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THE USE OF Pb-210/Ra-226 AND Th-228/Ra-228 DIS-EQUILIBRIA IN THE
AGEING OF OTOLITHS OF MARINE FISH

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ABSTRACT

Naturally-occurring isotopes of radium are ideally suited as tracers for chemical uptake in the calcified tissues of marine organisms since radium is a water soluble, bio-geochemical analogue for calcium. Assays designed to exploit this uptake mechanism can be used to determine the longevity of certain species of fish. Measurements of Pb-210/Ra-226 disequilibria in the otoliths of redfish have revealed that this species of fish can live to ages in excess of 75 years in coastal waters off Nova Scotia, Canada. Measurements of the Th-228/Ra-228 disequilibria in the otoliths of the much shorter-lived silver hake and flying fish may provide estimates of longevity on time scales of 0-10 years, which could then be used to evaluate the accuracy of currently-used ageing models based on otolith annulus counts. The radioisotopic ageing technique relies on the extraction of a rectangular block centred around the core of the otolith whose analysis for radionuclide ratios permits an unambiguous estimate of the age of the fish. Age determinations of fish based on natural radioisotopes can result in significant improvements in the assessment and management of certain fisheries resources.

INTRODUCTION

Fundamental to the study of marine biology is the age and growth rate of specific organisms and yet, for many fish this is a poorly known parameter. Most ageing techniques rely on the identification of annual increments associated with the growth of teeth, otoliths (ear bone), scales, vertebrae or other bony tissues of the organism. The difficulties in these techniques

generally lie with uncertainties in the regularity with which the growth increments occur and in the identification of the chronological markers themselves. For example, several north temperate fish species are thought to have lifespans over 60 y [1], but the reported maximum ages have varied widely depending upon the choice of ageing methodologies and the interpretation of the investigator [2]. Errors in estimates of fish ages can confound any attempt at optimal management of a fish stock and interfere with the most basic understanding of the life cycles of the species.

Natural radionuclide tracer measurements offer a new and comparatively accurate methodology for determining the growth rates of marine organisms. The techniques discussed in this report rely on the uptake by fish otoliths of either Ra-226 or Ra-228 from seawater and the subsequent decay of these isotopes to their daughter products (Pb-210 and Th-228, respectively) in a closed chemical system. Measurements of the parent and daughter product ratios then permit a determination of the fish age since the time of radium uptake. Pb-210/Ra-226 disequilibria provide a sensitive dating range of from 0-100 y while the Th-228/Ra-228 disequilibria is useful for fish ages of 0-20 y.

Radionuclide ageing techniques rely on two basic geochemical features of the naturally-occurring, U-238 decay series, (1) the high solubility of radium in seawater compared to its parent and daughter radio-isotopes, and (2) the relatively efficient uptake of radium from seawater by marine tissues as a proxy for calcium. These conditions combine to insure that an initial state of radioactive dis-equilibrium is established by the incorporation of radium isotopes into the tissues of organisms, unaccompanied by parent or daughter radio-isotopes. Successful applications of the method then rely on a sufficiently high signal to noise ratio in the daughter/parent activities as they decay to a state of radioactive equilibrium.

The measurement of radionuclide ratios in whole otoliths or other accreting structures does not generally supply conclusive evidence of the age of the fish. The basic problem is that as the otolith accretes, it continually forms a new outer sheath of material having a parent (eg. Ra-226) concentration equivalent to its initial, or t (time) = 0 value. However, the material formed in previous years will have already begun to generate Pb-210 by radioactive decay. As a result, a gradient in the Pb-210 activity will be formed along any axis from the centre of the otolith to an external surface. The Pb-210 activity at the otolith centre will have a maximum value while newly formed material at the external surface will have a minimum or zero value. Hence, the analysis of a whole otolith will give an intermediate Pb-210 activity having some value between zero and the maximum value representative of the core of the otolith. Upon introduction into the decay equation, this number will always give an under-estimate of the true age.

The first Pb-210/Ra-226 disequilibria measurements on otoliths were undertaken by Bennett et al. [3] on the whole otoliths of redfish (*Sebastes diploproa*). They attempted to overcome the above difficulties by formulating an otolith mass-growth model and then calculating the degree of Pb-210 dilution by the continuous addition of mass during the life time of the fish. The difficulty with this approach was that it involved a certain degree of circular reasoning. The accuracy of the age determination depended on the validity of the mass-growth model, but the formulation of a mass-growth model was based on an initial estimate of the fish age. Campana et al. [4] circumvented this entire issue by shaving away the outer layers of material leaving only the central core of the otolith formed near the time of hatch. Under these conditions, a simple, first order radioactive decay law can be used to determine fish age, since the rate of mass accumulation at the otolith surface, and, indeed, variations in the rate, have no impact on the properties of the core.

