# FIRDC FINAL REPORT GRANT 87/94:

# INVESTIGATION OF A TECHNIQUE FOR AGEING MARINE ANIMALS

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## 1. PROJECT OBJECTIVES:

The objective of the project was to investigate whether a relatively new technique for estimating age could be applied to fisheries problems. During the first year of the project FIRDC directed us to concentrate on deep-sea fish species, in particular orange roughy.

The technique of radionuclide analysis is potentially very valuable for determining age in animals in which age cannot be determined with any accuracy by other means. It relies on the unequal decay rates of naturally occurring radioisotopes incorporated into hard parts e.g. shell, otolith or bones, at the time of their formation. The nuclides $^{226}$Ra (1620 years half-life) and its progeny $^{210}$Pb (22.3 years half- life) occur naturally in seawater and are the most useful nuclide pair for ageing fish. The radioactive disequilibrium between these nuclides approaches equilibrium in a manner controlled by their respective decay constants (equilibrium would take about 100 years). The change in the extent of the disequilibrium is a measure of time elapsed since they were incorporated. Once we have evaluated this method we will attempt to calibrate some easily measured parameter e.g. dimension, mass or some other parameter of otolith growth, against calculated age.

## 2. ACHIEVEMENT OF OBJECTIVES:

*a) Development of the radiometric technique:*

The development of the analytical technique was achieved and analyses of whole otoliths can now be conducted routinely for ageing purposes. Prior to this study no laboratories in Australia had attempted to age fish using this technique.

*b) Blue Grenadier*

The first species analysed was blue grenadier. The radiometric analysis was successful, however a principle assumption, that of constant $^{226}$Ra uptake was violated and therefore ages could not be determined. The reason for violation of this assumption was thought to be due to the change in habitat that occurs between juvenile and adult life. The results although negative in terms of age determination provided

valuable information for selection of suitable species for analysis.

c) *Orange Roughy*
Analysis of orange roughy otoliths was successful and ages were estimated.

d) *Warty Dory*
The third species analysed was warty dory and ages estimated.

## 3. RESEARCH RESULTS AND BENEFITS

a) *Analytical method*

It was necessary to adapt existing procedures to measure the isotopes $^{226}Ra$ and $^{210}Pb$ at the extremely low levels encountered. The radiochemical analysis of $^{226}Ra$ and $^{210}Pb$ requires the best available ultra-low level techniques and rigorous allowance for all possible sources of significant error, such as in situ allogenic nuclide contamination (i.e. from within the fish but outside the aragonitic closed system of the otolith), chemical blank values, and instrumental backgrounds. Both isotopes were analysed using alpha-spectrometry: $^{226}Ra$ was measured directly, but $^{210}Pb$ was measured via its alpha emitting granddaughter, $^{210}Po$, which would be (one or more years after collection) within at least 5% of secular equilibrium with $^{210}Pb$.

Details of the radiochemical method are given in Fenton et al. (1990) in Appendix A. A simplified flow chart of the procedure is given in Fig. 1.

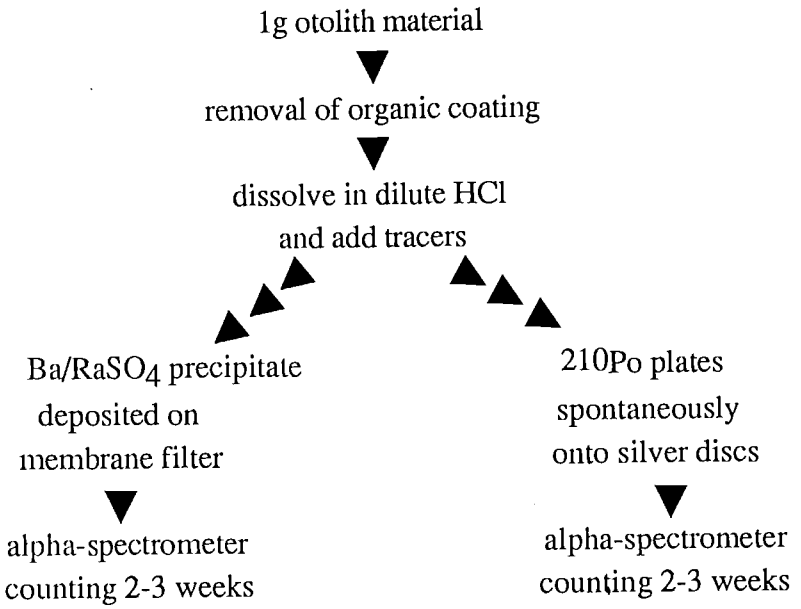


**Fig. 1 Simplified flow-chart of the radiometric analysis.**

*b) Sample preparation: Removal of the organic coating on the otoliths*

Trial dissolutions of whole otoliths in dilute HCl showed that they were enclosed within a chemically-resistant membrane, which forms a continuous sheath to which may be attached the remnants of the semi-circular canals. The removal of this adherent organic matter is of critical importance because it is generally recognised that marine tissue is capable of accumulating high levels of $^{210}Po$ (Cherry and Shannon 1974; Heyraud and Cherry 1979; Cherry and Heyraud 1982). If this step is not taken, contamination with allogenic $^{210}Po$ derived from sources external to the otolith, is potentially a serious problem given the low concentrations involved. The importance of thorough decontamination, particularly from the $^{210}Po$-rich tissue component, became evident during radioanalyses of blue grenadier Macruronus novaezelandiae otoliths. Published methods of pretreatment proved ineffective, therefore details of the trials to find a suitable sample preparation method are given here.

*i) Blue Grenadier*

Several pretreatments were used in an attempt to remove this organic layer. The otoliths were pretreated with "Fenton's Reagent", i.e. 30% $H_2O_2$ at 50°C in the presence of trace $Fe^{3+}$ catalyst for varying periods of time to remove the organic envelope, and then washed. The choice of Fenton's Reagent was based on the fact that it has been used successfully in radiochemistry for the destruction of organic matter (Sansoni and Kracke 1971; Bock 1979) including relatively resistant material such as ion-exchange resins (Kubota 1983). Also, Bennett et al. (1982) pretreated otoliths with $H_2O_2$ (without catalyst) but at an unspecified concentration, temperature, and period of exposure. In addition to the trials with "Fenton's Reagent", 2 samples were treated with 30% $H_2O_2$ (without catalyst) under strong UV illumination for 11 hours.

The results of these trials are given in Table 1 in Appendix A. It is evident that a minimum exposure of 16 hours is necessary to remove the organic coating. The UV treatment was not successful.

*ii) Orange roughy*

The pretreatment was improved further for cleaning orange roughy otoliths. The otoliths were cleaned by soaking in 30% $H_2O_2$ for 16-24 hours at 50°C (no $Fe^{3+}$ catalyst was added). They were then successively rinsed with water, 0.05M $Na_4EDTA$

(alkaline EDTA, pH10.5), water, 0.05M $Na_4$ EDTA, water (twice), 0.1MHCl (for less than 10 seconds) and finally water (twice). There was no evidence of contamination using this method and we would suggest this method be adopted for all otolith radioanalysis.

*c) Analysis of $^{210}Pb/^{226}Ra$ disequilibria in otoliths of Blue Grenadier Macruronus novaezelandiae.*

The abstract of the paper detailing the results of the blue grenadier study is given here. A copy of the complete paper is attached as Appendix A.

**Abstract**

Otoliths from Blue Grenadier (Macruronus novaezelandiae), which had been aged previously by annuli analysis, were analysed for the naturally occurring radionuclides $^{210}Pb$ and $^{226}Ra$ in an attempt to independently verify their age. However, the radiometric technique could not be applied to determine age because the results showed that $^{226}Ra$ was not incorporated at a constant rate throughout the life of M.novaezelandiae. Uptake of $^{226}Ra$ was greater in juveniles than in adult fish. This was probably due to the juvenile phase inhabiting inshore/estuarine waters.

*d) Age determination of Orange Roughy, Hoplostethus atlanticus (Pisces:Trachichthyidae) using $^{210}Pb/^{226}Ra$ disequilibria.*

The abstract of the paper detailing the results of the orange roughy study is given here. A copy of the complete paper is attached as Appendix B.

**Abstract**

Natural levels of $^{210}Pb/^{226}Ra$ in otoliths of orange roughy have been measured to determine fish ages radiometrically. Up to maturity, radiometric age estimates were consistent with a single constant otolith growth rate. Radiometric ages for juveniles were comparable with, but greater than, those obtained in a recent, validated New Zealand study which employed counts of annuli on the surface of otoliths. Beyond maturity, radiometric ages were obtained by modelling with an otolith growth rate set at 45% of the juvenile rate. Radiometric ageing confirms orange roughy is very slow-growing with age at maturity (32 cm SL) estimated to be ~32 years, and very long-lived, with fish 38-40 cm 77 - 149 years old. These results have important implications for the management of the fishery.

*e) Warty Dory Allocyttus verrucosus Analysis*

## Introduction

Warty dory Allocyttus verrucosus (Oreosomatidae) is a demersal fish species which is found on the continental slope in depths from 640-1100m (May and Maxwell 1986). It has a wide geographic distribution including south-eastern and western Australia, New Zealand, South Africa, California and Patagonia (Last et al ., 1984; James et al., 1988 ). It is often caught in the trawls targetted at orange roughy, sometimes in quite large quantities ( J. Kitchener, Division of Sea Fisheries Tasmania, pers. commun.). Although its potential as a commercial species is doubtful because most of the catch is composed of relatively small individuals (<1kg body weight) other oreo species e.g. black Allocyttus niger, smooth Pseudocyttus maculatus and spiky Neocyttus rhomboidalis are of commercial interest and the former two species currently form a significant fishery in New Zealand.

Little is known about the biology of A. verrucosus apart from two Russian papers, one on feeding and the other on age determination by Mel'nikov (1981a,b). He estimated age using growth zones on the scales since the zones were more clearly defined than on otoliths. The ages were not validated. The maximum age claimed was 15 years for females and 14 years for males although the bulk of the catches was composed of fish 6-9 years old. In contrast, recent attempts in Australia to age warty dory have not been successful (David Smith, MSL, pers. comm.).

