0.5 to 1.0 g by the ^{210}Pb determination procedures. Depending upon the weight of the otolith cores, the number of otoliths needed to perform the analysis was determined and a subsample of otoliths were held aside for radiometric analysis.

Traditional otolith ageing

Before the ageing criteria were adopted, an objective study of growth increments was performed. Sections were interpreted as many ways as possible under a range of magnifications (10x to 80x), where increments were split or lumped depending on what was visible for the magnification. A subjective decision was made based on several very clear sections that showed growth increments gradually becoming finer toward the margin (Figure 3). Criteria based on these sections provided the most consistent results and varied the least with respect to fish length. However, transverse sections were inherently difficult because section angle was very critical and dark patches, possibly proteinaceous inclusions, often obscured growth increments.

Otoliths selected for traditional ageing were transversely sectioned for growth increment analysis. Each otolith was centered and mounted on cards with casting resin.

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Transverse sections containing the nucleus were removed using a Buehler-Isomet low-speed bone saw with two Norton® low density diamond blades separated by acetate spacers (0.6 mm total). Sections were placed on a slide, mounted with Cytoseal, and allowed to dry overnight. Sections were thinned to optimal viewing thickness using a Buehler Ecomet III lapping wheel with 600 grit silicon-carbide paper. Growth increments were viewed with transmitted light at a magnification of 32 to 80 times under an Olympus dissecting scope. Each section was analyzed by counting concentric growth increments radiating from the nucleus out to the margin. The sulcal region was usually used, but the distal margin was also used for some samples. Otolith sections that did not have quantifiable increments were removed from the analysis. Readable otoliths were aged independently by two readers three times. Average percent error (APE) and index of precision (D) were used to test the reproducibility of ageing between readers (Beamish and Fournier 1981, Chang 1982). Consultation between readers over ageing criteria was used to resolve differences and final age estimates were assigned by the first reader. A von Bertalanffy growth function was fitted, using FISHPARM software (Elsevier Scientific Publishing Co.), to the final age estimates to describe growth parameters.

Age-group determination and core extraction

Because otoliths were difficult to age and the amount of otolith material must be maximized for radiometric analysis, age-groups were determined using otolith and fish parameters, instead of sectioning. For otoliths to be used in age-groups, sexes were separated and age was estimated based on a linear regression of otolith weight and

estimated age from the aged otoliths. To discriminate dissimilar fish and maximize similarities among the members of the potential age-groups, age was also estimated based on a multiple linear regression incorporating fish length (PAF), otolith weight, and an interaction term (Otolith length multiplied by PAF). A comparison of each regression age was made to determine if the otoliths for each fish would be included in the age-group. Fish with regression ages that differed by greater than 10% were not included in the age-group. The age range for each age-group was kept as narrow as was permitted by the amount of otolith mass needed. The average age of the age-group was a weighted average based on the estimated age of each core in the sample. An age-group weight of 1 gram was targeted, but lower amounts were used where insufficient sample was available.

The otoliths selected for each age-group were cored to the size of a 5 year old otolith. Dimensions determined from 3 juvenile otoliths were 5.0 mm long, 3.0 mm high, and 1.0 mm thick. Each core was extracted by cutting a centered transverse section and hand grinding to the core dimensions. This was performed by mounting the otolith on cards with casting resin and cutting with a Buehler-Isomet low-speed bone saw. The Norton® low density diamond blades were separated by an aluminum spacer (6 mm). Sections were ground down to core size by hand on a Buehler Ecomet III lapping wheel with 320 grit silicon-carbide paper. A weight of approximately 0.02 g per otolith core was targeted.

Radiometric analysis

To address some of the assumptions of this technique, juvenile otoliths and whole adult otoliths were analyzed. To determine if exogenous ^{210}Pb is a factor by being incorporated during otolith formation, a sample of juvenile fish was added to the age-groups. For comparative purposes, a whole-otolith pair was analyzed for ^{226}Ra to assess if an ontogenetic difference occurs in ^{226}Ra uptake.

Each pooled otolith core sample was analyzed for ^{210}Pb and ^{226}Ra. To determine ^{210}Pb, alpha-spectrometry of ^{210}Po, its daughter product, was performed. Determination of ^{226}Ra was performed using a new technique that utilizes isotope-dilution thermal ionization mass spectrometry (TIMS; Andrews et al. 1997). The details of ^{210}Pb determination are described here, but only the details of ^{226}Ra determination that vary from the established procedure are stated here.

Because of the extremely low levels of ^{210}Pb and ^{226}Ra, trace-metal precautions were exercised during sample cleaning and processing (Linn 1988, Fabry and Delaney 1989, Watters 1995). All acids used were double distilled (GFS chemicals®) and dilutions were made using Millipore® filtered Milli-Q water (18 MΩ cm^{-1}). Thorough cleaning and repeated dissolution of the core samples was performed prior to radiometric analysis (Andrews et al. 1997).

210Pb activity determination

To determine ^{210}Pb activity in the otolith core samples, the activity of ^{210}Po was used as a proxy for ^{210}Pb. Because background in the ^{210}Pb-^{210}Bi beta-counting technique via lead sulfate coprecipitation is relatively high (6 to 12 counts per hour), this technique was not employed (Koide and Bruland 1975). Due to the relatively short half-life and low activity of ^{210}Pb, the detection of ^{210}Pb is typically accomplished through the autodeposition and α-spectrometric determination of its daughter proxy, ^{210}Po (Flynn 1968). Differences in half-life for ^{210}Pb and ^{226}Ra lead to low ^{210}Pb:^{226}Ra atom ratios. Therefore direct mass determination methods are not feasible for ^{210}Pb at this time. Because of these constraints, the long counting times required, and the low backgrounds associated with α-spectrometry, autodeposition and α-spectrometric analysis of ^{210}Po was chosen to determine ^{210}Pb activity in this study.

To ensure that all of the ^{210}Po is due to ingrowth from ^{210}Pb, and that ^{210}Po:^{210}Pb was in secular equilibrium, all samples were at least 2 yr old. Samples prepared for ^{210}Po analysis were spiked with a yield tracer, ^{208}Po, calibrated against NBS and geological standards (Williams 1988). The amount added was estimated to be 5 times the activity of ^{210}Po in the otolith sample to reduce counting error in the determination of ^{210}Pb activity.

To isolate the polonium isotopes for the purpose of α-spectrometry, the isotopes were autodeposited onto a silver planchet. Spiked samples were redissolved in approximately 50 mL of 0.5N HCl on a hot plate covered with a watch-glass. Samples were completely dissolved and the temperature was elevated to 90-100°C before plating.

The ^{210}Po and ^{208}Po-tracer were autodeposited at this temperature (higher than suggested because of inaccurate temperature monitoring, 80°C) onto a purified silver planchet (99.999%, A.F. Murphy Die and Machine Co.) held in a rotating teflon holder over a 4 hour period (Flynn 1968). Planchets were counted using both silicon surface barrier detectors and ion implant detectors in eight Tennelec TC256 alpha-spectrometers interfaced with a multi-channel analyzer and an eight channel digital multi-plexer. Counts were collected with Nucleus® software on an IBM-PC. Radium blanks included these steps to account for any potential radium contamination. The sample remaining after polonium autodeposition was dried down and saved for ^{226}Ra analysis.

Activity of ^{210}Po was calculated in the following manner. Counts collected using α-spectrometry for ^{210}Po and ^{208}Po were corrected for background and reagent counts by subtracting the amount of counts calculated to occur for counting time using the rates measured for reagent blanks. Adjusted sample counts for ^{208}Po and ^{210}Po were corrected for decay during the counting period in two steps. First, the time in the count interval, where counts before and after were equal, was calculated,

$$t_{mid} = \frac{\ln\left(\left(1 + e^{\left(-\lambda^{2XX}Po * \, t_{fin}\right)}\right)\right)}{2} * \left(-\lambda^{2XX}Po\right),$$

where t_{mid} was the point in the counting interval, for each isotope, at which the counts collected before and after are equal; $\lambda^{2XX}Po$ was the decay constant for ^{208}Po ($\ln(2)/2.898$

yr) or ^{210}Po (ln(2)/0.3789 yr); and t_{fin} was the time from autodeposition to the end of the counting interval. Secondly, t_{mid} was used to calculate the ^{210}Po and ^{208}Po counts, corrected for decay since autodeposition,

$$Cts^{2XX}Po_u = \frac{Cts^{2XX}Po_m}{\left(e^{\left((-\lambda^{2XX}Po)\cdot t_{mid}\right)}\right)},$$

where $Cts^{2XX}Po_u$ was the decay corrected ^{210}Po or ^{208}Po counts to the time of autodeposition, $Cts^{2XX}Po_m$ was the measured ^{210}Po or ^{208}Po counts, and $\lambda^{2XX}Po$ was the decay constant for ^{210}Po or ^{208}Po.

Because the ^{210}Po/^{208}Po activity ratio was equal to the measured ^{210}Po/^{208}Po count ratio and the ^{208}Po yield-tracer activity was known, then ^{210}Po activity was determined using,

$$A^{210}Po_{unk} = A^{208}Po_{known} * \left(\frac{Cts^{210}Po}{Cts^{208}Po}\right),$$

where $A^{210}Po_{unk}$ was the unknown ^{210}Po activity of the sample, $A^{208}Po_{known}$ was the activity of the ^{208}Po yield tracer, and $Cts^{210}Po/Cts^{208}Po$ was the ratio of the corrected counts for ^{210}Po and ^{208}Po.