The underlying assumptions of the methodology employed by Campana et al. [4] are reviewed in the present paper. Comparisons are made between the whole otolith and core techniques. In addition, more recent applications of this aging technique to shorter-lived fish using a different isotope pair (Ra-228/Th-228) are discussed.

METHODOLOGY

Otolith Analyses

Redfish were collected from depths of 200-900 m along the edge of the Scotian Shelf in October, 1985 and 1986. Sagittal otolith pairs ($n=2775$) were extracted from fresh specimens and stored dry in paper envelopes. One otolith from each pair was cracked, charred and coated with oil for the determination of fish age via annulus counts. Annuli were counted under 50 x magnification at least twice by each of two independent readers, using criteria established by Beamish et al. [5]. The remaining otolith of each pair from fish of age categories; 6, 10-15, 20-25, 30-35, 40-45, 50-55 and 60+ y were selected for core extraction and radiochemical analyses.

Otolith cores were extracted as rectangular blocks centred around the otolith nucleus. The location of the nucleus was assessed in relation to intact otolith morphology and confirmed through sectioning of test samples. While a block is a crude approximation of otolith shape the relatively large annuli in young otoliths imply that slight errors in measurement or extraction would have little effect on the mean age of the core. Cores were isolated with a low-speed, diamond blade saw, thinned with a metallurgical polishing machine and cleansed of surface residue. Cores were subsequently pooled within age categories to form samples weighing approximately 1.0 g (21-28 cores).

Flying fish (*Hirundichthys affinis*) and silver hake (*Merluccius bilinearis*) specimens were obtained from commercial fisheries in Barbados and Nova Scotia, respectively in February, 1990. Otolith pairs were collected and examined by the methods noted above.

Radionuclide Measurements

For Pb-210/Ra-226 analyses, one gram otolith samples from redfish were pre-treated to remove organic or inorganic deposits by first soaking them in 30% hydrogen peroxide solution, followed by successive washings with 1 N HCl solution and distilled water, using high purity reagents. Po-208 tracer solution plus 10 ml of 50% conc. HCl + HNO₃ were added to the samples and then heated to dryness. Ten ml of conc. HCl were added, the solution again heated to dryness and then re-dissolved in successive additions of 1 ml of H₂O₂ and 30 ml of 0.5 N HCl. The polonium isotopes were plated at 85-90 deg. C. for approximately 4-6 hours onto a silver disc and the Po-208 and Po-210 activities (equal to the Pb-210 activity for samples several years old) subsequently measured by alpha particle spectrometry. The Pb-210 blank was 0.0005 dpm and background counts were less than 1/day.

Following plating of the polonium isotopes, the solution was transferred to an extraction cell connected to a radon gas extraction vacuum line. The radon was subsequently transferred (with a 95 % transfer efficiency) to a scintillation counting vial and then the radon (and Ra-226) activity determined using a photomultiplier assembly. The counting efficiency of the entire system for Ra-226, determined using NBS Ra-226 standard solutions, was $80 \pm 5\%$. Ra-226 analyses of both the core and whole otolith samples revealed no significant differences. As a result, the whole-otolith analyses of 3 samples (13 g each) were used to determine a mean Ra-226 concentration (0.033 ± 0.002 dpm/g) for the otolith material.

Following pre-treatment procedures, the flying fish and silver hake otolith samples for Th-228/Ra-228 analyses were digested in aqua regia for four hours, together with Ra-226 and Th-229 yield tracers, cooled and 20 ml of hydrogen peroxide were added drop wise. An additional 30 ml of aqua regia were then added and the samples evaporated to dryness. The residue was redissolved in 100 ml of conc. HNO_3 and 200 mg of $\text{Pb}(\text{NO}_3)_2$ carrier added. The solution was reduced in volume to 75 ml, 25 ml of fuming nitric acid were added, and the radium separated by co-precipitation with $\text{Pb}(\text{NO}_3)_2$ [6]. The precipitate was centrifuged (saving the supernatant for Th analyses), dried in a counting vial and the Ra-226 and Ra-228 activities determined by gamma counting using the 339 keV and 911 keV gamma energies of Ac-228 for the Ra-228 measurement and the 186 keV (Ra-226) and 295 keV (Pb-214) gamma energies for the Ra-226 measurement. The gamma analyses were conducted using a Canberra HPGE well detector having a relative efficiency of 20%, a 103.5 cc active volume and a well 20 mm in diameter and 35 mm deep.