Although no age data are currently available for closely related oreo dory species size-fecundity relationships for the smooth oreo Pseudocyttus maculatus and black oreo Allocyttus niger have been estimated by Conroy and Pankhurst (1989) from New Zealand waters. They found both species to have comparatively low absolute and relative fecundities (within the same order of magnitude as orange roughy). The depth and geographic distributions of these oreo species overlap with that of orange roughy. Similarities between the reproductive biology of oreo species and orange roughy raises the possibility that growth of oreo dories is slow similar to that of orange roughy.

Therefore, the radionuclide ageing technique is ideally suited to determine whether the ages estimated by Mel'nikov are realistic for warty dory or whether they are long-lived similar to orange roughy.

## Materials and Methods

Warty dory were collected off the west coast of Tasmania in the 800-1200m depth zone during cruises by the Tasmanian Department of Primary Industry, Division of Sea Fisheries in August 1988. Sagittal otoliths were removed and stored dry. Radiochemical analysis was restricted to female fish, except for fish <17cm total length TL. Individual otoliths were weighed to the nearest 0.1mg.

Samples of about 1g were required for radiochemical analysis, therefore pooling

of otoliths was necessary. Selection of the otoliths for analysis was based on similarity of fish length TL and otolith weight. The radiochemical analyses followed the method described in Fenton et al., (in press).

The activity of the $^{226}Ra$ reagent blank was 0.0192± 0.0019 dpm slightly lower than the value obtained for orange roughy 0.0255±0.0023 dpm (Fenton et al. in press), showing further improvement in the analytical technique. The $^{210}Po$ reagent blank was 0.0082±0.0015 dpm also slightly lower than that obtained for orange roughy 0.0103±0.0027 dpm (Fenton et al. in press).

**Results**

The specific activity of $^{226}Ra$ was similar in fish from all size classes. The $^{210}Po$ value measured from the sample of small fish <17cm TL was slightly contaminated and could not be used to determine age. A new sample of small fish has been obtained and is about to be analysed. The value of R (initial $^{210}Pb/^{226}Ra$ ratio), used in the calculation of age, cannot be accurately determined until a sample of small juveniles is analysed. However, the similarity of the $^{226}Ra$ measured for warty dory to that of orange roughy would suggest the R value will be close to that found for orange roughy i.e. 0.5. Warty dory ages have therefore been calculated using R= 0.5.The results are given in Table 1. Ages have been calculated using the average otolith age model Equ.1, linear growth model Equ. 2 and Double growth model Equ. 3. The equations are given in Appendix B.

An absolute minimum age for each sample can be estimated by doubling the average otolith age. Therefore the minimum age expected for the samples LH 1142 and LH 1143 are 120 and 102yr respectively. The linear growth model takes into account the mass growth of the otolith, although it assumes the rate of growth is constant throughout life. The ages estimated for these two samples are 180 yr and 136 yr respectively.

A two-stage growth model is thought to best represent the growth of the otolith and therefore the ages calculated using this model should be the most accurate. Otolith mass growth was assumed to be linear until maturity was reached i.e. 28cm TL for females and 24cm TL for males (J.Kitchener pers. commun). A value of 1.464mg/yr for the prematurity otolith mass growth rate was estimated from sample LH1349 using the linear growth model. Beyond maturity growth of the otolith was assumed to slow to 45% of the prematurity value (the value chosen for orange roughy otolith growth). It should be noted that these values of otolith mass growth represent rough estimates. The analysis of additional samples of prematurity fish is needed to improve these estimates. If the prematurity otolith growth-rate is indeed as slow as indicated here, the age at maturity would be about 30yr. For the calculation of ages using the two-stage growth model here an age of 30yr was assumed for the age at maturity. It must be stressed that the estimates of age given here are preliminary and the results of additional samples in the process of being analysed will refine these estimates.

Table 1. Allocyttus verrucosus.Physical and radiometric data for all otolith samples. Radiometric ages have been calculated using a simple $^{210}Pb$ ingrowth equation (which provides mean otolith age) (Eq.1), constant growth-rate model (Eq.2) and two-stage growth rate model (Eq.3). All ages were calculated using R = 0.05 ( where R = initial $^{210}Pb/^{226}Ra$ activity ratio at time of deposition). Maturity was set at 30yr. All errors expressed at the $\pm 1\sigma$ level.

| Sample Number | Mean Fish Length SL (cm) | Mean Otolith Mass (mg) | $^{210}Pb/^{226}Ra$ activity ratio | Mean Otolith Age (years) | | Constant growth rate otolith age (years) | | Double growth rate otolith age (years) |
|---|---|---|---|---|---|---|---|---|
| LH1349 | 22.9±0.6 | 20.2±2.8 n=50 | 0.228±0.051 | 9 | ±2 | 14 | +5,-4 | - |
| LH1350 | 29.8±0.9 | 47.4±9.7 n=25 | 0.808±0.021 | 56 | +4,-3 | 158 | +20,-16 | 56±1 |
| LH1351 | 32.2±0.6 | 81.5±6.3 n=20 | 0.815±0.019 | 57 | +4,-3 | 164 | +19,-16 | 92±2 |
| LH1143 | 34.5±0.5 | 129.6±10.0 n=8 | 0.779±0.115 | 51 | +24,-14 | 136 | +151,-52 | 139±20 |
| LH1142 | 34.9±0.8 | 87.9±13.3 n=12 | 0.831±.091 | 60 | +26,-15 | 180 | +211,-65 | 101±11 |

## Discussion

Our radiometric data for warty dory show that warty dory is a very long-lived species with adults 34-36cm TL estimated to be 101-139 years old. The results at this stage do not estimate the age of maturity accurately however, it appears that the age is about 30yr. The longevity of warty dory is quite similar to that of orange roughy (Fenton et al. in press).

It is interesting to consider that the largest fish analysed here averaged 34cm TL and yet specimens of warty dory up to 42 cm TL have been caught in Australian waters. Although these larger fish may represent faster growing individuals there is a high likelihood that they are extremely old.

In conclusion, the radiometric analysis of warty dory otoliths has demonstrated that this species is very long-lived. This raises the possibility that other oreo species are also long-lived. The radiometric analytical technique used here would be well suited to examine the age of these other oreo species in the future.

*f) Otolith weight as a predictor of age*

The need for a reliable and quick predictor of age for fish populations has resulted in many attempts at scaling, i.e. relating the size of structures to age.Two recent experimental studies designed to examine the effects of somatic growth on otolith size in guppies and striped bass respectively, both indicated the value of otolith weight as a predictor of age (Reznick et al 1989; Secor and Dean 1989). Otolith weight offers distinct advantages over otolith length measurements since it is easily measured and reader differences are minimal. Recently, Radtke and Hourigan (1990) demonstrated the value of otolith weight for the estimation of the age of the Antarctic fish Nototheniops nudifrons.

The blue grenadier otolith measurement data collected during this study (using ages calculated by Kenchington and Augustine, 1987) suggested a relationship between otolith weight and age. In addition the relationship we observed between otolith weight and fish age for orange roughy is potentially useful for the analysis of the population age structure. However the extent of the relationship could not be fully examined since otolith weight was used in the calculation of age using the two-stage growth model i.e. this model assumes that the heavier the otolith the older the fish. (However this whole problem could be avoided by analysing otolith cores -discussed later).It would be incorrect to assume that otolith weight is only determined by age, other factors such as the growth rate of the fish are important in determining the otolith weight. For example, several studies have demonstrated that slow-growing fish had small otoliths while comparably aged fast-growing fish had larger otoliths (Struhsaker and Uchiyama, 1976; Taubert and Coble 1977; Barkman, 1978). In contrast three recent studies (Mosegaard et al., 1988; Reznick et al., 1989; Secor and Dean, 1989) have

shown that slow-growing individuals in a population develop disproportionately large otoliths.

*g) Benefits*

Development of an alternative and independent method for ageing fish is an important benefit of the project. The ages obtained for orange roughy and warty dory have provided valuable data for the management of these fisheries.

## 4. DIFFICULTIES ENCOUNTERED:

*a) Removal of organic coating of otoliths*

It is necessary to remove all traces of the organic coating present on the otoliths prior to analysis since $^{210}Po$ can adsorb on the organic coating during dissolution of the otoliths and result in considerable inaccuracies. A wash with peroxide recommended by Bennett et al. (1982) was not effective. Therefore several trials were conducted (discussed in RESEARCH RESULTS AND BENEFITS) until a suitable method was found.

*b) Development of the analytical method*

Initially samples were sent to radiochemists at two laboratories: Dr. David Smith at the University of Melbourne and to Dr. Steve Short at ANSTO. The extremely low levels of radionuclides present in the otoliths could only be measured using the equipment at ANSTO. Therefore all analyses were conducted by Dr. Steve Short at ANSTO.

The extremely low levels of $^{210}Pb/^{226}Ra$ present in the otoliths required modification of existing methodology to allow the analysis. Apart from the development of a procedure to remove the organic coating on the otoliths all other chemical procedures were adapted to minimise the risk of contamination. Identifying the sources of contaminants in many cases was a time-consuming business considering the long counting times (2-3 weeks) involved per trial.

The analytical procedure has now been developed to the point where counting errors have been minimised. The improvement with time with our procedures can be seen by the reduction in errors associated with the ages estimated (see Orange Roughy results i.e. the higher the LH number the more recent the analysis).

*c) Speed of analysis/ number of spectrometers available for ageing*

In order to measure the extremely low levels of $^{210}Pb/^{226}Ra$ present in fish

otoliths counting times are 2-3 weeks per sample. We had access to 4 spectrometers at ANSTO (purchased by the grant ). Therefore it was only possible to analyse 2 samples (i.e. a separate spectrometer for $^{210}Po$ and $^{226}Ra$) at one time. In addition the need to run frequent blanks (counted under identical conditions as the samples) to check for possible contamination, particularly during the developmental stage, resulted in slow throughput of samples.

In addition it should be recognised that ANSTO was co-operative in analysing a number of samples as part of their own R&D and therefore it was not purely a contract arrangement. Note also that the spectrometers bought using FIRDC funds are still in use at ANSTO solely for analysing fish ages.

