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Age validation of deepwater fish species, with particular reference to New Zealand orange roughy, black oreo, smooth oreo, and black cardinalfish

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7. Executive Summary

Stock assessments used to define total allowable catch (TAC) levels depend upon estimates of biomass and on the species productivity; the latter often determined by age and growth analyses. While there is growing confidence in the current age interpretations of deepwater species most age estimates are based on otolith growth zone counts that remain unvalidated. Several ageing studies have been performed on the orange roughy (*Hoplostethus atlanticus*, Trachichthyidae), black and smooth oreo (*Allocyttus niger* and *Pseudocyttus maculates*, Oreosomatidae), and black cardinalfish (*Epigonus telescopus*, Apogonidae), but age validation for each species is still an unresolved issue to a lesser or greater degree. These ageing studies applied an established procedure of interpreting and counting growth zones in otoliths, maintaining the assumption that these zones, although narrow and somewhat different from those of more near shore species, represent annual growth. Otolith reading for these species has been by viewing burnt cross-sections (black cardinalfish), and thin sections, both longitudinal (orange roughy) and transverse (black oreo and smooth oreo).

One of the most promising validation procedures for long-lived fish is radiometric ageing (using the known decay rate of radioactive parent elements into daughter elements). Earlier radiometric studies of orange roughy and oreos used pooled samples of whole otoliths, which caused a problem of between-individual variability in otolith mass growth rates. This

problem can be overcome by using otolith cores and a refined radiometric technique used at Moss Landing Marine Laboratories (MLML) that uses thermal ionization mass spectrometry (TIMS) and smaller samples than previously possible. This report presents results of a radiometric age validation feasibility study conducted at MLML on the otolith core samples of these four species and results indicate application of the refined radiometric technique to each of the species is feasible to varying degrees.

For the orange roughy measurable ^{226}Ra was determined from one of the smallest samples ever processed. A juvenile sample weighed only 0.040 g and consisted of nine whole otoliths. The resultant ^{226}Ra activity was 0.209 ± 2.7% dpm/g. This sample size is lower than any sample reported in other orange roughy studies by more than 160 times with an error two to three times lower than reported for larger samples. This sample exemplifies the utility and advantages of the refined radiometric method and the use of TIMS. The limiting factor for this sample was the detection of ^{210}Pb. For the juvenile sample ^{210}Pb levels are expected to be low, given negligible incorporation of exogenous ^{210}Pb. The activity measured in this sample was near the limit of detection and demonstrated that exogenous ^{210}Pb is not a likely concern in radiometric age determination of the orange roughy. Calculated radiometric age was in fairly close agreement for all samples except one. However, the trend set by these samples and the strong agreement of the oldest sample (77 yr) indicate that applying the refined radiometric technique to core material of orange roughy otoliths is feasible. The findings of this feasibility study support the concept that orange roughy are a long-lived species.

For the black oreo a first time assessment of whether the refined technique can be applied to this species was the goal. Unfortunately all radium was lost from these samples and was consequently estimated. The estimated activity of ^{226}Ra was relatively low for this species (0.035–0.050 dpm/g) when compared to the other species in this study. The levels are also lower than reported in another study (0.0809-0.1782 dpm/g; Fenton n.d. 1996?). However, ^{210}Pb activities were measurable and did increase as expected with increasing estimated age. Based on the measured activities of ^{210}Pb and the estimated and measured activities in this and other studies, it is feasible that further study could yield an age validation for the black oreo. A full radiometric age validation study of this species, however, would be very challenging because 1) otoliths are very small, 2) use of extracted cores would require numerous fish in each age group, 3) there is a lack of available juvenile otoliths (black oreo juveniles (aged <4 years) are thought to inhabit the upper surface layers, very few have been caught, and consequently few otoliths are available), and 4) there is low sample availability through the range of age possibilities.

For the smooth oreo a first time assessment of whether the refined technique can be applied to this species was the goal. The opposite trend of what was expected for ^{210}Pb was measured. Instead of increasing with estimated age, the ^{210}Pb activity for the oldest sample was lower than the young sample. Unfortunately, radium was lost from the oldest sample and was consequently estimated. The measured ^{226}Ra activity from the young sample was relatively high (0.221 ± 6.2% dpm/g). This is similar to values reported for another study on the smooth oreo (0.2236-1.3409 dpm/g; Fenton n.d. 1996?). The estimated activity of ^{226}Ra for the oldest sample was high as well, but lower by a factor of about two (ca. 0.10 dpm/g). Because of the relatively high radium levels for this species it is feasible that further study could yield an age validation for the smooth oreo. A full radiometric age validation study of this species would be less challenging than the black oreo because the otoliths are slightly larger and radium levels are significantly higher, but challenging none the less because 1) the otoliths are still very small, 2) use of extracted cores would require numerous fish in each age group, 3) there is a lack of available juvenile otoliths (smooth oreo juveniles (aged <4 years) are thought to inhabit the upper surface layers, very few have been caught, and consequently few otoliths are available), and 4) there is low sample availability through the range of age possibilities.

A juvenile group and an old aged group of otoliths from the black cardinalfish were analyzed for ^{210}Pb and ^{226}Ra to determine the feasibility of applying a refined radiometric ageing technique. The study for this species was optimal because the low age group was actually for juvenile fish aged 3 to 5 years and the otolith for this species is large. As a result the juvenile sample consisted of only seven whole otoliths and fifteen otoliths for the adult group aged at 63 and 65 years. ^{210}Pb increased as expected with increasing age and the corresponding measured ^{226}Ra value (0.0222 ± 4.1% dpm/g) supported the trend. The estimated ^{226}Ra value (ca. 0.14 dpm/g) for the oldest age group was slightly lower than that measured in the young age group. Radiometric age was calculated based on the measured ^{210}Pb and ^{226}Ra values (4.7 –0.6/+0.7 yr), which was in close agreement with the estimated age of the group (4 yr). This indicates exogenous ^{210}Pb is not a significant factor in radiometric age determination of the black cardinalfish. Application of a full radiometric age validation study to the black cardinalfish is the most promising of all species examined in this study.

Age validation of deepwater fish species, with particular reference to New Zealand orange roughy, black oreo, smooth oreo, and black cardinalfish

1. Introduction

Stock assessments used to define TAC levels depend upon estimates of biomass and on the species productivity; the latter often determined by age and growth analyses. While there is growing confidence in the current age interpretations of deepwater species and proposed mechanisms for why deepwater fishes can attain such a high longevity (Cailliet et al. 2001), most age estimates are based on growth zone counts from otoliths that remain unvalidated. Several ageing studies have been performed on the orange roughy (*Hoplostethus atlanticus*), black and smooth oreo (*Allocyttus niger* and *Pseudocyttus maculates*), and black cardinalfish (*Epigonus telescopus*), but age validation for each species is still an unresolved issue to a lesser or greater degree, depending on the species (Tracey & Horn 1999; Paul et al. 2002). These ageing studies applied an established procedure of interpreting and counting growth zones in otoliths, maintaining the assumption that these zones, although narrow and somewhat different from those of more near shore species, represent annual growth. Otolith reading for these species has been by viewing burnt cross-sections (black cardinalfish), and thin sections, both longitudinal (orange roughy) and transverse (black oreo and smooth oreo).

Determining fish age and validating that age estimate are separate but related tasks. Validation studies can include one or some of the following applications: length frequencies, daily growth zones, annual growth zones, marginal increment or edge analysis, otolith weights, mark-recapture, biochemical ageing, elements and isotopes, radiometric ageing, and bomb radiocarbon. These methods were described in a literature review as part of this project (Paul et al. 2002).

More emphasis is now being placed on validating the counts from otolith growth zones using an age estimation technique that is independent of otolith appearance or structure. The most frequently used method, particularly for the deeper water and long-lived species, is radiometric age determination; use of the radioactive disequilibria of naturally occurring radioisotopes in calcified structures, such as otoliths. There is a considerable amount of literature on radiometric ageing (see literature list in Paul et al. 2002) and NIWA researchers were optimistic that further application of the radiometric technique to the orange roughy would begin to resolve issues with previous age validation studies, and that the feasibility study of the oreo species and black cardinalfish would prove useful for these commercially important deepwater species.