The supernatant was boiled to dryness and the residue dissolved in 8 N HNO_3 . The thorium isotopes were separated by ion exchange using BIORAD AG 1x8 resin, 100-200 mesh and eluted from the column with 12 N HCl. The sample was then redissolved in H_2SO_4 and plated onto a stainless steel disk at 1.1 amp and 50 volts for two hours. The alpha activities of Th-228 and the yield tracer, Th-229, were measured by alpha particle spectrometry. Blanks determined using the same procedures, reagents, carriers and tracers as in the otolith analyses gave values of 0.010 ± 0.005 dpm for Th-228 and 0.050 ± 0.020 for Ra-228.

AGEING MODELS

Model Assumptions

Radium disequilibria aging models for whole otoliths are generally based on the following assumptions: (1) There is negligible, post-formational, internal migration of radionuclides across internal otolith annuli, i.e. the otolith core constitutes a closed chemical system; (2) The uptake of the daughter radionuclide from seawater or other external sources is small compared to the parent (radium) uptake; (3) The uptake of parent and daughter radionuclides is in constant proportion to the mass accumulation rate of the otolith.

Condition (1) ensures that the chronological, radioactive disequilibria features of the otolith are preserved and is supported by numerous experiments indicating that internal chemical migration within the acellular, otolith structure is negligible [7]. However, it should be noted that this condition is apparently invalid in some cellular systems such as the vertebrae of sharks where post-formational transport of Pb-210 has been observed [8]. Condition (2) ensures that the signal to noise ratio of the daughter/parent disequilibria signal is sufficiently great to ensure the practical viability of the method. This assumption can be validated by measurements on the otoliths of young fish and small discrepancies can be accommodated within a given model. Condition (3) is the most problematic to whole-otolith applications of radium aging because it generally presupposes a knowledge of the otolith mass-growth rate as determined by unvalidated annulus counts. As noted above, the coring technique obviates the necessity of relying on condition (3), except for the initial period of core formation. Indeed, Fenton et al. [9] detected a major violation of Condition (3) in their measurements of Pb-210/Ra-226 disequilibria in the otoliths of blue grenadier from Southeastern Australian waters. They measured an order of magnitude decrease in the Ra-226 concentration of whole otoliths of fish having ages (determined by annulus counts) of 1 to 10

years old. They ascribed this variability to the life habits of the fish, with the younger, juveniles spending their time in radium-enriched, nearshore waters and the adults occupying the deeper, offshore waters, diminished in Ra-226.

Core Model

The processes governing radium uptake and decay in otoliths are;



where N_1 represents the parent radionuclide having a decay constant, λ_1 , and N_2 represents the daughter radionuclide having a decay constant, λ_2 . The relationship between the radionuclide activity in the otolith and its age can be determined by first considering the formation of a small nucleus or core of the otolith. The parent and daughter specific activities, A_1 and A_2 , and their ratio in this core at some future time or "age", t , are given by the standard equations for this general decay scheme;

$$A_1 = A_1^0 e^{-\lambda_1 t} \quad (3)$$

$$A_2 = A_1^0 \left(\frac{\lambda_2}{\lambda_2 - \lambda_1} \right) \{ e^{-\lambda_1 t} - e^{-\lambda_2 t} \} + A_2^0 e^{-\lambda_2 t} \quad (4)$$

As the otolith grows, new material is accreted to the outer surface having an initial activity ratio, $R^0 (= A_2^0/A_1^0)$, while the inner nucleus of the otolith has an activity ratio given by A_2/A_1 . In the general case, the total activity, S_1 , of N_1 (or S_2 , of N_2) in the otolith is calculated by integrating the product of the mass-growth rate, $G(t)$ and equation 3 (or equation 4) with respect to time (equation 5). In the study of Campana et al. [4], it was assumed that during the period of core formation (up to an age of T or approximately 10% of its full growth), condition 3 above was valid and the otolith underwent a linear increase of mass with time at a constant rate of Ra-226 incorporation. This constant mass-growth (CMG) model can be applied to the general case by substituting a constant mass-growth rate (equal to the total mass, M , of the core divided by its age, T) for $G(t)$ in equation 5, ie.

