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

Allen H. Andrews, Kenneth H. Coale, Jocelyn L. Nowicki, Craig Lundstrom, Zenon Palacz, Erica J. Burton, and Gregor M. Cailliet

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 incorporating an ion-exchange procedure followed by isotope-dilution thermal ionization mass spectrometry (TIMS) to determine ^{226}Ra. This technique counts ionized radium atoms directly; therefore, the uncertainty of the technique is superior to conventional radio-decay dependent techniques. Calcium and barium are major components of the otolith matrix that can interfere with TIMS analysis of radium. To remove these interferants, an ion-exchange separation procedure was developed. 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 that the procedure efficiently separates radium from calcium and barium. Measured ^{226}Ra activities for each species were similar to previous radiometric ageing studies, with the exception of one sample. When results were compared with traditional ^{226}Ra determination techniques, radon emanation and α-spectrometry, the separation procedure with isotope-dilution TIMS had significant advantages. Samples over three times smaller than attempted in other studies were processed with decreased uncertainty and processing time.

Résumé : Pour accroître l'exactitude et la précision de la détermination radiométrique de l'âge par le déséquilibre du ratio ^{210}Pb:^{226}Ra dans les otolithes des poissons, on a mis au point une technique utilisant une procédure d'échange d'ions suivie d'une analyse au moyen de la spectrométrie de masse à thermo-ionisation avec dilution des isotopes pour déterminer le ^{226}Ra. Cette technique compte directement les atomes de radium ionisés, de sorte que l'efficacité de cette technique est supérieure aux techniques classiques qui dépendent de la décroissance de la radioactivité. Le calcium et le baryum sont des composantes de la matrice des otolithes qui peuvent interférer avec l'analyse du radium par spectrométrie de masse à thermo-ionisation. Pour éliminer ces interférences, on a mis au point une technique de séparation par échange d'ions. On a testé cette technique en l'appliquant aux échantillons d'otolithes de trois espèces de poissons dans trois études distinctes de détermination radiométrique de l'âge. Les séparations résultantes et les déterminations par spectrométrie de masse à thermo-ionisation indiquent que cette technique sépare efficacement le radium du calcium et du baryum. Les activités du ^{226}Ra mesurées chez chaque espèce étaient semblables à celles obtenues dans d'autres études de détermination radiométrique de l'âge, à l'exception d'un échantillon. La comparaison de nos résultats avec ceux des techniques classiques de détermination du ^{226}Ra, de la technique utilisant l'émanation du radon et de l'α-spectrométrie a montré que notre technique de séparation suivie de l'analyse par spectrométrie de masse à thermo-ionisation avec dilution des isotopes comporte des avantages importants. On a pu traiter plus rapidement et avec un plus faible degré d'incertitude des échantillons plus de trois fois plus petits que ceux utilisés dans d'autres études.

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A.H. Andrews,[1] K.H. Coale, J.L. Nowicki, E.J. Burton, and G.M. Cailliet. Moss Landing Marine Laboratories, P.O. Box 450, Moss Landing, CA 95039-0450, U.S.A.
C. Lundstrom[2] and Z. Palacz.[3] University of California, Santa Cruz, Department of Earth Sciences, Santa Cruz, CA 95064, U.S.A.

[1]Author to whom all correspondence should be addressed. e-mail: andrews@mlml.calstate.edu
[2]Present address: Brown University, Geological Sciences, Providence, RI 02912, U.S.A.
[3]Present address: Micromass UK Ltd., Floats Rd., Wythenshawe, Manchester M23 9LZ, United Kingdom.

Introduction

Fisheries management strategies rely heavily on accurate age determinations. Age is typically determined in fishes by performing one of several techniques. The most common technique is counting growth increments in calcified structures (i.e., otoliths; Beamish and McFarlane 1987). The annual periodicity of growth increments in these structures is often assumed and, until recently, this assumption was rarely validated (Beamish and McFarlane 1983). The problem with typical 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 (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 that exploits the disequilibria of ^{210}Pb and ^{226}Ra in otoliths as a natural chronometer (Smith et al. 1991; Bergstad 1995).