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Because the activity of ^{210}Po was in secular equilibrium with ^{210}Pb, then the activity of ^{210}Po was equal to the ^{210}Pb activity. This activity was then corrected for ^{210}Pb ingrowth from the time of capture to autodeposition by applying,

$$A^{210}Pb_{tc} = A^{210}Pb_u - A^{226}Ra_{TIMS} * \left(1 - e^{(-\lambda dt)}\right)$$

where $A^{210}Pb_{tc}$ was the ^{210}Pb activity at the time of capture, $A^{210}Pb_u$ was the ^{210}Pb activity corrected to the time of autodeposition, $A^{226}Ra_{TIMS}$ was the ^{226}Ra activity measured using TIMS (determined later), λ was the decay constant for ^{210}Pb (ln(2)/22.26 yr), and dt was the time between capture and autodeposition.

^{226}Ra activity determination

Determination of ^{226}Ra was performed using the new elemental separation procedure and isotope-dilution TIMS described elsewhere (Andrews et al. 1997, Chapter 2). The only exception to the procedure used for these samples was the collection intervals used for each column separation. In the first two column passes, where calcium removal occurs, the collection interval was from 35 to 120 mL, instead of 40 to 120 mL. For the third column pass, where radium is isolated, the collection interval was from 300 to 700 μL, instead of 250 to 700 μL. These intervals were changed as a result of this study to improve radium separation and recovery and to further reduce calcium.

Radiometric age determination

Age determination was performed using the measured ^{210}Pb and ^{226}Ra activities in the following equations. Because the activities were measured using the same sample, the calculation was independent of sample mass. For adult samples, where estimated age was greater than the 5 yr core, radiometric age was calculated as follows to compensate for the ingrowth gradient of ^{210}Pb:^{226}Ra in the otolith core,

$$t_{age} = \frac{\ln\left(\dfrac{1 - \dfrac{A^{210}Pb_{tc}}{A^{226}Ra_{TIMS}}}{\dfrac{1 - e^{-\lambda T}}{\lambda T}}\right)}{-\lambda} + T,$$

where t_{age} was the radiometric age for the time of capture, $A^{210}Pb_{tc}$ was the ^{210}Pb activity corrected to time of capture, $A^{226}Ra_{TIMS}$ was the ^{226}Ra activity measured using TIMS, λ was the decay constant for ^{210}Pb, and T was the core age (5 yr). The radiometric age calculation for the juvenile age-group was much simpler because estimated age was lower than 5 years. Age was calculated using,

$$t_{age} = \frac{\ln\left(1 - \left(\dfrac{A^{210}Pb_{tc}}{A^{226}Ra_{TIMS}}\right)\right)}{-\lambda},$$

where t_{age} was the radiometric age, $A^{210}Pb_{tc}$ was the ^{210}Pb activity corrected to time of capture, $A^{226}Ra_{TIMS}$ was the ^{226}Ra activity, and λ was the decay constant for ^{210}Pb.

Analytical uncertainty calculation

Determination of error for each isotope was as follows. The uncertainties for the corrected ^{210}Pb activity were based on the total counts after correction for background and reagents:

$$\sigma A^{210}Pb = A^{210}Pb * \sqrt{\left(\frac{\sqrt{N^{208}Po}}{N^{208}Po}\right)^2 + \left(\frac{\sqrt{N^{210}Po}}{N^{210}Po}\right)^2},$$

where $\sigma A^{210}Pb$ is the standard deviation of the ^{210}Pb activity, $A^{210}Pb$ is the corrected ^{210}Pb activity, N is the number of counts corrected for background and reagents for ^{208}Po or ^{210}Po (Wang et al. 1975). Uncertainty for ^{226}Ra activity is calculated during sample analysis using TIMS (Andrews et al. 1997). These uncertainties were used to calculate high and low radiometric ages.

Age estimate accuracy

A comparison of estimated age versus radiometric age was performed in two plots and one statistical analysis. First, the estimated age of the age-groups and measured $^{210}Pb:^{226}Ra$ activity ratios were plotted with expected $^{210}Pb:^{226}Ra$ ingrowth curves.

Concordance of these data with the expected ingrowth curves were used as an indicator of age estimate accuracy. A direct comparison of estimated age was also performed by plotting the radiometric age opposite estimated age. A linear regression of these data were compared to a line of agreement or one-to-one. A paired two-sample *t*-test was used to determine if a significant difference existed between the line of agreement and the regression line.

Results

A total of 747 Pacific grenadier was collected from waters off California, Oregon, and Washington. Of these fish, 128 with complete tails were used in the partial length analysis. All three partial lengths (PAF, SD, and HL) had linear relationships which could be used to determine total length (Figure 4). Fish used in this analysis ranged in size from 124 mm to 688 mm TL. Because PAF had the largest proportion of total length, it was chosen as the length used in growth analyses and is used to determine total length.

Traditional ageing

Fish chosen for traditional age determination numbered 178 and ranged in size from 22 mm to 272 mm PAF. Transverse sections were very difficult to age. APE was comparable between readers at about 6%, but D indicates precision was better for the first reader (6 and 10% respectively). Forty-three percent of the readings between readers were within 1 year, 55% within 2 years, 80% within 5 years, and 95% within 10 years (Table 1). Most of the differences between readers resulted from lower age estimates made by the second reader on old otolith sections (69%). Precision estimates were 6.3% (APE) between readers. Consultation between readers usually resolved differences greater than 10%. Age estimates were ultimately finalized by the first reader. For 32 of the specimens, the second otolith of the pair was sectioned for another attempt at ageing. Twenty were still unageable after both sections. Ninety-two of the otoliths selected were ageable (52%). Left and right otoliths were not differentiated because there was no systematic difference between sides (t test, p>95%).

Three von Bertalanffy growth functions fitted to the PAF lengths and estimated ages to describe growth characteristics for females, males, and combined sexes, where juveniles were factored into each determination (Figure 5). The growth curves for males and combined sexes have comparable growth curves, but females appear to have slower growth and a higher maximum length.

Age-group determination and core extraction

The regression of otolith weight and estimated age indicate there is a linear relationship for females and males, with some scatter (Figure 6). These regressions were used to determine age for the otoliths considered for radiometric age determination. By comparing the multiple linear regression ages, dissimilar fish were identified by having regression ages that differed by greater than 10%. This process eliminated 9 to 33% of the potential age-group members.

A total of 6 adult age-groups (3 female, 3 male) and one juvenile age-group was selected for radiometric analysis (Table 2). Age-groups range from as low as 1 yr for the juvenile sample to a maximum of 56 yr for the oldest age-group. The number of fish in the adult age-groups ranged from 10 to 21, but the juvenile age-group numbered higher at 46 because the otoliths were small. Some otolith cores were lost in the coring process due to breakage. Average core weight for the female samples, processed first, were slightly higher (0.03 g) than the targeted core weight (0.02 g). Male samples were cored a little smaller and more closely approached the target core weight. Total sample weight for each

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age-group ranged from 0.4717 g to 1.2279 g. Mean length was also calculated for each age-group ($\pm\sigma$).

Radiometric analysis

The results of the radiometric determinations are given in the form of activity (dpm g^{-1}) and a ratio of activities (Table 3). The activity of ^{210}Pb increased, as expected, 25 times from 0.0023 dpm g^{-1} in the juvenile samples to 0.0568 dpm g^{-1} in to oldest adult sample. Error for this determination ranged from 4.4 to 7.8%. The activity of ^{226}Ra ranged from 0.0413 to 0.1232 dpm g^{-1}, where the highest activity was from the juvenile sample. This was 2.2 times higher than the mean adult sample activity (0.0560 ± 0.01) and nearly 3 times the lowest activity measured. The activity of the adult whole-otolith pair is comparable to the lowest adult sample. Error for ^{226}Ra was very low and ranged from 0.278 to 3.073% (1σ). The activity ratio of ^{210}Pb:^{226}Ra increased as predicted by estimated age from a low of 0.0186 for the juvenile age-group to 0.810 for the oldest adult age-group.

Radiometric age determination

Radiometric age determined using the activity ratio was comparable to the average estimated age of the age-groups (Table 4). Each age-group range overlaps the radiometric age range, with the exception of the juvenile age-group. The low radiometric age for the 1-3 yr age-group indicates ^{210}Pb uptake by juveniles was insignificant.

Therefore, radiometric age was determined based on the measured activity ratios and no adjustment was necessary.

Age estimate accuracy

A graphical comparison of the age-groups versus the measured ^{210}Pb:^{226}Ra activity ratio plotted with the expected ingrowth curves indicated age estimate accuracy (Figure 7). The 1-3 yr age-group was closely associated with the ingrowth curve for an R_o of 0.0, which indicates it was the most accurate model for what to expect in adult otolith cores. The strong concordance of the data points with the expected ingrowth curve indicates high age estimate accuracy.

A direct comparison of estimated age with radiometric age indicated ages are statistically in agreement (Figure 8). Data points were all in close proximity to the line of agreement. A regression of these data shows a high correlation and a slope very close to one with an intercept near the origin (adjusted $R^2 = 0.957$). A paired two-sample t-test comparison indicates there was no significant difference between estimated age and radiometric age ($t = 0.427$, $P = 0.684$).

Discussion

Partial length defined

Partial lengths are used to indicate total length of grenadier for the north Atlantic (Atkinson 1981 & 1991). The Pacific grenadier often has a tail that is apparently intact, but a few centimeters can be missing with little evidence. Most broken tails occurred during capture by trawling. In some cases, tails were lost during life and regenerated, where regenerated means growth of fin rays from the severed edge. For a few fish, approximately 10 to 20% of the total length was lost during life. In this study, approximately 80% of the fish collected had damaged, broken or regenerated tails. For fish greater than 500 mm TL, tail loss was as high as 18% of the total length.