$$S_1(T) = \int_0^T G(t) A_1 dt \quad (\text{CMG model}) = \frac{M}{T} \int_0^T A_1^0 e^{-\lambda_1 t} dt \quad (5)$$

Integration of equation 5, and the equivalent equation for S_2 , followed by dividing S_2 by S_1 gives for the activity ratio, A_2/A_1 , of the otolith core at $t = T$;

$$\frac{A_2(T)}{A_1(T)} = \frac{S_2}{S_1} = \frac{\lambda_2}{(\lambda_2 - \lambda_1)} + \{ R^0 - \frac{\lambda_2}{(\lambda_2 - \lambda_1)} \} \frac{\lambda_1 (1 - e^{-\lambda_2 T})}{\lambda_2 (1 - e^{-\lambda_1 T})} \quad (6)$$

Following the formation of the core at $t = T$, its future activity ratio remains unaffected by the changes in the otolith growth and is governed solely by the rate law. Since equation 6 gives the activity ratio, A_2/A_1 at $t = T$, then the activity ratio of the otolith core at any future time is given by substitution into equations 3 and 4;

$$\frac{A_2(t)}{A_1(t)} = \frac{\lambda_2}{(\lambda_2 - \lambda_1)} \{1 - e^{-(\lambda_2 - \lambda_1)(t - T)}\} + \left[\frac{\lambda_2}{(\lambda_2 - \lambda_1)} + \{R^\circ - \frac{\lambda_2}{(\lambda_2 - \lambda_1)}\} \frac{\lambda_1(1 - e^{-\lambda_2 T})}{\lambda_2(1 - e^{-\lambda_1 T})} \right] e^{-(\lambda_2 - \lambda_1)(t - T)} \quad (7)$$

for $t \geq T$. By cutting the otolith back to its core, future variability in either the otolith accretion rate or in the activity ratio, A°_2/A°_1 , of newly accreted material are eliminated as necessary considerations in the model. Errors associated with the use of a linear mass-growth model for the core can be minimized (to the extent possible consistent with maintaining a reasonable signal to noise ratio) by simply reducing the size and age of the core. In fact, recent evidence from flying fish and silver hake studies (S. Campana, unpubl. man.) indicates that throughout part of their life history, otolith mass increases exponentially with time, a functional relationship which would produce large errors in whole otolith studies based on linear mass-growth models, but would have a minor effect on core model results.

Pb-210/Ra-226 and Th-228/Ra-228

For the Pb-210 ($t_{1/2} = 22.3$ y)/Ra-226 ($t_{1/2} = 1620$ y) pair, the half-life of Ra-226 is sufficiently greater than that of Pb-210 that the parent does not appreciably decay during the period of Pb-210 ingrowth and equations 6 and 7 simplify to;

$$\frac{A_{\text{Pb-210}}}{A_{\text{Ra-226}}} = 1 - (1 - R^\circ) \frac{1 - e^{-\lambda t}}{\lambda t} \quad (8)$$

$$\frac{A_{\text{Pb-210}}}{A_{\text{Ra-226}}} = (1 - e^{-\lambda(t-T)}) + \{1 - (1 - R^\circ)(1 - e^{-\lambda T})\} e^{-\lambda(t-T)} \quad (9)$$

for the cases ($0 < t \leq T$) and ($T < t$), respectively and where λ is the decay constant for Pb-210. (Note that the last exponential term in equation 9 was inadvertently omitted from the equivalent equation (2) in [4]).

At large values of t , a condition of secular equilibrium is attained with the parent and daughter activities becoming equal. The Pb-210/Ra-226 ratio predicted using a linear mass-growth model for the formation of the otolith core at $T = 5$ y (eqn. 8), and the core model (eqn. 9), thereafter are illustrated in Figure 1b, for values of $T = 0-100$ y and values of R°

= 0, 0.1 and 0.2. For comparison, the results in Figure 1a were determined using the linear mass-growth model (eqn. 8) for the entire age range. The activity ratio for the core model is more sensitive to fish age over the 0-100 y range compared to the linear growth model, although both models have a limiting activity ratio of 1 at large ages.