Radiometric technique

Various radiometric studies were described in section three of the literature review on recent developments in deepwater fish ageing (Paul et al. 2002). The radiometric technique determines age from measurements of the relative activities of two radionuclides from the same decay series. The technique relies on the incorporation of one nuclide (parent) into the calcified structure where it remains and subsequently decays to the second nuclide (daughter product). It is a measure of the radioactive disequilibria of these radioisotopes that can be used as a measure of elapsed time or, as with fishes and in this study, a measure of age.

The most common radionuclide pair is radium-226 (^{226}Ra) and lead-210 (^{210}Pb), but thorium-228 (^{228}Th) and radium-228 (^{228}Ra) have also been used for a shorter-lived species (Campana et al. 1993). The technique using the disequilibria of ^{210}Pb:^{226}Ra was pioneered for fishes by Bennett et al. (1982), and gained acceptance during 1990s as an age validation technique. It is well suited to long-lived fishes up to about 100 to 120 years and has been useful in demonstrating the longevity of some species where there were conflicting interpretations of growth zones resulting in widely divergent age estimates (e.g. longevity of 6 yr vs. >60 yr for the Pacific grenadier (Family Macrouridae, *Coryphaenoides acrolepis*), Andrews et al. 1999a).

Through the development and use of this technique there have been several assumptions that make the application more or less subjective. The assumptions for early studies have been:
1) the constant uptake of ^{226}Ra within and among individuals, 2) no significant uptake of exogenous ^{210}Pb, and 3) no loss of either isotope or any of the decay products through the life of the fish and during otolith storage. It is because of limitations from instrument sensitivity in detecting ^{226}Ra that made it necessary for the first assumption. Typically large groups of whole otoliths, amounting to several grams of material, were required to determine ^{226}Ra activities with a relatively low degree of uncertainty using either alpha-spectrometry or radon emanation (e.g. Fenton et al. 1991, Watters 1993). This assumption is valid if uptake is fairly constant relative to otolith mass growth through the life of the fish and if there is little variation among fish used in the study. This assumption can be tested to a degree by measuring ^{226}Ra in a series of whole otolith samples ranging from young to old (e.g. longspine and shortspine thornyhead rockfishes, *Sebastolobus altivelis* and *S. alascanus*; Kline 1996). Making a radiometric age determination for a juvenile aged sample can test the second assumption best because there is less of a question about the age of juveniles (typically otoliths from the smallest fish available). The third assumption, in addition to the first two assumptions, is further discussed later in this paper.

When the technique was applied to orange roughy in a previous study (Fenton et al. 1991), it was necessary to pool a large number of whole otoliths to acquire enough material for measurement of ^{226}Ra. The technology used at the time was not sensitive enough to detect ^{226}Ra at the low levels typically present in otolith material. Hence, a necessary assumption was that ^{226}Ra was incorporated in constant proportion to mass growth. In addition, when considering the decay of ^{226}Ra to ^{210}Pb with respect to otolith growth, a gradient of ^{210}Pb activity is formed where core material has the highest ^{210}Pb: ^{226}Ra activity ratio (oldest part) decreasing to the outer layer (youngest part). It is because of the gradient that it is necessary to make an additional assumption; mass growth must be modelled, which introduces some circularity with respect to age and otolith growth.

To improve on the circumstances new technology needed to be developed for measuring low levels of radium. The development of a technique that uses thermal ionization mass spectrometry (TIMS) for measuring femtogram (10^{-15} g) quantities of radium in geological samples (Cohen & O'Nions 1991, Chabaux et al. 1994) prompted the development of its application to otolith material (Andrews et al. 1999b). The increased sensitivity of TIMS allowed for an application to very small samples of otolith material.

Because of the increased sensitivity and reduced sample size, application of the radiometric ageing technique to otolith core material, the first few years of growth, was now possible. In studies that followed there was no need for sample weight dependence in calculating the activity ratio, nor was it necessary to assume that ^{226}Ra uptake was constant, because ^{226}Ra and ^{210}Pb were measured from the same sample. In addition, TIMS significantly reduced the error and processing time associated with the measurement of ^{226}Ra, making the measurement of ^{210}Pb the limiting factor in radiometric age determination (Andrews et al. 1999b). By using core material to measure both the ^{210}Pb and ^{226}Ra activities from the same small sample the problems associated with mass growth assumptions or variable uptake of ^{226}Ra were largely circumvented (Kimura & Kastelle 1995).

The refined radiometric technique using TIMS has been applied to a number of species at MLML. Studies describing the method and its application to long-lived fishes have been published in several scientific papers (Andrews et al. 1997, 1999a, 1999b, 2001, 2002; Burton et al. 1999). In addition, the TIMS technique for measuring radium was applied to the ^{210}Pb dating of deep-sea corals (Andrews et al. 2002a; Wilson et al. 2002).

Orange roughy

There is general consensus that orange roughy are slow growing and long-lived with an age at maturity of about 30 yr and maximum ages approaching or exceeding 150 yr (Table 1). Ageing studies of orange roughy have been summarized by Tracey & Horn (1999) and Paul et al. (2002), which include the juvenile surveys of orange roughy that confirmed the estimates of size at age for the juveniles and indicated that a 5-year-old fish would be around 12.4 cm (Mace et al. 1990; Doonan unpub. data), as well as studies to update estimates of *M* (Doonan 1994; Doonan & Tracey 1997). No routine ageing of New Zealand orange roughy has been carried out at NIWA since age and growth estimates were made for the Northwest Chatham Rise (Doonan 1994) and Bay of Plenty stocks (Doonan & Tracey 1997); however, otoliths were aged by Francis & Horn (1997) to further study the transition zone and age at maturity, and by Horn et al. (1998) to look at regional differences in size at age at maturity. Age estimates for the North Atlantic Ocean orange roughy population have yielded similar results for longevity (Allain & Lorance 2000).

The validation studies to date for the orange roughy were modal length analysis and otolith edge analysis (Mace et al. 1990, Doonan unpub. data), radiometric age determination (Fenton et al. 1991, Smith et al. 1991), reinterpretation and support for the early radiometric studies (Francis 1995), and radiocarbon (^{14}C) dating (Morison et al. 1999; Sparks 2000). The reliability of the radiometric method has been questioned, for orange roughy in particular (West & Gauldie 1994; Gauldie & Cremer 1998), but their concerns have not been accepted by other workers because alternative explanations for the findings of each study that do not discredit the radiometric technique are not considered or discussed in these papers. Loss of radon-222 from the otolith has been the most commonly addressed potential problem, but no study to date has measured a significant loss from otoliths (Baker et al. 2001, Kastelle & Forsberg 2002). In addition, a study that specifically addressed this issue for the orange roughy was ignored in the applicable publication (Whitehead & Ditchburn 1995). Only the findings that support the author's conclusions are cited from Whitehead & Ditchburn (1996).

The practicality of applying the refined radiometric technique to orange roughy was discussed between NIWA and MLML (Tracey 2001) and the otolith characteristics made it appear suitable. The aim of this project was to apply the radiometric technique to four samples of pooled orange roughy otolith cores. Pooled samples would represent age classes from juveniles, pre-maturation (pre-transition zone) ages, adult post maturation, and "old" adults. The pilot study was to determine if the ages could be validated by comparing the measured radiometric ages with ages from growth zone counts in otolith thin sections.