Partial length for grenadier is poorly defined. "Anal-fin length" (defined as tip of snout to base of first anal fin ray) was adopted as the partial length for the roundnose grenadier by NAFO in 1980, where no acronym was assigned (Atkinson 1981). This length was adopted because the anus is often distorted by air-bladder expansion and extrusion of intestines caused by barotrauma. Pre-anal length (length anterior to the anus), however, was used instead of anal fin length in an age and growth study of the roundnose grenadier (Bergstad 1990). In two age and growth studies of the Pacific grenadier, "anal length" was used, where SVL (snout to vent length) was assigned (Wilson 1982, Matsui et al. 1990). In a partial length study of the roughhead grenadier (*Macrourus berglax*), "pre-anal fin length (AFL)" was used as the equivalent to anal fin length (Atkinson 1991). More recently, in another age and growth study of the roundnose

grenadier, pre-anus (PA) was used, but pre-anal fin length (PAF) was also noted and nicely illustrated (Kelly et al 1997).

Based on the advantages of using the anterior most fin ray of the anal fin over the anus and the unambiguous acronym (PAF), we chose PAF as the partial length for the Pacific grenadier. It is further proposed that partial length be standardized for grenadier in these terms, and that use of HL or SD be used where the anal fin is missing or badly damaged (Figure 4).

Traditional age estimation

A graphical comparison of growth curves from this study and other studies reveal both differences and similarities (Figure 9). Kulikova (1957) did not calculate growth parameters, but for comparative purposes, growth parameters were calculated using the average length given for each estimated age. In this case, the growth constant is very high and longevity is very low relative to validated estimates in this study (Table 5). This growth constant is unrealistically greater than many fast growing fishes (i.e. tuna and billfishes; Prince and Pulos 1983). The growth curve fitted to age estimates determined by Matsui et al. (1990) is very similar to the male and combined sexes growth curves in this study (Figure 9). It must be noted that Matsui et al. (1990) used snout to anus length (less than PAF by approximately 1%). The upper end of the female growth curve in this study is largely unsupported by data, but it is important to mention that some otoliths from females greater than 300 mm PAF were aged at up to greater than 90 yr. These otoliths were not used in this study because growth increments were very fine and confusing

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toward the margin and were also aged as low as 65 yr. The difficulty of ageing otolith sections for this species was comparable to recent findings for the roundnose grenadier (*Coryphaenoides rupestris*, Kelly et al. 1997). The asymptotic length of the female growth curve more closely approximates the maximum size reported (~900 mm TL, Cohen et al. 1990). Therefore, it is possible that females get larger than males with increasing age.

A comparison of historic and recent age estimates indicate the age and growth of the Pacific grenadier is controversial and age is usually underestimated (Table 6). The first Pacific grenadier age estimates are for specimens collected in the Bering and Okhotsk Seas and the Kuril-Kamchatka trench, where scales were used to estimate a longevity of 6 years (Kulikova 1957). This result underestimates the longevity determined in this study by an order of magnitude. Scales can, however, be unreliable as a conserved record of age (Mugiya 1974, Simkiss 1974, Ichii and Mugiya 1983, Beamish and McFarlane 1987, Yoklavich and Boehlert 1987). In a study of the microstructure of otoliths, extrapolation of daily increments were used to estimate the age of one fish (Brothers et al. 1976). This estimate is low by a factor of three for a fish of this length in this study. The first attempt at quantification of annual growth increments in transverse otolith sections utilized 15 fish collected off San Diego, California (Mulcahey et al. 1979). They found otoliths difficult to age and cautiously estimated age at 15 to 25 yr for the size range of fish used. In this study, comparably sized fish would be 2 to 3 times older. A detailed examination of scales, vertebrae, and otoliths from 130 fish collected off southern California resulted in an estimated longevity of approximately 20 yr (Wilson 1982). Otoliths sections were

determined, however, to be too ambiguous for age estimation. Because of the difficulty in ageing the otoliths in this study, we largely agree, but this longevity estimate is low by a factor of 3 to 4. Most recently, otolith break-and-burn was used on 60 fish collected off northern Baja California, Mexico to central California (Matsui et al. 1990). Longevity was estimated at greater than 60 years, which is comparable to the results of this study.

Radiometric analysis

Use of otolith cores for radiometric age determination is typically limited to a minimum of approximately 1 gram of material because of instrument detection limits (Kastelle et al. 1994, Fenton and Short 1995). Because of cleaner procedures and the application of isotope-dilution TIMS, sample size can be lower than 0.5 g (Table 2). Reduced error associated with ^{226}Ra determination make the analytical uncertainty of ^{210}Pb determination the limiting factor in radiometric ageing. In this study, uncertainty in ^{210}Pb activity contributes 7.8% of the error where counts are very low (juvenile samples) and decreases to approximately 5% as age and counts increase (Table 3). Typically the error for ^{226}Ra determination is lower than 1%. In this study, reported error greater than 1% is attributed to high calcium in the sample. An alteration of the technique remedied the problem (Andrews et al. 1997).

In some radiometric ageing studies ^{226}Ra activity is inferred, rather than measured directly, because of the detection limits of ^{222}Rn emanation (Bennett et al. 1982, Campana 1990, Watters 1995, Kline 1996). It is clear from the results of this study that ^{226}Ra activity can vary significantly among samples and, presumably, among individuals for some

fishes (Table 2). The ^{226}Ra activity for the juvenile sample was the highest activity

measured in this study. Some juvenile specimens were collected from upper slope areas

near the Columbia River mouth. Because freshwater is a source of ^{226}Ra it is possible that

environmental levels of ^{226}Ra, are elevated in this area (Osterberg et al. 1963, Fanning et

al. 1982, Moore 1996). This may explain the elevated ^{226}Ra activity in the juvenile

otoliths. Use of an average ^{226}Ra activity in this study causes significant changes to

radiometric age. This emphasizes the necessity of measuring ^{210}Pb and ^{226}Ra for each

sample in future studies.

Assumption clarification

The radiometric ageing technique requires several assumptions depending on the

analytical circumstances. The typical assumptions are as follows: 1) the otolith is a closed

system with no loss or migration of post-formational radionuclides in the ^{226}Ra decay

series through to ^{210}Po; this is necessary because loss of nuclides would result in lower

^{210}Pb activity and subsequently underestimated age, 2) uptake of exogenous nuclides in the

^{226}Ra decay series is negligible relative to ingrowth from ^{226}Ra; this is necessary in order to

attribute ^{210}Pb activity in the otolith to the decay of ^{226}Ra, 3) uptake of ^{226}Ra is in constant

proportion to the otolith mass growth-rate; this assumption is only necessary when whole

otoliths are used and is largely circumvented when otolith cores are used (Kimura and

Kastelle 1995). These assumptions can be more closely scrutinized with the increased

accuracy of the radiometric ageing technique using isotope-dilution TIMS (Andrews et al.

1997).

The two most important assumptions are that the otolith is a closed system and that incorporation of exogenous ^{210}Pb is negligible. Loss of ^{222}Rn and sources of exogenous ^{210}Pb or ^{210}Po are potential violations of these assumptions (West and Gauldie 1994). If ^{222}Rn loss was a problem, radiometric disequilibria of ^{210}Pb:^{226}Ra would consistently underestimate fish age. In this study it is very unlikely that the traditional age estimates of each age-group would be underestimated by the precise amount necessary to compensate for ^{222}Rn loss. Because the use of TIMS has increased the accuracy of the radiometric ageing technique, tolerance for such an error is very low. Based on the results of this study and other concurrent studies, ^{222}Rn loss is very improbable or insignificant. In the case of exogenous ^{210}Pb or ^{210}Po, ^{210}Pb was eliminated as a possible factor by ageing the juvenile age-group (1-3 yr). It is highly unlikely that uptake of ^{210}Pb in adults would be different from juveniles. If any exogenous ^{210}Po was incorporated into the core, it would decay away well before radiometric analysis (half-life = 138.4 days). Therefore, this study clearly supports these assumptions and concerns regarding these assumptions are less substantiated.

Age estimate accuracy

The strong concordance of measured ^{210}Pb:^{226}Ra with the expected ingrowth curves validates age estimation using growth increments in the Pacific grenadier (Figure 7). Because the 1-3 yr age-group shows extremely low ^{210}Pb activity, we believe the most applicable ingrowth model is for an R_o of 0.0. Variation of the data points around the ingrowth curve can be explained by the analytical uncertainty associated with the

technique and the age-group age range. An additional consideration is variation in the otolith weight models (Figure 6). Because otoliths were difficult to age, there is a fair amount of scatter associated with otolith weight as a predictor of age. It is clear, however, that these effects are minimal relative to the average age of each sample because anomalous otoliths were removed using the regression comparisons.

Statistical agreement between estimated age and radiometric age further supports validation of age estimates (Figure 8). Radiometric age determinations confirm a longevity of at least 56 years and validates the annual periodicity of the growth increments used to estimate age. Based on these growth increments, longevity of the Pacific grenadier is at least 73 years.