For the Th-228 ($t_{1/2} = 1.91$ y)/Ra-228 ($t_{1/2} = 5.76$ y) pair, equations 6 and 7 are applicable and the Th-228/Ra-228 activity ratio exponentially approaches a constant value of 1.5 as a function of t . The decay curves for $T = 1.5$ y and $R^0 = 0, 0.1$ and 0.2 are illustrated in Figure 1c. In contrast to the Pb-210/Ra-226 pair, the Th-228/Ra-228 pair are most useful over the fish age range of 0-10 y.

RESULTS AND DISCUSSION

Redfish

From their radiochemical assay of whole otoliths and the use of a two stage, linear otolith growth model, Bennett et al. [3] concluded that redfish lived to considerably greater ages than was evident from the annuli visible on the otolith surface. Attempts in the present study to reproduce the measurements of Bennett et al. [3] met with mixed success. Whole otoliths of the Atlantic redfish, *Sebastes mentella* from different age ranges were analyzed for their Pb-210/Ra-226 ratio. The results are compared to the predictions of a single stage, linear growth model (equation 8) in Figure 1a. The measurements from the younger fish are in good agreement with model predictions. However, the results for fish in excess of 20 y old are not consistent with model predictions, although the 2 sigma uncertainties of all the data generally overlap the $R^0 = 0$ curve. Discrepancies between the experimental results and the linear-growth model predictions may be due to non-linear growth, changes in the Ra-226 uptake rate or to some contamination of the otolith surface which was not completely removed in this initial set of experiments.

These above-mentioned sources of error were avoided in a subsequent study of Atlantic redfish by restricting the Pb-210/Ra-226 analyses to the otolith core [4]. The measured Pb-210/Ra-226 activity ratios for the cores are plotted against the age of the fish determined by annular counts. The agreement between the experimental results and the annulus counts using a core age of 5 y is very good. The core age of 5 y was chosen based on the relative benefits of having a young core age for modelling purposes, an adequate sample size and good confidence in the core age based on annulus counts. From their results with juvenile *Sebastes*, Bennett et al. [3] concluded that $R^0 < 0.2$ while the results of this study for the two youngest otolith cores (Figure 1b) are consistent with $R^0 < 0.1$.

The agreement between these results and the curve predicted from annulus counts indicates that the criteria outlined by Beamish et al. [5] for the interpretation of otolith annuli are correct. These criteria have been widely accepted by those ageing Pacific fishes [1], but have been rejected by those ageing some Atlantic species as inducing age overestimation. The core method results also indicate that previous suggestions of longevity in Atlantic redfish in excess of 70 y are correct [10] as the present examinations identified individuals that were 75 y old [4].

Silver Hake and Flying Fish

Flying fish (*Hirundichthys affinis*) is the main commercial species harvested from the Caribbean Sea. Although daily growth rings are evident in young flying fish, they become