Black oreo and smooth oreo

While age determination has proved difficult for deepwater oreos, there is consensus that these species are slow growing and long-lived, with growth parameters not unlike those of orange roughy (Doonan et al. 1995, 1997; McMillan et al. 1997). Maximum estimated age for black and smooth oreo is 153 yr and 86 yr, respectively (Table 1). Most ageing of oreo species has been by otolith growth zone counts. Radiometric age validation was successful for the warty oreo (*Allocytus verrucosus*; Stewart et al. 1995) and findings of measured ^{210}Pb:^{226}Ra ratios for the black, smooth, and spiky oreos supported the high estimated longevity of each species (Fenton n.d. 1996?). Applying the bomb radiocarbon dating method further supported these findings (Morison et al. 1999). The validation of age in these studies, however, was based on basic trends and the resolution of each application is in need of refinement. Use of otolith cores and the refined radiometric technique may provide for a more precise age validation.

The requirement of this study was to establish whether the relatively small otolith cores of black oreo and smooth oreo could successfully be isolated and then to determine the amount of material (number of otolith cores) required for each oreo species. The amount of material required is very much dependent upon the activity of ^{226}Ra. Higher activity means less material is needed. In addition, an age that is more than just a few decades is necessary for sufficient ingrowth of ^{210}Pb. In this study two small samples for each species were analyzed for ^{226}Ra and ^{210}Pb to determine the feasibility of validating black and smooth oreo age estimates.

Black cardinalfish

There is only one study of black cardinalfish using otolith growth zone counts to establish estimates of age and growth (Tracey et al. 2000). Interpretation of the zones in the whole juvenile otolith samples aided interpretation of the zones in the sections of adult black cardinalfish. The patterns of zones were reasonably good although toward the edge of the otolith counting was difficult due to the fine spacing of the zones. Based on growth zone counts the black cardinalfish has a slow growth rate, high age at recruitment (~45 yr), and a maximum age of 104 yr (Table 1). Validation of age estimates was possible for young fish because of clear marginal increment growth zones in juvenile otoliths. This provided some evidence for the annual periodicity of growth zone formation into adult ages, but is strictly an extrapolation to the old ages.

The requirement of this study was to determine if the cores of black cardinalfish could be isolated and if the radiometric ageing technique could be applied. The characteristics of the otolith for this species were similar to orange roughy, but black cardinalfish have a much larger otolith for the first few growth zones. This factor was reason for optimism because larger juvenile otoliths mean fewer otoliths would be needed to acquire enough material for the analysis (given ^{226}Ra levels were not unusually low) and isolation of core material would be relatively easy. MLML were provided with black cardinalfish otolith samples to explore the feasibility of validating ages for this species by processing a minimum number of samples to determine if radiometric ages could be established. In this study two samples were analyzed for ^{226}Ra and ^{210}Pb to determine the feasibility of validating age estimates.

This report addresses Milestones 2 and 3 of Ministry of Fisheries Project DEE2000/02, a feasibility study on the age validation of New Zealand deepwater fish species, particularly orange roughy, black oreo, smooth oreo, and black cardinalfish. Milestone 2 was a pilot study to applying the refined radiometric technique to four samples of pooled orange roughy otolith cores to determine if ages can be validated. The measured radiometric ages were to be compared with ages from growth zone counts in otolith thin sections. Milestone 3 was a feasibility study to determine if otolith cores from black oreo, smooth oreo, and black cardinalfish can be isolated and then measured to ascertain what amount of material (and hence the size of otolith cores that represent the first few years of growth) is required to perform radiometric age validation and then, determine if radiometric ages can be obtained.

2. Methods

Data sources

Otolith samples were collected from research voyages, the Scientific Observer Programme (SOP), and the market sampling programme (SMP). The data sources were the Ministry of Fisheries "age" and "trawl" databases managed by staff at NIWA. Otoliths were selected for each species from the archived collection held in storage by NIWA for the Ministry of Fisheries. A detailed map of the otolith-sampling sites for the 4 study species is given in Figure 1.

Sample availability and selection

I. Orange roughy

A wide range of otoliths was made available for the orange roughy study. Juvenile orange roughy otoliths collected on *James Cook* voyages JCO8806 and JCO8911 from the Northwest Chatham Rise region (Figure 1) were selected to provide "low" age class data for analysis. The samples comprised otoliths aged predominantly 0–2 yr. Adult orange roughy otoliths were selected from otoliths collected and aged from the Northwest Chatham Rise region (COR9002). Samples comprised 33 otoliths aged between 19 and 26 yr, 34 between 36–38 yr, and 15 between 84–144 yr. A few samples collected from the 1984 Northwest Chatham Rise (BUC8401) were also selected. To ensure adequate core material was available for the 3 age groups, additional adult orange roughy otoliths were selected from the aged Bay of Plenty fishery SOP samples. Numbers selected were 40 otoliths from ages 15–24 yr, 41 otoliths from 35–39 yr, and 48 otoliths from 84–182 yr. From the otoliths made available, two sets of juvenile otoliths and one adult age group (34–38 yr) were selected from the Northwest Chatham Rise collections for analysis. The remainder of the adult age groups were from the Bay of Plenty region.

II. Black oreo

Black oreo samples were made available from a sample collected in 1992 from the Puysegur Bank (TAN9208). A sample size of 46 was taken from younger low age fish (4–21 yr) and 44 otoliths were selected from the 62–128 yr age group. From the otoliths made available, one young age group (4–21 yr) and one old age group (62–80 yr) were chosen for the feasibility study.

III. Smooth oreo

Smooth oreo otoliths were made available from samples collected on the Puysegur Bank (TAN9208). From the aged sample 39 otoliths were sampled between 16–33 yr and 33 otoliths between 49–75 yr. From these samples an adult age group was formed (49–59 yr). Nineteen additional otoliths between 8–15 yr from the South Chatham Rise (COR9004) were made available to enable an analysis of younger ages. Thirteen otoliths were used from this sample to form a young age group (8–15 yr).

IV. Black cardinalfish

Black cardinalfish SMP otolith samples from the East Coast North Island were made available for analysis. The collection comprised 33 otoliths from juvenile fish, 27 otoliths between 60–66 yr, and 8 otoliths from the maximum ages of 80–104 yr. The young age group consisted of seven 3–5 yr otoliths and the adult age group was 63–65 yr.

<u>Sample preparation and processing</u>

Three types of samples were processed in this study; whole otoliths, hand cored otoliths and milled otolith cores. Regardless of the sample type all were cleaned of any adhering contamination by following specific cleaning procedures described elsewhere (Andrews et al. 1999b). Whole and hand cored otolith samples were cleaned once selected or ground down to core size. For milled core samples only the whole otolith could be cleaned prior to milling because the end result was a powdered sample. These clean samples were placed in acid cleaned 100 mL Teflon® PFA Griffin beakers and dried at 85° C for 24–48 hr.

Sample coring

I. Orange roughy

Three types of samples were processed for the orange roughy, whole juvenile otoliths and two types of cored adult otoliths. Based on the dimensions and weight of 4 yr whole otoliths from several samples made available and from Mace et al. (1990), a target core sample size of 5.5L x 2.5W x 0.8D mm weighing approximately 0.012 g was chosen for a trial extraction using a milling machine. In preparation for coring, whole otoliths were mounted on PVC discs with fiberglass resin distal side up. Cores were extracted using a milling machine with a titanium coated end mill (3.2 mm or 1/8 inch diameter). Because the growth and structure of the orange roughy otolith is pyramid-like, the end mill was brought down onto the top of the otolith (distal and youngest portion) to a depth of 0.8 mm. It was then panned horizontally across the otolith until the top (first 4 years of growth) was planed off. Coring produced a powdered sample that was placed into an acid cleaned 100 mL Teflon® PFA Griffin beaker. The weight of the core material was measured to the nearest 0.1 mg. Subsequent samples were hand cored because most of the otoliths would crack during the milling extraction. These samples were simply ground down to the dimensions of the 4-year core on a Buehler Ecomet III lapping wheel with 120 to 300-grit wet-dry silicon-carbide paper by grinding away the proximal surfaces.

