



Age validation of orange roughy and black cardinalfish using lead-radium dating

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EXECUTIVE SUMMARY

This report describes an application of an independent ageing technique, lead-radium dating, to orange roughy and black cardinalfish otoliths. The method applied follows that of the preliminary study (DEE200301) described in Andrews and Tracey (2003).

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 was confidence in the current age interpretations of deepwater species most age estimates were based on otolith growth zone counts that remain unvalidated. Ageing studies have been carried out on orange roughy (*Hoplostethus atlanticus*, Trachichthyidae) and black cardinalfish (*Epigonus telescopus*, Apogonidae), but age validation for both species was still an unresolved issue to a lesser or greater degree. The age estimation studies for both species have utilized interpretation of otolith growth zone counting that maintains the assumption that the growth zones, although narrow and somewhat different from those of more near shore species, represent annual growth.

One of the most promising age validation procedures for long-lived fishes is a radiometric technique called lead-radium dating. This method uses the known properties of radioactive decay for radium-226 (^{226}Ra) and lead-210 (^{210}Pb) and has proved useful as a natural chronometer in otoliths. Earlier radiometric studies of orange roughy used pooled whole-otolith samples, which incorporated the potential problem of introducing circularity by way

of calculated otolith-mass growth rates. This potential complication, however, can be overcome by using otolith cores and a refined radiometric technique used at Moss Landing Marine Laboratories (MLML). Advances in mass spectrometry have led to the application of thermal ionization mass spectrometry (TIMS) and inductively coupled plasma mass spectrometry (ICPMS) to ^{226}Ra determination on smaller samples than previously possible in lead-radium dating (Lundstrom *In Preparation*).

Application of lead-radium dating using otolith cores was successful in this study for both orange roughy and black cardinalfish and the goals established by the feasibility study have been accomplished. The measurement of lead-radium ratios for a series of putative age groups for each species showed a high degree of correlation to the expected ingrowth curve, with some indication of where improvements are needed in the growth-zone age estimation procedures.

In general, the findings of this study provide support for 1) growth-zone age estimation procedures for each species, with information on where improvements can be made, 2) longevity exceeding 100 years, and 3) the findings of Fenton et al. (1991).

For orange roughy, there was 95% confidence that the oldest fish aged in this study were greater than 74 years old. In addition, we can be confident that growth zone counts were not over ageing fish with growth-zone counts by more than 25%. Radiometric age determinations made in this study were similar to the age findings of the seminal lead-radium dating paper on orange roughy (Fenton et al. 1991), and provided additional support for centenarian longevity.

For black cardinalfish, the agreement between growth zone derived age and radiometric age was not as good as with orange roughy; however, the independent age series provided by lead-radium dating indicated where improvements might be made. Growth-zone counts less than about 60 y were consistent with radiometric ages, but higher counts appeared to be under-estimates. Hence, the lead-radium findings provide support for the great longevity for this species and there was 95% confidence that maximum age was not less than 94 years. These results complement the findings from a bomb radiocarbon age validation study on black cardinalfish (Neil et al. 2007).

OBJECTIVES

1. To carry out radiometric age validation of deepwater fish species, with particular reference to New Zealand orange roughy and black cardinalfish.

SPECIFIC OBJECTIVE:

1. To provide independent age determinations and an validated basis for age estimation studies of New Zealand orange roughy (~ 6 pooled otolith samples) and black cardinalfish (~10 pooled otolith samples) using lead-radium dating on otolith core material.

1. INTRODUCTION

Orange roughy (*Hoplostethus atlanticus*) is most important and valuable of the deepwater species in New Zealand with current annual landings of about 14,600 t. The main fishery is concentrated on the Chatham Rise where the current total allowable catch (TACC) is 12,700 t (Anon. 2006). Black cardinalfish (*Epigonus telescopus*) is a lower value species with the TACC currently set at 3,760 t; in 2004–05 only about 40% of this TACC was caught (Dunn in prep.).

Stock assessments used to define TACC 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 and many remain unvalidated. Numerous age estimation studies have been performed on New Zealand orange roughy, (Kalish et al. in Annala 1993; Doonan 1994; Francis & Horn 1997; Horn et al. 1998; Tracey & Horn 1999; Paul et al. 2002; Ackerman & Green 2003; Tracey et al. 2004). For black cardinalfish there are only two age and growth studies to date (Pshenichny et al. 1986, in Abramov 1991; Tracey et al. 2000). These ageing studies applied procedures of interpreting and counting growth zones in otoliths, maintaining the assumption that these zones represent annual growth. Otolith reading for these species was performed by viewing longitudinal thin sections (orange roughy), which is an interpretation procedure that has been developing for over 25 years, and baked and embedded transverse sections (black cardinalfish), a routine otolith preparation procedure. The accuracy and precision of age estimation of sectioned orange roughy otoliths has been the focus of recent discussions (Hicks 2005; Francis 2006). Processes are now in place to refine and agree on between-agency protocols for ageing orange roughy, so the context of this study must be considered an independent age determination that can provide validated age guidelines for future inter-agency calibration studies.

Age validation techniques that can be applied to deep water fishes range widely in efficacy and age precision (Campana 2001). Some rely on establishing a temporal context to early growth by measuring changes in otolith zones or fish length (e.g. marginal increment analysis and length frequency analysis); however, these techniques require an extrapolation of the age and growth information to older fish because of a loss of zone or length resolution. Other methods rely on marking and recapturing older fish (e.g. oxytetracycline injection and/or tagging), but deep-water fish usually have problems with survival upon return due to barotrauma and the techniques suffer because of low returns. Advances in the use of naturally occurring radioactive proxies for age have provided opportunities for independent age validation of deep water fishes and the primary techniques currently in use are bomb-radiocarbon age validation method and lead-radium dating techniques.

Lead-radium dating relies on the incorporation of naturally occurring radium-226 (^{226}Ra) from the environment into the otolith and its subsequent decay to lead-210 (^{210}Pb). By

measuring the disequilibria of these two radioisotopes in otolith core material (first few years of life), an independent estimate of age can be determined based on the known ingrowth rate of ^{210}Pb from ^{226}Ra (Campana et al. 1990, Smith et al. 1991, Kimura and Kastle 1995). This technique works well as a tool for determining the validity of age interpretations from growth zone counts that differ considerably, but its application is limited by relatively low resolution at ages approaching 100 years. The typical end result for this kind of study is that it provides strong support for a given age estimation technique and establishment of an independent estimate of longevity that supports one age estimation technique over another. For example, it is certain the Pacific grenadier (*Coryphaenoides acrolepis*) can attain an age exceeding 50 yr based on lead-radium dating and that an early estimate of approximately 6 years as maximum longevity was proven incorrect (Andrews et al. 1999a). At ages near the middle of the ingrowth curve, from approximately 10 years to approximately 60 years (depending on the sample activity and approach used), radiometric age resolution is better and can support or refute growth-zone counting age estimates and procedures to a greater degree (Francis 2003b).

Through the development and use of lead-radium dating 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 et al. 2006). 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 lead-radium dating was applied to orange roughy in a previous study, it was necessary to pool a large number of whole otoliths to acquire enough material for measurement of ^{226}Ra (Fenton et al. 1991). 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 by assuming growth rates are known, which introduces some circularity. This has been used as a point to argue against the validity of radiometric age

determinations made by Fenton et al. (1991), but was later scrutinized in detail by Francis (1995) and found to be more robust than originally thought. Based on reasonable assumptions that were supported by orange roughy growth data (Smith et al. 1995), the radiometric data presented by Fenton et al. (1991) support a maximum age that exceeds 84 years (Francis 1995).

To improve on the circumstances new technology was needed 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. These advances have since led to an application of ICPMS to measure radium, a process that is less sensitive to ionization interference (Lundstrom et al. *In preparation*).

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 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 (Andrews et al. 1999b). In addition, TIMS/ICPMS 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 & Kastle 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. 1999a, 1999b, 2001, 2002, 2005; Burton et al. 1999; Stevens et al. 2004). Use of the preferable ICPMS method has since taken its place in this study.

This project has focused on the application of the improved radiometric procedure to provide independent ages and validated age guidelines for future inter-laboratory calibration/agreement studies for both orange roughy and black cardinalfish. This study builds on the Ministry of Fisheries Project DEE2000/02, a feasibility study investigating the application of lead-radium dating to New Zealand deepwater fishes, including orange roughy and black cardinalfish (Andrews & Tracey 2003).

1. 1. 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), and include reference to the estimates of size at age studies for juvenile orange roughy that showed a 5-year-old fish would be approximately 12.4 cm (Mace et al. 1990; Doonan unpublished data), as well as to ageing studies to update estimates of M (Doonan 1994; Doonan & Tracey 1997). No routine growth-zone 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 from several regions 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.

More recently, growth-zone ageing work has been carried out by NIWA and Central Ageing Facility (CAF). Tracey et al (2004) presented ages for additional Northeast Chatham Rise orange roughy otoliths and several unpublished reports have been presented at the MFish Deepwater Working Group meetings on orange roughy growth data for the Northwest and Northeast Chatham Rise, and Mid-East Coast North Island over time (Green & Ackerman (2004); Robertson & Green (2004); Ackerman & Green (2004a, 2004b).

Along with the New Zealand and Australian studies, age estimates have yielded similar results for longevity for the North Atlantic Ocean orange roughy population (Allain & Lorange 2000), Chilean (Gili et al. 2002), and Namibian fisheries (Butterworth & Brandao, 2005)

The validation studies to date for orange roughy have been 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 bomb radiocarbon (^{14}C) dating (Morison et al. 1999), which was critiqued in Sparks (2000). Bomb radiocarbon trials for deepwater species found the technique to be unsuitable for orange roughy because it lacks a pelagic phase in its life-cycle, a requirement if the timing of the influx of bomb radiocarbon is to provide a temporal marker (Kalish 2001). We note however that NIWA is currently building information toward obtaining a deepwater radiocarbon calibration curve for New Zealand, information that could be used to apply the bomb radiocarbon technique to validate orange roughy ages. In the Allain & Lorange (2000) study, the lead-radium radiometric technique was used but it was considered that the age estimates were not validated by the method.

The reliability of the lead-radium dating radiometric method has been questioned, for orange roughy in particular (West & Gauldie 1994; Gauldie & Cremer 1998), but their concerns have not been widely accepted by other researchers because alternative explanations for their findings, interpretations that do not discredit lead-radium dating, were not considered or discussed in these papers. Loss of radon-222 (^{222}Rn) 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, Kastle & 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 supported the author's conclusions were cited from Whitehead & Ditchburn (1996). It is also

important to point out that even if ^{222}Rn is measured as having emanated from otolith material, it must be considered that the origin is probably from the surface of the otolith and is not lost from the deeply buried core material (i.e. adsorptive properties of radon and a short half-life of 3.84 days).

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 Ministry of Fisheries recognised the need for further refinement of growth-zone age estimates for these species and a feasibility study took place in 2002 (Andrews & Tracey 2003). The aim of the feasibility study was to apply the refined radiometric technique to three samples of pooled adult orange roughy otolith cores and one pooled whole otolith juvenile group to determine if growth-zone age estimates can be validated using an improved technique that circumvents some of the potential problems with the Fenton et al. (1991) study.

In the feasibility study, pooled samples consisting of growth-zone derived age groups, represented age classes from juveniles, pre-maturation (pre-transition zone) ages, adult post maturation, and “old” adults. Juvenile samples were collected from the Chatham Rise and the adults were sourced from growth-zone aged otoliths from the Bay of Plenty study. (Measurable ^{226}Ra was determined from one of the smallest samples ever processed; a juvenile sample that weighed only 0.040 g and consisted of nine whole otoliths. The resultant ^{226}Ra activity was $0.209 \pm 2.7\%$ dpm/g. This sample size was 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 exemplified 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.

