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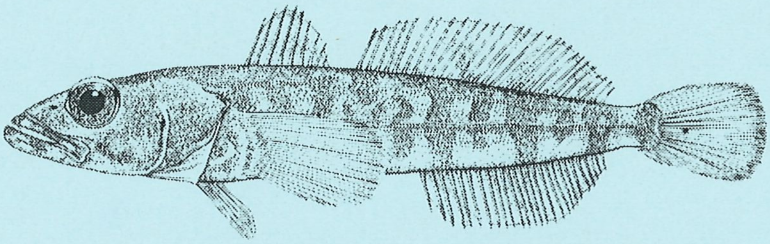

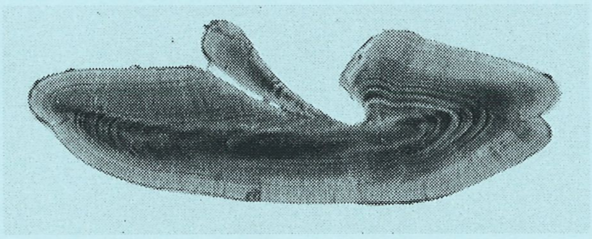

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Cover illustration: (Upper) Drawing of a Patagonian toothfish from Fischer, W. and Hureau, J. (eds) 1983. FAO Species Identification Sheets for Fishery Purposes. Southern Ocean (Fishing Areas 48, 58 and 88) (CCAMLR Convention Area). Rome, FAO, Vol. 2: 233-470. (Lower) Transverse section of an otolith from a Patagonian toothfish estimated to be 15 years of age.

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ISBN 0 646 39950 0

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1. Non-technical Summary

97/123 Determination of Patagonian toothfish age, growth and population characteristics based on otoliths

Catches of Patagonian toothfish, *Dissostichus eleginoides*, in the southern oceans have increased dramatically during the past decade and, with the inclusion of illegal, unregulated and unreported fishing may exceed 70,000 tonnes in some years (CCAMLR 1998). The impact of current catches on toothfish stocks is unknown due to the lack of information on the biology of this species. Effective management of toothfish requires significant research in a range of areas including age and growth, life history, migration and movements, stock discrimination and others. Without this research stocks of toothfish are threatened with overfishing and there would be considerable risk to the viability of toothfish resources within the Australian Fishing Zone surrounding Macquarie Island, Heard Island and McDonald Island.

This project involved the analysis of morphological and chemical data in otoliths to investigate several parameters critical to effective assessment of toothfish stocks. Otoliths are calcium carbonate structures in the inner ear of fish and they act as gravity and auditory receptors. Aside from these critical physiological functions, otoliths are extremely useful in studies of fish biology due to the fact they are deposited throughout the life of the fish and record events in the fish's life from birth to death. These events are recorded in both the structure and chemical composition of the otolith. Otoliths deposit rings analogous to tree rings and these rings can be used to determine the fish's age. In addition, the calcium carbonate matrix of the otolith incorporates elements that can provide information about the fish's past life and environment.

Fish age is among the key parameters required to carry out an effective stock assessment. The most common method used to determine fish age is based on the interpretation of opaque and translucent zones in the thin sections of otoliths. Although this method of age estimation is widely accepted, validation of the methodology is critical due to the uniqueness of each fish species and the variability of otoliths.

Validation of age estimation procedures is required to ensure that estimated ages of fish are accurate. Several procedures exist to validate ages, but the majority are not feasible for Patagonian toothfish. This study employed a novel method of age validation based on the 'bomb radiocarbon chronometer'. This method is based on the fact that radiocarbon produced by the atmospheric detonation of atomic weapons during the late 1950s and early 1960s produced elevated levels of radiocarbon in the ocean, and otoliths, and these levels can be related to time.

Otolith cores prepared from the otoliths of 37 Patagonian toothfish collected between 1979 and 1996 from the Chilean slope (between 47°S and Cape Horn), Falkland Islands, Heard and McDonald Islands, Kerguelen Island, Macquarie Island, Prince Edward Islands and South Georgia. These cores were analysed for radiocarbon to determine the accuracy of the otolith thin section method for estimation of toothfish age. The otolith core is the earliest formed portion of the otolith and the level of radiocarbon measured in these samples provides an independent estimate of the year a fish was spawned.