II. Black oreo

The young sample was to be whole otoliths from the lowest ages available. These otoliths were to be grouped with enough otoliths to provide a sufficient amount of material for analysis while keeping the age range as low as possible. Based on ^{226}Ra levels measured in other deepwater species it was estimated that 0.3 to 0.5 g of material consisting of 10 to 20 otoliths would be sufficient. However, an error was made when this group was compiled and the age range and sample size was much greater than planned. For the adult sample cores were extracted using the milling machine. Based on dimensions and weights published in Morison et al. (1999) a 4 year otolith core measuring 3.2L x 2.7W x 0.6D mm weighing 0.0065 g was targeted. In preparation for coring, whole otoliths were mounted on PVC discs with fiberglass resin distal side up. Cores were extracted using a milling machine with a titanium coated end mill (3.2 mm or 1/8 inch diameter), which was centered and moved straight into the center of the whole otolith to a depth of 0.6 mm. Coring produced a powdered sample that was placed into an acid cleaned 100 mL Teflon® PFA Griffin beaker. The weight of the core material was measured to the nearest 0.1 mg.

III. Smooth oreo

The young sample was whole otoliths from the lowest ages available grouped with enough otoliths to provide a sufficient amount of material for analysis while keeping the age range as low as possible. Based on ^{226}Ra levels measured in other deepwater species and the considerably smaller otolith relative to the black oreo it was estimated that about 30 otoliths would be necessary to provide a sufficient sample. This was more than was available within a narrow age range; therefore 13 otoliths were selected which amounted to about 0.2 g. This is lower than typically needed for radiometric analysis. However, ^{226}Ra levels were not known and relatively high ^{226}Ra can make smaller sample size possible (Andrews et al. 2001). For the adult sample cores were extracted using the milling machine. Based on dimensions and weights published in Morison et al. (1999) a 4 year otolith core measuring 2.4L x 1.8W x 0.5D mm weighing 0.005 g was targeted. In preparation for coring, whole otoliths were mounted on PVC discs with fiberglass resin distal side up. Cores were extracted using a milling machine with a titanium coated end mill (2.4 mm or 3/32 inch diameter), which was centered and moved straight into the center of the whole otolith to a depth of 0.5 mm. Coring produced a powdered sample that was placed into an acid cleaned 100 mL Teflon® PFA Griffin beaker. The weight of the core material was measured to the nearest 0.1 mg.

IV. Black cardinalfish

The young sample was whole otoliths from the lowest ages available grouped with enough otoliths to provide a sufficient amount of material for analysis while keeping the age range as low as possible. Based on the relatively large size of these otoliths at such a low age (3–5 yr) it was easy to come up with a sample that had sufficient material with low age and age range. Based on dimensions and weights measured from 7 otoliths aged 3 to 6 yr a 5 year otolith core measuring 9.0L x 6.5W x 1.4D mm weighing 0.08 g was targeted. Because the otoliths were so large hand coring was chosen for simplicity. Each was ground down around the edges and on the proximal side using a Buehler Ecomet III lapping wheel with 120 to 300-grit wet-dry silicon-carbide paper.

Radiochemical protocol

A detailed protocol describing sample preparation, chromatographic separation of ^{226}Ra from barium and calcium, and analysis of ^{226}Ra using TIMS is described elsewhere (Andrews et al. 1999b). Only an overview of the ^{226}Ra procedures is given here with details on the determination of ^{210}Pb activity. Because the levels of ^{226}Ra and ^{210}Pb typically found in otoliths were extremely low (from femtograms (10^{-15} g) for ^{226}Ra and attograms (10^{-18} g) for

^{210}Pb) and the great potential for contamination from calcium, barium and lead, trace-metal clean procedures and equipment were used throughout sample preparation, separation, and analysis. All acids used were double distilled (GFS Chemicals®) and dilutions were made using Millipore® filtered Milli-Q water (18 MΩ cm^{-1}).

To determine ^{226}Ra activity using TIMS, the sample must be clean of naturally occurring organics (such as otolin). Organic residues elevate background counts in the ^{226}Ra region and increase the analytical uncertainty during TIMS analysis. Dried and weighed samples were dissolved in beakers on hot plates at 90°C by adding 8N HNO_3 in 1–2 mL aliquots. Alternation between 8N HNO_3 and 6N HCl, with an aqua regia transition, several times resulted in complete sample dissolution. The dried sample, after dissolution, formed yellowish foam. To further reduce any remaining organics, and to put the residue into the chloride form required for the ^{210}Pb activity determination procedure, the samples were redissolved in 1 mL 6N HCl and taken to dryness five times at 90–120° C. A whitish residue indicated that sufficient amounts of the organics have been removed. These samples were used to determine ^{210}Pb activity prior to TIMS analysis.

Determination of ^{210}Pb Activity

To determine ^{210}Pb activity in the otolith samples, the α-decay of ^{210}Po was used as a daughter proxy for ^{210}Pb. To ensure that activity of ^{210}Po was due solely to ingrowth from ^{210}Pb, the time elapsed from capture to ^{210}Pb determination was greater than 2 yr. Samples prepared for ^{210}Po analysis were spiked with ^{208}Po, a yield tracer. The amount of ^{208}Po added was estimated based on observed ^{226}Ra levels in other studies of deepwater fishes. This amount was adjusted to 5 times the expected ^{210}Po activity in the otolith sample to reduce error in the ^{210}Pb activity determination. The spiked samples were redissolved in approximately 50 mL of 0.5N HCl on a hot plate at 90°C covered with a watch glass. The ^{210}Po and ^{208}Po-tracer was auto deposited for 4 hours onto a silver planchet. The activities of these isotopes were determined by using α-spectrometry on the plated samples. Additional procedural and system details are described elsewhere (Andrews et al. 1999a). Quantification of the ^{210}Po was made by subtracting a detector blank and reagent counts from each peak region-of-interest, multiplying the ^{210}Po:^{208}Po count ratio by the known ^{208}Po activity, and correcting for decay back to the time of plating. To attain sufficient counts and reduce counting error, samples were counted for 28 to 67 days. The solution remaining after polonium plating was dried and saved for ^{226}Ra analyses.

Determination of ^{226}Ra Activity

To prepare the samples for ^{226}Ra activity determination using TIMS, each sample was spiked with ^{228}Ra, a yield tracer, and a recently developed ion-exchange separation technique was used to isolate radium from calcium and barium (Andrews et al. 1999b). The final samples were processed using TIMS and the measured ratios of ^{226}Ra:^{228}Ra were used to calculate ^{226}Ra activity.

Radiometric age determination

To assess the feasibility of applying the radiometric ageing technique to each of the four species, uptake of ^{210}Pb and ^{226}Ra was assessed in otoliths from juvenile or young fish. Because the age of juvenile or young fish is better constrained than that of adults, age was determined using ^{210}Pb:^{226}Ra disequilibria in whole otoliths from juvenile or young fish. For the juvenile otolith samples, the age determined would be higher than expected if a significant amount of exogenous ^{210}Pb was incorporated into the otolith.

Age was estimated from the measured ^{210}Pb and ^{226}Ra activities (Equation 1). Because the activities were measured using the same sample, the calculation was independent of sample mass. Radiometric age was calculated as follows using an equation derived from Smith et al. (1991) to compensate for the ingrowth gradient of ^{210}Pb:^{226}Ra in the otolith core,

$$t_{age} = \frac{\ln\left(\dfrac{1 - \dfrac{A^{210}Pb_{tc}}{A^{226}Ra_{TIMS}}}{(1 - R_0)\left(\dfrac{1 - e^{-\lambda T}}{\lambda T}\right)}\right)}{-\lambda} + T, \qquad \text{Eq. 1}$$

where t_{age} was the radiometric age at 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, R_0 was the activity ratio of ^{210}Pb:^{226}Ra initially incorporated, λ was the decay constant for ^{210}Pb (ln(2)/22.26 yr), and T was the core age. A radiometric age range, based on the analytical uncertainty, was calculated for each sample by applying the calculated error for ^{210}Pb and ^{226}Ra activity determinations to the measured ^{210}Pb:^{226}Ra. Calculated error included the standard sources of error (i.e., pipetting, spike and calibration uncertainties), alpha-counting statistics for ^{210}Pb (Wang et al. 1975), and an analysis routine used to run ^{226}Ra samples on the thermal ionization mass spectrometer (Andrews et al. 1999b).