The measured radiometric ages for all samples in the feasibility study was compared with ages from growth zone counts in whole otoliths for juveniles, and in otolith thin sections for the pre-recruit (pre-transition zone) and adult samples. Calculated radiometric age was in fairly close agreement for all samples except one. The trend of increasing age determined by these samples and the age agreement of the oldest sample (77 yr) indicated an application of the refined radiometric technique to core material of orange roughy otoliths was feasible. The findings of the feasibility study supported the concept that orange roughy are a long-lived species and, because of the positive results, the current radiometric age validation study of orange roughy was approved.

1.2. Black cardinalfish

Several samples of black cardinalfish otoliths had been collected from various areas in the New Zealand EEZ before the ageing study by Tracey et al. (2000), but no detailed age and growth studies had been carried out. In the early 1990s a small sample of black cardinalfish

otoliths were collected from the Bay of Plenty as part of a study of mercury levels in black cardinalfish (Tracey 1993). These otoliths were transversely sectioned and baked, and age estimated using counts of dark and light zones. The age data indicated that fish of 45–72 cm FL were 15 to 42 years (Tracey 1993). Pshenichny *et al.* (in Abramov 1991) examined whole otoliths and considered that *Epigonus telescopus* on the North Atlantic Ridge attained a length of about 70 cm at only 10 years. In addition, Pshenichny *et al.* (in Vinnichenko 1997) stated that *E. telescopus* become sexually mature at age 7.

The New Zealand study of black cardinalfish using otolith growth zone counts to establish estimates of age and growth was carried out by 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 in this study. The patterns of zones were reasonably clear 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 the radiometric feasibility study carried out by Andrews and Tracey (2003) was to determine if otolith cores from black cardinalfish could 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.

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 in the feasibility study 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. Two samples were analysed for ^{226}Ra and ^{210}Pb , a juvenile group and an “old” aged group of otoliths. 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. Lead-210 increased as expected with increasing age and the corresponding measured ^{226}Ra value ($0.222 \pm 4.1\%$ 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 growth zone age of the group (4 yr). This indicated exogenous ^{210}Pb is not a significant factor in radiometric age determination of the black cardinalfish and that future studies would utilise an initial uptake ratio (R_0) of zero. The results for the feasibility study of the black cardinalfish were optimal and an application of a full radiometric age validation study to black cardinalfish was the most promising of all species

examined in Andrews & Tracey (2003). Based on these results the project results presented here was approved.

The aim of the project reported here was to apply lead-radium dating to a full series of age groups for each species. The target for orange roughy in this study was to acquire enough cored otolith material for six age groups that span the growth-zone derived age range to near 100 years (approaching the maximum resolution of the technique). The target for black cardinalfish was more comprehensive because the larger otoliths had advantages in terms of acquiring enough otolith core sample material; ten age groups were targeted to span the estimated age range with sample replication where possible. Ultimately, the ages from growth zone counts for each species would be compared with radiometric age estimates to provide support or disagreement for age estimation procedures. These results could be compared with the Ministry of Fisheries bomb radiocarbon validation project (Neil et al. 2007), which will be the first instance of applying two radiometric validation methods in tandem to a New Zealand species.

2. METHODS

Samples used in this study were collected previously and otolith samples had already been aged from growth zone counts for both species. The sister otolith was available for radiometric analyses. Age groups were selected to ensure full coverage of the growth curves for both species and otolith samples were comprised of sister otoliths of aged otoliths. The number of otoliths required for orange roughy and black cardinalfish age groups were based on the lead-radium information determined from the feasibility study (Andrews & Tracey 2003).

The archived collection and age database maintained for Ministry of Fisheries at NIWA was investigated and it was ascertained that insufficient sister otoliths of NIWA aged orange roughy samples were available for the study. This was a likely scenario highlighted in the tender document. The backup was to seek SeaFic's permission to obtain the sister otoliths of Central Ageing Facility (CAF) aged orange roughy held in the NIWA collection. Permission was obtained from SeaFic to use sister otoliths of CAF aged otoliths for the validation study. Results from the feasibility study were included in this report, which were aged by NIWA. Otoliths selected for each species came from the archived collection at NIWA for the Ministry of Fisheries and from SeaFic (sister otoliths from CAF aged fish).

Orange roughy otoliths were selected to form a range of age groups from various regions (Appendix 1). Most of the samples for the age groups were from the Spawning Box and Northwest Chatham Rise region (Figure 1). For the “older” age groups, otolith samples from other parts of the Northeast Chatham Rise and from the Mid-East Coast area were added to acquire sufficient sample weight. Adult otoliths from earlier years were provided, but could not be combined because the sampling periods were too far apart. Feasibility study sample areas were Northeast Chatham Rise (juvenile otolith group 0-3 yr) and Bay of Plenty (adult samples).

Black cardinalfish otoliths, Ministry of Fisheries owned and NIWA aged, were available in adequate numbers from the Mid-East Coast region and had been collected as part of the Stock Monitoring Programme (SMP) in 1998 and 1999 (Appendix 1). Juvenile black cardinalfish otolith samples were sourced from the Chatham Rise region, having been collected opportunistically on Ministry of Fisheries funded trawl surveys of hoki and middle depth fish.

2.1.1. Orange roughy

The approach was to analyse six additional pooled otolith samples consisting of adult orange roughy otolith cores for measurable lead-210 and radium-226 activity levels and to provide independent estimates of age. Because a series of samples from the feasibility study was successful, additional age groups considered for analysis in this study were aimed at filling in the older age groups. Otoliths for these samples came from four regions (Spawning Box, Northeast and Northwest Chatham Rise, and Mid-East Coast) and all were collected during the year 2003. Age groups were filled with otoliths from these regions and resulted in the following age groups: 25 – 30 yr (ORH 1), 40 – 45 yr (ORH 2), 60 – 69 yr (ORH 3), 70 – 80 yr (ORH 4), 81 – 88 yr (ORH 5), 90 - 108 yr (ORH 6). Samples from the Spawning Box contributed exclusively to ORH 1 and 2, and composed part of ORH 3 with Mid-East Coast samples. The oldest age groups (ORH 4, 5, 6) consisted of otolith samples from all regions. Fish sample details for the otoliths used in this study were tabulated (Appendix 1).

2.1.2. Black Cardinalfish

The approach was to analyse eleven samples of pooled otolith samples consisting of adult black cardinalfish otolith cores for measurable lead-210 and radium-226 activity levels and to provide independent estimates of age. Because the analysis from the feasibility study indicated radium levels were relatively high, small age groups and sample replication was possible for most of the age range. The adult black cardinalfish otolith samples were selected from fish collected in 1998 and mostly 1999 from the Mid-East coast region. Sample replicates were denoted as A and B resulting in the following age groups: 20 – 23 yr (EPT 1A/B), 35 – 40 yr (EPT 2A/B), 50 – 55 yr (EPT 3 A/B), 60 – 65/66 yr (EPT 4A/B), 70 – 78 yr (EPT 5A/B), 80 - 102 yr (EPT 6). Replicate samples for EPT 1 and EPT 5 consisted of otoliths that were randomly selected. Replicate groups for EPT 2 through EPT 5 consisted of low (A) and high (B) otolith weight groups because the weights varied considerably within each age group. This approach was used to potentially elucidate age estimation problems that may be indicated by a wide otolith weight range. The oldest age sample was not replicated because of low sample availability. Fish sample details for the otoliths used in this study were tabulated (Appendix 1).

2.2. Sample preparation and processing

All otoliths used in this study were ground down by hand on a Buehler Ecomet III lapping wheel with 120 to 320-grit wet-dry silicon-carbide paper to the dimensions and weight of an otolith with a target estimated age. Hand grinding was chosen for this study over the use of a micromilling machine primarily to follow the method used in the feasibility study and also because of the ease at which each species could be cored. All cores were individually measured and weighed for consistency. Samples were cleaned of any adhering contamination, the details of which are described elsewhere (Andrews et al. 1999b). The cleaned samples were placed in acid cleaned 100 mL Teflon® PFA Griffin beakers and dried at 85°C for 24-48 hr.

2.3. Sample coring

2.3.1. Orange roughy

Based on the dimensions and weight of 4 yr whole otoliths from several samples made available during the feasibility study, and from information on juvenile otolith weight data available 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. This was achieved by grinding away the proximal surfaces of the whole otolith; the result was a core that looked very similar to a juvenile otolith in shape (pyramidal) and size. It was ascertained from the feasibility study that dimensions alone were not enough to be certain the core was not heavier than targeted size because the average weight of cored otoliths was higher than expected (~0.024g). To correct for this in this study, each core was ground down using otolith weight as a guide; regrinding was a common result once the dimensions were met. A weight of just under 0.02 g was targeted to avoid the loss of too much material and to allow for mass loss during the cleaning process (typically 3-5%). The weight of the cleaned cores and pooled otolith age groups were measured to the nearest 0.1 mg.

2.3.2. Black cardinalfish

Refined dimensions were determined from 5 yr otoliths that better approximated the shape of a juvenile otolith. Added to the coring procedure from the feasibility study, the edges of the core were ground down to a taper leading to the edge. Adding a taper to the end with a thickness of 0.9 mm better approximated the target weight and shape of the juvenile otolith. Revised target dimensions and weight were 8.0L x 5.5-6.0W x 1.1-1.2D mm (tapering to 0.9 mm at the edge) and weighing 0.105 g. Because the otoliths were so large hand coring was chosen for simplicity and to follow the method used in the feasibility study. The weight of the cleaned cores and pooled otolith age groups were measured to the nearest 0.1 mg.

2.4. Radiochemical protocol

A detailed protocol describing sample preparation, chromatographic separation of ^{226}Ra from barium and calcium, and analysis of ^{226}Ra using mass spectrometry was described elsewhere (Andrews et al. 1999b). These procedures have not changed for this study, save two aspects of the analysis; 1) radium recovery was improved by shifting the collection interval on the final chromatography column to begin at 200 μL (as opposed to 250 μL), and 2) purified radium samples were analysed using an improved ICPMS technique (Lundstrom *In Preparation*). Other than these details, 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 (femtograms (10^{-15} g) for ^{226}Ra and attograms (10^{-18} g) for ^{210}Pb) and the great potential for contamination of various forms and sources, 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 ($18\text{ M}\Omega\text{ cm}^{-1}$).

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 reduce remaining organics (otolin), 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 $90\text{-}120^\circ\text{C}$. A whitish residue indicated that sufficient amounts of the organics have been removed. These samples were used to determine ^{210}Pb activity prior to ICPMS analysis.

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 about 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). To attain sufficient counts and reduce counting error, samples were counted for 61 to 81 days for orange roughy and 42 to 52 days for black cardinalfish. The solution remaining after polonium plating was dried and saved for ^{226}Ra analyses.

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

Radiometric age was determined 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,

$$t_{\text{age}} = \frac{\ln \left(\frac{1 - \left(\frac{A^{210}\text{Pb}}{A^{226}\text{Ra}} \right)}{(1 - R_0) \left(\frac{1 - e^{-\lambda t}}{\lambda T} \right)} \right)}{-\lambda} + T,$$

where t_{age} was the radiometric age at the time of analysis, $A^{210}\text{Pb}$ was the lead-210 activity at time of analysis, $A^{226}\text{Ra}$ was the radium-226 activity measured using ICPMS, 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. An initial uptake ratio was assumed as $R_0 = 0.0$ based on the close agreement of the juvenile age group ratios with the expected ingrowth curve for each species. A radiometric age range, based on the analytical uncertainty, was calculated for each sample by using error propagation through to the final age determinations (2 SE). Calculated error included the standard sources of error (i.e. pipetting, spike and calibration uncertainties, etc.), alpha-counting statistics for ^{210}Pb (Wang et al. 1975), and the ICPMS analysis routine.

The 95% confidence intervals (2 SE) from lead-radium dating were used to make age estimate interpretations for growth zone counts, and determine the strength and limits of age determinations for each species. To describe the trend between age estimation from growth zone counts and lead-radium dating a simple linear regression was applied to the data. Age agreement or disagreement between the methods, in terms of potential ageing bias, were given in terms of a bias value (b), defined as the potential difference from true age as the percent deviation of the growth zone derived age from radiometric age.