Life histories

Longevity estimates for two similar grenadier species of the North Atlantic differ considerably. The roundnose grenadier (*Coryphaenoides rupestris*) has longevity estimates that range from 50 to 72 yr, which are comparable to the Pacific grenadier (Bergstad 1990, Kelly et al. 1997). Age validation of these estimates is limited to young fish using marginal increment analysis (Gordon and Swan 1996). This species can attain a greater length (1.5 to 2 m TL) and appears to have a faster growth rate than the Pacific grenadier. In contrast, the roughhead grenadier (*Macrourus berglax*) has a relatively low estimated longevity of 13 yr for males and 22 yr for females (Savvatimsky 1994). Females exceed the length of males and approach 1 m TL, which is comparable to the Pacific grenadier. Age estimates, however, are based largely on scales and validation has yet to

be performed. Grenadier from other parts of the world are thought to have similarly low longevity, but validation is necessary (Rannou 1976, Middleton and Musick 1986, Morales-Nin 1990). It is possible that the grenadier family has a series of species complexes that differ in growth characteristics, as is thought to be the case for the eastern Pacific Ocean rockfishes (*Sebastes* spp.; Boehlert and Kappenman 1980, O'Connell and Funk 1986, Leaman and Nagtegaal 1987, Pearson et al. 1991).

Maturity at length from several studies can be used to estimate age at maturity for the Pacific grenadier. Length at maturity is estimated at 500 mm TL (ca. 170 mm PAF) for males and between 460 to 650 mm TL (ca. 153 to 230 mm PAF) for females (Stein and Pearcy 1982, Matsui et al. 1990). Based on the growth functions calculated for each sex, maturity may occur between 20 to 40 yr for females and at 20 yr for males. This is much later than the typically larger and faster growing roundnose grenadier, where 50% maturity occurs at 8 to 11 yr (Kelly et al. 1997).

It is important to consider the potential age of the fish being landed because landings for the Pacific grenadier have increased substantially (Figures 1 and 2). Landings for Oregon have been about 91% male, where most range in size from 450 to 650 mm TL (Mike Hosie, Oregon Department of Fish and Wildlife, PO Box 5430, Charleston, OR 97420, personal communication). The small percentage of females landed are typically larger at about 550 to 650 mm TL. Based on the calculated growth functions, fish being landed may range in age from 20 to 50 yr for males and 30 to 40 yr for females. Landings for Monterey Bay, California consist of fish ranging in size from 550 to 650 mm TL (~95%) with some small individuals at about 300 mm TL (~5%). Sex composition is not

known. Based on both growth functions, age of the large fish may be between 30 to 50 yr and the small fish may be about 10 yr. Because maturity occurs late in life, many of the fish being landed may be sexually immature.

The depth distribution of the Pacific grenadier may provide a viable management strategy. Because size and abundance appear to increase to a depth of approximately 1500 m (Stein and Pearcy 1982), it may be possible to implement a size refuge for the Pacific grenadier by limiting fishing to a depth range (i.e. 1200 to 1800 m). By fishing in this depth range, juvenile and old adults could be protected in a strategy comparable to that of white sturgeon (*Acipenser transmontanus*) in California, where small and large fish are released. With this strategy, recruitment and growth of immature fish on the upper slope could be more protected, and larger, older fish at depth could provide an undisturbed source of productivity for the population. In addition to this limitation, a seasonal restriction should be implemented to reduce to impact on individuals during spawning season. A moratorium on fishing for the early fall (August) to late winter (February) would encompass the period where reproduction appears to hit a peak (Matsui et al. 1990).

Much remains unknown about the Pacific grenadier that is necessary to develop a sustainable fishery. Population size and distribution, and the movement of individuals in the population, are probably the most important aspects of this fishery that need research. Population size and individual movement may be assessed using recently developed acoustical techniques (Priede et al. 1990). Catch per unit effort information needs to be compiled and analyzed for abundance estimates in areas already being fished. Other

studies including tag and recapture coupled with deep trawling and submersible transects

could be used to estimate population characteristics at depths greater than current

commercial fishing depths.

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new method of validation. Fish. Bull. 85(4):826-832.

Table captions

Table 1. A comparison of the agreement between readers revealed the difficulty of reading otolith sections from Pacific grenadier. Less than half were within one year of each other. Readings with the widest disagreement (> ±5 yr) were from otolith sections of large fish and higher estimated age.

Table 2. Composition of age-groups used for radiometric age determination. Both otoliths were used from each fish, except where otoliths were lost in the coring process. Target samples size was 1 g. Some samples were closer to 0.5 g because of insufficient sample. Mean length (PAF mm) was given for comparison.

Table 3. Results of radiometric analysis for ^{210}Pb and ^{226}Ra activity determinations for each age-group. A single otolith pair from one fish was also listed for comparison with core results.

Table 4. Comparison of estimated ages and radiometric ages for each age-group. High and low radiometric ages were determined using analytical uncertainty extremes.

Table 5. Comparison of von Bertalanffy growth function parameters for other studies with the results of this study.

Table 6. Comparison of historic and recent age and longevity estimates for the Pacific

grenadier. Technique and number of fish used in each study were listed with fish lengths

and age estimates.

Table 1. Comparison of reader agreement.

Age difference	Percent of observations	Agreement ±1 yr	±2 yr	±5 yr	±10yr
- >10 yr	5				
- 6 to 10 yr	14				
- 3 to 5 yr	19				
- 2 yr	9				
- 1 yr	12				
0	16	43%	55%	80%	95%
+ 1 yr	15				
+ 2 yr	3				
+ 3 to 5 yr	6				
+ 6 to 10 yr	1				
+ >10 yr	0				

Table 2. Composition of age-groups.

Age group (yr)	Sex	Number of fish	Number of otoliths	Sample size (g)	Mean length ± σ (PAF mm)
1-3	Juvenile	46	92	0.9147	44 ± 2
14-20	Female	19	35	1.2279	143 ± 14
30-41	Female	15	29	0.9823	224 ± 19
43-54	Female	10	19	0.6242	245 ± 13
34-38	Male	21	39	0.5315	196 ± 12
40-45	Male	21	41	0.7458	207 ± 16
46-56	Male	12	23	0.4717	216 ± 19

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Table 3. Radiometric results for each age-group and an otolith pair.

Age group	Sample size (g)	^{210}Pb activity (dpm g^{-1})	^{210}Pb % error	^{226}Ra activity (dpm g^{-1})	^{226}Ra % error	^{210}Pb:^{226}Ra activity ratio
1-3	0.9147	0.0023	7.8	0.1232	1.455	0.0186
14-20	1.2279	0.0124	5.9	0.0413	0.730	0.300
30-41	0.9823	0.0321	4.4	0.0536	0.616	0.599
43-54	0.6242	0.0386	5.1	0.0503	0.617	0.766
34-38	0.5315	0.0391	5.4	0.0575	0.278	0.680
40-45	0.7458	0.0418	5.5	0.0631	3.073	0.663
46-56	0.4717	0.0568	4.8	0.0702	1.461	0.810
Otolith pair[a]	1.2826	N.M.	--	0.0413	1.345	N.A.

[a] Otolith pair from individual adult fish.

N.M. Not measured.

N.A. Not applicable.

Table 4. Comparison of estimated age with radiometric age.

Age group (yr)	Average estimated age (yr)	Radiometric age (yr)	Radiometric age range (yr)
1-3	2	0.6	0.5-0.7
14-20	17	14.0	13.1-14.9
30-41	36	31.9	29.6-34.4
34-38	36	39.2	35.5-43.3
40-45	43	37.5	32.6-43.7
43-54	48	49.2	43.7-55.9
46-56	51	55.8	48.4-65.9

Table 5. Comparison of von Bertalanffy growth function parameters for the Pacific grenadier.

	Kulikova (1957)[a] Combined sexes	Matsui et al. (1990)[b] Combined sexes	This study Combined sexes	Females	Males
L_∞	318 mm	242 mm	272 mm	372 mm	268 mm
±CI	N.C.	N.R.	26 mm	136 mm	25 mm
k	0.435	0.063	0.041	0.024	0.040
±CI	N.C.	N.R.	0.010	0.015	0.010
t_0	-0.166	1.093	0.25	-1.79	0.20
±CI	N.C.	N.R.	1.52	2.51	1.36
n	5 (average)	60	92[c]	62[c]	57[c]

[a] The von Bertalanffy growth function was fitted to published average length-at-age

values, where total length was converted to pre-anal fin length (PAF).

[b] Partial length used was snout-to-anus. This length was approximately 1% less than

PAF.

[c] Immature fish were included in each determination.

N.C. Not calculated.

N.R. Not reported.

Table 6. Historic and recent age estimates for Pacific grenadier.

Author	Technique	Number of fish	Length (TL mm)	Age at length (yr)
Kulikova (1957)	Scales	92	820 (max)	6+
Brothers et al. (1976)	Daily increment extrapolation	1	580	10-11
Mulcahey et al. (1979)	Sagittal otolith sections	15	640-840	15-25
Wilson, R.R. Jr. (1982)	Scales and vertebrae	130	820 (max)	18-20
Matsui et al. (1990)	Sagittal otolith break and burn	60	600-700	>60

Figure captions

Figure 1. Landings of Pacific grenadier for California and Oregon from 1972 to 1996 given in metric tons (t). Landings for California up to 1992 are primarily from Eureka, California. After 1992, landings substantially increased from Monterey Bay, California. Total landings for California and Oregon for 1996 exceeded 1500 t (3.3 million pounds).

Figure 2. Pacific Grenadier landings in metric tons (t) reported for each of the 3 ports in Monterey Bay, California from 1980 to 1996. Through the 1980's the landings have been negligible, with the exception of 1984 where 6.2 t was brought in to Moss Landing. Since 1992 landings have substantially increased to nearly 900 t (~2 million pounds) in 1996.

Figure 3. Three views of a transverse otolith section at different magnifications. The full section view is at a magnification of 10X. The two successive views are at 32X and 50X. Markers indicate quantified growth increments which rapidly become compressed toward the margin. This increment pattern was used as a search image in ageing other otolith sections. This section was aged at 41 yr.