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 femtograms (10^{-15} g) for ^{226}Ra to attograms (10^{-18} g) for ^{210}Pb. Detection of ^{210}Pb is typically accomplished through the autodeposition and α-spectrometric determination of its daughter, ^{210}Po (Flynn 1968). Because ^{210}Pb is the result of ingrowth from ^{226}Ra, low ^{210}Pb:^{226}Ra atom ratios exist in otolith material. Direct mass determination methods, therefore, are not feasible for ^{210}Pb at this time. Two techniques traditionally used to determine ^{226}Ra are scintillation counting of its daughter ^{222}Rn via radon emanation and direct α-spectrometry of ^{226}Ra in a filtered precipitate.

The radon emanation technique has been applied to otoliths, as part of the radiometric ageing technique, of six fish species: *Sebastes diploproa* (Bennett et al. 1982); *Sebastes mentella* (Campana et al. 1990); *Anoplopoma fimbria* (Kastelle et al. 1994); *Sebastes rufus* (Watters 1995); and *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 radiometric ageing technique used whole otoliths for both ^{210}Pb and ^{226}Ra determination (Bennett et al. 1982). Use of whole otoliths, however, can be imprecise and requires application of mass-growth models that are not always robust (Francis 1995). To circumvent the need for mass-growth models, a method was developed to extract the oldest part of the otolith, the core, which represents the first few years of growth (Campana et al. 1990). Because otolith cores can be small (i.e., 0.01 to 0.05 g), cores from similar age/size-classes must be pooled to attain measurable activity. A sample size of approximately 1 g, however, can produce a large error for the radon emanation technique (Bennett et al. 1982). Therefore, ^{226}Ra determination is usually performed on pooled whole otoliths with the necessary assumption that ^{226}Ra uptake is in constant proportion to the otolith mass growth rate.

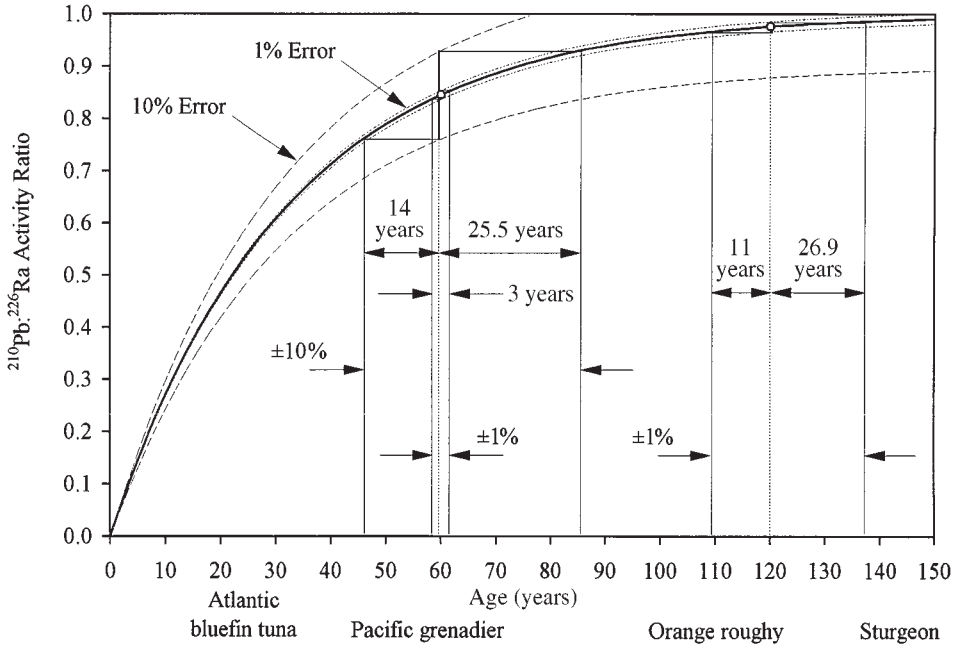
To determine ^{226}Ra, direct α-spectrometry has also been used as part of the radiometric ageing technique for six fish species: *Hoplostethus atlanticus* (Fenton et al. 1991; Smith et al. 1995); *Allocyttus verrucosus* (Stewart et al. 1995); three species of the family Lutjanidae (Milton et al. 1995); and *Macruronus novaezelandiea* (Fenton and Short 1995). In this technique, ^{226}Ra is isolated by co-precipitation 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 has a lower background and, therefore, a lower detection limit, a smaller sample size can be used (~1 g). 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 the radon emanation and direct α-spectrometry 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 a low uncertainty using radon emanation, however, make the use of cores problematic because the pooling of cores from hundreds to thousands of similarly aged fish would be necessary (e.g., >10 g; Campana et al. 1990; Watters 1995; Kline 1996). For the small sample sizes typically associated with the use of otolith cores, the determination of ^{226}Ra via direct α-spectrometry has produced the lowest uncertainty for samples with low mass and ^{226}Ra activity (Fenton et al. 1991). Both techniques (radon emanation and α-spectrometry), however, are time-consuming (3–5 weeks) and can lead to large analytical uncertainties (e.g., range of ~4–150%; 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).