3. Results

Orange roughy pilot study

Eight samples of pooled orange roughy otoliths were analyzed for ^{210}Pb and ^{226}Ra as a pilot study in applying a refined radiometric ageing technique. The result was two sets of four age groups consisting of either pooled whole otoliths or pooled cored otoliths (Table 2). The age groups within each set the samples covered a wide range of age from juveniles to old adults. Because of problems with the chemical separation protocol radium was lost in the first set of four samples. Hence, a second set was processed once the problem was solved to retrieve radium. The number of otoliths used in each sample ranged from 9 to approximately 50 with sample weight ranging from 0.040 g to 1.270 g. The two juvenile samples were processed whole and core material, consisting of the first few years of growth, was extracted from adults by either a milling machine or by hand grinding. Average otolith weight for the cored samples ranged from 0.0076 g to 0.0326 g.

The activity of ^{210}Pb was measured in all samples for each set of samples, but ^{226}Ra was lost for the first sample set (Table 3). For the first sample set, the activity per gram for ^{210}Pb did not increase with increasing age, as would be expected if ^{226}Ra levels were relatively constant. Based on the activity of ^{210}Pb and the assumption that age estimates were correct, the potential activity of ^{226}Ra in the sample was estimated. The result was ^{226}Ra levels that ranged widely from a calculated 0.0550 to 0.170 dpm/g. For the second sample set, ^{210}Pb activities were variable, as with the first sample set; however, the corresponding measured ^{226}Ra activities effectively compensated for the lack of a trend. The activity ratio calculated from the measured ^{210}Pb and ^{226}Ra values created an expected trend of an increasing activity ratio with increasing age. The trend was similar to that calculated for the first sample set.

Estimated age of the age groups, based on counting the growth zones, was compared with the radiometric ages determined from the measured 210Pb:226Ra activity ratios (sample set 2; Table 4). Two age groups were in close agreement (youngest and oldest) and two medium aged groups had a radiometric age that was lower than expected. The radiometric age of the juvenile group was 6 ± 4 yr, which overlapped the estimated age range (0–2 yr). The 34–38 yr age group was only slightly lower than expected. When taking into consideration the CV of 10%, the ranges nearly overlap. The 61–71 yr age group was lower than expected by at least 19 yr. This can be explained by the possibility that a small amount of 228Ra tracer was lost during the addition of the spike. For this particular sample there was a build up of static electricity in the Teflon beaker, which caused the droplets to disperse onto the paraffin film covering the beaker. Droplets that were seen were washed back into the beaker, but a small amount could have been lost. A loss of only 10% (a fraction of a droplet) would explain the lower than expected radiometric age. The radiometric age determined for the oldest group (77 18/+51 yr) was in very close agreement with the mean age estimated from growth zone counts (76 yr).

A graphical comparison of these results with the expected ingrowth curve provides a visual means to assess the level of agreement between the age estimation from growth zone counts and the radiometric method (Figure 2). Concordance of the measured ^{210}Pb:^{226}Ra ratios with the expected ingrowth curve is an indication that age estimates are precise. It must be noted that this plot is only possible for actual sample age (age from growth zone counts plus the time since capture). In two out of the four samples the placement of the data points may indicate estimated age was higher than actual age; however, one of the two is not far from expected and one data point is in strong agreement. It is important to note that the juvenile sample (total sample age of almost 20 yr) is in close agreement with the expected ratio, indicating the effect of exogenous ^{210}Pb is low or negligible.

Black oreo and smooth oreo feasibility study

Two otolith samples from both the black and smooth oreos were analyzed for ^{210}Pb and ^{226}Ra to determine the feasibility of applying a refined radiometric ageing technique. The study for each species consisted of two otolith age groups, the lowest available and oldest available with the narrowest age ranges possible (Table 5). For the black oreo the youngest group was erroneously made to range from 4 to 21 yr and contained 43 whole otoliths. This group should have had a narrower age range and lower sample weight amounting to about 0.3 to 0.5 g instead of 1.527 g. The adult age group averaged an age of 64 yr and consisted of 24 milled otolith cores. Average core weight (0.0033 ± 0.0013 g) was less than the target core weight for a 4-year otolith core (0.0065 g, Morison et al. 1999). For the smooth oreo the youngest otoliths available began at 8 yr. To get enough of a sample, assuming usual levels of radium (~0.05 dpm/g), 18 otoliths were selected and ranged from 8 to 15 yr. Sample weight was low, but was adequate for an initial estimate of ^{210}Pb and ^{226}Ra. The adult age group averaged 54 yr and consisted of 15 milled otolith cores. Average core weight (0.0053 ± 0.0019 g) was close to the target core weight for a 4-year otolith core (0.0048 g, Morison et al. 1999).

The activity of ^{210}Pb was measured in all samples for each species, but ^{226}Ra was lost for three of the four samples (Table 6). ^{210}Pb activities for the black oreo increased with estimated age, as would be expected if ^{226}Ra levels were relatively constant. No ^{226}Ra was recovered for the black oreo. Based on the measured ^{210}Pb activities and the assumption that age estimates were correct the activity of ^{226}Ra would have been relatively low and from a calculated 0.035 to 0.051 dpm/g. Only the young sample for the smooth oreo had measurable ^{226}Ra, which was relatively high (0.221 ± 6.2% dpm/g). This provided for an unexpectedly high activity ratio of 0.910 making for an unrealistic radiometric age of about 72 yr. Estimated ^{226}Ra activity for the older sample (ca. 0.10 dpm/g) was less than half that measured for the young sample.

Black cardinalfish feasibility study

Two otolith samples from the black cardinalfish were analyzed for ^{210}Pb and ^{226}Ra to determine the feasibility of applying a refined radiometric ageing technique. This study consisted of two otolith age groups, the lowest available and oldest available with the narrowest age ranges possible (Table 4). The youngest group ranged from 3 to 5 yr and consisted of seven whole otoliths. Total sample weight was relatively high for the number of otoliths at 0.484 g. The adult age group averaged 64 yr with a narrow age range of 63 to 65 yr. Fifteen hand cored otolith cores amounted to a more than sufficient 1.166 g and an average core weight of 0.0777 g, close to the target weight of 0.08 g for the first 5 yr of growth.

The activity of ^{210}Pb was measured within each sample for the black cardinalfish, but ^{226}Ra was lost for the oldest age group (Table 6). ^{210}Pb increased as expected with increasing age and the corresponding measured ^{226}Ra value (0.0222 ± 4.1% dpm/g) supported the trend. The estimated ^{226}Ra value (ca. 0.14 dpm/g) for the oldest age group was slightly lower than that measured in the young age group. Based on the measured activities of ^{210}Pb and ^{226}Ra a radiometric age was determined for the juvenile age group (4.7 –0.6/+0.7 yr), which was in close agreement with the estimated age (4 yr).