*d) Collection of juvenile Orange Roughy*

Juvenile orange roughy <15cm SL were rarely caught by CSIRO or Tasmanian Sea Fisheries Division. Therefore our requirement for a 1g sample of otoliths was difficult to achieve. Coupled with this is the necessity for an absolute minimum period of 6 months, preferably 1 year to elapse after capture and before analysis of the samples to allow for ingrowth of $^{210}Po$ i.e. to equilibrate with $^{210}Pb$. The analysis of juvenile fish is necessary to establish the R value (initial uptake ratio of $^{210}Pb/^{226}Ra$ in the otolith) which is fundamental to the estimation of age.

## 5. RECOMMENDATIONS FOR FURTHER RESEARCH:

*a) Development of an otolith coring method to enable analysis of only the oldest material within the otolith.*

By analysing only the cores the problems associated with modelling otolith mass-growth rates and analysis of juveniles would be avoided. A recent paper by Campana et al., (1990) described the successful use of a coring method for radionuclide analysis of otoliths of Sebastes mentella. The ages they obtained compared closely to ages estimated by counting annuli on the otoliths (using the criteria recommended by Beamish 1979).

The development of a coring method although potentially a very useful approach will require careful sample preparation. Contamination has to be avoided. Furthermore careful selection of species suitable for analysis by coring will be necessary. For example, the shape, size (mass), number of primordia etc. of the otolith will largely determine whether it is possible to remove the otolith core. Quite different approaches to coring may be necessary for different species.

Therefore, development of a coring method will involve careful experimentation however the successful development of such a method offers great rewards. In particular it will enable ageing of fish species which have a major change in habitat during their lifecycle e.g. Blue Grenadier.

Dr. Fenton has received an ARC small grant of $9000 for 1991 to develop the coring technique for blue grenadier.

b) *Studies of the mass-growth of fish otoliths.*

Although this is not necessary when otoliths can be cored there will be species for which coring is not possible e.g. species with small otoliths. The requirement of 1g of otolith material for each analysis would be very difficult to achieve in many species. Therefore whole otolith analysis will still remain a valuable approach to ageing but its value could be substantially improved by better data on the mass-growth rates of otoliths. The majority of studies of otolith growth have dealt with changes in the linear dimensions of the otolith and not weight. For those studies which have used weight it has generally been restricted to studies of larval or juvenile fish. Data on the mass-growth of otoliths at maturity and beyond are rare and when present are often in studies where ages were not validated and therefore are of little use.

c) *Application of the technique to a wide range of fisheries species.*

Radionuclide analysis of fish species from different habitats e.g. coastal, tropical, pelagic, Antarctic etc. In addition there is wide scope for ageing of sharks, corals, echinoderms (e.g. starfish, urchins) and molluscs (e.g. scallops, abalone, giant-clams) by radionuclide analysis.

d) *Development of analytical techniques to use other isotope pairs for ageing.*

There are several different isotope pairs ($^{228}Th/^{228}Ra$, $^{210}Po/^{210}Pb$ and $^{210}Pb/^{226}Ra$) from the uranium and thorium decay series that are biologically useful . For example, the pair $^{228}Th/^{228}Ra$, useful in the range 0-10 years, has been used to verify annual banding in modern corals (Dodge and Thomson 1974), to age vesicomyid clams growing in hydrothermal areas (Turekian et al. 1983) and to age the carapaces of spider crabs and the European lobster ( LeFoll et al., 1989). The pair $^{210}Po/^{210}Pb$ , useful for only 0-2 years, has been used to measure the growth rate of Nautilus pompilius (Cochran et al. 1981) and to age sharks (Weldon et al., 1987). Therefore development of the analytical procedures to enable routine analysis of these different isotope pairs would be advantageous for ageing short-lived species.

e) *Contract analyses*

It is anticipated that the cost of further development of the ageing technique will be partially offset by income generated from contract analyses. Radiometric ageing of whole otoliths can now be realistically offered as a service. We have received expressions of interest from within Australia and New Zealand to conduct analyses.

## 6. APPLICATION OF RESULTS TO INDUSTRY:

The results obtained for orange roughy have had direct and immediate implications for the fishery. The great age at which maturity is reached and the longevity of orange roughy requires careful management of the resource in order to achieve a sustainable fishery.

The orange roughy results have been made available to scientific and management committees in Australia and New Zealand, CSIRO Fisheries Research, and the Tasmanian Department Primary Industry, Division of Sea Fisheries for use in the development of management models. The results for warty dory also have immediate implications for the developing fishery on oreo dory species.

## 7. PUBLICATIONS RESULTING FROM THE PROJECT:

*a) Scientific journal articles*

Fenton, G.E., Ritz, D. A. and Short, S.A. (1990) $^{210}Pb/^{226}Ra$ disequilibria in otoliths of Blue Grenadier Macruronus novaezelandiae; Problems associated with radiometric ageing. Australian Journal of Marine and Freshwater Research **41**: 467-473

Fenton, G.E., Short, S.A. and Ritz, D. A. (1990) Age determination of Orange Roughy, Hoplostethus atlanticus (Pisces:Trachichthyidae) using $^{210}Pb/^{226}Ra$ disequilibria. Marine Biology in press

*b) Published conference proceedings*

Fenton, G.E., Ritz, D.A. and Short, S.A. (1990) Ageing fish using radionuclide analysis. In: Proceedings of the Australian Fish Biology Workshop on Age and Growth, August 1990, Lorne, Victoria.

Short , S.A. and G.E. Fenton (1990) $^{210}Pb/^{226}Ra$ disequilibria ageing of Orange Roughy. In: Proceedings of the Workshop on Environmental radiochemistry and radionuclide measurements 18th-20th September 1990, Adelaide, South Australia.

*c) Abstracts of conference papers*

Fenton, G.E., Ritz, D.A. and Short, S.A. (1988) Age determination using naturally occurring radionuclides. Australian Marine Sciences Association Conference, Sydney, N.S.W.

Fenton, G.E., Ritz, D.A. and Short, S.A. (1989) How old is Orange Roughy?

Australian Marine Sciences Association Conference, Wirrina, South Australia. (Poster)

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Fenton, G.E., Short, S.A. and Ritz, D. A. (in press) Age determination of Orange

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# APPENDIX A: RADIOCHEMICAL ANALYSIS OF BLUE GRENADIER OTOLITHS

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## $^{210}Pb/^{226}Ra$ Disequilibria in Otoliths of Blue Grenadier, *Macruronus novaezelandiae*; Problems Associated with Radiometric Ageing

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*Abstract*

Otoliths from blue grenadier (*Macruronus novaezelandiae*), which had been aged previously by annuli analysis, were analysed for the naturally occurring radionuclides $^{210}Pb$ and $^{226}Ra$ in an attempt to independently verify their age. However, the radiometric technique could not be applied to determine age because the results showed that $^{226}Ra$ was not incorporated at a constant rate throughout the life of *M. novaezelandiae*. Uptake of $^{226}Ra$ was greater in juveniles than in adult fish. This was probably due to the juvenile phase inhabiting inshore/estuarine waters.

### Introduction

An accurate knowledge of the age structure of a fish population is one of the fundamental criteria necessary to manage a fishery. One of the most widely used methods to determine fish age is based on counting annuli in otoliths. However, it is often difficult to independently validate the ages obtained (Beamish and McFarlane 1987). Kenchington and Augustine (1987), in a study of the age and growth of blue grenadier (*Macruronus novaezelandiae*) in south-eastern Australian waters, found that none of the conventional techniques of validating their otolith annuli data could be applied to this relatively new fishery. For example, tagging is not feasible, growth is too slow to enable modal analysis, the otolith annuli are too narrow and complex to support marginal increment analysis or edge-type analysis, and the fishery has not been underway long enough to be able to follow strong year classes.

An alternative, independent method for age determination that might prove useful for ageing blue grenadier is radionuclide analysis. Over recent years, analysis of several different isotope pairs ($^{228}Th/^{228}Ra$, $^{210}Po/^{210}Pb$ and $^{210}Pb/^{226}Ra$) from the uranium and thorium decay series has been used in ageing studies. For example, the pair $^{228}Th/^{228}Ra$, useful in the range 0–10 years, has been used to verify annual banding in modern corals (Dodge and Thomson 1974) and to age vesicomyid clams growing in hydrothermal areas (Turekian *et al.* 1983). The pair $^{210}Po/^{210}Pb$, useful only in the range 0–2 years, was used to measure the growth rate of *Nautilus pompilius* (Cochran *et al.* 1981). The pair $^{210}Pb/^{226}Ra$ is potentially very useful since it provides age data in the range 0–100 years. This pair was used in the only other radiometric ageing study of a teleost fish that has been published, to confirm the longevity of the splitnose rockfish, *Sebastes diploproa* (Bennett *et al.* 1982).

Radiometric age determination using $^{210}Pb/^{226}Ra$ is based on the fact that these isotopes are in radioactive disequilibrium when they are first incorporated into an otolith. Thereafter, they gradually approach the equilibrium state, in which the rates of decay of the two nuclides reach equality, which in this case takes about 100 years. It is the extent of the

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disequilibrium that acts as a natural chronometer. For the radiometric ages to be valid, several assumptions must be met. These assumptions were detailed by Bennett *et al.* (1982), but it is important to restate them here:

1. Both $^{226}$Ra and $^{210}$Pb are taken into the otolith at a rate that is always in a constant ratio to the rate of mass increase of the otolith.
2. The rate of uptake of $^{226}$Ra significantly exceeds that of any $^{210}$Pb; i.e. allogenic $^{210}$Pb is much less than $^{226}$Ra and can therefore be distinguished from radiogenic (authigenic) $^{210}$Pb.
3. No losses or gains of $^{226}$Ra and $^{210}$Pb occur after uptake, other than by radioactive decay or ingrowth.

Any violation of assumptions 1 and 2 can be identified from the results of the assays, particularly by analysing juveniles of the species. The specific activity (activity per unit mass) of $^{226}$Ra in the otoliths should be invariant with increase in mass of the otolith (i.e. invariant with age). The specific activity of $^{210}$Pb would be a function of the decay of any allogenic $^{210}$Pb and ingrowth of authigenic $^{210}$Pb (from the decay of $^{226}$Ra). Thus, the specific activity of $^{210}$Pb would be expected to increase with time. Assumption 3 is based on the observations of numerous authors (reviewed by Veeh and Burnett 1982) that $^{226}$Ra and $^{210}$Pb distributions in modern aragonitic corals are not compatible with any significant loss of $^{222}$Rn, or other nuclides, in the $^{238}$U decay chain between $^{226}$Ra and $^{210}$Pb.