3. RESULTS

3.1. Orange roughy

Six samples of pooled orange roughy otoliths were analysed for ^{210}Pb and ^{226}Ra as a follow up to the feasibility study. The age groups covered a range of early to old adults. These age groups consisted of groups of individual otolith cores numbering 13 to 32, with an age group sample weight of 0.227 to 0.464 g (Table 2). Average core weight was consistent and close to the target weight of a 4 year old otolith (~0.12 g). In addition to these findings, the results from the four age groups from the feasibility study were added to this study for further consideration.

The activity of ^{210}Pb and ^{226}Ra was measured for all samples (Table 3). The activity of ^{210}Pb was lowest for the youngest sample and was inconsistent for the oldest samples. The activity of ^{226}Ra , however, compensated for what might be a random distribution relative to age by combining with ^{210}Pb activity for a steady increase in the ^{210}Pb : ^{226}Ra activity ratio.

Estimated age of the samples, based on growth zone counts, was compared with radiometric ages determined from the measured ^{210}Pb : ^{226}Ra activity ratios (Table 4). There was considerable agreement for the sample series and only two age groups resulted in radiometric ages that were outside the age estimate interval (2 SE).

A graphical comparison of the estimated age versus the measured ^{210}Pb : ^{226}Ra activity ratio, plotted with the expected ingrowth curve, provided a visual means to assess the level of agreement between the two methods (Figure 2). Correspondence of the measured ^{210}Pb : ^{226}Ra ratios with the expected ingrowth curve indicated growth-zone derived age estimates were precise, within a level of uncertainty that increases with age, and that findings provide support for orange roughly age estimation procedures. It must be noted that this plot is only possible for actual sample age (age from growth zone counts plus the time since capture). For all but one of the samples the measured ratio falls within 2 SE of the expected ingrowth curve. This margin of error translates into a radiometric age estimation error that becomes larger as the ratio approaches the top of the curve (Table 4). Data from the feasibility study were included for further consideration and comparison. It is important to note that the juvenile sample (total sample age of about 14 yr) was in close agreement with the expected ratio, indicating the effect of exogenous ^{210}Pb is low or negligible. This was the basis for choosing an initial uptake ratio (R_0) equal to 0.0.

An age agreement plot allows for direct comparison of growth zone age with radiometric age (Figure 3). Age data were plotted with respect to each other and relative to a line of agreement (1:1). All data could be considered in this manner, except one data point (ORH 6) because the measured ratio exceeded 1.0 (undefined age). Correlation between the two ageing methods was good, both with ($R^2 = 0.827$) and without ($R^2 = 0.975$) the feasibility study data. The slope of the regression was close to 1.0 (regression slope = 0.997), indicating there was, in general, an agreement between the methods; however, this finding does not take into consideration the change in radiometric age uncertainty as the measured ^{210}Pb : ^{226}Ra activity ratio increases. Treating the radiometric age determinations as independent estimates of age, there was 95% confidence that fish aged in this study were greater than 74 years old. In addition, there was 95% confidence that age estimation from growth zone counts was not over ageing fish by $b > 25$ (Figure 3).

3.2.Black cardinalfish

Eleven samples of pooled black cardinalfish otoliths were analysed for ^{210}Pb and ^{226}Ra as a follow up to the feasibility study. The age groups within each set the samples covered a range of age from young fish (minimum 3 years) to old adults (maximum 102 years). These age groups consisted of otolith cores numbering 7 to 12 with an age group sample weight of 0.761 to 1.174 g (Table 5). Average core weight was consistent and close to the target

weight of a 5 year old otolith (~0.105 g). In addition to these findings, the results from the feasibility study were added to this study for further consideration.

The activity of ^{210}Pb and ^{226}Ra was measured for all samples (Table 6). The activity of ^{210}Pb was lowest for the youngest sample, highest for the middle aged adult samples, and lower for the oldest samples. The activity of ^{226}Ra , however, compensated for what might be a random distribution relative to age by combining with ^{210}Pb activity for a steady increase in the ^{210}Pb : ^{226}Ra activity ratio.

The average estimated age of the age groups, based on growth zone counts, was compared with radiometric age determined from the measured ^{210}Pb : ^{226}Ra activity ratios (Table 7). Agreement was variable for the sample series; the youngest age groups were relatively close to expected radiometric age values, but the trend for radiometric age was toward older than growth-zone age for samples beyond approximately 60 years.

A graphical comparison of the growth zone age versus the measured ^{210}Pb : ^{226}Ra activity ratio, plotted with the ingrowth curve, provided a visual means to assess the level of agreement between the two methods (Figure 4). Correspondence of the measured ^{210}Pb : ^{226}Ra ratios with the expected ingrowth curve indicated age estimates were relatively precise at ages below approximately 60 years and older than expected for older age groups. It must be noted that this plot is only possible for total sample age (age from growth zone counts plus the time since capture). In general, the measured ratios followed the expected trend established by the ingrowth curve; however, within the constraints of 95% confidence, about half the samples differed from the expected ingrowth ratios. This margin of error translated into a radiometric age error that becomes larger as the ratio approaches the top of the curve (Table 7). Data from the feasibility study were included for further consideration and comparison. It is important to note that the juvenile sample measured in the feasibility study (total sample age of about 8 yr) was in close agreement with the expected ratio, indicating the effect of exogenous ^{210}Pb is low or negligible. This was the basis for choosing an initial uptake ratio (R_0) equal to 0.0.

An age agreement plot allowed for direct comparison of growth zone age with radiometric age (Figure 5.). Age data were plotted with respect to each other and relative to a line of agreement (1:1). All data could be considered in this manner, except one data point (EPT 5B) because the measured ratio exceeded 1.0 (undefined age). Correlation between the two ageing methods was good ($R^2 = 0.828$), when considering all data for this species (including the juvenile sample from the feasibility study), but an older than expected trend for radiometric age relative to growth zone age was evident (regression slope = 1.326). Within the clustering of older than expected adult age groups, there was 95% confidence that fish aged in this study were greater than 94 years old. In addition, there was 95% confidence that age estimation from growth zone counts underestimated ageing by at least 19 yr ($b < -21$) for one age group.

Radiometric determinations on the age groups separated into low and high whole otolith weight groups (EPT 3A/B, 4A/B, and 5A/B) revealed there were significant differences the weight groups (Table 8). All low weight age groups had lower ^{210}Pb : ^{226}Ra activity ratios,

and consequently radiometric age, than the corresponding high weight age group and each differed by 2 to 3.4 SEs.

The findings provided support for the method used to derive growth zone age estimates up to about 50 or 60 years; however, there was an indication that age estimation under aged the oldest fish and fish with the heaviest whole otolith weight.

4. DISCUSSION

4.1. Orange roughy

A full application of lead-radium dating to orange roughy was successful with good agreement between the age groups and the lead-radium ingrowth curve. Use of otolith core material to enhance radiometric age determinations made in the past by avoiding the application of otolith mass-growth models provided more convincing age determinations. No difference was found between age estimation techniques and, despite the increased level of uncertainty at ratios approaching a level of 1.0, the findings provided continued support for age estimates that exceed 100 years. What remains uncertain is the degree of age validation for the methods used to estimate age (Francis 2006). A documented drift in estimated ages over time may explain the outlier from the feasibility study age group (61-71 yr), which was over aged in 1990 by at least 21 yr ($b > 33$; 95% CI). Most of the radiometric ages presented provide support for the age estimation procedures used by facilities that utilize longitudinal thin sections to determine the age of orange roughy. Independent of age estimation procedures there was 95% confidence that fish aged in this study were greater than 74 years old. In addition, there was 95% confidence that age estimation from growth zone counts was not over ageing fish used in this study by more than approximately 25% for all but three age groups.

The seminal radiometric study on orange roughy by Fenton et al. (1991) used pooled whole otolith samples, which incorporated potential problems; negligible between-individual variability in otolith-mass growth rates and variability in radium uptake with time may not be valid assumptions (West & Gauldie 1994, Francis 1995, Kimura & Kastle 1995). These potential problems can be overcome by using otolith cores (Campana et al. 1990), but limits on radium detection technology at the time prevented an analysis of otolith core material from orange roughy because of the small core size. While it is possible that otoliths with consistent levels were measured in the Fenton et al. (1991) study, more support was necessary to alleviate concerns about the assumptions. Based on recent findings, ^{226}Ra uptake can vary by a factor of up to about 3 times (Whitehead & Ditchburn 1996, Andrews & Tracey 2003); however, further ^{226}Ra sampling from this study provides additional support that ^{226}Ra activity levels can also be relatively consistent (average activity of $^{226}\text{Ra} = 0.0753 \pm 8\%$). It is uncertain if the assumption was violated for the samples used by Fenton et al. (1991); however, radiometric age determinations made in that study were similar to the age findings of this study and provide additional support for centenarian longevity.

4.2. Black cardinalfish

A full application of lead-radium dating to black cardinalfish was successful. As suspected from the evidence presented in the feasibility study, small otolith samples with relatively high radium-226 levels were analysed and age was determined. The agreement of the age groups with the lead-radium ingrowth curve was not as good as with orange roughy, but the independent age series provided an indication of where improvement can be made. Agreement between the two methods for ages less than 60 years provides support for the age estimation procedures used by NIWA. Ages exceeding 60 years tended toward an underestimation of age, which was supported by the significant difference between age groups that were separated by low and high whole otolith weights. Age estimation of black cardinalfish was known to be very difficult at high ages and the findings of this study indicate age was underestimated by at least 19 yr ($b < -21$) for one of the oldest age groups. Further investigation is necessary in the analysis of otolith sections and it is suggested that thin section ageing be considered to increase the chances of detecting growth zones that were missed. In addition, otoliths used in the high otolith weight age-groups should be reinvestigated; the otolith sample that was used in each age group was highlighted in the appendix to facilitate this follow up analysis. There was 95% confidence that fish aged in this study were greater than 94 years old and that age estimation from growth zone counts tends to underestimate fish age at ages exceeding approximately 60 yr.

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6. REFERENCES

- Ackerman, J.; Green, C. (2004a). Age composition of orange roughy (*Hoplostethus atlanticus*), spanning 20 years of sampling from the Northwest Rise, New Zealand. Report to the New Zealand Seafood Industry Council Ltd. Deepwater Fishery Assessment Working Group Document (WG04/06).
- Ackerman, J.; Green, C. (2004b). Age composition of orange roughy (*Hoplostethus atlanticus*) from two New Zealand survey samples. Report to the New Zealand Seafood Industry Council Ltd. Deepwater Fishery Assessment Working Group Document (WG04/07) 21 p.