Because some fish species have a longevity that may exceed 100 years (4 to 5 half-lives of ^{210}Pb), the utility of the radiometric ageing technique becomes increasingly dependent on the analytical uncertainty of the technique. As the activity of ^{210}Pb asymptotically approaches the activity of ^{226}Ra, the analytical uncertainty becomes a larger proportion of the age estimate (Fig. 1). For example, a 10% error for a radiometric age of 60 years would be +25.5 to –14 years. A hypothetical reduction of this error to 1% significantly reduces the uncertainty to +1.7 to –1.3 years. Additionally, a reduction in error increases the applicable age range. A 10% error for a radiometric age of 120 years becomes undefined at the upper limit (the ^{210}Pb:^{226}Ra ratio can not exceed 1.0) and –52.3 years at the lower limit. A reduction of the error to 1% reduces the age uncertainty where the upper limit becomes defined (+26.9 and –11 years). A reduction of the error inherent in the technique would increase the applicability of the radiometric ageing technique to fish approaching 150 years and would reduce the age estimate uncertainty. As an example, reduced error in accelerator mass spectrometry was highly significant where age estimate uncertainty was reduced and the applicable age range was increased (Vogel et al. 1995).

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). By using isotope-dilution TIMS, radium is measured directly by counting the ionized atoms with low detection

Fig. 1. Conceptual view of the effect of error in the measurement of ^{210}Pb and ^{226}Ra on the age estimate uncertainty. Given are two hypothetical scenarios where the ^{210}Pb:^{226}Ra activity ratio yields a 10% error and a reduced error of 1%. Note that a reduction from 10% to 1% reduces the age uncertainty considerably and increases the utility of the technique to fish that approach 150 years. Examples of fish species that show how longevity can vary among fishes are listed along the age axis. The Atlantic bluefin tuna (*Thunnus thynnus*) is an example of a fish that has relatively fast growth and an estimated longevity of 20 to greater than 30 years (Prince and Pulos 1983). The longevity of the Pacific grenadier (*Coryphaenoides acrolepis*) has been validated to at least 46 years and may approach 70 years, and the orange roughy (*Hoplostethus atlanticus*) has been validated to approximately 100 to 120 years (Smith et al. 1995; Andrews et al. 1999). Candidates for the greatest longevity in fishes are members of the sturgeon family at over 150 years (Anonymous 1954).



limits and high analytical precision (Volpe et al. 1991). The elemental composition of otoliths, however, is very different from that of volcanic rocks. Of concern 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 technique to determine ^{226}Ra directly in otoliths using an adapted ion-exchange procedure (Chabaux et al. 1994) and isotope-dilution TIMS. To test the application of this technique, ^{226}Ra activity was determined 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 will be discussed in future publications. The focus of this paper is the determination of ^{226}Ra activity in otoliths using the new technique and a comparison with the traditional techniques, where the benefits of TIMS are discussed.

Materials and methods

Determination of ^{226}Ra activity was performed on sagittal otoliths from three fish species. The species analyzed were shortspine thornyhead (*Sebastolobus alascanus*) collected off California in Monterey Bay and in the Gulf of the Farallones, Atlantic tarpon (*Megalops atlanticus*) from inshore waters of Florida, and yelloweye rockfish (*Sebastes ruberrimus*) from off southeastern Alaska. Whole adult *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, NOAA, 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 (Collier-Seminole and Jack Island State Parks in southern Florida) and date to determine if exogenous ^{210}Pb would present a problem in ageing adults. Because traditional age is more reliable for young-of-the-year, radiometric age can be used to determine if exogenous ^{210}Pb is incorporated. Otolith cores (first 4 years of growth) of *S. ruberrimus* were extracted using procedures given elsewhere (Andrews et al. 1999) and were pooled into age-groups. A sample weight of 0.5 to 1.0 g was targeted in this study. In addition, one whole otolith sample was processed for a comparison of whole versus core ^{226}Ra activities.