These three assumptions were met in the study by Bennett *et al.* (1982), but in a recent study in which sharks were aged by $^{210}$Pb, serious problems were experienced with violation of the 'closed system' and 'constant uptake' assumptions (Weldon *et al.* 1987). Of the 4 species of sharks analysed, the radiometric ages for only 2 were consistent with estimates based on other methods. The variability observed was thought to be due both to the analytical limitations of low-level radioactive analyses and to physiological processes within the calcified cartilage of the vertebral centra used for analysis (Weldon *et al.* 1987).

In the present study, the applicability of the $^{210}$Pb/$^{226}$Ra radionuclide ageing technique to blue grenadier has been tested, using otoliths contralateral to those analysed by Kenchington and Augustine (1987).

## Materials and Methods

Otoliths (sagittae) from blue grenadier were collected during 1984–85 by the CSIRO Division of Fisheries and the Tasmanian Department of Primary Industry, Division of Sea Fisheries. The analysis was restricted to female fish whose otoliths had been stored dry and for which collection date and age data (estimated from annuli) were available. (Details of the annuli analysis are given in Kenchington and Augustine 1987.) Samples of about 1 g were selected for radiochemical analysis. This involved pooling otoliths, but only otoliths recorded as having the same annuli age were pooled together. Since at least 1 g of material was required for each analysis, pooled sample sizes ranged from 30 otoliths (age class 0+ years) to 2 otoliths (age class 21+ years). Details of fish collection data and otolith sample sizes are given in Table 1.

Radiochemical analysis of $^{226}$Ra and $^{210}$Pb requires the best available ultra-low-level techniques and rigorous allowance for all possible sources of significant error, such as *in situ* allogenic nuclide contamination (i.e. from within the fish but outside the aragonitic closed system of the otolith), chemical blank values, and instrumental backgrounds. Both isotopes were analysed by alpha spectrometry: $^{226}$Ra was measured directly, but $^{210}$Pb was measured via its alpha-emitting granddaughter, $^{210}$Po, which would be (at 1 or more years after collection) within at least 5% of secular equilibrium with $^{210}$Pb.

Details of the analytical method are given below since it was necessary to adapt existing procedures to measure these isotopes at the extremely low levels encountered.

### *Radiochemical Analysis*

Gamma spectra were collected with a lead-shielded Ortec Gamma-X 50-mm-diameter, high-purity-germanium, coaxial gamma detector interfaced (via an NIM bin-based Ortec Model 572 amplifier) to

a Lecroy Model 3500 multichannel analyser. Some 4000 channels were dedicated to the full 0–2 MeV gamma spectrum. Alpha spectrometric measurements were made with either Tennelec Model TC256 or Ortec Model 676 single-chamber spectrometers also interfaced (via a Lecroy Model 3542 mixer–router and Lecroy Model 3511 ADC) to the Lecroy multichannel analyser. Some 512 channels were dedicated to each alpha spectrometer, covering the 3–8 MeV energy range.

Computation of otolith $^{226}Ra$ and $^{210}Po$ activities was carried out on an IBM AT-compatible personal computer fitted with an 80287 maths coprocessor. The programs correctly propagated all known systematic errors (counting errors, errors in computed chemical yield, errors in tracer calibration, etc.) to the final computed assay result. This is absolutely essential for such ultra-low-level analytical determinations so that the precision of those determinations is not overestimated.

All alpha spectrometers used had been modified to incorporate the anti-recoil-contamination system described by Sill and Olson (1970). By employing this system, alpha instrumental backgrounds were maintained at the very low levels necessary for the 2–3 week counting periods required here. Background rates in the four alpha spectrometers used here were invariably less than 24 counts per day over the full MeV range. These background rates are approximately 10 times lower than for the emanation method used by Bennett *et al.* (1982) for $^{226}Ra$.

All reagents used were analytical reagent grade. The mean $^{210}Po$ activity of reagent blanks, spiked with $^{208}Po$ tracer and processed as though they were samples, was $0{\cdot}0103 \pm 0{\cdot}0027$ dpm. This finding is in marked contrast to the claim of Bennett *et al.* (1982) that no blank $^{210}Po$ activity was detectable above instrumental background. The mean $^{226}Ra$ activity of reagent blanks was $0{\cdot}0125 \pm 0{\cdot}0043$ dpm. This value should be compared with the mean blank $^{226}Ra$ activity of 0·039 found by Bennett *et al.* (1982).

Trial dissolutions of whole otoliths in dilute HCl showed that they were enclosed within a chemically resistant membrane, which formed a continuous sheath to which may have been attached the remnants of the semicircular canals. The removal of this adherent organic matter is of critical importance because it is generally recognized that marine tissue is capable of accumulating high levels of $^{210}Po$ (Cherry and Shannon 1974; Heyraud and Cherry 1979; Cherry and Heyraud 1982). If this step is not taken contamination with allogenic $^{210}Po$ derived from sources external to the otolith is potentially a serious problem, given the low concentrations involved. Several pretreatments were used in an attempt to remove this organic layer. The otoliths were pretreated with 'Fenton's reagent' (30% $H_2O_2$ at 50°C in the presence of trace $Fe^{3+}$ catalyst) for varying periods of time to remove the organic envelope, and then washed. The choice of Fenton's reagent was based on the fact that it has been used successfully in radiochemistry for the destruction of organic matter (Sansoni and Kracke 1971; Bock 1979), including relatively resistant material such as ion-exchange resins (Kubota 1983). Bennett *et al.* (1982) also pretreated otoliths with $H_2O_2$ (without catalyst), but at an unspecified concentration, temperature and period of exposure. In addition to the trials with Fenton's reagent, 2 samples were treated with 30% $H_2O_2$ (without catalyst) under strong ultraviolet illumination for 11 h.

After removal of the organic layer, two tracers ($^{208}Po$ and $^{133}Ba$) and 100 μg of Ba carrier were added. The otoliths were then completely dissolved in dilute HCl. A decontaminated Teflon stirrer-bar was added and the beaker placed on a magnetic stirrer hotplate. The temperature was then raised to 80–90°C and the pH adjusted to 1·5 with concentrated ammonium hydroxide. After addition of 1·00 g of hydroxylamine hydrochloride, a polished silver disc mounted in a custom-made, high-density polythene holder was placed upside down in the hot solution and stirred evenly for 3 h. At the end of this autodeposition period, the silver disc was removed, washed carefully with water and ethanol, and dried prior to submission to alpha spectrometry for a period of 2–3 weeks. Total counts under both the $^{208}Po$ and the $^{210}Po$ alpha peaks were determined.

The otolith solution was then transferred to a clean 1 L beaker and diluted to 1 L, and 10 mL of concentrated $H_2SO_4$ and 10 g of $K_2SO_4$ were added. Ba and Ra were precipitated by slowly adding, with stirring, 100 mg of Pb [as 10 mL of a 10 mg $mL^{-1}$ Pb carrier solution made from $Pb(NO_3)_2$ in 0·1 M $HNO_3$] to co-precipitate Ba and Ra with $PbSO_4$. In order to maximize Ba and Ra recovery, settling was allowed to continue for 16 h. The Pb/Ba/Ra$SO_4$ precipitate was then collected on a 47 mm, 0·45 μm filter (Millipore), washed with 0·5% $H_2SO_4$, and placed face up in a clean 80 mL beaker. The precipitate was fully solubilized by the addition of 10 mL of 0·2 M alkaline diethylenetriamine penta-acetic acid (pH 10) with alternate warming and ultrasonic agitation. The solution was then filtered through a plastic syringe fitted with a disposable Millex 0·45 μm filter (Millipore) into a clear polystyrene vial containing 1 mL of 20% $Na_2SO_4$ and 1 drop of methyl red indicator (to confirm alkaline conditions). The solution was then cooled to room temperature. In quick succession, 2 mL of 10% $H_2SO_4$ and 200 μL of preformed $BaSO_4$ (125 μg Ba $mL^{-1}$) seeding suspension were added (Sill

1983). The suspension was left at room temperature for 30 min, then filtered through a smooth-surface cellulose nitrate filter 0·05 μm (Sartorius), and the precipitate was gently washed with a little 0·5% $H_2SO_4$. The filter was placed on a clean glass Petri dish and dried under an infrared lamp. Once dry, the filter was first transferred to a small plastic Petri dish and placed on top of a high-resolution crystal gamma detector. The gamma spectrum from this source was measured for exactly 10 min and the area under the 356 keV emission peak determined. The Ba (and Ra) recovery was then determined by reference to a standard source made by evaporating $^{133}Ba$ tracer solution onto a similar filter membrane. The Ra membrane source was then pressed with a thin film of pure petroleum jelly onto a normal alpha spectrometric source holder and inserted into the alpha spectrometer. The alpha spectrum was recorded between 3 and 8 MeV for 2–3 weeks and the $^{226}Ra$ counts determined between 3 and 4·85 MeV.

Using these simple chemical separation techniques, workers at the Environmental Radiochemistry Laboratory, Australian Nuclear Science and Technology Organisation, have verified that recoveries of Ba and Ra are esentially identical. This is in accordance with the findings of other workers (e.g. Sill 1983; Dean and Chiu 1984).

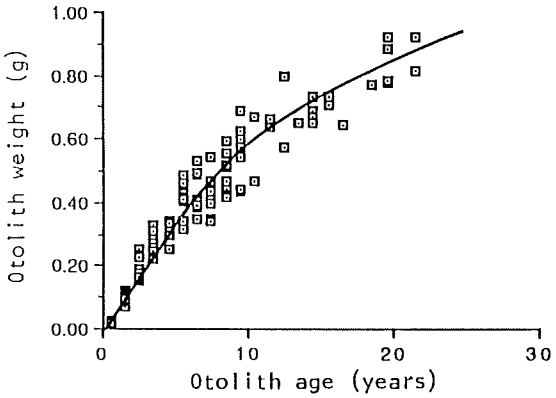


Fig. 1. Otolith mass growth history *M. novaezelandiae* ($n$ = 158).