- Allain, V.; Lorance, P. (2000). Age estimation and growth of some deep-sea fish from the northeast Atlantic Ocean. *Cybium* 24(3) suppl.: 7–16.
- Andrews, A.H.; Cailliet, G.M.; Coale, K.H. (1999a). Age and growth of the Pacific grenadier (*Coryphaenoides acrolepis*) with age estimate validation using an improved radiometric ageing technique. *Canadian Journal of Fisheries and Aquatic Sciences* 56(8): 1339–1350.
- Andrews, A.H.; Coale, K.H.; Nowicki, J.L.; Lundstrom, C.; Palacz, Z.; Burton, E.J.; Cailliet, G.M. (1999b). 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. *Canadian Journal of Fisheries and Aquatic Sciences* 56(8): 1329–1338.
- Andrews, A.H.; Burton, E.J.; Coale, K.H.; Cailliet, G.M.; Crabtree, R.E. (2001). Application of radiometric age determination to the Atlantic tarpon, *Megalops atlanticus*. *Fisheries Bulletin (U.S.)* 99(3): 389–398.
- Andrews, A.H.; Tracey, D.M. (2003). Age validation of deepwater fish species, with particular reference to New Zealand orange roughy, black oreo, smooth oreo, and black cardinalfish. Final Research Report for Ministry of Fisheries Research Project DEE2000/02 Objective 1. National Institute of Water and Atmospheric Research, Wellington, New Zealand. 25 p.
- Andrews, A.H.; Burton, E.J.; Kerr, L.A.; Cailliet, G.M.; Coale, K.H.; Lundstrom, C.C.; Brown, T.A. (2005). Bomb radiocarbon and lead-radium disequilibria in otoliths of bocaccio rockfish (*Sebastes paucispinis*): a determination of age and longevity for a difficult-to-age fish. *Marine and Freshwater Research: Proceedings of the 3rd International Symposium on Otolith Research and Application*. 56: 517–528
- Annala, J.H. (1993). Deepwater projects review 1992. MAF Fisheries Greta Point Internal Report No. 200. (Unpublished report held in NIWA library.) 57 p.
- Baker, M.S. Jr; Wilson, C.A.; VanGent, D.L. (2001). Testing assumptions of otolith radiometric aging with two long-lived fishes from the northern Gulf of Mexico. *Canadian Journal of Fisheries and Aquatic Sciences* 58(6): 1244–1252.
- Bennett, J.T.; Boehlert, G.W.; Turekian, K.K. (1982). Confirmation of longevity in *Sebastes diploproa* (Pisces: Scorpaenidae) from $^{210}\text{Pb}/^{226}\text{Ra}$ measurements in otoliths. *Marine Biology* 71(2): 209–215.
- Burton, E.J.; Andrews, A.H.; Coale, K.H.; Cailliet, G.M. (1999). Application of radiometric age determination to three long-lived fishes using $^{210}\text{Pb}/^{226}\text{Ra}$ disequilibria in calcified structures: a review. *In Life in the Slow Lane: ecology and conservation of long-lived marine animals. Proceedings of the symposium Conservation of Long-Lived Marine Animals held at Monterey, California, USA 24 August 1997. American Fisheries Society Symposium* 23. pp 77–87.
- Butterworth, D.; Brandao, A. (2003). Aspects of the Assessments of Orange Roughy off Namibia and Patagonian Toothfish off the Prince Edward Islands. In: Report on DEEP SEA 2003, An International Conference on Governance and Management of Deep-sea Fisheries. FAO Fisheries Report No. 772. Vol. 2. 84 p.
- Cailliet, G.M.; Andrews, A.H.; Burton, E.J.; Watters, D.L.; Kline, D.E.; Ferry-Graham, L.A. (2001). Age determination and validation studies of marine fishes: do deep-dwellers live longer? *Experimental Gerontology* 36(3–4): 739–764.

- Campana, S.E.; Zwanenburg, K.T.C.; Smith, N.J. (1990). $^{210}\text{Pb}/^{226}\text{Ra}$ determination of longevity in redfish. *Canadian Journal of Fisheries and Aquatic Sciences* 47(1): 163–165.
- Campana, S.E.; Oxenford, H.A.; Smith, J.N. (1993). Radiochemical determination of longevity in flyingfish *Hirundichthys affinis* using Th-228/Ra-228. *Marine Ecological Progress Series* 100: 211-219.
- Chabaux, F.; Othman, D.B.; Brickman, J.L. (1994). A new Ra-Ba chromatographic separation and its application to Ra-mass spectrometric measurement in volcanic rocks. *Chemical Geology* 119: 191-197.
- Cohen, A.S.; O’Nions, R.K. (1991). Precise determination of femtograms quantities of radium by thermal ionization mass spectrometry. *Analytical Chemistry* 63: 2705-2708.
- Doonan, I.J. (1994). Life history parameters of orange roughy: estimates for 1994. New Zealand Fisheries Assessment Research Document 94/19. 13 p.
- Doonan, I.J.; McMillan, P.J.; Kalish, J.M.; Hart, A.C. (1995). Age estimates for black oreo and smooth oreo. New Zealand Fisheries Assessment Research Document 95/14. 26 p.
- Doonan, I.J.; McMillan, P.J.; Hart, A.C. (1997). Revision of smooth oreo life history parameters. New Zealand Fisheries Assessment Research Document 97/9. 11 p.
- Doonan, I.J.; Tracey, D.M. (1997). Natural mortality estimates for orange roughy in ORH 1 (Bay of Plenty). New Zealand Fisheries Assessment Research Document 97/26. 9 p.
- Dunn, M.R. (200#). Analysis of catch and effort data from New Zealand black cardinalfish (*Epigonus telescopus*) fisheries up to the 2004–05 fishing year. *New Zealand Fisheries Assessment Report* 200#/##. XX p.
- Fenton, G.E. (n.d. 1996). Age determination of oreo dory species by radiometric analysis. School of Zoology, University of Tasmania, Hobart. Final Report FRDC Grant Project 92/41. (Unpublished report.)
- Fenton, G.E.; Short, S.A. (1992). Fish age validation by radiometric analysis of otoliths. *Australian Journal of Marine and Freshwater Research* 43(5): 913–922.
- Fenton, G.E.; Short, S.A.; Ritz, D.A. (1991). Age determination of orange roughy *Hoplostethus atlanticus* (Pisces: Trachichthyidae) using ^{210}Pb : ^{226}Ra disequilibria. *Marine Biology* 109(2): 197–202.
- Francis, R.I.C.C. (1995). The longevity of orange roughy: a reinterpretation of the radiometric data. New Zealand Fisheries Assessment Research Document 95/2. 13 p.
- Francis, R.I.C.C. (2003). The precision of otolith radiometric ageing of fish and the effect of within-sample heterogeneity. *Canadian Journal of Fisheries and Aquatic Sciences* 60(4): 441-447.
- Francis, R.I.C.C. (2006). Some recent problems in New Zealand orange roughy assessments. *New Zealand Fisheries Assessment Report* 2006/43. 65 p.
- Francis, R.I.C.C.; Horn, P.L. (1997). The transition zone in otoliths of orange roughy (*Hoplostethus atlanticus*) and its relationship to age at maturity. *Marine Biology* 129(4): 681–687.
- Gauldie, R.W.; Cremer, M.D. (1998). Loss of ^{222}Rn from otoliths of orange roughy, *Hoplostethus atlanticus*, invalidates old ages. *Fisheries Science* 64(4): 543-546.

- Gili, R.; Cid, L.; Pool, H.; Young, Z.; Tracey, D.; Horn, P.; Marriott, P. (2002). Estudio de edad, crecimiento y mortalidad natural de los recursos orange roughy y alfonsino. Informe Final, FIP 2000–12. Investigacion y Fomento Pesquero, Instituto de Fomento Pesquero, Valparaiso. (Unpublished report held in NIWA Library, Greta Point, Wellington.)
- Green, C.; Ackerman, J. (2004). Age composition of orange roughy *Hoplostethus atlanticus* from the mid east coast of New Zealand (Ritchie Hill). Report to the New Zealand Seafood Industry Council Ltd. Deepwater Fishery Assessment Working Group Document (WG04/04).
- Hicks, A. (2005). Between-reader and between-lab ageing errors for orange rough otoliths aged at NIWA and CAF. WG-DW-05/23(revised). 18 p. (Unpublished report held by Ministry of Fisheries, Wellington.)
- Horn, P.L.; Tracey, D.M.; Clark, M.R. (1998). Between-area differences in age and length at first maturity of the orange roughy *Hoplostethus atlanticus*. *Marine Biology* 132(2): 187–194.
- Kastelle, C.R.; Forsberg J.E. (2002). Testing for loss of ^{222}Rn from Pacific halibut (*Hippoglossus stenolepis*) otoliths. *Fisheries Research* 5(1): 93–98.
- Kimura, D.K.; Kastelle, C.R. (1995). Perspectives on the relationship between otolith growth and the conversion of isotope activity ratios to fish ages. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 2296-2303.
- McMillan, P.J.; Doonan, I.J.; Hart, A.C. (1997). Revision of black oreo life history parameters. New Zealand Fisheries Assessment Research Document 97/8. 13 p.
- Kline, D.E. (1996). Radiochemical age verification for two deep-sea rockfishes *Sebastolobus altivelis* and *S. alascanus*. M.Sc. Thesis, San Jose State University, California, Moss Landing Marine Laboratories.
- Lundstrom, C.C; Glessner, J., Zhang, Z.; Huang F.; Bopp C.; Ianno. (In Preparation) Rapid, accurate and precise measurement of U-series nuclides by multicollector ICP-MS. To be submitted to *Journal of Mass Spectrometry and Ion Processes*.
- McMillan, P.J.; Doonan, I.J.; Hart, A.C. (1997). Revision of black oreo life history parameters. New Zealand Fisheries Assessment Research Document 97/8. 13 p.
- Mace, P.M.; Fenaughty, J.M.; Coburn, R.P.; Doonan, I.J. (1990). Growth and productivity of orange roughy (*Hoplostethus atlanticus*) on the north Chatham Rise. *New Zealand Journal of Marine and Freshwater Research* 24(1): 105–119.
- Ministry of Fisheries, Science Group (Comps.) 2006: Report from the Fishery Assessment Plenary, May 2006: stock assessments and yield estimates. 875 p. (Unpublished report held in NIWA library, Wellington.)
- Morison, A.K.; Kalish, J.M.; Green, C.P.; Johnston, J.M. (1999). Estimation of age and growth of orange roughy, black oreo and smooth oreo, and natural mortality of black and smooth oreo. Final Report to the New Zealand Ministry of Fisheries (99/45). Marine and Freshwater Resources Institute, Queenscliff, Victoria, Australia. 67 p.
- Paul, L.J.; Tracey, D.M.; Francis R.I.C.C. (2002). Age validation of deepwater fish species, with particular reference to New Zealand orange roughy and oreos: a literature review. Final Research Report for Ministry of Fisheries Research Project DEE200002. NIWA. 33 p.

- Robertson, S.; Green, C. 2004. Age composition of orange roughy *Hoplostethus atlanticus* 2002/03. Report to the New Zealand Seafood Industry Council Ltd. Deepwater Fishery Assessment Working Group Document (WG04/05).
- Smith, J.N.; Nelson, R.; Campana, S.E. (1991). The use of Pb-210/Ra-226 and Th-228/Ra-228 dis-equilibria in the ageing of otoliths of marine fish. *In* Kershaw, P.J.; Woodhead, D.S. (eds), Radionuclides in the study of marine processes. Elsevier, New York, pp. 350–359.
- Sparks, R. (2000). Review of ‘Estimation of age and growth of orange roughy, black oreo and smooth oreo, and natural mortality of black and smooth oreo’, by Morison, A.K.; Kalish, J.M.; Green, C.P.; Johnston, J.M. Unpublished report to the New Zealand Ministry of Fisheries Deepwater Working Group, 00/12. 6 p.
- Stevens, M.M.; Andrews, A.H.; Cailliet, G.M.; Coale, K.H.; Lundstrom, C.C. (2004). Radiometric validation of age, growth, and longevity for the blackgill rockfish, *Sebastes melanostomus*. *Fisheries Bulletin* 102: 711-722
- Stewart, B.D.; Fenton, G.E.; Smith, D.C.; Short, S.A. (1995). Validation of otolith-increment estimates for a deepwater fish species, the warty oreo *Allocyttus verrucosus*, by radiometric analysis. *Marine Biology* 123(1): 29–38.
- Tracey, D. (2001) A visit to the Moss Landing Marine Laboratories, Monterey, USA, to investigate the improved radiometric fish ageing technique (FSF2000/04). *Travel Report*. (Unpublished report held in NIWA library, Wellington) 16 p.
- Tracey, D.M.; George, K.; Gilbert, D.J. (2000). Estimation of age, growth, and mortality parameters of black cardinalfish (*Epigonus telescopus*) in QMA 2 (east coast North Island). New Zealand Fisheries Assessment Report 2000/27. 21 p.
- Tracey, D.M.; Horn, P.L. (1999). Background and review of ageing of orange roughy (*Hoplostethus atlanticus*) from New Zealand and elsewhere. *New Zealand Journal of Marine and Freshwater Research* 33(1): 67–86.
- Tracey, D.M.; Francis, R.I.C.C.; George, K.; Horn, P.L.; Hart, A.C.; Marriott, P. (2004). Age composition of orange roughy in the Northeast Chatham Rise spawning box from *Otago Buccaneer* 1984 and *Cordella* 1990 samples. Final Research Report for Ministry of Fisheries Research Project MOF2003/03J).
- Wang, C.H.; Willis, D.L.; Loveland, W.D. (1975). Radiotracer Methodology in the Biological, Environmental, and Physical Sciences. Prentice Hall, Englewood Cliffs, New Jersey, USA, 480 p.
- Watters, D.L.; Kline, D.E.; Coale, K.H.; Cailliet, G.M. (2006). Radiometric age confirmation and growth of a deep-water marine fish species: The bank rockfish, *Sebastes rufus*. *Fisheries Research* 81: 251–257
- West, I.F.; Gauldie, R.W. (1994). Perspectives: Determination of fish age using Pb-210:Ra-226 disequilibrium methods. *Canadian Journal of Fisheries and Aquatic Sciences* 51(10): 2333–2340.
- Whitehead, N.E.; Ditchburn, R.G. (1995). Two methods of determining radon diffusion in fish otoliths. *Journal of Radioanalytical and Nuclear Chemistry* 198(2): 399-408.
- Whitehead, N.E.; Ditchburn, R.G. (1996). Dating hapuku otoliths using $^{210}\text{Pb}/^{226}\text{Ra}$, with comments on dating orange roughy otoliths. Institute of Geological and Nuclear Sciences, Science Report 96/15. 17 p.