Figure 4. Linear relationships of three partial lengths were calculated as an indicator of total length (TL; n = 128). Head length (HL), snout to dorsal fin length (SD), and pre-anal fin length (PAF) can be used to indicate TL. PAF was the primary partial length used in this study because it is the largest proportion of TL.

Figure 5. Von Bertalanffy growth functions were fitted to estimated ages and pre-anal fin lengths (PAF) for females ($\times$), males(O), and immature($\bullet$). Immature fish were used in each growth function. Total length (TL) was calculated based on PAF (Figure 3).
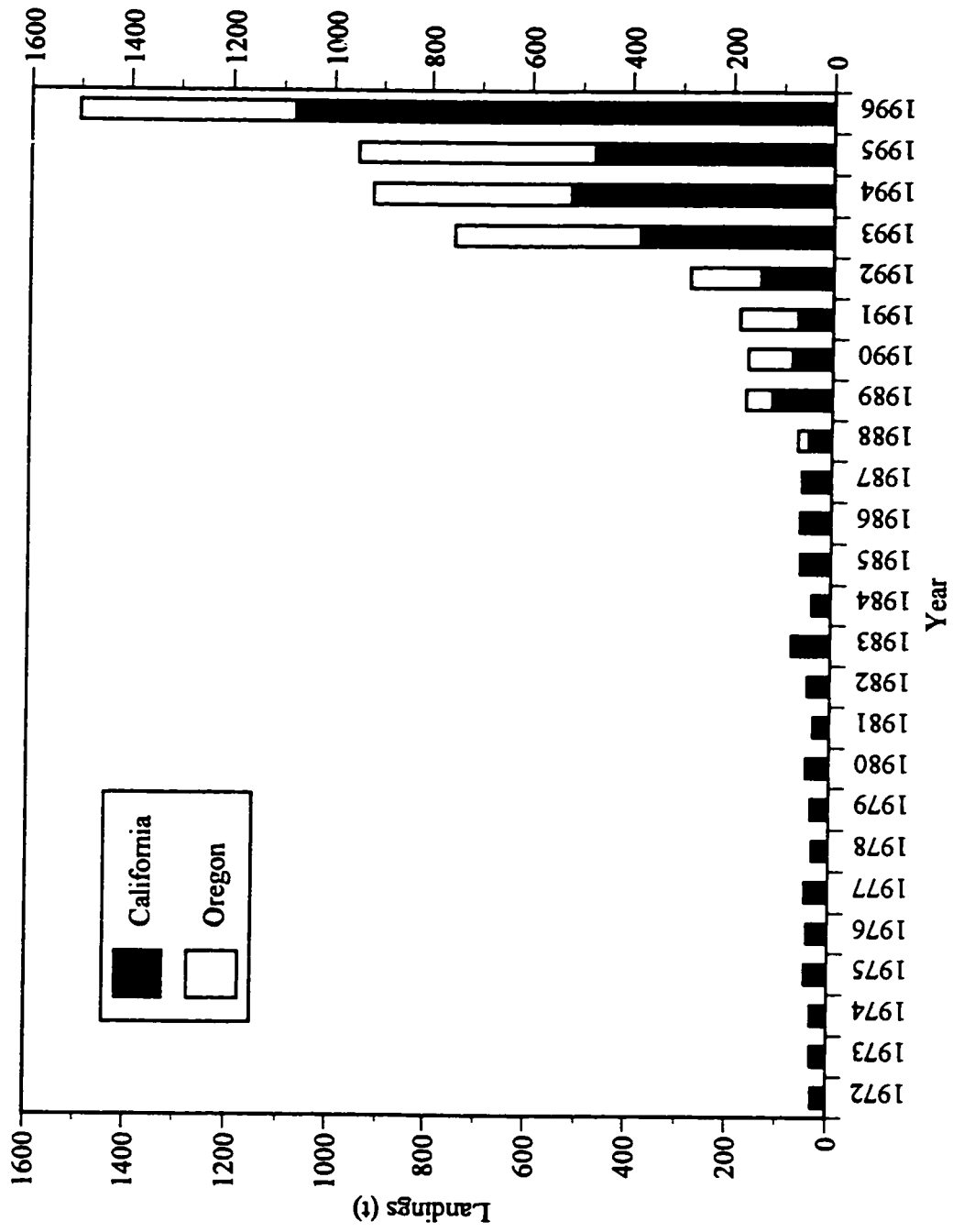
Figure 6. Linear relationships for females and males were calculated using estimated age and otolith weight. These equations were used to determine potential age-group members for unaged otoliths used in the radiometric age determinations.
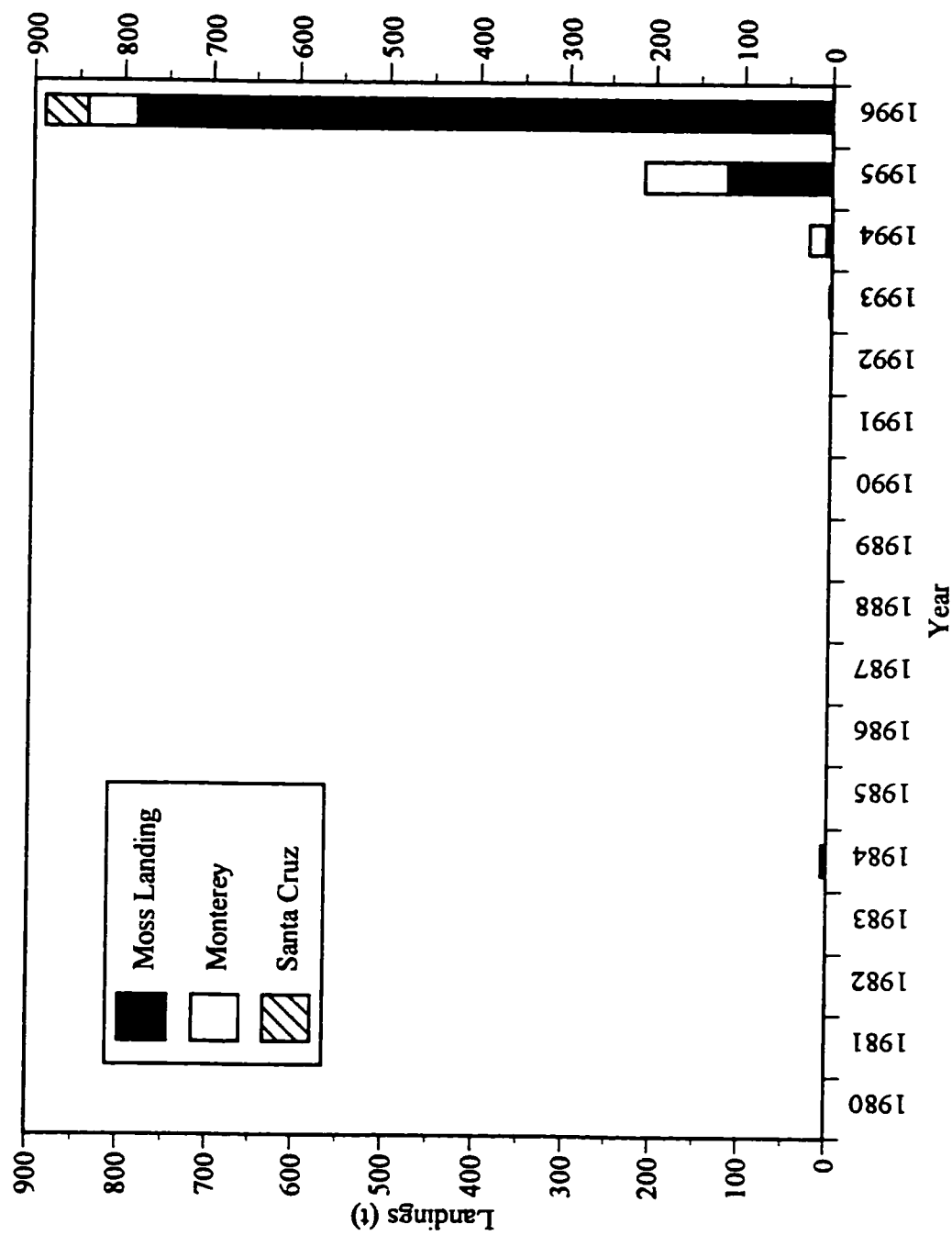
Figure 7. Observed ^{210}Pb:^{226}Ra activity ratios for age-groups plotted with expected activity ratio ingrowth curves. Strong concordance of the observed activity ratios (data points) with the expected activity ratio ingrowth curves was an indication of age estimate accuracy. Horizontal error bars represent age range of otolith core samples. Vertical error bars indicate analytical uncertainty i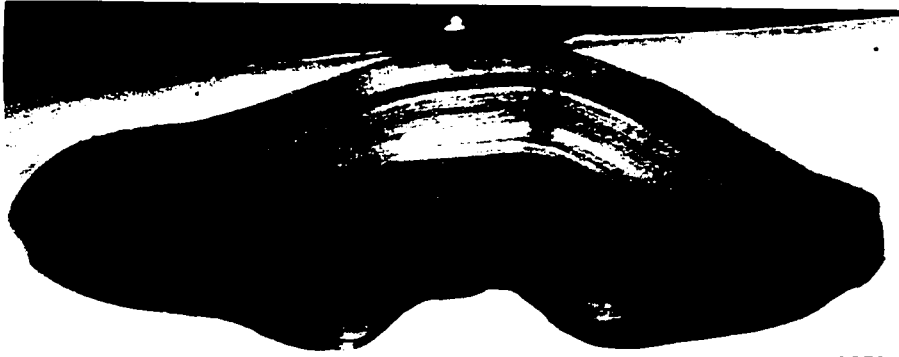n the determination of ^{210}Pb and ^{226}Ra activities. Expected activity ratio ingrowth curves represent possible initial uptake ratios (R_o) of ^{210}Pb:^{226}Ra.