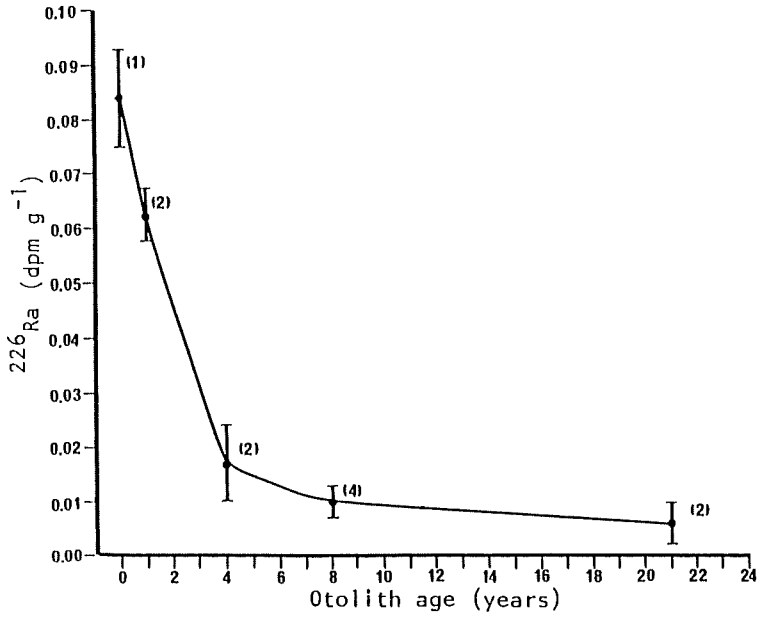


Fig. 2. $^{226}Ra$ specific activity of *M. novaezelandiae* otoliths *v.* age determined by annuli analysis. Numbers in parentheses indicate numbers of individual analyses. Uncertainties are 1$\sigma$ (1 s.d.).

**Table 1. $^{226}Ra$ and $^{210}Po$ assays of otoliths of blue grenadier (*M. novaezelandiae*) from waters around Tasmania**

Annuli-based age determinations are taken from Kenchington and Augustine (1987). All errors are quoted at the level of $1\sigma$ (1 s.d.) and are fully propagated as described in the text. n.a., Not available.

| Collection date, locality, depth | Age from annuli (years) | Number of otoliths in sample | Ra chemical recovery (%) | Specific activity $^{226}Ra$ (dpm $g^{-1}$) | Specific activity $^{210}Po$ (dpm $g^{-1}$) | Pretreatment |
|---|---|---|---|---|---|---|
| 30.i.85, Derwent River estuary, depth n.a. | 0+ | 30 | 79·9 ± 1·0 | 0·084 ± 0·009 | 0·009 ± 0·005 | Fenton's reagent, 16 h |
| 10.ix.84, Eastern coast off Maria Island, 195–460 m[A] | 1+ | 7 | 85·5 ± 1·1 | 0·075 ± 0·008 | 0·028 ± 0·007[B] | Fenton's reagent, 16 h |
| | 1+ | 7 | 65·4 ± 1·0 | 0·050 ± 0·005 | 0·003 ± 0·003 | Fenton's reagent, 24 h |
| 12.ix.85, Eastern coast off St Helens, 450–550 m | 4+ | 5 | 42·8 ± 0·7 | 0·016 ± 0·010 | 0·004 ± 0·006 | $H_2O_2$/UV, 11 h |
| | 4+ | 4 | 78·9 ± 1·0 | 0·018 ± 0·009 | 0·010 ± 0·009 | Fenton's reagent, 16 h |
| 14.viii.85, Western coast, depth n.a. | 8+ | 3 | 39·5 ± 0·7 | 0·014 ± 0·010 | 0·017 ± 0·006[B] | $H_2O_2$/UV, 11 h |
| | 8+ | 3 | 93·6 ± 1·1 | 0·018 ± 0·005 | 0·007 ± 0·004 | Fenton's reagent, 16 h |
| | 8+ | 3 | 87·5 ± 1·1 | 0·004 ± 0·006 | 0·030 ± 0·009[B] | Fenton's reagent, 1 h |
| | 8+ | 3 | 85·7 ± 1·1 | 0·005 ± 0·004 | 0·005 ± 0·005 | Fenton's reagent, 16 h |
| 14.viii.85, Western coast, depth n.a. | 21+ | 2 | 79·9 ± 1·0 | 0·005 ± 0·005 | 0·007 ± 0·004 | Fenton's reagent, 16 h |
| | 21+ | 2 | 85·5 ± 1·1 | 0·006 ± 0·006 | 0·015 ± 0·007[B] | Fenton's reagent, 1 h |

[A] Mid-water trawl. [B] Incomplete removal of adherent organic matter.

## Results

A mass growth curve for otoliths of blue grenadier is presented in Fig. 1. The results of the $^{226}$Ra and $^{210}$Po assays for blue grenadier otoliths are presented in Table 1, and the $^{226}$Ra specific activities are plotted in Fig. 2. No increase in $^{210}$Po specific activity was observed with increasing age of blue grenadier.

It is evident from the variability of the $^{210}$Po data that the organic layer is extremely resistant to chemical oxidation. An exposure period of not less than 16 h to Fenton's reagent appears to be required for its complete removal. The $H_2O_2$/ultraviolet treatment for 11 h was clearly not successful. The importance of pretreatment to remove the adherent organic layer on otoliths should not be underestimated.

## Discussion

The possibility that the ages determined by annuli analysis (Kenchington and Augustine 1987) are incorrect must be considered, but the radionuclide data obtained in the present study cannot be used to either verify or reject those results. This is because blue grenadier did not accumulate $^{226}$Ra in its otoliths at a constant rate throughout its life. Uptake of $^{226}$Ra was greatest during its first few years of life. Thus, since the assumption of constant uptake of $^{226}$Ra has been violated, age cannot be determined by the radiometric method.

Juveniles less than 40 cm long, besides being caught in the same habitat as adults, are common in the large bays and estuaries of southern Tasmania, with some individuals even penetrating upstream into fresh water. On the other hand, adults are found in 500–700-m-deep water on the continental slope (Last *et al.* 1983). Environmental levels of $^{226}$Ra are generally substantially higher in estuarine/inshore coastal waters than in shelf waters (Cochran 1982; Levy and Moore 1985). Fish in the 0+ age class whose otoliths were analysed in the present study were captured in shallow water in the Derwent River estuary, and high $^{226}$Ra levels might thus be expected. However, all other samples analysed were taken in slope water off the eastern or western coast of Tasmania (Table 1). Acquisition of a high level of $^{226}$Ra early in life would seem to be confirmed by the similarly high levels in 1+ fish from eastern Tasmania, where ambient levels would be considerably lower. This would explain why the $^{226}$Ra specific activity of the otoliths declines systematically with age at a rate that probably is largely dictated by the rate of mass increase (growth) of the otolith (Fig. 2).

Furthermore, the $^{226}$Ra specific activities for fish older than approximately 10 years are so low ($<0{\cdot}010$ dpm g$^{-1}$) that their precision is dubious above that age. In fact, the low levels of $^{226}$Ra measured in adult fish are at least 4 times lower than that found in another deep-sea species, the splitnose rockfish (Bennett *et al.* 1982). This suggests a metabolic difference in the rate of uptake of $^{226}$Ra in blue grenadier compared with the splitnose rockfish.

The $^{210}$Po data suggest that the assumption of a constant ratio of initial $^{226}$Ra to allogenic $^{210}$Pb deposited in each growth zone being invariant with time also may not be strictly true for blue grenadier, again invalidating the radiometric method. Weldon *et al.* (1987) also found that the uptake of $^{210}$Pb was not constant in the shark species they examined, and they suggested that this was due to either habitat or diet changes with increasing age.

In conclusion, the failure of the $^{210}$Pb/$^{226}$Ra radiometric method to determine the age of blue grenadier could be attributed largely to a major change in habitat that occurs between the juvenile and adult stages of the fish. The result, although a negative one in terms of age determination, is valuable in that it indicates a type of species that could not be profitably aged by this method (i.e. a species known to have a major change in habitat during its life cycle).

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# APPENDIX B: RADIOCHEMICAL ANALYSIS OF ORANGE ROUGHY OTOLITHS- PAPER IN PRESS MARINE BIOLOGY 1991

## Age determination of Orange Roughy, Hoplostethus atlanticus (Pisces:Trachichthyidae) using $^{210}Pb/^{226}Ra$ disequilibria.

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### Abstract

Natural levels of $^{210}Pb/^{226}Ra$ in otoliths of orange roughy were measured to determine fish ages radiometrically. Up to maturity, radiometric ages estimates were consistent with a single constant otolith growth rate. Radiometric ages for juveniles were comparable with, but greater than, those obtained in a recent, validated New Zealand study which employed counts of annuli on the surface of otoliths. Beyond maturity, radiometric ages were obtained by modelling with an otolith growth rate set at 45% of the juvenile rate. Radiometric ageing confirms orange roughy is very slow-growing with age at maturity (32 cm SL) ~32 years, and is very long-lived, with fish 38-40 cm 77 - 149 years old. These results have important implications for the management of the fishery.

### Introduction

Orange roughy (Hoplostethus atlanticus) forms the basis of a major trawl fishery in south-east Australia and in New Zealand waters. Commercial catches are taken in the mid-slope region, in water mainly in the 900-1100m depth zone. In order to manage the fishery, it is essential that the age of the species can be determined. However, orange roughy has proven very difficult to age.