Comparisons of age estimates from reading otoliths and radiocarbon data indicated that the estimates of age from otolith thin sections were accurate and likely to be within about five years of the true fish age. Toothfish otoliths analysed for radiocarbon yielded otolith section ages from 2 to 43 years and associated birth dates between 1940 and 1988. The relationship between otolith radiocarbon and otolith-based estimates of fish age was consistent with pre-bomb and post-bomb radiocarbon measured in Southern Ocean surface waters. There were significant differences in otolith core radiocarbon for the toothfish from different localities, consistent with the broad geographic separation of the collections and suggestive of stock separation for toothfish in the southern oceans.

The appearance of opaque and translucent zones in transverse sections of Patagonian toothfish otoliths was highly variable among specimens from a single locality; however, this variability appeared to be consistent among the many localities sampled. A total of 994 otoliths collected from the trawl fishery targeting toothfish in the Australian Fishing Zone surrounding Macquarie Island during the 1995/96 and 1996/97 were used to determine length at age and growth rates. The majority of the fish in the sample were estimated to be less than 15 years old, far younger than the maximum age in excess of 40 years estimated in the validation study. This was due most likely to the restricted size range (~30 cm SL to 150 cm SL) of toothfish sampled by trawl gear fished at moderate depths (<800 m).

Otolith sections prepared from toothfish collected at Macquarie Island were read by four independent readers at different laboratories to determine if age estimation procedures were consistent among laboratories. There was evidence of bias between some readers, but individual readers were consistent in their interpretation of presumed annual increments Where bias was evident it indicated mean differences in age estimates of around 1 to 2 years.

Otolith shape and otolith chemistry were used to discriminate among putative stocks of Patagonian toothfish in the Southern Ocean region. Otolith samples were collected from fishing vessels engaged in longlining or trawling activities in toothfish fisheries around Chile, Falkland Islands, Heard and McDonald Islands, Kerguelen Island, Macquarie Island, Prince Edward Islands and South Georgia. Otolith shapes were described with mathematical shape descriptors and otolith chemistry was determined with laser ablation-inductively coupled plasma mass spectrometry.

Based on the trace element composition of otolith cores, 73% of the toothfish could be assigned to the correct location of capture. Otolith chemistry was more effective than otolith shape for the identification of stocks although both methods provided good evidence for stock separation.

Analysis of chemical data from the cores of toothfish otoliths collected in several fisheries suggests that there are at least four stocks in the Southern Ocean. These stocks include a South American group, Falkland Islands group, Macquarie Island group and an Indian Ocean group comprised of toothfish collected from islands in the Indian Ocean sector of the Southern Ocean (Prince Edward Islands, Kerguelen Island and Heard and McDonald Islands). Stock differences were determined on the basis of the composition of otolith cores and this suggests that there are at least four distinct spawning areas or nursery grounds for Patagonian toothfish and minimal dispersal among these areas. The lack of separation of toothfish from the Indian Ocean group may be due to movement of larval or adult toothfish between these islands or the inability of the otolith chemistry method employed to separate these potential stocks.

This research supports several conclusions that are critical to the management of Patagonian toothfish stocks. The results provide conclusive evidence that toothfish are a long-lived species with maximum ages of more than 40 years. Furthermore, there are several stocks of toothfish in southern oceans and these may be separated due to limited dispersal of larval, juvenile and adult life history stages. These stocks should be considered isolated units for the purposes of stock assessment and management.