Figure 8. Comparison of estimated age versus radiometric age using a paired two-sample t test indicate the ages were not significantly different ($t = 0.427$, $P = 0.684$). The solid line represents agreement between age estimates and had a slope of 1. The dashed line was a regression of the compared ages. Bars for estimated ages represent the age range of each age-group. Bars for the radiometric ages represent the range between high and low age based on analytical uncertainties.

Figure 9. Comparison of von Bertalanffy growth functions for two other studies with the results of this study. The growth function for Kulikova (1957) was calculated using the published average length-at-age where total lengths were converted to PAF.
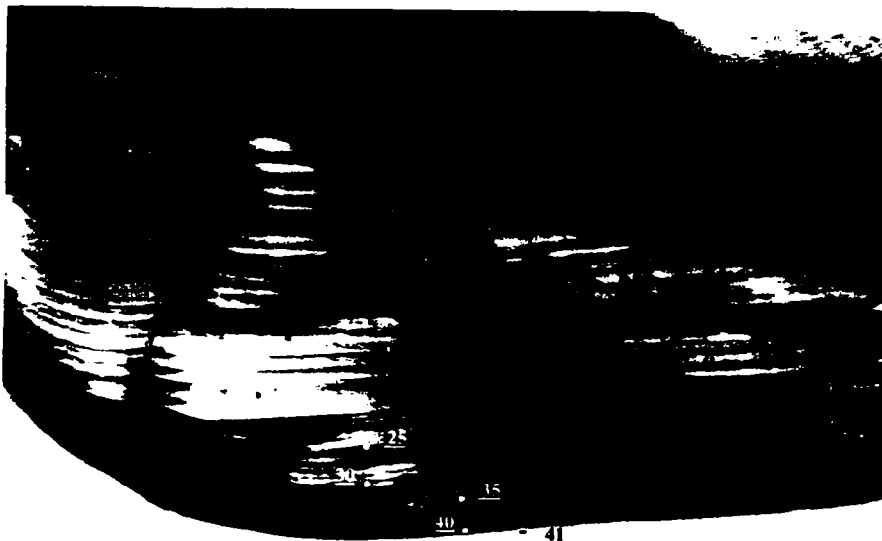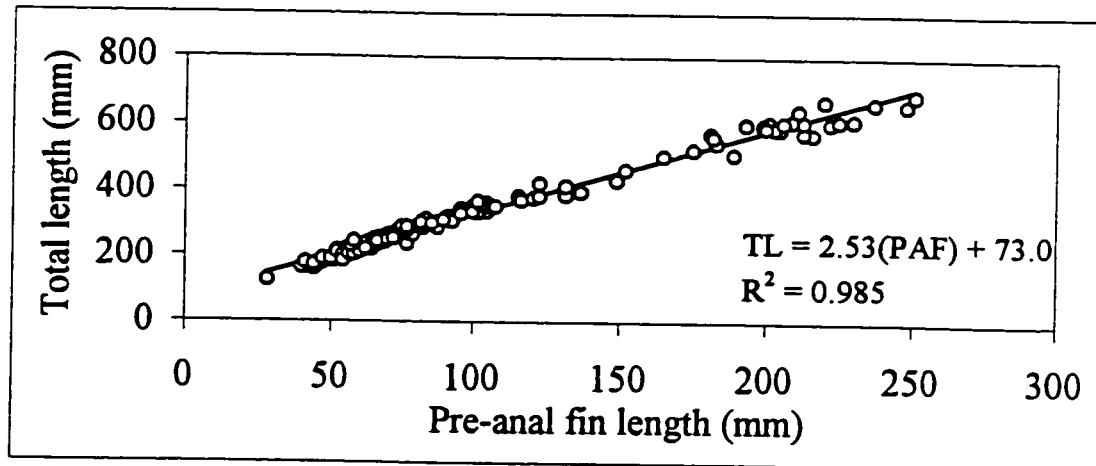
Year

Landings (t)

California
Oregon

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10X

32X

50X

$TL = 4.69(HL) + 20.2$
$R^2 = 0.988$

$TL = 4.31(SD) + 21.2$
$R^2 = 0.987$

$TL = 2.53(PAF) + 73.0$
$R^2 = 0.985$

	Female	Male	Combined
L_∞	372 mm	268 mm	272 mm
k	0.024	0.040	0.041
t_o	-1.79 mm	0.20 mm	0.25 mm
n	57	62	92

Female regression age = 88.6(Oto wt) + 4.8
$R^2 = 0.863$

Male regression age = 91.3(Oto wt) + 4.6
$R^2 = 0.927$

Otoltih weigth (g)

Estimated age (yr)

Female ×
Male ○
Immature •

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Figure: $^{210}Pb{:}^{226}Ra$ activity ratio plotted against Radiometric and estimated age (yr), showing model curves for $R_o = 0.2$, $R_o = 0.1$, and $R_o = 0.0$.