Table 1. Von Bertalanffy growth parameters and estimates of maturity and maximum age 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 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 and the previous feasibility study. Estimated age composition and average time since capture for each group, with resultant pooled sample number and weight are given. An average core weight for each otolith in each sample is given to show consistency of coring.

Sample	Age group (yr)	Average age (yr)	Time since capture (yr)	Number of otoliths	Sample weight (g)	Average core weight (g)
ORH 1	25 - 30	28	2.67	31	0.426	0.0137
ORH 2	40 - 45	42	2.67	32	0.464	0.0145
ORH 3	60 - 69	65	3.00	24	0.402	0.0168
ORH 4	70 - 80	74	3.07	26	0.339	0.0130
ORH 5	81 - 88	85	2.86	14	0.242	0.0173
ORH 6	90 - 108	98	2.75	13	0.227	0.0175
Feasibility	0 - 2	2	13.34	9	0.040	0.0044
Study	34 - 38	36	18.68	37	0.842	0.0228
	61 - 71	66	12.68	20	0.474	0.0237
	70 - 81	76	7.01	28	0.701	0.0250

Table 3. Radiometric results for orange roughly. Listed are the estimated age range from growth-zone counts and measured ^{210}Pb and ^{226}Ra activities for all samples. Two standard errors were expressed as an error percentage for the measured activities. Calculated activity ratios and their corresponding margin of error (based on error propagation and the delta method) are given to provide a 95% confidence interval.

Sample	Age group (yr)	^{210}Pb (dpm/g) \pm % error	^{226}Ra (dpm/g) \pm % error	^{210}Pb : ^{226}Ra activity ratio	2 SE
ORH 1	25 - 30	0.0426 \pm 12.1	0.0784 \pm 2.20	0.5437	0.0669
ORH 2	40 - 45	0.0591 \pm 9.8	0.0755 \pm 2.17	0.7824	0.0782
ORH 3	60 - 69	0.0696 \pm 10.5	0.0795 \pm 2.18	0.8761	0.0941
ORH 4	70 - 80	0.0759 \pm 10.5	0.0826 \pm 2.24	0.9189	0.0985
ORH 5	81 - 88	0.0624 \pm 19.0	0.0672 \pm 3.19	0.9285	0.1757
ORH 6	90 - 108	0.0693 \pm 10.9	0.0688 \pm 2.56	1.0067	0.1126

Table 4. Summary of growth-zone ages and radiometric ages for orange roughly. Average growth-zone age (with 2 SE of the age group mean) are given with radiometric age, calculated from the measured ^{210}Pb : ^{226}Ra activity ratios and corrected for time since capture. Radiometric age range was based on the analytical uncertainty and error propagation (2 SE). Radiometric age estimates from the feasibility study were adjusted slightly because of a spike recalibration.

Sample	Age group (yr)	Average growth zone age (yr)	Radiometric age (yr)	Radiometric age range (yr)
ORH 1	25 - 30	28 (0.5)	24	20 – 29
ORH 2	40 - 45	42 (0.7)	48	38 – 62
ORH 3	60 - 69	65 (1.0)	66	47 – 111
ORH 4	70 - 80	74 (1.1)	79	54 – undefined
ORH 5	81 - 88	85 (1.4)	83	44 – undefined
ORH 6	90 - 108	98 (3.0)	undefined	74 – undefined
Feasibility	0 – 2	2 (0)	1	0 – 6
Study	34 – 38	36 (0.3)	29	21 – 39
	61 – 71	66 (0.5)	32	24 – 44
	70 – 81	76 (1.4)	83	54 – undefined

Table 5. Summary of characteristics for the black cardinalfish samples processed in this study and the previous feasibility study. Estimated age composition (from growth-zone counts) and average time since capture for each group with resultant pooled sample number and weight are given. An average core weight for each otolith in each sample is given to show consistency of coring. Replication method was how the age group was split into two age groups. Low and high weight samples were segregated based on whole otolith weight to determine if there was any difference in radiometric age for the age group that could be traced to otolith weight. Results are tabulated and compared in Table 8 once radiometric age is revealed.

Sample	Age group (yr)	Average age (yr)	Time since capture (yr)	Otolith number	Sample weight (g)	Average core weight (g)	Replication method
EPT 1A	20 - 23	21.5	7.31	12	1.173	0.0978	Random
EPT 1B	20 - 23	21.6	7.47	12	1.174	0.0978	Random
EPT 2A	35 - 40	37.3	6.61	10	1.053	0.105	Low weight
EPT 2B	35 - 40	36.8	6.74	10	1.062	0.106	High weight
EPT 3A	50 - 55	52.0	6.98	7	0.681	0.0973	Low weight
EPT 3B	50 - 55	52.8	6.84	9	0.940	0.104	High weight
EPT 4A	60 - 65	62.0	6.99	8	0.785	0.0981	Low weight
EPT 4B	60 - 66	61.4	6.70	8	0.761	0.0951	High weight
EPT 5A	70 - 78	74.1	6.89	9	0.907	0.101	Random
EPT 5B	70 - 78	73.2	6.79	9	0.897	0.0997	Random
EPT 6	80 - 102	90.0	6.70	9	0.917	0.102	N/A
Feasibility	3 - 5	4	3.05	7	0.484	0.0691	N/A
Study	63 - 65	64	3.24	15	1.166	0.0777	N/A

Table 6. Radiometric results for black cardinalfish. Listed are the estimated age range from growth-zone counts and measured ^{210}Pb and ^{226}Ra activities for all samples. Two standard errors were expressed as an error percentage for the measured activities. Calculated activity ratios and their corresponding margin of error (based on error propagation and the delta method) are given to provide a 95% confidence interval.

Sample	Age group (yr)	^{210}Pb (dpm/g) \pm % error	^{226}Ra (dpm/g) \pm % error	^{210}Pb : ^{226}Ra (R) activity ratio	2 SE
EPT 1A	20 - 23	0.0297 \pm 9.3	0.0659 \pm 2.09	0.450	0.043
EPT 1B	20 - 23	0.0468 \pm 9.4	0.0889 \pm 2.08	0.527	0.051
EPT 2A	35 - 40	0.0760 \pm 8.4	0.1084 \pm 2.06	0.701	0.061
EPT 2B	35 - 40	0.1446 \pm 7.8	0.1790 \pm 2.06	0.808	0.065
EPT 3A	50 - 55	0.1297 \pm 8.6	0.1727 \pm 2.07	0.751	0.066
EPT 3B	50 - 55	0.1170 \pm 7.8	0.1430 \pm 2.07	0.818	0.066
EPT 4A	60 - 65	0.1162 \pm 8.3	0.1380 \pm 2.06	0.842	0.072
EPT 4B	60 - 66	0.1058 \pm 7.9	0.1103 \pm 2.07	0.959	0.078
EPT 5A	70 - 78	0.0718 \pm 7.7	0.0738 \pm 2.08	0.974	0.078
EPT 5B	70 - 78	0.0890 \pm 8.8	0.0848 \pm 2.07	1.050	0.095
EPT 6	80 - 102	0.0797 \pm 8.1	0.0825 \pm 2.09	0.965	0.080

Table 7. Comparison of growth-zone age and radiometric age for black cardinalfish. Average growth-zone age (with 2 SE of the age group mean) are given with radiometric age, calculated from the measured ^{210}Pb : ^{226}Ra activity ratios and corrected for time since capture. Radiometric age range was based on the analytical uncertainty and error propagation (2 SE).

Sample	Age group (yr)	Average growth zone age (yr)	Radiometric age (yr)	Radiometric age range (yr)
EPT 1A	20 - 23	21.5 (0.8)	15	12 - 17
EPT 1B	20 - 23	21.6 (0.8)	19	16 - 23
EPT 2A	35 - 40	37.3 (1.0)	34	28 - 41
EPT 2B	35 - 40	36.8 (1.4)	48	39 - 62
EPT 3A	50 - 55	52.0 (1.6)	40	32 - 50
EPT 3B	50 - 55	52.8 (1.2)	50	40 - 65
EPT 4A	60 - 65	62.0 (1.0)	55	43 - 74
EPT 4B	60 - 66	61.4 (1.4)	98	64 - undefined
EPT 5A	70 - 78	74.1 (1.8)	112	68 - undefined
EPT 5B	70 - 78	73.2 (1.8)	undefined	94 - undefined
EPT 6	80 - 102	90.0 (4.3)	103	65 - undefined
Feasibility study	3 - 5	4	6	5 - 7

Table 8. Age groups that were separated based on otolith weight and the resultant ^{210}Pb : ^{226}Ra activity ratio. Activity ratios within age groups differed by 2 to 3.4 SEs.

Sample	Age group (yr)	Average otolith weight (g)	Otolith weight range (g)	^{210}Pb : ^{226}Ra activity ratio	2 SE
EPT 2A	35 – 40	0.409	0.328 - 0.462	0.701	0.061
EPT 2B	35 – 40	0.521	0.467 – 0.571	0.808	0.065
EPT 3A	50 – 55	0.524	0.483 – 0.560	0.751	0.066
EPT 3B	50 - 55	0.653	0.592 – 0.704	0.818	0.066
EPT 4A	60 – 65	0.502	0.352 – 0.587	0.842	0.072
EPT 4B	60 – 66	0.711	0.637 – 0.792	0.959	0.078

Table 9. Summary of the study characteristics and ^{226}Ra results for four orange roughy studies. In the feasibility study, whole otoliths refer to uncored juvenile otoliths. The error for both this study and the feasibility study radium activity results. It is unclear what kind of sample was used in the analyses of Whitehead & Ditchburn (1996); they cite successive dissolutions from the same whole otolith sample and seven size fractions of 1-4 otoliths each (0.5 – 1 g), but also mention an 8 g sample.