Because of the extremely low levels of ^{226}Ra and ^{210}Pb, trace-metal precautions were exercised during sample processing (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 the technique used for ^{210}Pb determination had only a minor modification and was performed before ^{226}Ra analysis, the details of the procedure were described elsewhere (Andrews et al. 1999).

Otolith cleaning and dissolution

Otolith samples were cleaned, dried, and weighed before dissolution in the following manner. Rough cleaning began with hydrating the otoliths in de-ionized water for at least 5 min. Samples were agitated and rinsed three times with each of the following:

Table 1. Overview of column separation procedures.

	Calcium removal			Barium removal	
		Volume (mL)			
	Reagent	First pass	Second pass	Reagent	Volume (μL)
Ion-exchange resin	AG® 50W-X8 (Bio-Rad)	13[a]	13[a]	Sr® resin (EiChroM Ind.)	150[a]
Clean and	MQ water	100	100	MQ water	1000
condition	2.5–6.0 N HCl	50	50	1.1 N HNO_3	800
Introduction	Sample[b]	5	1	Sample[d]	50
Beaker rinse	6.0 N HCl	5	1	1.1 N HNO_3	50
Wash	6.0 N HCl	30	38	1.1 N HNO_3	150
Elution	6.0 N HCl	80[c]	80[c]	1.1 N HNO_3	450[e]

[a]Rinsed resin added in MQ water.
[b]Sample dissolved in 6.0 N HCl.
[c]Radium and barium fraction.
[d]Sample dissolved in 1.1 N HNO_3.
[e]Radium fraction.

(*i*) a mixture of de-ionized water and Micro® laboratory cleaner, (*ii*) de-ionized water, and (*iii*) 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 (*i*) MQ water (10 min), (*ii*) 0.15 N HNO_3 (1 min), (*iii*) basic 1:1 mixture of 30% H_2O_2:0.4 N NaOH (10 min), (*iv*) MQ water (10 min), and (*iv*) 0.001 N HNO_3 (3 times at 1 min). After the final rinse, samples were dried to constant weight (at least 24 h) in an oven at 80°C, cooled in a desiccator, and weighed to ±0.0001 g.

Dried and weighed samples were placed in acid-cleaned 100 mL Teflon® PFA griffin beakers. While on a hot plate at 80°C, 8.0 N HNO_3 was added to the otoliths in 1 mL aliquots until dissolved. The dissolved sample was dried, redissolved with 1 mL of 8.0 N HNO_3, and dried again. This was repeated 5 times. Before drying the fifth dissolution completely, 1 mL of 6.0 N HCl was added to form an aqua regia solution. This solution was dried, redissolved with 1 mL 6.0 N HCl, and dried again. Dissolution in 1 mL of 6.0 N HCl was repeated 5 times, which left the final sample in the desired chloride form. The repeated drying and dissolution enhanced the oxidation of otolin, a potential interferant.

Determination of ^{210}Pb activity

To determine ^{210}Pb activity in some of the otolith samples (*M. atlanticus* and *S. ruberrimus*) as part of independent radiometric ageing studies, α-spectrometry was performed on plated ^{210}Po samples spiked with ^{208}Po as a yield tracer (Andrews et al.1999). In previous studies, ascorbic acid was added to inhibit autodeposition of divalent iron (Kline 1996). Because ascorbic acid is an organic that can raise the background count levels in the TIMS analysis and because iron content in otoliths is low (Dannevig 1956), it was not used in this study. Radium blanks included the polonium plating steps to account for any potential radium contamination. The solution remaining after polonium autodeposition was recovered for ^{226}Ra analysis.

Radium separation

The separation of radium from calcium and barium is essential for obtaining good radium ionization efficiency during TIMS. This was achieved by applying a three column, ion-exchange separation procedure. Elution characteristics for each column separation were determined using otolith samples and calcium and barium standards, where the collection intervals were optimized using flame atomic absorption spectrophotometry (AA). Solid/solute 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).