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Radiometric age = 1.08 (Estmated age) - 3.42
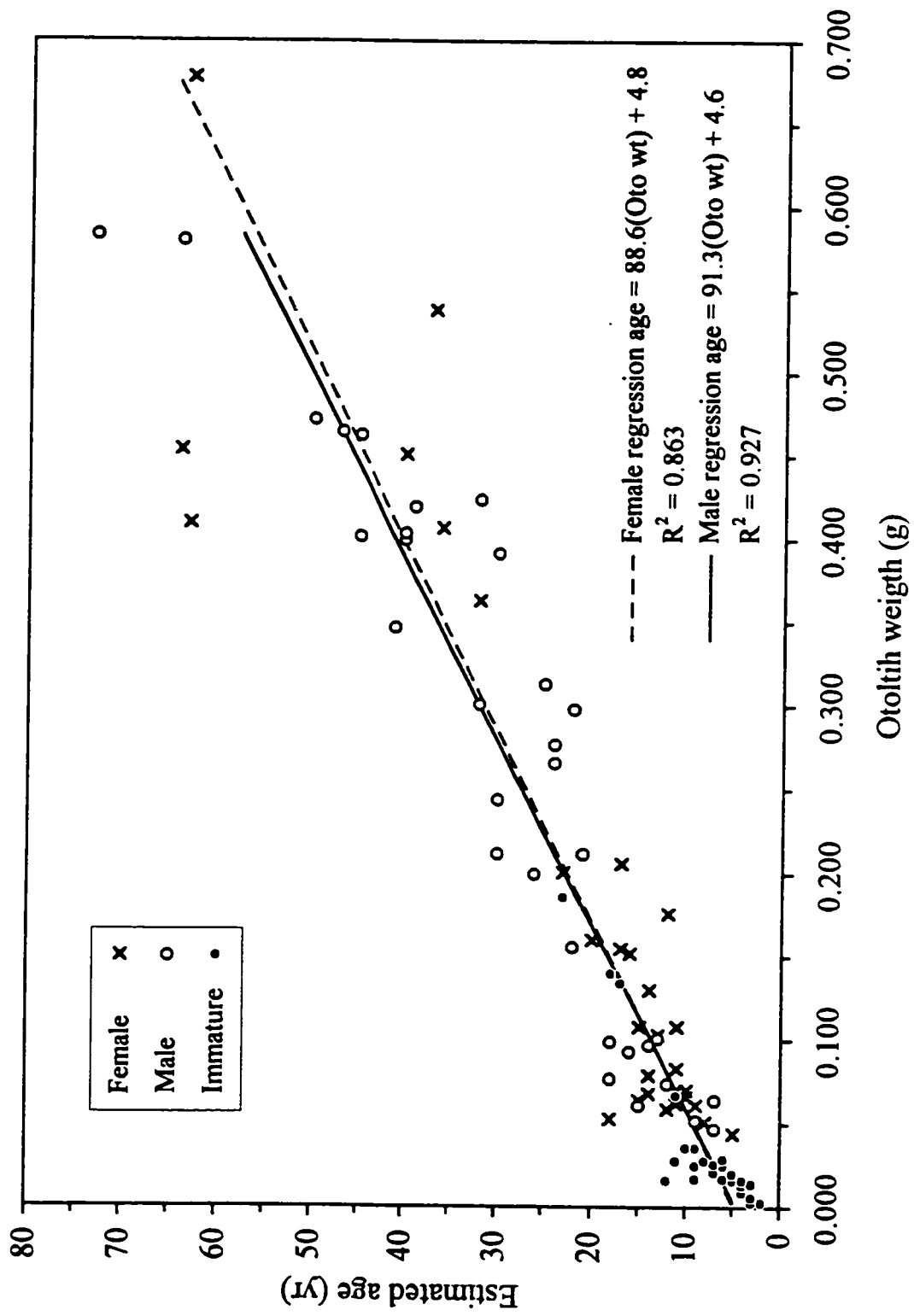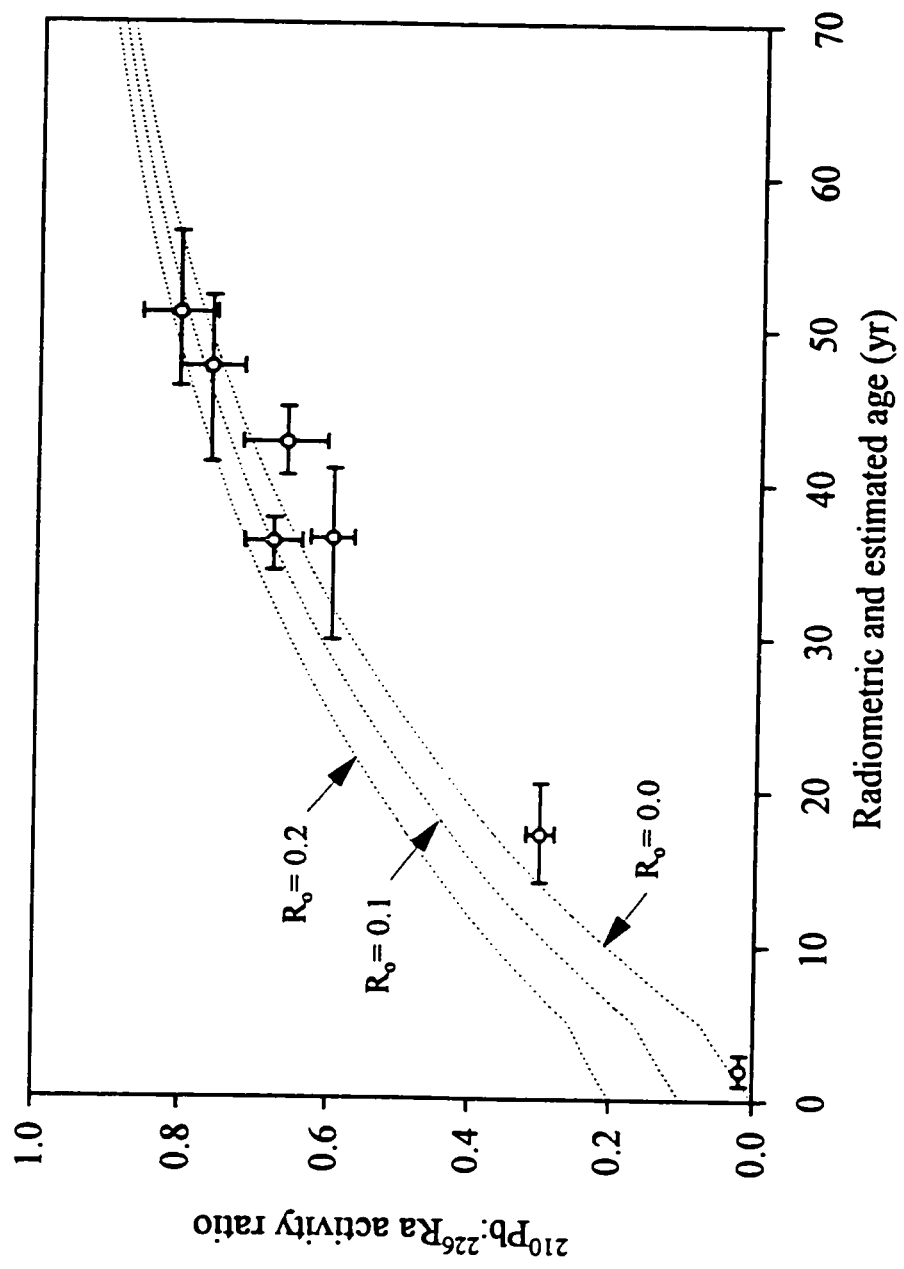adjusted r^2 = 0.957
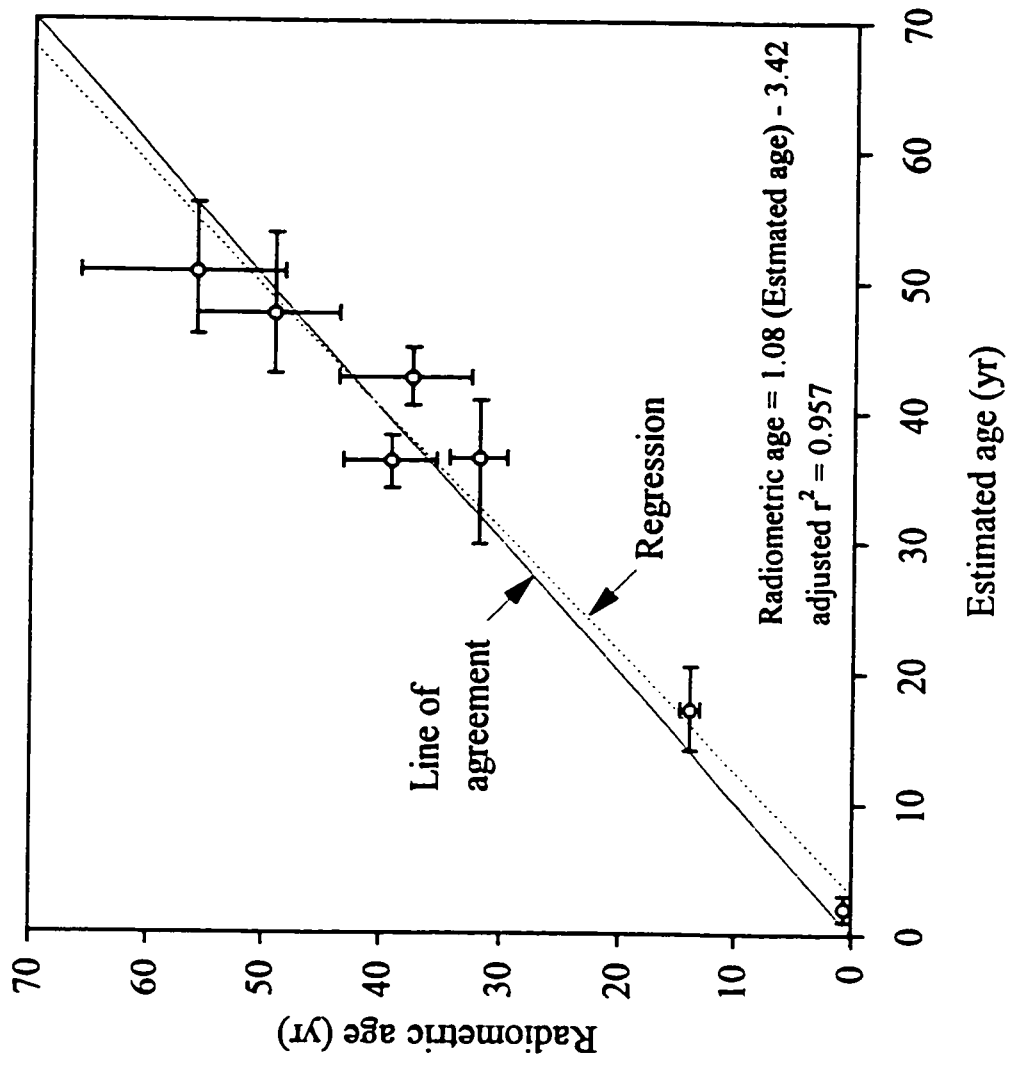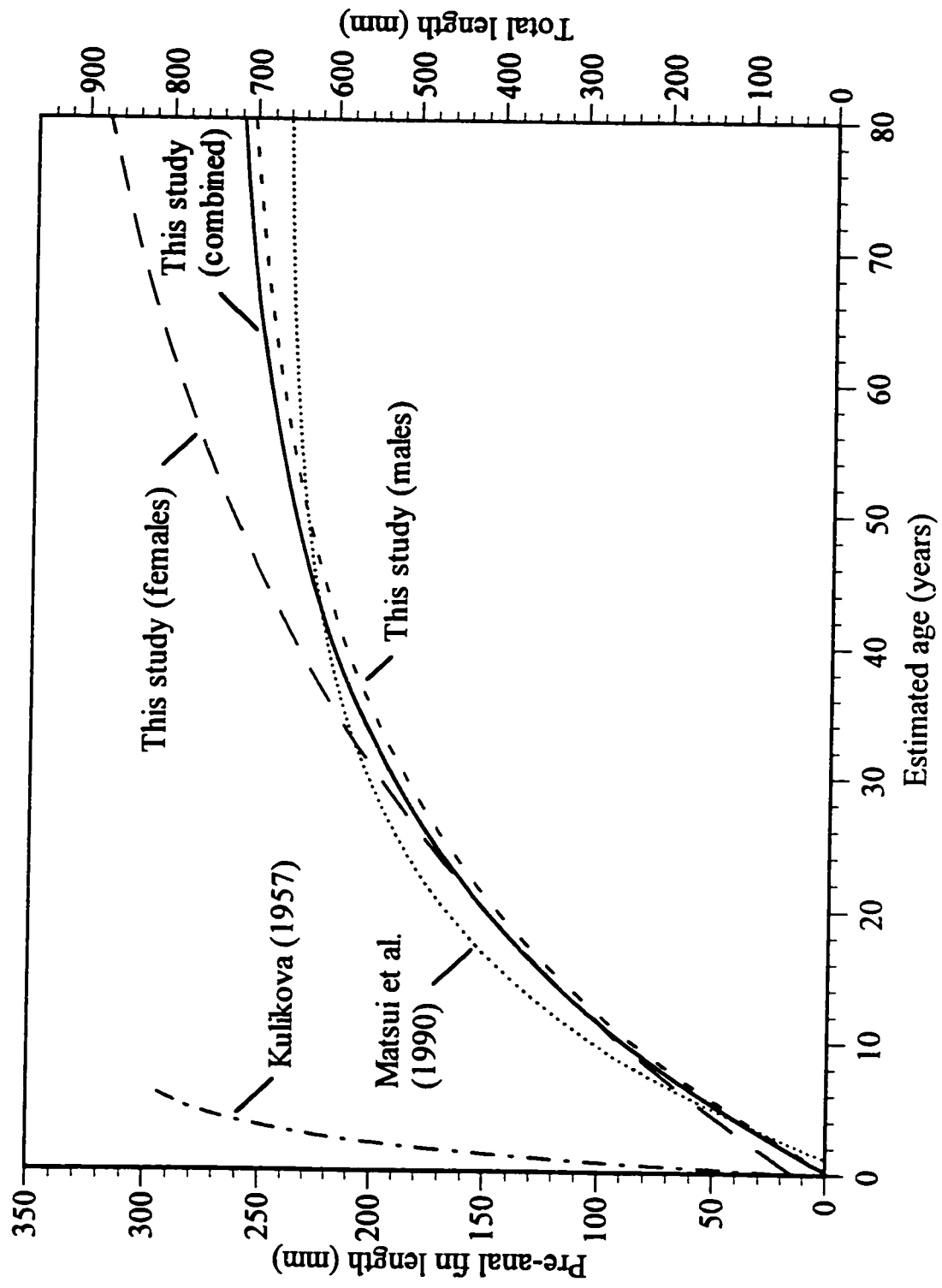
CHAPTER 4