	Technique	Sample type	Otolith number	Sample weight (g)	^{226}Ra activity (dpm/g)	Error (%)
This study	ICPMS	Cores	13 – 32	0.227 – 0.464	0.0672 – 0.0826	2.2 – 3.2
Andrews & Tracey (2003)	TIMS	Whole & cores	9 – 37	0.040 – 0.842	0.0599 – 0.209	3.0 – 5.4
Whitehead & Ditchburn (1996)	alpha- spectrometry	Whole	Not specified	~8	0.0560 – 0.137	5.5 – 7
Fenton et al. (1991)	alpha- spectrometry	Whole	28 – 315	6.06 – 6.793	0.0522 – 0.625	5 – 7

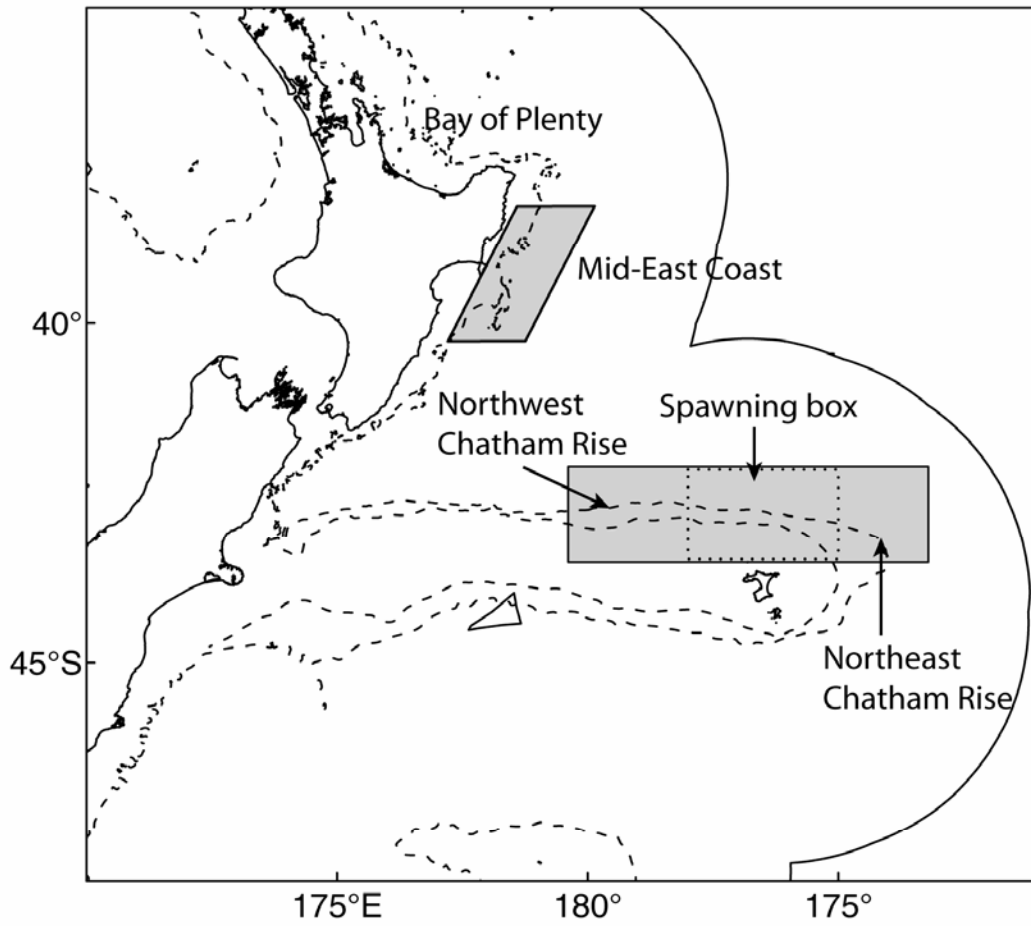


Figure 1: Areas within the New Zealand Exclusive Economic Zone (EEZ) referred to in the text from which orange roughy and black cardinalfish otolith samples were collected.

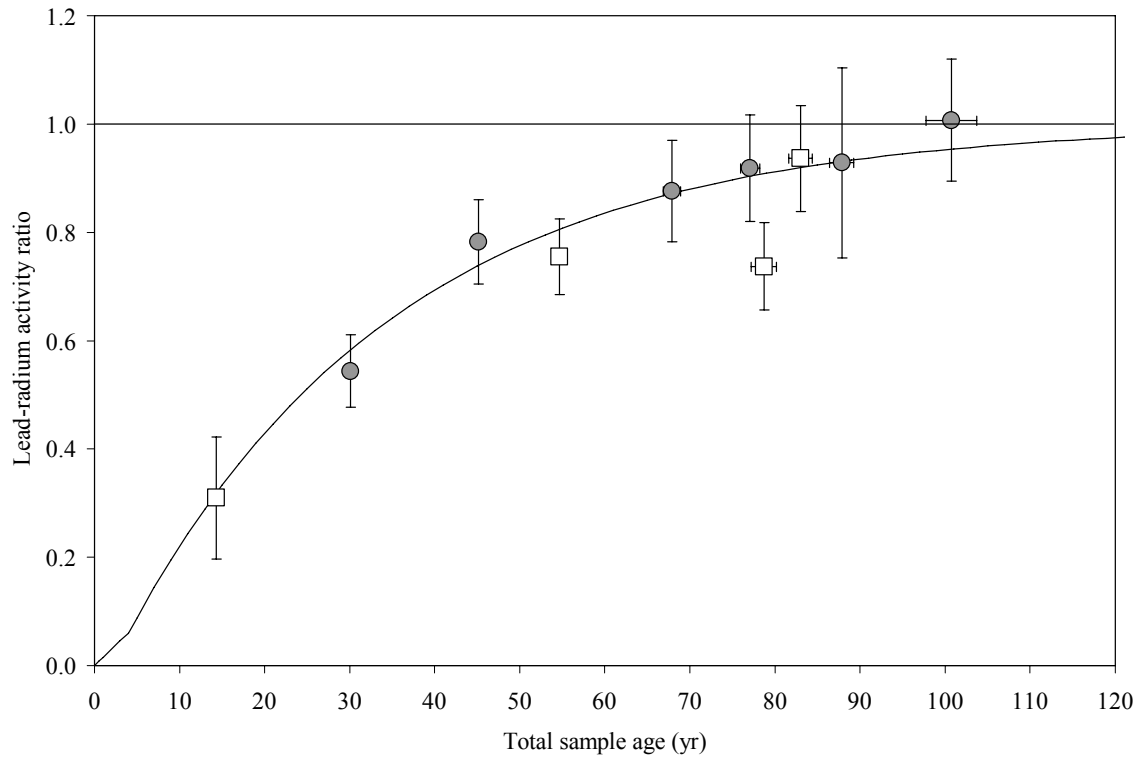


Figure 2. Plot of the measured $^{210}\text{Pb}:$ ^{226}Ra ratios with respect to total sample age (growth-zone age plus the time since capture) for orange roughly samples processed in this study (grey filled circle) and the previous feasibility study (open square), plotted with the $^{210}\text{Pb}:$ ^{226}Ra ingrowth curve. Horizontal error bars (some within the symbol size) represent 2 SE for the mean growth-zone age. The vertical error bars represent the analytical uncertainty associated with measuring ^{210}Pb and ^{226}Ra (2 SE).

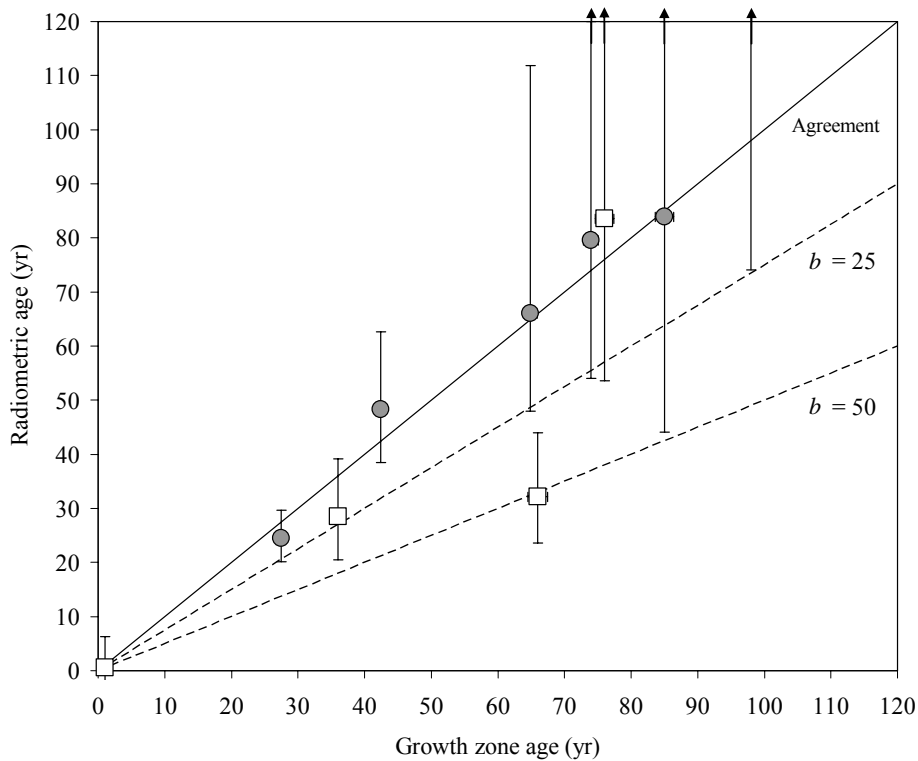


Figure 3. Orange roughly age agreement plot of growth zone derived age estimates versus radiometric age estimates (corrected to time of capture) for samples processed in this study (grey filled circle) and the previous feasibility study (open square), plotted with a line of agreement for comparison. Dashed lines provide a reference for potential over ageing bias of 25% ($b = 25$) and 50% ($b = 50$). Horizontal error bars (some within the symbol size) represent 2 SE for the age group mean. The vertical error bars represent the analytical uncertainty associated with measuring ^{210}Pb and ^{226}Ra (2 SE). Arrows at the upper end of some error bars indicate age (or its upper bound) was undefined because the $^{210}\text{Pb}:^{226}\text{Ra}$ ratio (or its upper bound) exceeded 1.0.

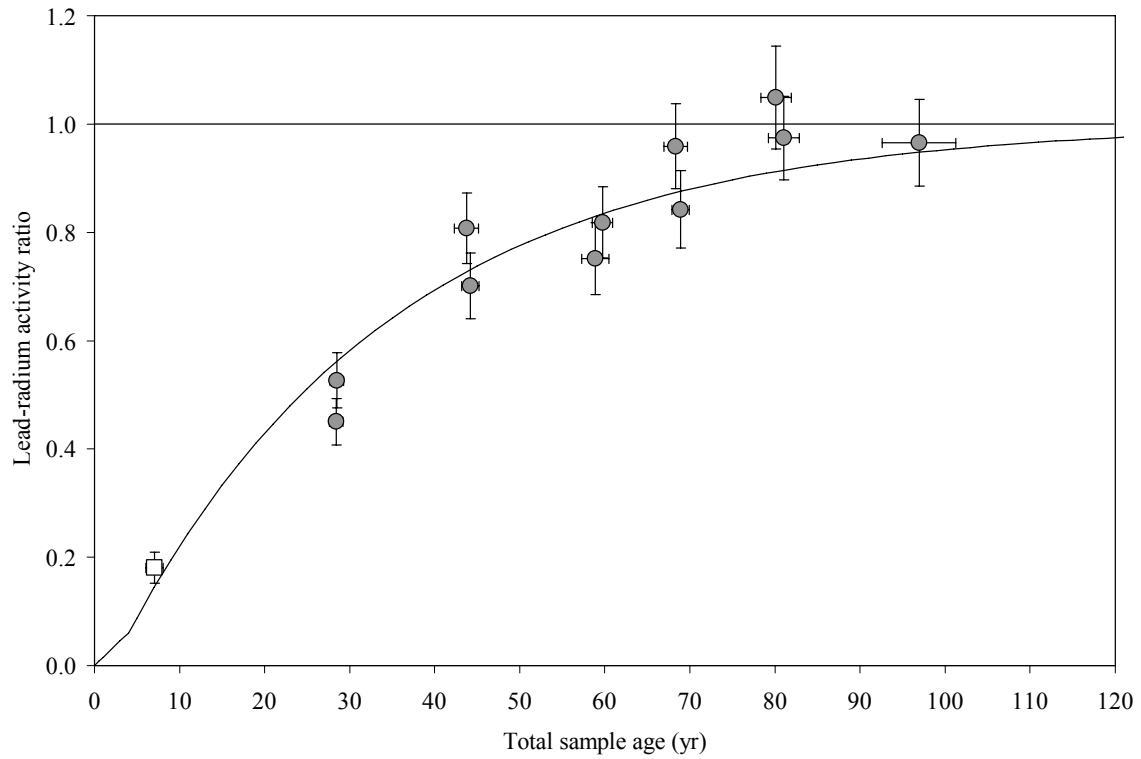


Figure 4. Plot of the measured $^{210}\text{Pb}:^{226}\text{Ra}$ ratios with respect to total sample age (age estimate plus the time since capture) for black cardinalfish samples processed in this study (grey filled circle) and the previous feasibility study (open square), plotted with the $^{210}\text{Pb}:^{226}\text{Ra}$ ingrowth curve. Horizontal error bars (some within the symbol size) represent 2 SE for the age group range. The vertical error bars represent the analytical uncertainty associated with measuring ^{210}Pb and ^{226}Ra (2 SE).