Several unvalidated attempts to age orange roughy by counting growth rings (annular and/or daily) on the otolith surface have produced quite different results (Kotylar, 1980; van den Broek 1983; Williams 1987; Gauldie, 1988a). Estimates of the age at maturity c. 30cm standard length (SL) have ranged from 5-12 years (presented in Fig. 1 in Mace et al. 1990 ). The interpretation of the rings seen on orange roughy otoliths as annuli has, however been questioned in studies of the fine structure of the otoliths (Gauldie, 1987, 1988b and 1990) and in a study of their morphology (Linkowski and Liwoch, 1986). Techniques capable of validating ages for all age groups have not been possible for orange roughy, e.g. mark-recapture experiments and capture of known-age fish (Beamish and McFarlane 1983). In

addition, methods which can validate ages during the initial period of fastest growth, e.g. analysis of length-frequency modes, monitoring of a strong year class, comparison of back-calculated lengths with observed lengths of the corresponding age group, examination of the edge of a structure, and analysis of growth (Beamish and McFarlane 1983), have also been unsuccessful for orange roughy. However, Mace et al. (1990) have recently managed to relate hyaline rings, present on the surface of otoliths, to the progression of length-frequency modes in a population of small juvenile fish <10cm standard length (SL) on the north Chatham Rise New Zealand. They provided validated ages for the age classes 0+, 1+ and 2+; however, age could not be validated beyond the 2+ age class. Furthermore, Mace et al. (1990) experienced increasing uncertainty with the interpretation of otolith rings with more than 7-8 hyaline zones. They found growth to be slow, with age at maturity estimated to be 20 years. The maximum (unvalidated) age Mace et al. (1990) estimated by counting surface annuli was 42 years, for a 36cm fish.

An alternative method of age determination that might be applicable to establish the longevity of orange roughy is radiometric analysis. Recently, Bennett et al. (1982) and Campana et al. (1990), analysed the naturally occurring radionuclides $^{210}Pb$ and $^{226}Ra$ in fish otoliths to determine the age of the deep-sea Sebastes species, S.diploproa and S.mentella respectively. The technique is based on the fact that $^{226}Ra$ (half-life 1600 years) is incorporated into the otolith, where it decays to $^{210}Pb$ (half-life 22.3 years). The ratio of $^{210}Pb/^{226}Ra$, present in an otolith, is dictated by the decay rates of these two isotopes and therefore provides an independent measure of the time elapsed since $^{226}Ra$ was incorporated.

The radiometric ageing technique when applied to whole otoliths assumes:
1) a constant ratio of uptake of $^{226}Ra$ to $^{210}Pb$ taken into the otolith during growth,
2) uptake of $^{226}Ra$ significantly exceeds that of any $^{210}Pb$, and
3) the aragonite of the otolith acts as a closed system for incorporated $^{226}Ra$ and all isotopes in its decay chain down to and including $^{210}Po$.
Any violation of the first two assumptions can be easily identified in the results of the assays. The third assumption is based on the studies of aragonitic coral structures by numerous authors (reviewed by Veeh and Burnett 1982). A more detailed discussion of these assumptions is given in Fenton et al. (1990) in which violation of assumption 1 was observed during radiometric analysis of Blue Grenadier (Macruronus novaezelandiae) otoliths. This violation, in the case of blue grenadier, was thought to be related to a major shift between juvenile and adult life from an inshore/estuarine habitat to an offshore/ deepsea habitat.

Unfortunately the life history of orange roughy is poorly understood. In particular, little is known about the early juvenile stage. The first spawning aggregation in Australian waters was only discovered in winter 1989, off the east coast of Tasmania (Lyle et al. 1989a). Fertilised orange roughy eggs are positively buoyant, but it it not

known whether they rise above the thermocline (at a depth of 200-300m off the east coast of Tasmania). No larvae have been found in Australian waters (Lyle et al. 1989a) and only two have been found in New Zealand waters (Mace et al. 1990). Furthermore, juveniles less than 10-15cm are rarely caught. However, the results of stock discrimination studies (Lester et al. 1988, Ovenden et al. 1989) have indicated that orange roughy is a sedentary species, and dietary studies (Rosecchi et al. 1988, Bulman pers. comm.) suggest that it does not undergo vertical migration. Therefore, all available evidence regarding the life history of orange roughy indicate that it should be suitable for radiometric ageing analysis.

In the present study, orange roughy otoliths from a range of size classes have been analysed using the radionuclide ageing technique.

## Materials and Methods

Orange roughy were collected during cruises by the Tasmanian Department Primary Industry, Division of Sea Fisheries and the CSIRO Division of Fisheries during 1988-1989. Sagittal otoliths were removed and stored dry. All otolith samples were collected from fish caught off the west coast of Tasmania in the 800-1200m depth zone, with the exception of two samples composed of small fish (<15cm SL) LH752 and LH753, since fish in this size range were rarely caught. Sample LH752 (n=135 otoliths) included 2 otoliths from fish caught off the east coast of Tasmania and 41 otoliths from fish collected near Kangaroo Island, South Australia. Sample LH753 (n= 102) included 8 otoliths from fish caught off the east coast of Tasmania.

Size frequency analysis of orange roughy populations has indicated female fish grow larger than male fish (Newton 1988, Mace et al. 1990). Therefore radiochemical analysis was restricted to female fish, except for samples of small fish (<15cm SL).

Individual otoliths were weighed to the nearest 0.1mg. Samples of at least 1g were required for radioanalysis. This required pooling of otoliths. The relationship between fish length and otolith weight (plotted in Fig. 1), showed that otoliths from adult fish exhibited a large variation in weight, between 0.1500 and 0.4300g. Therefore, samples for radioanalysis were pooled on the basis of similarity of otolith weight and fish length.

### Radioanalysis

Otoliths were cleaned by soaking in 30% $H_2O_2$ for 16-24 hours at 50°C (no $Fe^{3+}$ catalyst was added). They were then successively rinsed with water, 0.05M $Na_4EDTA$ (alkaline EDTA, pH10.5), water, 0.05M $Na_4$ EDTA, water (twice), 0.1M HCl (for less than 10 seconds) and finally water (twice). This extensive pretreatment is necessary to ensure the removal of the adherent, transparent organic sheath of the otolith and any associated allogenic $^{210}Po$ and $^{226}Ra$. The importance of thorough decontamination, particularly from the $^{210}Po$-rich tissue component, was established in

our earlier radioanalyses of blue grenadier Macruronus novaezelandiae otoliths (Fenton et al. 1990).

The analysis of $^{210}Pb$ via its alpha-emitting, short-lived daughter proxy $^{210}Po$, followed the method we have previously described (Fenton et al. 1990), employing high resolution alpha-spectrometry. Polonium-210 was assumed to be in equilibrium with $^{210}Pb$ in all samples. Even if no polonium was deposited with the proteinaceous fraction of the developing otolith, non-equivalence between $^{210}Pb$ and $^{210}Po$ could have been marginally significant (introducing a negative error of up to one year) only for the very youngest sample (LH752), which was analysed about 11 months (2.5 half-lives of $^{210}Po$) after collection. A $^{208}Po$ tracer was employed to measure chemical yield. Mean $^{210}Po$ reagent blank was 0.0103±0.0027 disintegrations per minute (dpm). Recovery of $^{210}Po$ was invariably >90%. Instrumental background counts (for $^{208}Po$ and $^{210}Po$) were less than 1 count per day.

Analysis of radium-226 was made using a direct alpha spectrometry technique (Fenton et al. 1990). Chemical yield through these few simple steps was measured by gamma-spectrometry of a $^{133}Ba$ tracer (Fenton et al. 1990). Mean activity of the $^{226}Ra$ blanks was 0.0255±0.0023 dpm, significantly lower than the 0.033-0.043 dpm found by Bennett et al. (1982). Recovery of $^{226}Ra$ (as estimated by the recovery of $^{133}Ba$ tracer) was always >85% except for sample LH569 (19.9±0.5%) and LH570 (47.2±0.8%).

Both the silver disk Po sources and the 0.05µm membrane filter, colloidal $Ba/RaSO_4$ alpha sources were counted for periods of up to 3 weeks to accumulate sufficient counts for acceptable statistics. Very low and constant background count rates were maintained over these extended count times by use of an electrostatic anti-recoil system devised by Sill and Olson (1970). Using the method the $^{226}Ra$ instrumental background (2-5 counts/day between 3 and 4.8 MeV) was approximately 10x lower than obtainable in the radon gas emanation techniques used by Bennett et al. (1982) and Campana et al. (1990).

## Results

The specific activity of $^{226}Ra$ was similar in fish from all size classes. However, there appeared to be a weak trend in $^{226}Ra$ specific activities with age. The younger group (samples numbers 569, 664, 751, 752, and 753) had a mean specific activity of 0.0522±0.0036 $dpm.g^{-1}$ and the older group (sample numbers 570, 666, 668, 748, 749, 750, and 1240) had a mean activity of 0.0625±0.0030 $dpm.g^{-1}$. This weak trend is possibly due to decreasing total protein content of otoliths with age (Kalish, 1989). These activities were used to compute corrected $^{210}Po$ specific activities and $^{210}Pb/^{226}Ra$ activity ratios for the two groups respectively.

Activity ratios are given in Table 1. The range of activity ratios measured (0.06 - 0.87) covers essentially the entire possible range for curves described by simple models

based on one or two linear growth phases (for the otolith) such as Bennett et al. (1982) described. The $^{210}Pb/^{226}Ra$ initial activity (uptake) ratio (R) must be commensurate with or less than that found in the youngest sample (sample 752). The activity ratio of the youngest sample suggests the value of R should be 0.05 or less; a value of 0.05 was chosen. We believe there is some likelihood of trace lead incorporation into the proteinaceous fraction of the otolith. This assumption also has the effect of rendering all following estimates of longevity conservative.

The average age of the otoliths can be calculated quite simply from the degree of $^{210}Pb$ ingrowth (assuming an initial activity ratio of 0.05) :

$$t = \frac{-1}{\lambda_p}\left[\frac{\ln(1-A)}{1-R}\right] \qquad (1)$$

where $t$ = age (years)

$\lambda_p$ = decay constant for $^{210}Pb$ (0.03114 years$^{-1}$)

$A = (^{210}Pb/^{226}Ra)_t$ = activity at time t

$R = (^{210}Pb/^{226}Ra)_o$ = initial activity ratio at time of deposition

The values obtained are given in Table 2. These values do not represent fish age since Eq. (1) takes no account of the mass-growth of the otolith .