4. Discussion

Orange roughy pilot study

Two sample sets consisting of a total of eight otolith samples were analyzed for ^{210}Pb and ^{226}Ra as a pilot study in applying a refined radiometric ageing technique. Analysis of the first sample set resulted in measured ^{210}Pb activity and lost ^{226}Ra because of problems with the radiochemical separation protocol. Once the problem was solved a second set of samples was processed. An analysis of the first sample set to determine the expected ^{226}Ra activity levels, based on the measured ^{210}Pb activity and given the age estimates were correct, revealed the activity of ^{226}Ra could be quite variable among samples. This was confirmed in the second set of samples where measured ^{226}Ra activity was similar to the calculated activity in the first samples set. It is uncertain why the activity of the whole juvenile otoliths is considerably higher than that measured and calculated for the adult samples, but the juvenile samples were from a different region (Northwest Chatham Rise vs. Bay of Plenty). Contrary to this possible geographical explanation was the result from a cored adult sample from Bay of Plenty collections. The measured ^{226}Ra was relatively low when compared to the juvenile activities. An alternative interpretation is that geographical variation alone is not significant and that changes in ^{226}Ra among the samples are an artifact of life history (e.g., occupying surface waters vs. deep waters) and core extraction. The juvenile otoliths used in this study were considerably smaller than the extracted cores. By extracting a 4-year core (in some cases the material extracted was more than targeted) there may be a dilution of the more active juvenile material with material deposited later in life with lower ^{226}Ra activity. These findings are similar to the results for the Pacific grenadier where the juvenile sample was two to three times greater than the adult samples (Andrews et al. 1999a).

The study on orange roughy by Fenton et al. (1991) used pooled whole otolith samples. This caused a problem with between-individual variability in otolith mass growth rates, but these problems can be overcome by using otolith cores (Campana et al. 1990). In addition, the rate of incorporation of ^{226}Ra in the otolith must be constant through the life of the fish for the whole otolith method to work. Based on the findings of this study and by Whitehead & Ditchburn (1996), ^{226}Ra uptake can varying by a factor of up to 2.5 to 3.5 times through the life history of orange roughy.

Based on the results from the second set of otoliths it is apparent that the refined radiometric ageing technique can be applied successfully to otolith core material of the orange roughy. In this study, measurable ^{226}Ra was determined from one of the smallest sample ever processed. The juvenile sample for the second sample set weighed only 0.040 g and consisted of 9 whole otoliths. The resultant ^{226}Ra activity was 0.209 ± 2.7% dpm/g. This sample size is lower than any sample reported in other orange roughy studies by more than 160 times with an error 2 to 3 times lower than reported (Table 7). This sample exemplifies the utility and advantages of the refined radiometric method and the use of TIMS. The limiting factor for this sample was the detection of ^{210}Pb (^{210}Po by proxy) in alpha-spectrometry. Being a juvenile sample where levels are expected to be low, given negligible incorporation of exogenous ^{210}Pb, and being a very small sample a count time of 48 days was required to acquire a total of 100 counts. Based on the measured background for this detector, 14 of these counts came from background leaving 86 counts total from the sample. This is near the limit of detection and exemplifies that exogenous ^{210}Pb in not a likely concern in radiometric age determination of the orange roughy.

Calculated radiometric age was in fairly close agreement for all samples except one (Figure 2). In most radiometric age validation studies there is a fair amount of dispersion associated with the agreement between the estimated age and radiometric age (e.g., Andrews et al. 2002b). Studies performed to date usually have a few outliers, but a general pattern of agreement associated with the expected activity ratio and estimated age is apparent and supported by more than just four samples. The age group that had a lower than expected radiometric age may be explained by the loss of a very small amount of 228Ra tracer (*see* Results) or discrepancies in age estimation. It has been noted that there is a point in ageing the otoliths of orange roughy where agreement is lower than elsewhere in the otolith. However, the trend set by these samples and the strong agreement of the oldest sample indicate that applying the refined radiometric technique to core material of orange roughy otoliths is feasible and it supports the concept that orange roughy are a long-lived species.

<u>Black oreo and smooth oreo feasibility study</u>

Young and old otolith samples from both the black and smooth oreos were analyzed for ^{210}Pb and ^{226}Ra to determine the feasibility of applying a refined radiometric ageing technique. Each of these species had very small otoliths and few samples to choose from. Ideally juvenile otoliths would have been used, but this kind of sample was not available. The best-case scenario for this feasibility study was to choose the otoliths from the youngest fish available.

For the black oreo the age range (4–21 yr) of the juvenile sample should have been lower; however, a first time assessment of whether the refined technique can be applied to this species was the goal. Hence, a simple comparison of whole young otoliths with cores from old otoliths was performed to get a basic idea of what the levels of ^{210}Pb and ^{226}Ra. Unfortunately all radium was lost from these samples and was consequently estimated. The estimated activity of ^{226}Ra was relatively low for this species (0.035–0.050 dpm/g) when compared to the other species in this study. The levels are also lower than reported in another study (0.0809–0.1782 dpm/g; Fenton n.d.1996?). However, ^{210}Pb activities were measurable and did increase as expected with increasing estimated age. Based on the measured activities of ^{210}Pb and the estimated and measured activities in this and other studies, it is feasible that further study could yield an age validation for the black oreo. A full radiometric age validation study of this species, however, would be very challenging because 1) otoliths are very small, 2) use of extracted cores would require numerous fish in each age group, 3) there is a lack of available juvenile otoliths, and 4) there is low sample availability through the range of age possibilities.

For the smooth oreo the age range (8–15 yr) of the juvenile sample was as low as the available sample would permit; however, a first time assessment of whether the refined technique can be applied to this species was the goal. Hence, a simple comparison of whole young otoliths with cores from old otoliths was performed to get a basic idea of what the levels of ^{210}Pb and ^{226}Ra. The opposite trend of what was expected for ^{210}Pb was measured. Instead of increasing with estimated age, the ^{210}Pb activity for the oldest sample was lower than the young sample. Unfortunately, radium was lost from the oldest sample and was consequently estimated. The measured ^{226}Ra activity from the young sample was relatively high (0.221 ± 6.2% dpm/g). This is similar to values reported for another study on the smooth oreo (0.2236–1.3409 dpm/g; Fenton n.d. 1996?). The estimated activity of ^{226}Ra for the oldest sample was high as well, but lower by a factor of about two (ca. 0.10 dpm/g). Based on the measured ^{226}Ra levels here and in another study is it conceivable that radium uptake is just as variable for the smooth oreo as with the orange roughy. Because of the relatively high radium levels for this species it is feasible that further study could yield an age validation for the smooth oreo. A full radiometric age validation study of this species would be less challenging because the otoliths are slightly larger and radium levels are significantly higher, but challenging none the less because 1) the otoliths are still very small, 2) use of extracted cores would require numerous fish in each age group, 3) there is a lack of available juvenile otoliths, and 4) there is low sample availability through the range of age possibilities.

Black cardinalfish feasibility study

A juvenile group and an old aged group of otoliths from the black cardinalfish were analyzed for ^{210}Pb and ^{226}Ra to determine the feasibility of applying a refined radiometric ageing technique. The study for this species was optimal in several ways. The low age group was for actually juvenile fish aged 3 to 5 yr as opposed to the oreo species. Having a sample in this age range is currently the best way to determine if exogenous ^{210}Pb is a factor. In addition, the otoliths for this species grow rapidly and are quite large at 3 to 5 yr (~0.08 g) relative to the other species in this and other studies. This is especially important in reducing the number of fish required for an age group. As a result the juvenile sample consisted of only seven whole otoliths for a total sample weight of 0.484 g. For the adult age group it was possible to hand core the otoliths with ease. A narrow age range was available for an old age group. Fifteen otoliths were chosen between 63 and 65 yr of age amounting to 1.166 g of core material. The activity of ^{210}Pb was measured each sample for the black cardinalfish, but ^{226}Ra was lost for the oldest age group. ^{210}Pb increased as expected with increasing age and the corresponding measured ^{226}Ra value (0.222 ± 4.1% dpm/g) supported the trend. The estimated ^{226}Ra value (ca. 0.14 dpm/g) for the oldest age group was slightly lower than that measured in the young age group. Radiometric age was calculated based on the measured ^{210}Pb and ^{226}Ra values (4.7–0.6/+0.7 yr), which was in close agreement with the estimated age of the group (4 yr). This indicates exogenous ^{210}Pb is not a significant factor in radiometric age determination of the black cardinalfish.