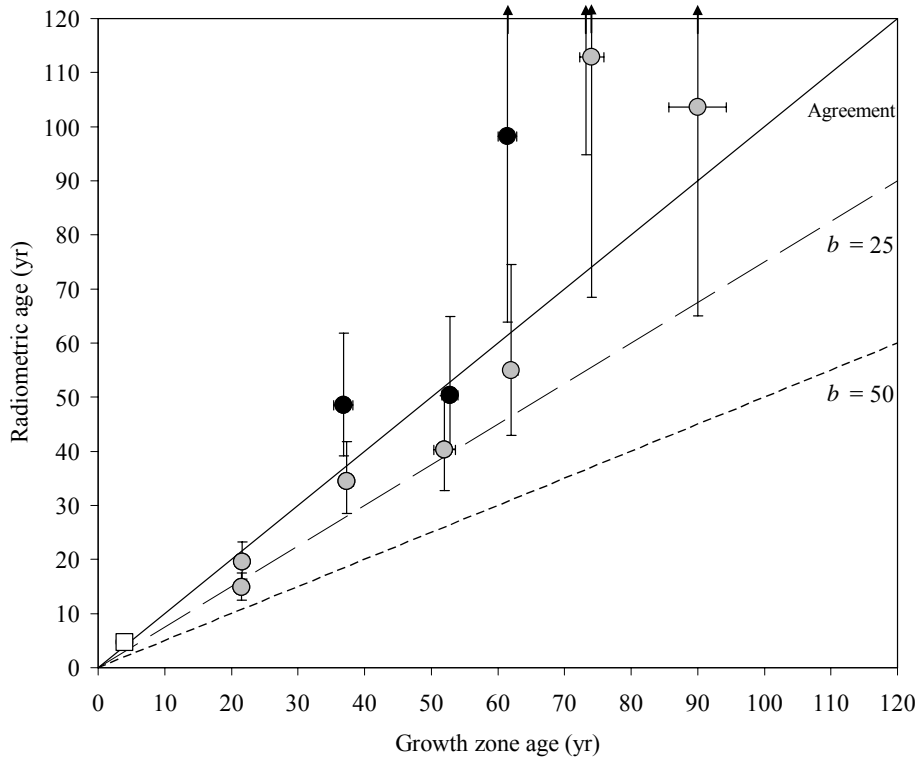


Figure 5. Black cardinalfish age agreement plot of growth zone derived age estimates versus radiometric age estimates (corrected to time of capture) for samples processed in this study (all circles) and the previous feasibility study (open square), plotted with a line of agreement for comparison. High otolith weight age groups were differentiated in the plot (black filled circle). Dashed lines provide a reference for potential under or over ageing bias of 25% ($b = 25$) and under ageing bias of 50% ($b = 50$). Horizontal error bars (some within the symbol size) represent 2 SE for the age group range. The vertical error bars represent the analytical uncertainty associated with measuring ^{210}Pb and ^{226}Ra (2 SE). Arrows at the upper end of some error bars indicate age (or its upper bound) was undefined because the ^{210}Pb : ^{226}Ra ratio (or its upper bound) exceeded 1.0.

7. APPENDICES

List of orange roughy (ORH) and black cardinalfish (EPT) samples used in the radiometric analyses with the collection date and biological details listed for each fish. Otolith weight was provided for only some of the samples used, therefore, all otoliths were weighed independently at MLML. Each age group is separated to define sample specificity. Replicated black cardinalfish samples were also specifically defined. Black cardinalfish collected from Mid-East Coast region of North Island that were aged by NIWA and selected for lead-radium dating. Otoliths were collected by Stock Monitoring Programme (SMP) in 1998 and 1999 from area CEE. N.D. represents no data of this type was recorded for this sample.

ORH SAMPLES								
25-30 yr group								
Batch	Specimen	Collection date	Fish Length (SL cm)	Fish wt. (kg)	Otolith wt. (NIWA)	Otolith wt. (MLML)	Sex	Estimated age (yr)
155	39	8-Jul-03	25	0.58	0.067	0.069	1	25
155	45	8-Jul-03	31	1.00	0.116	0.116	1	25
155	94	8-Jul-03	32	0.82	0.097	0.099	1	25
155	97	8-Jul-03	31	0.76	0.099	0.100	1	25
155	155	9-Jul-2003	29	0.66	0.076	0.079	1	25
155	138	9-Jul-2003	29	0.66	0.094	0.095	2	26
155	202	10-Jul-2003	29	0.80	0.107	0.107	1	26
155	220	10-Jul-2003	32	1.08	0.13	0.132	1	26
155	226	10-Jul-2003	34	1.18	0.158	0.156	2	26
155	54	8-Jul-03	34	1.10	0.128	0.131	1	27
155	58	8-Jul-03	29	0.88	0.097	0.097	1	27
155	154	9-Jul-2003	29	0.84	0.092	0.097	2	27
155	175	9-Jul-2003	35	1.26	0.12	0.125	2	27
155	13	8-Jul-03	33	1.08	0.124	0.123	2	28
155	14	8-Jul-03	31	1.04	0.122	0.103	2	28
155	32	8-Jul-03	31	0.86	0.101	0.106	1	28
155	44	8-Jul-03	33	1.16	0.108	0.105	1	28
155	87	8-Jul-03	31	0.94	0.119	0.118	1	28
155	140	9-Jul-2003	34	1.36	0.12	0.119	2	28
155	145	9-Jul-2003	27	0.52	0.085	0.083	1	28
155	152	9-Jul-2003	30	0.84	0.118	0.120	2	28
155	189	10-Jul-2003	36	1.38	0.123	0.124	1	28
155	209	10-Jul-2003	33	1.22	0.133	0.131	1	28
155	56	8-Jul-03	35	N.D.	0.173	0.171	2	29
155	91	8-Jul-03	30	0.80	0.102	0.098	1	29
155	96	8-Jul-03	32	0.92	0.139	0.134	1	29
155	98	8-Jul-03	33	1.12	0.148	0.157	1	29
155	130	9-Jul-2003	36	1.50	0.141	0.141	2	29
155	143	9-Jul-2003	36	1.50	0.163	0.153	1	29
155	57	8-Jul-03	34	1.16	0.11	0.105	1	30
155	182	10-Jul-2003	36	1.46	0.156	0.15	1	30

ORH SAMPLES								
40-45 yr group								
Batch	Specimen	Collection date	Fish Length (SL cm)	Fish wt. (kg)	Otolith wt. (NIWA)	Otolith wt. (MLML)	Sex	Estimated age (yr)
155	46	8-Jul-03	35	1.36	0.141	0.141	2	40
155	157	9-Jul-2003	35	1.34	0.196	0.200	2	40
155	166	9-Jul-2003	37	1.38	0.197	0.207	2	40
155	212	10-Jul-2003	36	1.50	0.155	0.160	2	40
155	263	10-Jul-2003	33	1.28	0.144	0.150	1	40
155	271	10-Jul-2003	34	1.18	0.158	0.153	1	40
155	361	11-Jul-2003	36	1.50	0.195	0.197	2	40
155	390	11-Jul-2003	38	1.64	0.22	0.211	2	40
155	113	9-Jul-2003	38	1.90	0.256	0.249	2	41
155	324	10-Jul-2003	33	1.11	0.133	0.129	1	41
155	347	11-Jul-2003	37	1.75	0.243	0.234	2	41
155	354	11-Jul-2003	37	1.70	0.167	0.164	2	41
155	252	10-Jul-2003	34	1.32	0.155	0.147	1	42
155	255	10-Jul-2003	32	1.12	0.132	0.138	2	42
155	408	11-Jul-2003	38	1.42	0.24	0.229	2	42
155	74	8-Jul-03	33	1.08	0.15	0.155	1	43
155	149	9-Jul-2003	30	0.86	0.129	0.130	2	43
155	274	10-Jul-2003	33	1.18	0.165	0.161	1	43
155	336	10-Jul-2003	36	1.40	0.205	0.185	1	43
155	362	11-Jul-2003	37	1.55	0.191	0.190	2	43
155	193	10-Jul-2003	33	1.48	0.187	0.157	1	44
155	195	10-Jul-2003	36	1.44	0.245	0.231	2	44
155	208	10-Jul-2003	34	1.20	0.146	0.155	2	44
155	246	10-Jul-2003	34	1.46	0.166	0.163	2	44
155	253	10-Jul-2003	39	1.58	0.223	0.215	2	44
155	299	10-Jul-2003	35	1.32	0.11	0.118	2	44
155	337	10-Jul-2003	36	1.30	0.191	0.189	1	44
155	12	8-Jul-03	39	1.84	0.201	0.193	2	45
155	112	9-Jul-2003	36	1.54	0.174	0.180	2	45
155	120	9-Jul-2003	37	1.72	0.225	0.212	2	45
155	153	9-Jul-2003	36	1.34	0.19	0.182	2	45
155	381	11-Jul-2003	41	1.82	0.237	0.228	2	45

ORH SAMPLES								
60-69 yr group								
Batch	Specimen	Collection date	Fish Length (SL cm)	Fish wt. (kg)	Otolith wt. (NIWA)	Otolith wt. (MLML)	Sex	Estimated age (yr)
155	510	18-Jul-2003	35	1.38	0.162	0.165	1	60
155	409	11-Jul-2003	37	1.50	0.254	0.258	1	61
155	6	8-Jul-03	39	1.90	0.239	0.247	2	62
155	356	11-Jul-2003	34	1.15	0.167	0.175	1	63
155	764	20-Jul-2003	33	1.04	0.16	0.162	1	63
155	201	10-Jul-2003	38	1.84	0.19	0.198	2	64
155	293	10-Jul-2003	37	1.76	0.207	0.191	2	64
155	587	18-Jul-2003	38	1.80	0.238	0.260	2	65
155	205	10-Jul-2003	37	1.58	0.28	0.292	1	66
155	210	10-Jul-2003	39	1.76	0.33	0.335	2	66
155	281	10-Jul-2003	40	1.96	0.31	0.297	2	66
155	652	19-Jul-2003	38	1.52	0.294	0.328	2	67
155	248	10-Jul-2003	38	1.92	0.236	0.236	2	68
155	655	19-Jul-2003	37	1.50	0.229	0.240	2	69
155	770	20-Jul-2003	39	1.68	0.324	0.344	2	69
137	69	12-Jul-02	39.8	0.90	N.D.	0.259	2	60
137	42	11-Jul-02	37	1.64	N.D.	0.338	2	63
137	160	17-Jul-02	35.2	1.42	N.D.	0.185	2	65
137	247	18-Jul-02	39	2.10	N.D.	0.254	2	65
137	408	22-Jul-02	37.5	1.68	N.D.	0.293	2	65
137	98	13-Jul-02	36.2	1.88	N.D.	0.239	2	66
137	220	18-Jul-02	36.2	1.38	N.D.	0.337	1	66
137	25	N.D.	37	1.50	N.D.	0.273	1	67
137	219	18-Jul-02	38.5	1.84	N.D.	0.315	1	67