To determine ^{226}Ra using isotope-dilution TIMS, the dissolved otolith sample was 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 was greater than 1.6 million, producing a ^{228}Ra to ^{226}Ra ratio in the yield tracer of 0.2855 ± 0.0057 as of June 1, 1995. The ^{228}Ra spike solution was calibrated against NBS and geological standards. The spike was added to the sample after polonium plating, which avoided autodeposition of ^{228}Th (daughter of ^{228}Ra) and subsequent contamination of the α-detector from ^{228}Th recoil (Sill and Olson 1970). 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 8.0 N HNO_3 and dried. This was repeated until a white residue was obtained. Before the final drying was complete, an aqua regia transition to 6.0 N HCl was created by adding 1 mL of 6.0 N HCl. This solution was dried. Dissolution with 6.0 N 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

The first column type was a 10 mL chromatography column with a 20 mL reservoir (Bio-Rad Laboratories, Econo-Pac 10 Column). A rinsed slurry of Bio-Rad AG® 50W-X8 cation exchange resin and MQ water was added into the acid-cleaned 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 2.5 N HCl, 5 mL 4.0 N HCl, and 40 mL 6.0 N HCl through the column. Conditioning the column shrinks the resin volume to 10 mL with a length:width aspect ratio of 3.9.

The sample was redissolved in 5 mL of 6.0 N 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 6.0 N 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 6.0 N 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 6.0 N 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 6.0 N 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 for a second pass, as in the first preparation, and the separation procedure was repeated from the beginning of this paragraph.

Barium removal: third column separation

The second column type was a custom 150 μL microcolumn made of TFE heat-shrink tubing (6.35 mm ID) shrunk over a hand-machined stainless steel die. Die dimensions were 6.35 mm (0.25 in.) in diameter by 57 mm (2.25 in.) in length for the reservoir which tapers down to 2.38 mm (0.094 in.) in diameter by 35 mm (1.38 in.) 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 acid-cleaned microcolumn was prepared for sample processing by adding a slurry of MQ water and 50–100 μm Sr® resin (EiChroM Industries, Darien, Ill.). For best results, the column was filled with MQ water first and 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 1.1 N HNO_3 through the column. The conditioned microcolumn contained 150 mL of Sr® resin and had a length:width aspect ratio of 13.1.

To prepare a sample for introduction to the third column, the sample was dissolved with 100 μL of 8.0 N 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 8.0 N HNO_3 and 10–30 μL of 6.0 N HCl, dried at 90–100°C, and redissolved in 10–30 μL of 8.0 N HNO_3. The last two steps were repeated until the sample was as white as possible.

The sample spot was redissolved with 50 μL of 1.1 N 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 1.1 N HNO_3 was added to the column. A column wash of 150 μL of 1.1 N HNO_3 was added and allowed to settle into the resin. The eluant (250 μL of 1.1 N HNO_3) was discarded. Radium elution was performed by adding 450 μL of 1.1 N HNO_3 to the column, while the barium remained on the column, and the sample fraction was collected in an acid-cleaned 3 mL Teflon® PFA sample vial. 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 8.0 N HNO_3 and 1 drop of 6.0 N 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 redissolved two times in 1 drop of 6.0 N 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 use TIMS, the final sample from the separation procedure must be loaded onto a metallic filament. The design was a 1-cm-long single-filament assembly made of 4 pass zone-refined 99.999% rhenium (0.020 in. wide and 0.001 in. thick; H. Cross Co., Weehawken, N.J.). Before 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. Tantalum activator solution (2 μL of 1% Ta solution, Birck 1986) were evaporated onto 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 1 N HCl. The tantalum activator and the samples 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 off 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 counts per minute (cpm) and dead time was 22 ns. The accuracy and linearity of this detector at signals up to 1 million counts per second (cps) has been verified by repeated measurements of NBS standard U-010. The following 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 cps at this point. Once focused, unspiked samples (samples containing no added ^{228}Ra) were scanned to determine if measurable natural ^{228}Ra existed in the otoliths. In all samples analyzed, no natural ^{228}Ra was recovered and, therefore, no adjustment to the ^{226}Ra determination was required.