Application of a full radiometric age validation study to the black cardinalfish is the most promising of all species examined in this study. High otolith weight coupled with relatively high ^{226}Ra makes for optimal conditions. Fewer samples can be used in forming age groups, providing the opportunity to reduce the uncertainty of sample age composition and making it possible to cover the full age range, even when few otolith samples exist.

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Table 1. Von Bertalanffy growth parameters and estimates of maturity for study species.

Species	M(Z)	L_∞	K	t_0	A_r	A_m^f	Max. age
Orange roughy (F)	0.045	38.0	0.061	-0.6	29	29	130
Orange roughy (M)	0.045	36.4	0.070	-0.4	29	29	
Black oreo (F)	0.044	39.9	0.043	-17.6	–	27	153
Black oreo (M)	0.044	37.2	0.056	-16.4		–	–
Smooth oreo (F)	0.063	50.8	0.047	-2.9	21	31	86
Smooth oreo (M)	0.063	43.6	0.067	-1.6	21	–	
Black cardinalfish (F)	0.034	70.9	0.038	-4.62	45	36.4	104
Black cardinalfish (M)	0.034	67.8	0.034	-8.39	45	34.5	

A_m^f = Age at maturity, included for comparative purposes.

Table 2. Summary of characteristics for the orange roughy samples processed in this study. Estimated age characteristics for each group with resultant sample weight and the average weight of the material from each otolith sample is given. The three sample types are based on the whether the otoliths used were whole, extracted with a milling machine or cored by hand by grinding.

Sample	Age group (years)	Average age (years)	Capture period	Number of otoliths	Sample weight (g)	Sample type	Average weight (g)
Orange roughy	0 – 2	2	11/1989	50	0.967	Whole	0.0193
(Set 1)	15 – 26	22	3/1996	39	0.297	Milling	0.0076
	35 – 39	36	3/1996	39	1.270	Hand	0.0326
	84 – 105	93	3/1996	30	0.948	Hand	0.0316
Orange roughy	0 – 2	2	11/1989	9	0.040	Whole	0.0044
(Set 2)	34 – 38	36	7/1984	37	0.842	Hand	0.0228
	61 – 71	66	7/1990	20	0.474	Hand	0.0237
	70 – 81	76	3/1996	28	0.701	Hand	0.0250

Table 3. Radiometric results for orange roughy. Listed are the estimated age ranges and the measured lead-^{210}Pb activities for all samples. Calculated activity ratios and their corresponding low and high values (based on calculated error) are given where applicable.

Sample	Age group (years)	^{210}Pb (dpm/g) ± % error[1]	^{226}Ra (dpm/g) ± % error[2]	^{210}Pb:^{226}Ra activity ratio	^{210}Pb:^{226}Ra (low)	^{210}Pb:^{226}Ra (high)
Orange roughy	0 – 2	0.0591 ± 4.1	0.170[3]	0.35[4]	n.a.[5]	n.a.[5]
(Set 1)	15 – 26	0.0456 ± 7.4	0.0800[3]	0.57[4]	n.a.[5]	n.a.[5]
	35 – 39	0.0394 ± 4.6	0.0550[3]	0.72[4]	n.a.[5]	n.a.[5]
	84 – 105	0.0651 ± 3.8	0.0680[3]	0.96[4]	n.a.[5]	n.a.[5]
Orange roughy	0 – 2	0.0901 ± 11.8	0.209 ± 2.7	0.431	0.370	0.495
(Set 2)	34 – 38	0.0505 ± 3.7	0.0692 ± 1.5	0.730	0.692	0.768
	61 – 71	0.0609 ± 4.4	0.0847 ± 2.4	0.719	0.671	0.768
	70 – 81	0.0552 ± 4.0	0.0599 ± 2.6	0.922	0.863	0.984

1 Calculation based on standard deviation of ^{210}Pb activity the delta method (Wang et al. 1975, Knoll 1989).
2 Calculation based on TIMS analysis routine (± 1 SE) and the delta method (Knoll 1989).
3 Estimated activity because radium-226 was not recovered from these samples.
4 Ratio based on estimated radium-226 activity.
5 Not applicable because radium-226 activities were estimated.

Table 4. Comparison of estimated ages and radiometric ages for orange roughy with the coefficient of variation (CV) of 10% taken into consideration. The radiometric age range was based on low and high activity ratios from analytical uncertainty calculations. The degree of age-range agreement is noted where the radiometric age range either overlapped or fully encompassed the age-group age range with the 10% CV.

Species	Age group (yr)	Age group (CV = 10%)	Average age (yr)	Radiometric age (yr)	Radiometric age range (yr)	Age range agreement
Orange roughy	0 – 2	0 – 2	2	6	2 – 10	Overlap
(set 2)	34 – 38	31 – 42	36	25	21 – 30	Low
	61 – 71	55 – 78	66	30	25 – 36	Low
	70 – 81	63 – 89	76	77	59 – 128	Agreement

Table 5. Summary of characteristics for the black and smooth oreos and black cardinal fish samples processed in this study. Estimated age characteristics for each group with resultant sample weight and the average weight of the material from each otolith sample is given. The three sample types are based on the whether the otoliths used were whole, extracted with a milling machine or cored by hand by grinding.

Sample		Age group (yr)	Average age (yr)	Number of otoliths	Sample weight (g)	Sample type	Average core weight (g)
Black oreo	Young	4 – 21	13	43	1.527	Whole	0.0355
	Adult	62 – 80	64	24	0.077	Milled	0.0033
Smooth oreo	Young	8 – 15	13	18	0.203	Whole	0.0113
	Adult	49 – 59	54	22	0.118	Milled	0.0053
Black cardinal	Young	3 – 5	4	7	0.484	Whole	0.0691
	Adult	63 – 65	64	15	1.166	Hand	0.0777

Table 6. Radiometric results for black and smooth oreos and black cardinal fish. Listed are the estimated age ranges and the measured lead-210 activities for all samples. Calculated activity ratios and their corresponding low and high values (based on calculated error) are given for the two samples where ^{226}Ra was recovered.

Sample	Age group (yr)	^{210}Pb (dpm/g) ± % error[1]	^{226}Ra (dpm/g) ± % error[2]	^{210}Pb:^{226}Ra activity ratio	^{210}Pb:^{226}Ra (low)	^{210}Pb:^{226}Ra (high)
Black oreo	4 – 21	0.0143 ± 6.3	0.035[3]	0.409[4]	n.a.[5]	n.a.[5]
	62 – 80	0.0466 ± 4.1	0.051[3]	0.914[4]	n.a.[5]	n.a.[5]
Smooth oreo	8 – 15	0.201 ± 4.6	0.221 ± 6.2	0.910	0.817	1.015
	49 – 59	0.0856 ± 7.1	0.10[3]	0.856[4]	n.a.[5]	n.a.[5]
Black cardinal	3 – 5	0.0353 ± 7.0	0.222 ± 4.1	0.159	0.142	0.177
	63 – 65	0.119 ± 2.8	0.14[3]	0.865[4]	n.a.[5]	n.a.[5]

[1] Calculation based on standard deviation of ^{210}Pb activity the delta method (Wang et al. 1975, Knoll 1989).
[2] Calculation based on TIMS analysis routine (± 1 SE) and the delta method (Knoll 1989).
[3] Estimated activity because radium-226 was not recovered from these samples.
[4] Ratio based on estimated radium-226 activity.
[5] Not applicable because radium-226 activities were estimated.

Table 7. Summary of study characteristics and ^{226}Ra results for the orange roughy.

	Technique	Sample type	Number of otoliths	Sample weight (g)	^{226}Ra activity (dpm/g)	Error (%)
This study	TIMS	Whole[1]/cores	9 – 37	0.040 – 0.842	0.0599 – 0.209	1.5 – 2.7
Fenton et al. (1991)	alpha-spectrometry	Whole	28 – 315	6.06 – 6.79[2]	0.0522 – 0.625	5 - 7
Whitehead and Ditchburn (1996)	alpha-spectrometry	Whole[3]	Not specified[4]	~8[4]	0.0560 – 0.137	5.5 - 7

[1] Whole juvenile otoliths.
[2] Calculated based on number of otoliths and the average weight published.
[3] Successive dissolutions from the same whole otolith sample.
[4] Authors state seven size fractions of 1-4 otoliths each (0.5–1 g), but also mention a single 8 g sample.