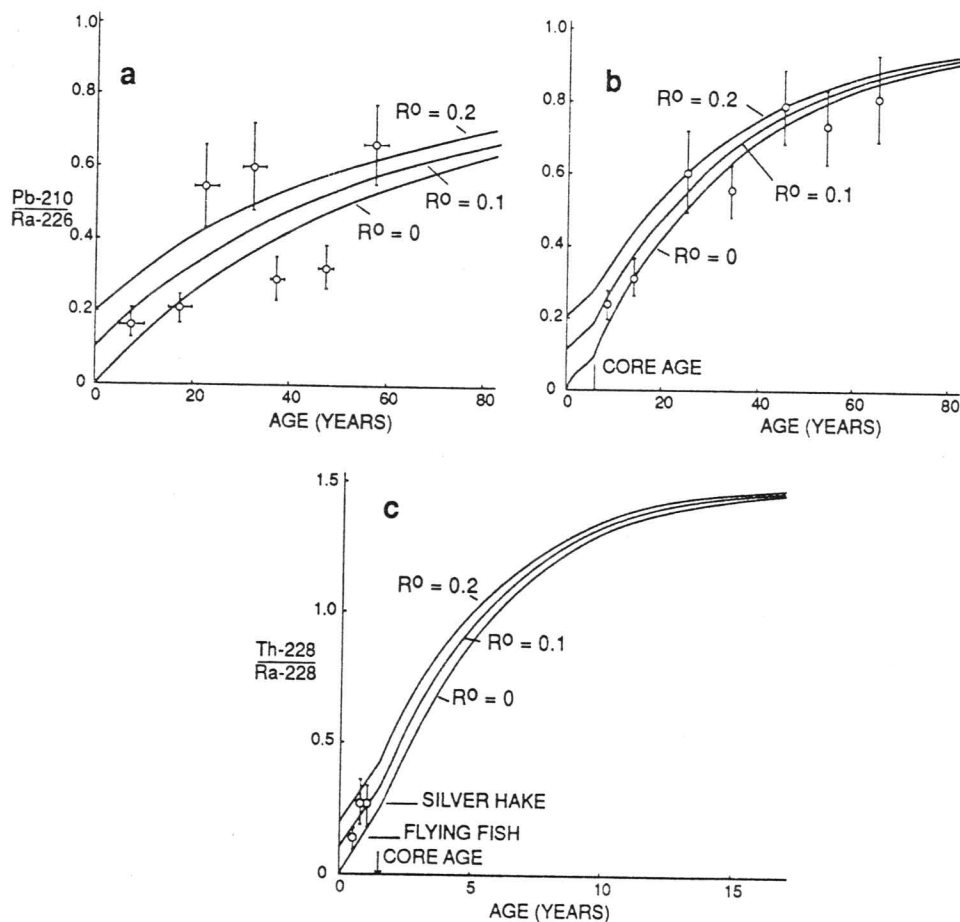


Figure 1. (a) Decay curves for the Pb-210/Ra-226 activity ratio as a function of fish age for the linear growth model ($T = 0-80$) and for given initial ratios, R^0 . Experimental results for whole otoliths from different age classes or redfish are also shown (error bars reflect 1 sigma uncertainties). (b) Decay curves for the Pb-210/Ra-226 activity ratio as a function of fish age for given initial ratios, R^0 . A linear growth model was employed during the period of $T = 5$ y for the formation of the otolith core. Experimental results for sets of otolith cores taken from redfish in given age classes are also shown. (c) Decay curves for the Th-228/Ra-228 activity ratio as a function of fish age for given initial ratios, R^0 . A linear growth model was employed during the period of $T = 1.5$ y for the formation of the otolith core. Initial results for whole otoliths of silver hake and flying fish are also shown.

too narrow in adults to be useful as an ageing tool. As a result, no technique has been previously available to determine growth rates which are the most significant parameter for fishery models. The single result of a Th-228/Ra-228 activity measurement on otolith samples from adult flying fish is illustrated in Figure 1c. Adult flying fish are thought to be short-lived, having ages of less than 2 years. The present results are consistent with activity ratios predicted by the linear growth model for the Th-228/Ra-228 pair (equation 6) for an age of 0.5 y. These initial whole otolith results provide the first evidence of a short lifetime (1-2 y) for flying fish. Measurements are presently being conducted on otolith cores of older flying fish to confirm their relatively short life spans.

Silver hake (*Merluccius bilinearis*) is also a commercially important fish, harvested mainly by Soviet and Cuban fishermen, and constitutes one of the largest fish biomasses off Eastern Canada. Although age estimates have been made on annuli, the annuli are unvalidated. Silver hake are thought to live longer than flying fish, but have ages of less than 10 y old. Whole otolith samples from juvenile silver hake collected off the Scotian Shelf, having ages of 1-2 y based on length frequencies, were analyzed for the Th-228/Ra-228 activity ratio. The two results of these analyses are generally consistent with a linear growth model over the initial stage of otolith growth (Figure 1c). However, an exponential growth model would provide better agreement between the experimental and predicted results, and this functional relationship will be incorporated into future models. The results for both flying fish and silver hake tend to confirm the applicability of the Th-228/Ra-228 ageing technique to short-lived fish.

CONCLUSIONS

(1) Otoliths constitute a closed chemical system owing to their acellular composition, and thus are suitable for radioisotopic dating. However, their masses and specific radionuclide activities are low and it is necessary to have low and stable detector backgrounds and long counting times in order to obtain useful results. (2) Radioisotopic dating techniques have important applications to the confirmation or rejection of fish ages determined using traditional techniques based on annulus counts. The otolith core model approach has major advantages over the multi-stage, linear growth model previously utilized to age redfish. (3) Pb-210/Ra-226 activity ratios have proven effective in determining the longevity of long-lived fish species such as redfish. Initial applications of the otolith core ageing method to juvenile silver hake and adult flying fish indicate that the Th-228/Ra-228 activity ratios are consistent with model predictions and thereby support the potential utility of this isotopic pair in ageing short lived fish.

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