To obtain estimates of fish age it is therefore necessary to incorporate a model describing otolith mass-growth in to the calculation. Ages have been calculated on the basis of a single constant (linear) growth rate (Table 2) using the equation derived by Bennett et al. (1982):

$$A = 1 - (1-R)\frac{1-e^{-\lambda_p t}}{\lambda_p t} \qquad (2)$$

where $A = (^{210}Pb/^{226}Ra)_t$ = activity at time t

$R = (^{210}Pb/^{226}Ra)_o$ = initial activity ratio at time of deposition

$\lambda_p$ = decay constant for $^{210}Pb$ ( 0.03114 year$^{-1}$)

The ages of the 34cm (LH664) and 37cm (LH1240) samples are 36 and 47 years respectively which compare closely to the oldest (unvalidated) age estimated from the Mace et al. (1990) study of annuli on the surface of otoliths for a 36cm fish of 42 years. (Note that this single estimate is at some variance from the age at 36 cm SL (32 years) predicted by the Von Bertalanffy equation chosen by Mace et al. (1990) from their annuli counts for juvenile fish.) If the mass-growth rate of orange roughy otoliths

remains linear throughout the lifespan, ages in excess of 200 years are to be found.

Ages for fish up to about 34 cm SL correlated very well with otolith mass, giving an otolith growth rate of 3.94 mg.y$^{-1}$ by weighted regression of age on mass ($r^2$ = 0.9417). The relationship clearly changes beyond this fish length which is in accordance with maturation occurring (32cm SL in Lyle et al., 1989b). Our data suggests that maturity occurs at about 32 years.

However, the growth of fish otoliths (as determined by its linear dimensions) generally slows after maturity is reached (Pannella, 1980) and continues at a reduced rate until maximum fish size is achieved (Williams and Bedford, 1974). Thereafter the thickness (and hence weight) of the otolith increases (Boehlert, 1985). Reliable values of the mass-growth rates of otoliths throughout a species lifespan are difficult to find in the literature. Bennett et al., (1982) in their radiometric ageing study of Sebastes diploproa estimated growth slowed to 30% of its initial rate beyond age 14 years. We conclude that ages computed using a single growth rate throughout the lifespan are not appropriate for orange roughy beyond maturity. A slower growth for the otolith beyond this age is inferred.

Therefore the ages for the older group have been recomputed on the basis of a two phase growth rate model essentially as derived by Bennett et al. (1982):

$$A e^{-\lambda_R t} = \frac{t_1 G_1}{M_t}\left[1-(1-R)\left\{\frac{1-e^{-\lambda_p t_1}}{\lambda_p t_1}\right\} e^{-\lambda_p (t-t_1)}\right] + \frac{(t-t_1)\,G_2}{M_t}\left[1-(1-R)\left\{\frac{1-e^{-\lambda_p (t-t_1)}}{\lambda_p (t-t_1)}\right\}\right] \quad (3)$$

where $A = (^{210}Pb/^{226}Ra)_t$ = activity ratio at time t

$R = (^{210}Pb/^{226}Ra)_0$ = initial activity ratio at time of deposition

$\lambda_p$ = decay constant for $^{210}$Pb ( 0.03114 year$^{-1}$)

$\lambda_R$ = decay constant for $^{226}$Ra ( 0.00043 year $^{-1}$)

$G_1$ = otolith mass growth rate until maturity (mg.y$^{-1}$)

$G_2$ = otolith mass growth rate after maturity (mg.y$^{-1}$)

$t_1$ = age at maturity (year)

$M_t$ = otolith mass at age t

The extra term $e^{-\lambda_R t}$ accounts for the minor decay of $^{226}$Ra from the specific activity laid by the aragonite at time of deposition. The resultant ages are shown in Table 2.

These ages have been calculated conservatively. An initial activity ratio (R) of 0.05 was assumed. Pre-maturity growth rate ($G_1$) was set at 3.94 mg.y$^{-1}$ as determined from the younger sample group (described above) using the linear growth model. Maturity ($t_1$), where otoliths are presumed to change from the first growth rate to the second is clearly near the age of sample LH664. For the purposes of the model maturity was set at 32 years (corresponding to a length of 32cm SL, Lyle et al. 1989b). A

growth rate ($G_2$) after maturity of 45% of the pre-maturity value (1.77 mg.y$^{-1}$) was also assumed. The selection of $G_2$ is however, an arbitrary value and it should be stressed that the validity of this value cannot be independently confirmed from our data. The rate of 45% chosen here for orange roughy is probably conservative compared to the value of 30% measured for the splitnose rockfish (Bennett et al. 1982). Our data shows that values of $G_2$ significantly lower than 45% of $G_1$ can only result in ages >>149 and >>127 years respectively for the oldest samples (LH 749 and 750) which have the most precisely known activity ratios and also the greatest mean otolith mass. Furthermore, for $G_2$ <<1.77 mg.y$^{-1}$, double growth rate model ages begin to significantly exceed ages estimated from the single growth rate model (156 and 117 years for these samples respectively) (Table 2). We believe this is not reasonable. Regardless of the otolith growth model adopted, mean otolith ages of the oldest samples (51 and 42 years respectively for these samples - see Table 2) should still be much less than 1/2 of the actual fish age. This is verified by the comparison of mean otolith age and single-growth rate otolith age for the six youngest samples (LH752, 753, 569, 751, 664 and 1240). Note that for samples LH 664 and 1240 the single-growth rate otolith age begins to exceed twice the mean otolith age. Thus we predict absolute minimum ages for samples LH 749 and 750 of about 102 and 84 years respectively. From these considerations we suggest that ages of 149±12 and 127±11 years respectively (for samples 749 and 750) found from the double-growth rate model, by setting the post-maturity growth rate at 1.77 mg.y$^{-1}$ or 45% of the pre-maturity rate, are not unreasonable.

Our radiometric ages versus fish standard length are plotted in Figure 2. We have fitted a Von Bertalanffy growth equation to our data:

$$L_t = L_\infty [1 - e^{-K(t-t_0)}] \qquad (4)$$

where
$L_t$ = length at age t (SL cm)
$L_\infty$ = maximum average length (SL cm)
K = Brodie Growth Coefficient (year$^{-1}$)
$t_0$ = intercept age at $L_t$ = 0 (years)

In view of the high level of error associated with the radiometric analysis of youngest sample (LH 752) this point was omitted from the calculation of the Von Bertalanffy equation. The resulting Von Bertalanffy growth curve is plotted in Fig. 2, with the parameter estimates $L_\infty$ = 40.05±0.53; K = 0.044±0.004; $t_0$ =-2.663±1.347. For comparison, the Von Bertalanffy equation chosen by Mace et al. (1990) from these studies of juvenile fish, with the parameter estimates of $L_\infty$ = 42.5±2.11 cm; K = 0.059±006; $t_0$ = -0.346±0.153 years is also plotted in Figure 2. Comparison of the two Von Bertalanffy growth curves needs to take into account the type of data used to

derive each. A larger error is associated with the estimation of $t_0$ using the radiometric data compared to Mace et al.'s (1990) data which is to be expected since their data is largely based on juvenile fish whereas we have no data for fish <10cm. Differences in the $L_\infty$ value are to be expected since the largest fish we analysed was 40.40cm SL. Nevertheless the difference between the Mace et al. (1990) estimates for ages of immature fish and our (radiometric) estimates is not great. However, the radiometric results suggest growth is slower than that proposed by Mace et al. (1990).

## Discussion and conclusions

Our radiometric results show that orange roughy is a slow growing and long-lived species, with fish 38-40 cm SL in excess of 77 years old. The fact that our radiometric results for orange roughy compare closely to the annuli counts on the surface of otoliths estimated by Mace et al. (1990) for immature fish indicates that radiometric analysis is providing realistic estimates of age for orange roughy. Our radiometric data suggests that orange roughy mature at about 32 years old ( i.e. 32cm SL) while Mace et al. (1990) suggested maturity occurs at about 20 years (maturity at a slightly smaller size of 30cm SL). Radiometric analysis has also been used to establish longevity in two species of Sebastes. Bennett et al. (1982) using radionuclide analysis of whole otoliths reported an age of 80 years for Sebastes diploproa, and also showed that otolith section age corresponded to the radiometric ages. Campana et al. (1990), analysed the central cores of otoliths from the Atlantic redfish Sebastes mentella and identified fish 75 years old. In their study they counted annuli (using the sectioning criteria recommended by Beamish 1979) which confirmed their radionuclide ages. It is important to note that by analysing otolith cores the need to know the mass growth rate of the otoliths is largely avoided. However, the relatively small size and complex shape of orange roughy otoliths would make coring extremely difficult.

The radiometric results confirm that slow-growth beyond maturity is responsible for the bimodal size distribution pattern (in which the modes apparently remain stationary) that is frequently observed in orange roughy populations (Williams 1989). The study of juvenile fish by Mace et al. (1990) also found orange roughy to have a slow-growth rate. Their estimates of growth suggested a slightly faster growth rate than indicated by the radiometric analysis. However this is not surprising since their data is based on juvenile fish i.e. during the period of fastest growth.

Mace et al. (1990) pointed out that only 4 out of 165 species for whom Von Bertalanffy equations have been derived have a Brodie Growth Coefficient K <0.06 $year^{-1}$. Our results obtained using the described double-growth rate model for the otolith confirm their suggestion of a K <0.06 $year^{-1}$ and suggest that it may even lie between 0.04 and 0.05 $year^{-1}$.

The longevity of orange roughy has not been established with any certainty prior to radiometric analysis. Mace et al. (1990), on the basis of the few mature fish where ring

counts were possible (but unvalidated), suggested that maximum age may exceed 50 years. The radiometric analysis found that ages for fish 38-40cm SL ranged from 77-149 years, making this species one of the longest-lived yet studied. Beamish and McFarlane (1987) provide a list of the maximum ages reported for commercially important ground fish off the west coast of Canada in which 14 of the 23 listed are >40 years old, 8 > 70 years old and two species Sebastes borealis and S. aleutians reaching 120 and 140 years respectively. It is worth noting that the largest orange roughy we analysed were 39-40cm SL , yet individuals in excess of 50cm SL are occasionally caught. Although these larger individuals may represent faster growing members of the population, our study suggests that they are extremely old.

The implications of the great age at maturity and the longevity of orange roughy are profound for the fishing industry. Combined with the low fecundity of orange roughy (Pankhurst and Conroy 1987), this makes the species particularly susceptible to overfishing.