Spiked samples and blanks 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 began. 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 (background compensation). A measurement cycle consisted of a 5 s integration on amu 228, a 10 s 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, residual 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.

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 was determined, after correcting for ^{226}Ra contributed by the spike, 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 (Fig. 2). 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

Fig. 2. Elution characteristics of the first pass using 6.0 N HCl on the AG® 50-X8 cation exchange column for calcium (○) 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 was within the calcium tail and, therefore, the collection interval for the radium and barium fraction was from 40 to 120 mL.

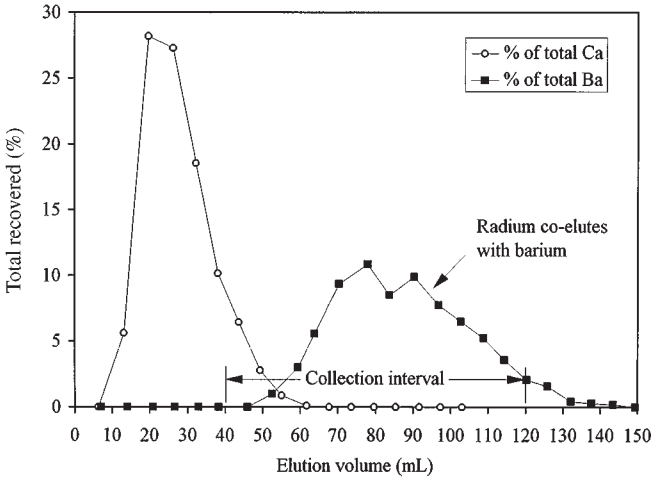
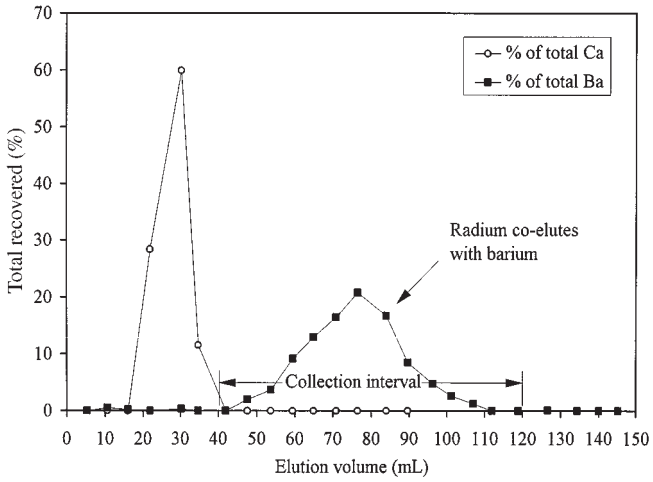


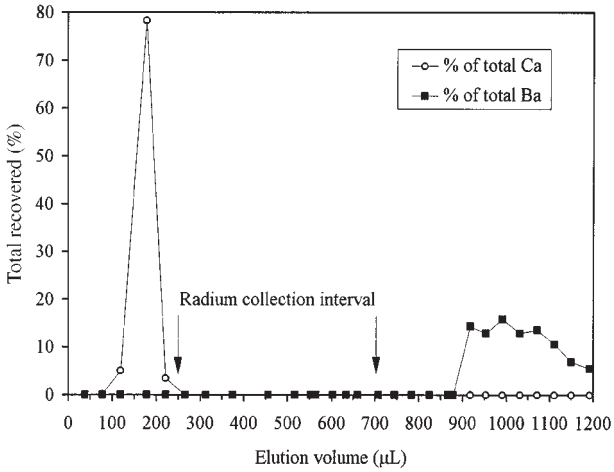
Fig. 3. Elution characteristics of the second pass using 6.0 N HCl on the AG® 50-X8 cation exchange column for calcium (○) and barium (■). To more clearly evaluate the separation, calcium and barium elutions were performed separately but were plotted together. The collection interval for the radium and barium fraction was from 40 to 120 mL



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 (Fig. 3). The selected sample collection interval was the same as in the first column pass. A calculated 1% 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%.

Fig. 4. Elution characteristics of the third column pass using 1.1 N HNO_3 on the Sr® resin microcolumn for calcium (○) and barium (■). Based on the distribution coefficients provided by EiChroM Industries, Darien, Ill., radium eluted between calcium and barium. The optimal collection interval which minimizes calcium and barium was determined to be 250–700 µL using TIMS.