Conclusions and recommendations

Isotope-dilution thermal ionization mass spectrometry

Isotope-dilution thermal ionization mass spectrometry is an effective tool for measuring ^{226}Ra in otolith material. The three column ion-exchange separation technique effectively isolates radium from interfering quantities of calcium and barium. Because thermal ionization mass spectrometry counts ionized radium atoms directly, determination of ^{226}Ra activity is no longer dependent upon activity or decay. This significantly improves detection limits and analysis time relative to existing techniques (Chapter 2, Table 2). Smaller samples than previously attempted with lower analytical uncertainty have improved the error associated with radiometric age determination. Because of these advances, the most significant source of error is measurement of ^{210}Pb activity by α-spectrometry (Chapter 3, Table 3). In this study, uncertainty in ^{210}Pb activity contributes 7.8% of the error where counts are very low (juvenile samples) and decreases to approximately 5% as age and counts increase. Recovery of ^{226}Ra can still be optimized by tracing radium through the full separation procedure. Experimentation with new ion-exchange resins may also provide increased efficiency and recovery (Dietz et al. 1997).

Clarifying assumptions

The radiometric ageing technique requires several assumptions depending on the analytical circumstances. The typical assumptions are as follows: 1) the otolith is a closed system with no loss or migration of post-formational nuclides in the ^{226}Ra decay series through to ^{210}Po; this is necessary because loss of nuclides would result in lower ^{210}Pb

activity and subsequently underestimated age, 2) uptake of exogenous nuclides in the ^{226}Ra decay series is negligible relative to ingrowth from ^{226}Ra; this is necessary in order to attribute ^{210}Pb activity in the otolith to the decay of ^{226}Ra, 3) uptake of ^{226}Ra is in constant proportion to the otolith mass growth-rate; this assumption is only necessary when whole otoliths are used. These assumptions can be more closely scrutinized with some background research and the increased accuracy of the radiometric ageing technique using isotope-dilution TIMS (Chapter 2).

The assumption that an otolith is a closed system is well supported by studies of coraline carbonate properties (a much less controlled matrix than the otolith) and of otolith structure and formation. A proposed violation of this assumption is the loss of ^{222}Rn, the daughter of ^{226}Ra, from the otolith via diffusion as a noble gas (West and Gauldie 1994). While loss of ^{222}Rn seems possible because it is a non-reactive gas that can enter the interstitial lamellar spaces via alpha-recoil (Moazed et al. 1973, Fleischer et al. 1975) and diffuse out of the otolith, there is strong evidence that indicates this does not occur in the *invivo* otolith. Any ^{222}Rn that enters the lamellar spaces is entering a matrix of water and otolin, a secreted neuroprotein (Gauldie and Nelson 1988, Panella 1980). A recent study of the sorption characteristics of ^{222}Rn in the presence of organic matter indicate the ^{222}Rn would tend to remain in the sorbent otolin (Wong et al. 1992, Morawska and Phillips 1993). The nature of coraline carbonates also supports the closed system assumption by finding little or no loss of ^{222}Rn from the carbonate matrix (Bender 1973, Moore et al. 1973, Dodge and Thompson 1974).

The lack of Ostwald ripening and cementation in otoliths support the assumption that there is no post-formational migration of nuclides. Ostwald ripening, a process in which small crystals (more soluble than larger ones) dissolve and reprecipitate onto larger crystals (Parker 1984), has been demonstrated not to occur in corals and otoliths. Studies using ^{45}Ca indicate that once calcium is incorporated it will not exchange with sea water in corals (Goreau and Goreau 1960) and is taken up and remains immobilized in otoliths (Mugiya 1974, Simkiss 1974, Campana 1983, Ichii and Mugiya 1983, Yoklavich and Boehlert 1987, Mugiya 1993). Cementation, precipitation of binding material, may occur in corals (Benninger and Dodge 1986), but probably does not occur in otoliths because porosity is conserved with increasing size, as is indicated by dye penetration (Richter and McDermott 1990). This suggests that the otolith is a well regulated structure, which is supported by the existence of polyanions in the organic matrix, called nucleation barriers, which create directional growth by inhibiting crystal growth to one direction (Wheeler 1993).

The assumption that uptake of exogenous nuclides in the ^{226}Ra decay series is negligible is only necessary for ^{210}Pb. All nuclides from ^{226}Ra through to ^{210}Pb are very short lived (seconds to minutes) Therefore, incorporation of these isotopes is highly improbable. Because ^{210}Pb exists in the natural environment, it must be assessed as a potential contaminant. Measuring ^{210}Pb in juvenile otoliths is currently the most applicable technique for assessing the possibility of direct incorporation. Epipelagic and inshore fishes may encounter the same problem as with corals, where uptake of ^{210}Pb varies more than ^{226}Ra due to precipitation and runoff (Moore and Krishnaswami 1972, Moore et al.

1973, Dodge and Thompson 1974, Veeh and Green 1977, Benninger and Dodge 1986). For deepwater fishes, however, the potential for uptake is far less because dissolved ^{210}Pb is depleted relative to ^{226}Ra with increasing depth (Nozaki and Tsumogai 1976, Thomson and Turekian 1976, Chung 1987).

West and Gauldie (1994) propose that ^{210}Pb and ^{210}Po may diffuse into the otolith through the pore structure. Migration of these nuclides into the otolith is very improbable because these species are extremely particle reactive and readily adsorb onto surfaces. The most probable location for either species to be deposited, if they are biologically transported this far, is the actively accreting surface. Therefore, ^{210}Po would not be a factor because of its short half life (138.4 d) and ^{210}Pb can be assessed in juvenile otoliths.

The assumption that ^{226}Ra uptake is in constant proportion to mass growth-rate is only necessary when whole otoliths and an otolith mass growth-model are used (Bennett et al. 1982). A violation of this assumption was documented for the blue grenadier (Gadidae: *Macruronus novaezelandiae*; Fenton et al. 1990). The radiometric ageing technique could not be used on whole otoliths because the activity of ^{226}Ra decreased in otoliths from juveniles to adults. This was attributed to an ontogenetic movement from estuarine and inshore waters, where environmental ^{226}Ra can be significantly higher (Fanning et al. 1982, Moore 1996), to deeper, offshore waters. A radiometric analysis of otolith cores from this species, however, was successful (Fenton and Short 1995).

In further support of the effectiveness of the radiometric ageing technique, it has been applied successfully to 13 fish species, including the Pacific grenadier (Bennett et al. 1982, Campana et al. 1990, Fenton et al. 1991, Kastelle et al. 1994, Fenton and Short

1995, Milton et al. 1995, Stewart et al. 1995, Watters 1995, Kline 1996). In each of these studies, radiometric age determinations are in close agreement with age estimates derived from traditional ageing techniques. It was said, however, that concerns about the assumptions are so significant that the radiometric ageing technique is "invalid" (West and Gauldie 1994). This perspective was biased because much of what was written in the manuscript did not consider all possibilities. The radiometric analysis results for the Pacific grenadier are the most accurate thus far and further supports the validity of this technique.

Pacific grenadier age and growth

The Pacific grenadier is confirmed as a long-lived fish that needs special attention because of the rapidly developing fishery (Chapter 3, Figures 1 & 2). According to radiometric age determinations, the Pacific grenadier can attain an age of at least 56 yr and may have a longevity greater than 73 yr based on growth increments (Chapter 3, Figures 4 & 7). Because the Pacific grenadier matures late in life at a large size and population distribution and abundance is unknown, conservative regulatory measures need to be taken.

The depth distribution of the Pacific grenadier may provide a viable management strategy. Because size and abundance appear to increase to a depth of approximately 1500 m (Stein and Pearcy 1982), it may be possible to implement a size refuge for the Pacific grenadier by limiting fishing to a depth range (i.e. 1200 to 1800 m). By fishing in this depth range, juvenile and old adults could be protected in a strategy comparable to

that of white sturgeon (*Acipenser transmontanus*) in California, where small and large fish are released. With this strategy, recruitment and growth of immature fish on the upper slope could be more protected, and larger, older fish at depth could provide an undisturbed source of productivity for the population. In addition to this limitation, a seasonal restriction should be implemented to reduce to impact on individuals during spawning season. A moratorium on fishing for the early fall (August) to late winter (February) would encompass the period where reproduction appears to hit a peak (Matsui et al. 1990).

Much remains unknown about the Pacific grenadier that is necessary to develop a sustainable fishery. Population size and distribution, and the movement of individuals in the population, are probably the most important aspects of this fishery that need research. Population size and individual movement may be assessed using recently developed acoustical techniques (Priede et al. 1990). Catch per unit effort information needs to be compiled and analyzed for abundance estimates in areas already being fished. Other studies including tag and recapture coupled with deep trawling and submersible transects could be used to estimate population characteristics at depths greater than current commercial fishing depths.

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