ORH SAMPLES								
70-80 yr group								
Batch	Specimen	Collection date	Fish Length (SL cm)	Fish wt. (kg)	Otolith wt. (NIWA)	Otolith wt. (MLML)	Sex	Estimated age (yr)
155	186	10-Jul-03	37	1.62	0.248	0.250	1	71
155	321	10-Jul-03	37	1.56	0.252	0.245	1	71
155	633	19-Jul-03	37	1.66	N.D.	0.292	2	74
155	270	10-Jul-03	34	1.28	0.204	0.204	1	75
155	618	19-Jul-03	38	1.74	0.238	0.243	2	75
155	663	19-Jul-03	39	1.60	0.217	0.222	2	76
137	192	17-Jul-02	36	1.42	N.D.	0.276	1	70
137	251	18-Jul-02	39.5	1.94	N.D.	0.428	1	70
137	152	17-Jul-02	36.5	1.76	N.D.	0.230	2	72
137	184	17-Jul-02	33	1.00	N.D.	0.240	1	73
137	218	18-Jul-02	38.5	1.74	N.D.	0.306	1	73
137	296	19-Jul-02	33.2	1.24	N.D.	0.227	2	73
137	37	N.D.	31.1	0.98	N.D.	0.158	1	74
137	393	22-Jul-02	32	1.67	N.D.	0.336	2	75
137	156	17-Jul-02	40	1.94	N.D.	0.237	2	77
137	167	17-Jul-02	37.5	2.08	N.D.	0.307	2	79
154	213	13-Jul-03	40.5	2.39	0.22	0.242	2	70
154	484	19-Jul-03	36	1.44	0.268	0.266	1	70
154	421	16-Jul-03	36.5	1.46	0.285	0.273	2	72
154	357	16-Jul-03	38.5	1.74	0.278	0.274	2	73
154	517	19-Jul-03	36.5	1.52	0.308	0.316	2	77
154	485	19-Jul-03	37.5	1.94	0.399	0.366	1	78
144	344	N.D.	34.8	1.30	0.31	0.300	1	71
144	541	N.D.	35	1.37	0.304	0.315	1	73
144	342	N.D.	36.8	1.57	0.423	0.413	1	78
144	461	N.D.	38	1.71	0.279	0.287	1	78

ORH SAMPLES								
81-88 yr group								
Batch	Specimen	Collection date	Fish Length (SL cm)	Fish wt. (kg)	Otolith wt. (NIWA)	Otolith wt. (MLML)	Sex	Estimated age (yr)
155	291	10-Jul-03	39	2.08	0.335	0.340	2	81
155	236	10-Jul-03	41	2.28	0.347	0.342	2	83
155	700	19-Jul-03	38	1.66	0.219	0.232	2	83
155	330	10-Jul-03	40	1.80	0.318	0.333	1	87
155	499	12-Jul-03	38	1.80	0.216	0.219	2	87
155	176	9-Jul-03	40	1.96	0.311	0.323	2	88
137	353	21-Jul-02	40	1.86	N.D.	0.322	2	83
137	164	17-Jul-02	37.3	1.98	N.D.	0.190	2	88
154	260	13-Jul-03	36.5	1.38	0.318	0.301	1	82
154	311	15-Jul-03	33	1.27	0.249	0.242	1	87
144	492	N.D.	36.8	1.65	0.379	0.378	1	85
144	537	N.D.	37.7	1.58	0.401	0.378	1	85
144	221	N.D.	39.7	2.37	0.35	0.354	2	88
144	532	N.D.	33.6	1.39	0.295	0.285	1	88
90-108 yr group								
Batch	Specimen	Collection date	Fish Length (SL cm)	Fish wt. (kg)	Otolith wt. (NIWA)	Otolith wt. (MLML)	Sex	Estimated age (yr)
155	351	11-Jul-03	36	1.60	0.22	0.232	2	90
155	288	10-Jul-03	37	1.52	0.373	0.377	1	91
155	481	12-Jul-03	40	1.70	0.287	0.299	2	92
154	286	14-Jul-03	37.5	1.51	0.288	0.293	1	93
154	491	19-Jul-03	36.5	1.45	0.368	0.378	1	96
155	258	10-Jul-03	38	1.78	0.269	0.268	2	98
154	511	19-Jul-03	40	2.10	0.28	0.289	2	98
154	74	10-Jul-03	37.5	1.72	0.344	0.351	2	101
144	463	N.D.	37.4	1.83	0.497	0.482	2	101
144	504	N.D.	34.5	1.28	0.294	0.294	1	101
144	524	N.D.	33.7	1.16	0.3	0.302	1	102
137	178	17-Jul-02	39.5	1.58	N.D.	0.280	2	104
155	702	19-Jul-03	43	2.16	0.357	0.359	2	108

EPT SAMPLES							
20-23 yr							
Group	Packet	Fish no.	Collection	Length (FL cm)	Sex	Age (yr)	Otolith wt.
EPT 1A	981801	45	11-Sep-98	40	2	20	0.243
	991802	80	28-May-99	39	1	20	0.243
	981801	9	11-Sep-98	40	1	20	0.270
	981801	2	11-Sep-98	43	2	21	0.261
	991802	63	28-May-99	42	1	21	0.282
	991802	71	28-May-99	48	2	21	0.312
	981801	34	11-Sep-98	42	2	22	0.266
	981801	15	11-Sep-98	46	2	22	0.282
	991802	7	28-May-99	45	1	23	0.280
	981801	14	11-Sep-98	46	2	23	0.288
	981801	10	11-Sep-98	49	2	23	0.296
	991802	48	28-May-99	48	1	23	0.298
EPT 1B	981801	50	11-Sep-98	42	1	20	0.272
	981801	49	11-Sep-98	44	2	20	0.280
	981801	39	11-Sep-98	47	2	20	0.294
	991801	6	11-Sep-98	47	1	21	0.260
	991802	21	28-May-99	41	1	21	0.266
	991802	57	28-May-99	49	2	21	0.305
	991801	6	11-Sep-98	47	1	21	0.337
	991802	54	28-May-99	43	2	22	0.268
	981801	29	11-Sep-98	43	2	23	0.287
	981801	43	11-Sep-98	48	2	23	0.289
	991801	8	11-Sep-98	45	2	23	0.297
	991801	2	11-Sep-98	48	1	23	0.334

EPT SAMPLES							
35-40 yr (weight segregated)							
Group	Packet	Fish no.	Collection	Length (FL cm)	Sex	Age (yr)	Otolith wt.
EPT 2A	991811	82	20-Sep-99	50	1	36	0.328
	991811	43	20-Sep-99	51	2	39	0.346
	991811	19	20-Sep-99	56	2	37	0.367
	991811	7	20-Sep-99	54	2	36	0.401
	991811	70	20-Sep-99	56	2	36	0.410
	991811	5	20-Sep-99	60	2	35	0.426
	991811	87	20-Sep-99	56	1	37	0.445
	991811	36	20-Sep-99	54	2	38	0.453
	991803	98	1-Jun-99	57	2	40	0.455
	991811	86	20-Sep-99	54	1	39	0.462
EPT 2B	991811	34	20-Sep-99	56	1	35	0.467
	991811	4	20-Sep-99	54	2	40	0.471
	991803	13	1-Jun-99	59	2	35	0.498
	991803	49	1-Jun-99	56	2	35	0.503
	991803	82	1-Jun-99	55	2	37	0.503
	991811	33	20-Sep-99	57	1	39	0.530
	991811	2	20-Sep-99	64	1	37	0.532
	991811	28	20-Sep-99	58	1	35	0.566
	991811	50	20-Sep-99	60	2	40	0.568
	991803	94	1-Jun-99	57	1	35	0.571
50-55 yr							
Group	Packet	Fish no.	Collection	Length (FL cm)	Sex	Age (yr)	Otolith wt.
EPT 3A	981821	27	6-Feb-99	57	2	53	0.483
	991811	31	20-Sep-99	61	1	54	0.483
	991803	41	1-Jun-99	61	2	52	0.509
	981821	22	6-Feb-99	61	1	50	0.527
	991811	100	20-Sep-99	63	2	55	0.549
	981821	26	6-Feb-99	61	1	50	0.554
	981821	11	6-Feb-99	59	1	50	0.560
EPT 3B	991803	22	1-Jun-99	63	2	53	0.592
	991802	9	28-May-99	60	2	50	0.601
	991811	54	20-Sep-99	60	1	55	0.619
	991825	9	20-Nov-99	65	2	55	0.649
	991811	17	20-Sep-99	65	2	54	0.662
	991803	8	1-Jun-99	63	2	52	0.671
	991803	84	1-Jun-99	67	2	53	0.676
	991821	21	6-Feb-99	69	2	52	0.699
	991803	73	1-Jun-99	68	2	51	0.704

EPT SAMPLES							
60-65/66 yr (weight segregated)							
Group	Packet	Fish no.	Collection	Length (FL cm)	Sex	Age (yr)	Otolith wt.
EPT 4A	981821	14	6-Feb-99	48	2	63	0.352
	981821	20	6-Feb-99	50	2	65	0.425
	991807	23	1-Jun-99	57	1	62	0.489
	991825	81	20-Nov-99	62	1	61	0.515
	991823	5	7-Apr-99	64	2	61	0.537
	991823	14	7-Apr-99	59	1	62	0.545
	991821	10	6-Feb-99	65	2	62	0.568
	991807	15	1-Jun-99	61	1	60	0.587
EPT 4B	991803	30	1-Jun-99	65	2	60	0.637
	991825	54	20-Nov-99	65	1	60	0.650
	991803	12	1-Jun-99	58	1	61	0.661
	991825	40	20-Nov-99	66	2	60	0.690
	991811	26	20-Sep-99	70	2	60	0.727
	991811	35	20-Sep-99	68	2	62	0.743
	991811	23	20-Sep-99	68	2	62	0.788
	991802	94	28-May-99	69	2	66	0.792
70-78 yr							
Group	Packet	Fish no.	Collection	Length (FL cm)	Sex	Age (yr)	Otolith wt.
EPT 5A	991822	6	12-Mar-99	67	2	70	0.728
	991824	17	18-May-99	63	1	71	0.585
	991807	85	1-Jun-99	70	2	73	0.717
	991825	58	20-Nov-99	69	2	73	0.785
	991807	63	1-Jun-99	67	2	73	0.791
	981821	1	6-Feb-99	64	1	75	0.619
	991803	1	1-Jun-99	71	2	77	0.802
	991825	60	20-Nov-99	67	2	77	0.812
	991807	93	1-Jun-99	60	1	78	0.672
EPT 5B	991825	13	20-Nov-99	67	2	70	0.741
	991807	58	1-Jun-99	72	2	70	0.825
	991825	36	20-Nov-99	66	2	71	0.647
	991825	29	20-Nov-99	66	2	72	0.627
	991803	45	1-Jun-99	68	1	72	0.772
	991807	30	1-Jun-99	67	2	75	0.636
	991807	24	1-Jun-99	68	2	75	0.855
	991823	28	7-Apr-99	64	2	77	0.621
	991802	44	28-May-99	71	2	77	0.869

EPT SAMPLES							
80-102 yr							
Group	Packet	Fish no.	Collection	Length (FL cm)	Sex	Age (yr)	Otolith wt.
EPT 6	991823	13	7-Apr-99	63	2	80	0.695
	991823	31	7-Apr-99	67	2	83	0.826
	991825	32	20-Nov-99	62	1	86	0.669
	991825	71	20-Nov-99	68	2	89	0.679
	991807	94	1-Jun-99	72	2	89	0.798
	991825	25	20-Nov-99	76	2	90	0.783
	991825	73	20-Nov-99	67	2	92	0.734
	991825	24	20-Nov-99	67	1	95	0.721
	991807	82	1-Jun-99	66	1	102	0.809