An acid strength of 1.1 N 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 (Fig. 4). A small sample spot (<0.5 mm) remained after drying and was used for radium determination on the TIMS.

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 0.0255 ± 0.0004 dpm·g^{-1} to 1.65 ± 0.03 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 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 (Ta-

Table 2. Summary of sample types for each species.

Species	Sample type (whole or core)	Number of otoliths	Sample weight (g)	^{226}Ra (dpm·g^{-1})	^{226}Ra % error
Sebastolobus alascanus	Whole	7	1.0150	0.0306	1.44
	Whole	8	1.2607	0.0323	2.29
Megalops atlanticus	Whole[a]	35	0.5068	0.201	1.38
	Whole[a,b]	26	0.525	0.306	1.05
	Whole[a]	28	0.564	1.65	1.60
Sebastes ruberrimus	Core	10	0.303	0.0305	2.49
	Core	10	0.319	0.0255	1.75
	Core	14	0.471	0.0298	1.91
	Core	19	0.561	0.0331	3.66
	Core	23	0.587	0.0296	2.26
	Core	20[c]	0.592	0.0283	4.88
	Whole	1	0.8819	0.0263	6.70

Note: Number of otoliths used corresponds to the number of fish in each sample because one otolith of each pair was sectioned for traditional ageing.
[a]Young-of-the-year.
[b] ^{210}Pb data taken to estimate age and assess exogenous ^{210}Pb in the otolith material.
[c]Approximate number.

ble 2). Whole and cored otolith samples from *S. alascanus* and *S. ruberrimus* contained the lowest ^{226}Ra activities and have similar 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 deepwater species. The ^{210}Pb activity for the juvenile *M. atlanticus* sample to be aged was 0.00243 ± 0.00017 dpm. Calculated age based on the ratio of ^{210}Pb:^{226}Ra activities was 0.51 ± 0.04 years.

To allow direct comparison of these results with existing studies, some reported ^{226}Ra uncertainties were recalculated as a percentage 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 whole otolith samples. Because the *S. ruberrimus* samples are otolith-core samples, the sample size and ^{226}Ra measurements were compared with results from two recent radiometric ageing studies on two different fish species (*Anoplopoma fimbria* and *Macruronus novaezelandiae*) 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), in addition to barium (~5–10 ppm; Edmonds et al. 1991), a recently developed ion-exchange separation technique was applied (Chabaux et al. 1994). With modifications, calcium and barium were successfully reduced and measurable radium was recovered.

There were problems with organic contamination in the developmental stages of the ion-exchange separation procedure. Samples analyzed using TIMS had an elevated and noisy background (high counts at amu 225.5) which was attributed to organic interference from otolin (0.2–10% in otoliths by weight; Degens et al. 1969). This observation led to the rigorous sample dissolution and oxidation procedures reported here. As a result of this procedure, the observed organic interference on the thermal ionization mass spectrometer was reduced to very low levels (<1 cps).

The samples used in this analysis were very diverse in composition and resultant ^{226}Ra activity (Table 2). Whole otoliths from adult *S. alascanus*, whole young-of-the-year

Table 3. Comparison of results for *S. alascanus* otoliths using each technique.

	Technique	Sample size range (g)	^{226}Ra activity range (dpm·g^{-1})	Analytical uncertainty (%)
This study	TIMS	1.0150–1.2607	0.0306–0.0323	1.44–2.29
Kline (1996)	Radon emanation	15.631–16.588	0.0387–0.0504	3.4–5.2
Butler[a] (unpublished data)	α-spectrometry	1.23–1.65[b]	0.0495–0.0697	5.8–6.6

Note: Otolith samples were similar but from different fish samples.
[a]John Butler, Southwest Fisheries Science Center, P.O. Box 271, La Jolla, CA 92038-0271, personal communication.
[b]Before cleaning.

Table 4. A comparison of results for *S. ruberrimus* cores with two studies using otolith cores and one of the other two ^{226}Ra assessment techniques.