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Table 1. Hoplostethus atlanticus. Radiometric analysis of pooled otolith samples for fish of similar length class. All errors expressed at the 1σ level. $^{210}Pb$ was measured as daughter $^{210}Po$ which was assumed to equal the $^{210}Pb$ activity. The youngest sample (LH752) was analysed approximately 2.4 half-lives of $^{210}Po$ after collection, hence the measured $^{210}Po$ in this sample should have differed from that of $^{210}Pb$ by no more than 20% - well within the precision of analysis. The difference in all other samples would be very much less than this. $^{210}Pb$ activity at collection time ($^{210}Pb_0$) was back-calculated from $^{210}Pb$ at analysis time ($^{210}Pb_a$) and mean $^{226}Ra$ activity. Mean $^{226}Ra$ specific activity of the "younger group" (samples LH569, 664, 751, 752 and 753) was 0.0522±0.0036 dpm.g$^{-1}$ (n = 315) and for the "older group" (samples LH570, 666, 668, 748, 749, 750 and 1240) was 0.0625±0.0030 (n = 28). All activity ratios are computed using these values for the $^{226}Ra$ specific activity.

| Sample Number | No of otoliths in sample | Mean Fish Length SL (cm) | $^{210}Pb$ activity at collection time (dpm.g$^{-1}$) | $^{210}Pb$/ $^{226}Ra$ activity ratio |
|---|---|---|---|---|
| LH752 | 135 | 10.87 | 0.0033±0.0032 | 0.063±0.061 |
| LH753 | 102 | 14.20 | 0.0070±0.0023 | 0.134±0.045 |
| LH569 | 54 | 16.51 | 0.010±0.0050 | 0.193±0.097 |
| LH751 | 16 | 25.40 | 0.0174±0.0033 | 0.333±0.067 |
| LH664 | 8 | 33.90 | 0.0223±0.0046 | 0.427±0.093 |
| LH1240 | 6 | 37.30 | 0.0312±0.0038 | 0.499±0.065 |
| LH570 | 4 | 38.90 | 0.0472±0.0124 | 0.755±0.202 |
| LH666 | 4 | 39.05 | 0.0509±0.0079 | 0.814±0.132 |
| LH748 | 4 | 39.20 | 0.0542±0.0046 | 0.867±0.085 |
| LH750 | 4 | 39.30 | 0.0466±0.0036 | 0.746±0.068 |
| LH668 | 2 | 40.30 | 0.0519±0.0099 | 0.830±0.163 |
| LH749 | 4 | 40.40 | 0.0504±0.0036 | 0.806±0.069 |

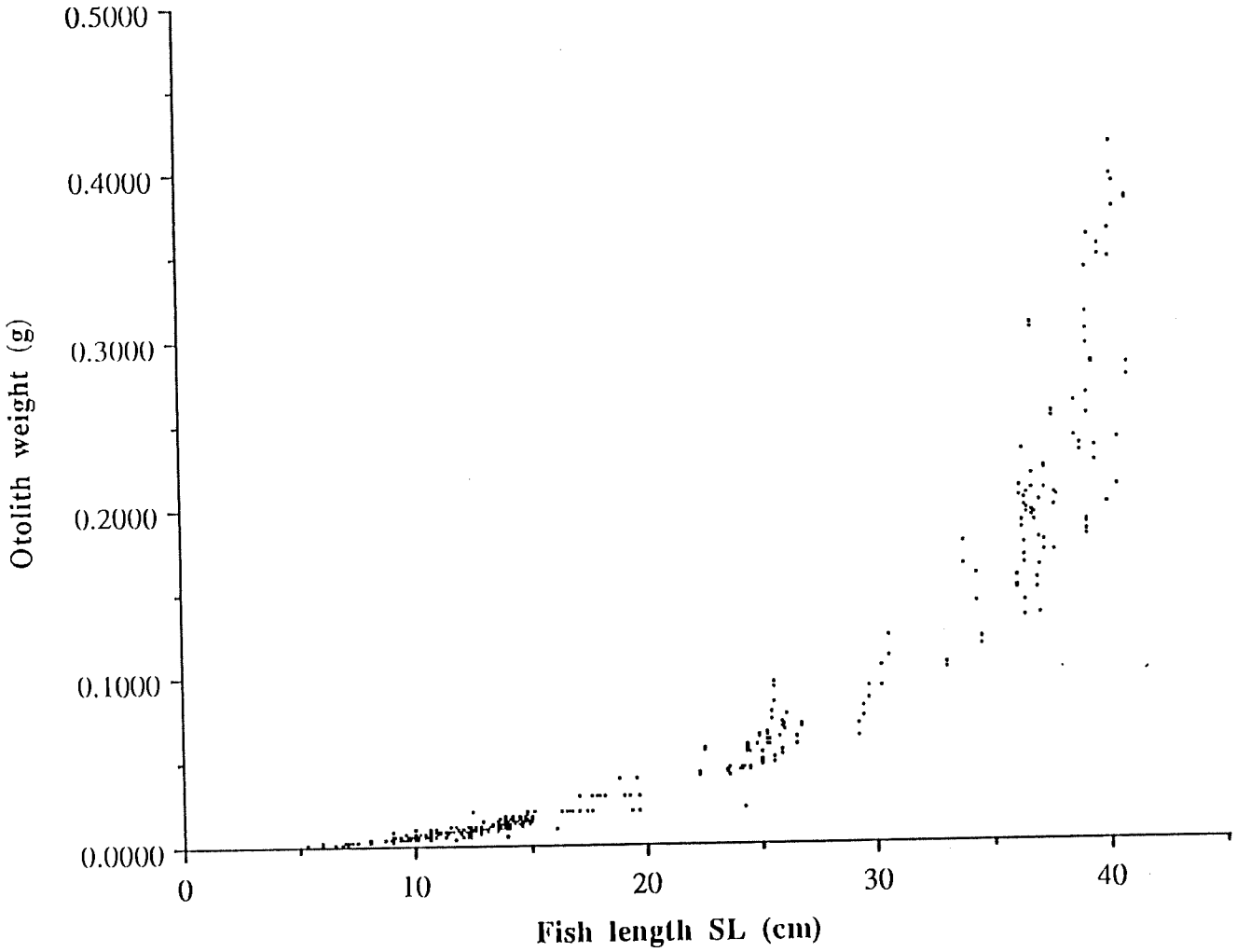


Fig. 1 Hoplostethus atlanticus. Relationship between fish length and otolith weight, n=497 (otoliths measured).

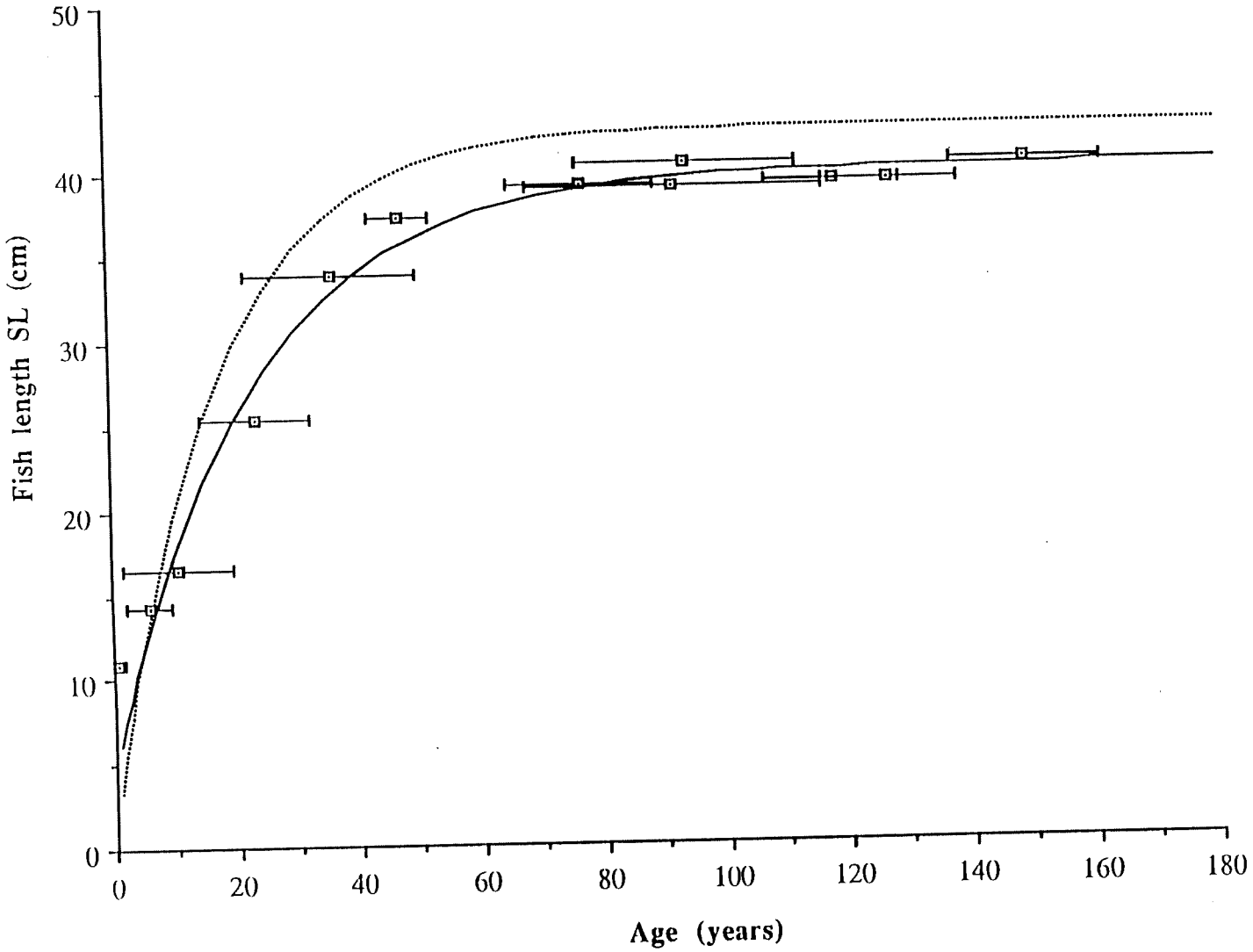


Fig. 2 Hoplostethus atlanticus. Mean fish length versus radiometric age of otolith samples. A von Bertalanffy growth curve (solid line) has been fitted to the data. The von Bertalanffy growth curve (dotted line) calculated by Mace et al. (1990) is plotted for comparison.