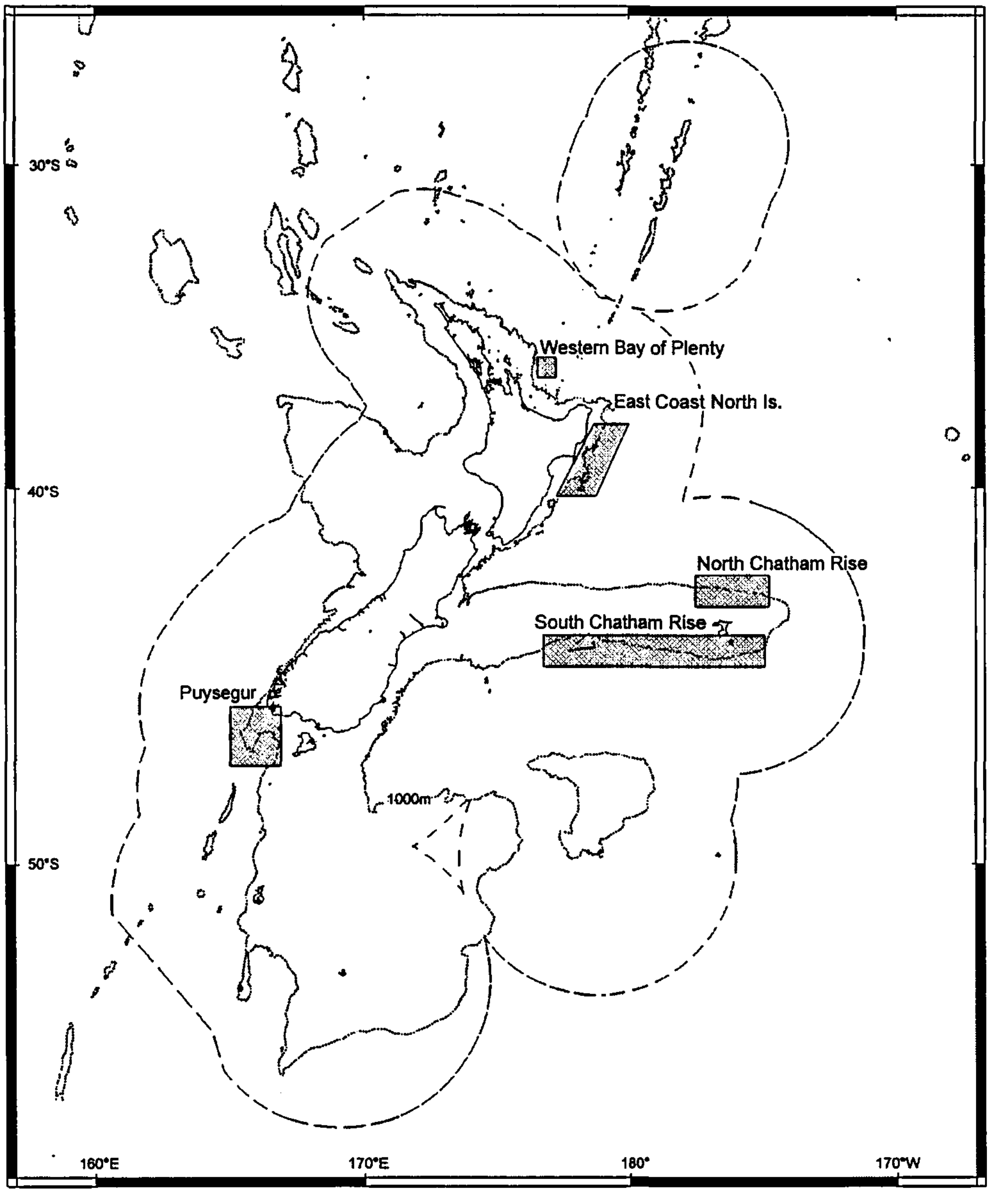

Figure 1: Areas within the New Zealand Exclusive Economic Zone (EEZ) referred to in the text from which otolith samples were collected.

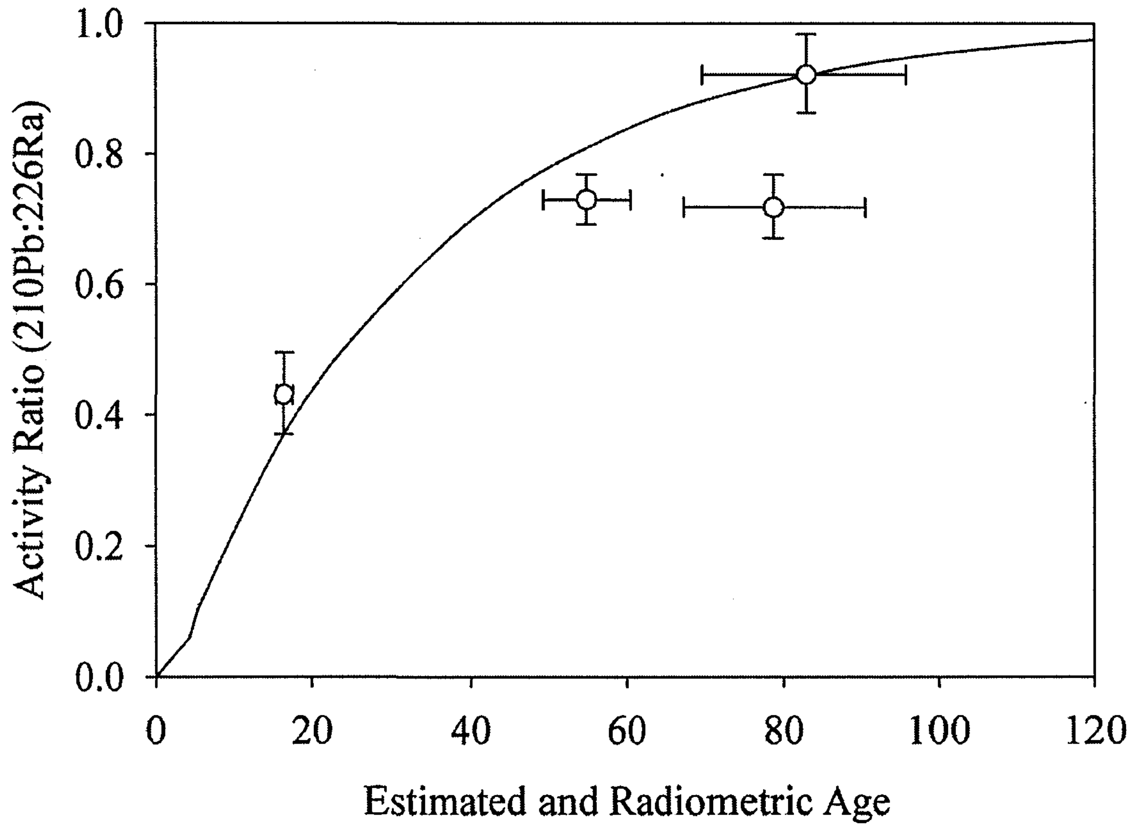

Figure 2. Plot of the measured ^{210}Pb:^{226}Ra ratios, plotted with respect to total sample age (age estimates plus the time since capture) for the orange roughy, with the expected ^{210}Pb:^{226}Ra ratio (ingrowth curve). Horizontal error bars represent the age estimate range with the 10% CV taken into consideration. The vertical error bars represent the analytical uncertainty associated with measuring ^{210}Pb and ^{226}Ra. <u>Note</u>: the estimated and radiometric ages are the total age from time of otolith collection, hence, "real" age requires subtraction of the elapsed time since collection.