	Technique	Sample size range (g)	^{226}Ra activity range (dpm·g^{-1})	Analytical uncertainty (%)	Processing time
This study *S. ruberrimus*	TIMS	0.303–0.592	0.0255–0.0331	1.75–4.88	7–10 days
Kastelle et al. (1994) *A. fimbria*	Radon emanation	0.9227–1.3748	0.288–0.517	~4	5–6 weeks[a]
Fenton and Short (1995) *M. novaevelandiae*	α-spectrometry	~1[b]	0.0179–0.0290	~12–21	3.5–4 weeks[c]

Note: Kastelle et al. (1994) used radon emanation on sablefish (*Anoplopoma fimbria*) and Fenton and Short (1995) used direct α-spectrometry on hoki (*Macruronus novazelandiae*).

[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.

otoliths from *M. atlanticus*, and cored otoliths from *S. ruberrimus* ranged from greater than a gram to less than half of a gram with ^{226}Ra activities covering almost 2 orders of magnitude. The error for these samples varies from optimal (~1–2.5%) to greater than 3%. The high error for some of the *S. ruberrimus* samples (3.66 to 6.70%) was attributed to high calcium and low radium counts. The ^{226}Ra activity results were used as part of separate radiometric ageing studies for each fish species, which are in progress or in preparation for publication.

Differences in the ^{226}Ra levels determined for whole otoliths of *S. alascanus* 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 the other two techniques, but more similar to Kline (1996). Kline (1996) pooled many otoliths to attain a very large sample size that provided for a low error but may have obscured individual differences. The α-spectrometry on similar-sized samples had higher error than the TIMS results. TIMS results are similar to Kline (1996) and differences between direct α-spectrometry and TIMS may indicate varying ^{226}Ra activities between fish from different locations. This seems possible because some of the samples used in this study were taken from a location off the Farallon Islands, California, north of Monterey Bay, California, where Kline (1996) collected. Geographical variation of radium in the natural environment is supported by the findings in otoliths of juvenile *M. atlanticus* and Pacific grenadier, *Coryphaenoides acrolepis* (Andrews et al. 1999).

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. To determine if exogenous ^{210}Pb would be a factor in radiometric age determination of adult *M. atlanticus*, age was calculated for a young-of-the-year sample using the measured ^{210}Pb and ^{226}Ra activities. If exogenous ^{210}Pb was accumulated in significant quantities, then the calculated radiometric age would be greater than the known age of these juveniles (<1 year). Measurements of ^{210}Pb in this sample resulted in a radiometric age that agreed with the expected average age of about 6 months (0.51 ± 0.04 years). The conclusion was that exogenous ^{210}Pb was not incorporated into juvenile otoliths of *M. atlanticus*.

The range of ^{226}Ra activity among the *M. atlanticus* samples can be attributed to the variable water chemistry of inshore waters where the juveniles were collected (Fanning et al. 1982; Crabtree et al. 1995; Moore 1996). The sample with the highest activity (1.65 ± 0.03) was collected in Collier-Seminole State Park, Florida, whereas the two more similar samples (0.201 ± 0.003 and 0.306 ± 0.003) were collected in Jack Island State Park, Florida. This variability, however, was not a detrimental factor for this technique because ^{226}Ra and ^{210}Pb were determined from the same sample. 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. 1997).

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

Because ^{226}Ra activities observed for *S. alascanus* and *M. atlanticus* varied considerably among the samples (Tables 2 and 3), the potential for variation of ^{226}Ra uptake among the individuals in a sample must be considered in future radiometric ageing studies. To increase the accuracy of radiometric age determination using otolith cores and to avoid the assumption of constant ^{226}Ra uptake, ^{210}Pb and ^{226}Ra activities should be measured in each sample. 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 inherent in the TIMS technique, the assumption of constant uptake becomes unnecessary and radiometric age determination be-

comes more accurate. In a companion study, this technique was successfully applied to the radiometric age determination of the Pacific grenadier (Andrews et al. 1999).

Because this technique has improved the accuracy and processing time of ^{226}Ra determination, the most significant source of error and analytical processing time in radiometric age determination is now associated with the ^{210}Pb analysis via α-spectrometry. For the radiometric age determined for the juvenile *M. atlanticus* sample, approximately 87% of the error contribution came from the ^{210}Pb analysis.

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 (1.65 ± 0.03 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. 1999) and Atlantic tarpon (Andrews et al. 1997), 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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