2. Background

Patagonian toothfish *Dissostichus eleginoides* is the most important commercial fish species in the Southern Ocean and legal catches have exceeded 20,000 tonnes in recent years and are likely to increase. With inclusion of illegal, unregulated and unreported (IUU) fishing catches of toothfish may have exceeded 70,000 tonnes in some years (CCAMLR 1998). The impact of current catches on toothfish stocks is unknown due to the lack of information on the biology of this species. Furthermore, a fishery for toothfish is in the development phase within the Australian Fishing Zone (AFZ) around Macquarie Island and both Heard and McDonald Islands. Biological data relevant to the assessment and management of toothfish resources in the Southern Ocean and adjacent regions are sparse. Due to the potential for rapid mobilisation of fishing fleets to harvest toothfish and the unknown impacts of IUU fishing, it is essential that biological data on this species be gathered, particularly within the AFZ where exploratory fishing is still underway.

Several studies have investigated age and growth of Patagonian toothfish (Yukhov 1971, Frolkina 1977, Hureau and Ozouf-Costaz 1980, Cassia 1998). Age estimates were based on scales, and both whole and sectioned otoliths; however, methods of age estimation used in these studies were not validated. Validated age estimates across the full size range of toothfish are required to assess the productivity of toothfish stocks. Although several methods have been used to estimate ages, it is infrequent that an age estimation method is proven to be accurate. Several methods are used to validate (sensu Kalish et al. 1995) fish ages; however, these possess many shortcomings (Kalish 1995b).

Validation is a critical component of the age estimation procedure, however, there are no reported studies of age validation for any nototheniid or Antarctic fish species. Validation is a process whereby the accuracy of an age estimation method is determined and, in the first instance, can be linked to confirming the temporal significance of zones that are counted when estimating age (Kalish et al. 1995). Age validation is a difficult process, even for temperate and tropical species. The remoteness of the southern oceans, the biology of the targeted fish species and the nature of the fishing and processing operations further complicate the validation process. Potentially, these issues make a method based on mark and recapture a poor candidate for age validation of nototheniid fishes. Methods based on the chemical composition of otoliths, including the radiometric method (Campana et al. 1990, Fenton and Short 1992) and the bomb radiocarbon chronometer (Kalish 1993, Kalish 1995a, Kalish et al. 1996) are more suitable as they do not entail marking and recapturing of fish; they simply require otolith samples. Research (funded by the Antarctic Science Advisory Committee (ASAC) and FRDC) in the Division of Botany and Zoology, Australian National University (ANU) has applied the bomb radiocarbon chronometer to validate age estimation procedures for several fish species.

In addition to applications dependent on age estimation, otoliths can provide data relevant to numerous aspects of fish biology, many of which are germane to assessment of toothfish populations in the Southern Ocean. Among these applications are stock discrimination and movement/migration studies. Toothfish are widely distributed in the Southern Ocean and adjacent waters and may form discrete stocks or there may be significant emigration and immigration among geographic localities. The rate of exploitation of toothfish is increasing and occurring over a wider range of fishing grounds. These circumstances may increase the likelihood that fishing operations in other exclusive economic zones (EEZ) or on the high seas may affect the sustainability of toothfish fisheries in the AFZ. Under these circumstances, stock discrimination studies are essential.

The identification of unique management units or stocks is central to the effective regulation of commercial fisheries (e.g., Ihssen et al.1981, Begg et al. 1999). The identification of stocks is, however, problematic due to the difficulty in uniformly defining these units and the lack of universal methods suitable for the investigation of stock-related issues. Established methods for the identification of fish stocks include methods based on capture-mark-recapture studies, molecular biology (e.g. allozyme electrophoresis, mtDNA analysis, DNA sequencing), meristics, morphometrics, parasites, and the trace element chemistry of calcified tissues. Stock identification based on allozyme electrophoresis, meristics, and morphometrics are all believed to provide some indication of the degree of relatedness among individuals on the basis of phenotypic characteristics. Capture-mark-recapture and parasite studies may provide an indication of the degree of mixing and are largely based on the geographical isolation of populations. These methods are, however, less definitive if extensive mixing has occurred during the egg, larval, or early juvenile stages.

This study aims to determine if separate stocks of Patagonian toothfish are present in the Southern Ocean. Two otolith-based methods, otolith morphometrics and otolith chemistry, will be employed due to the availability of otolith samples from a broad range of sites and the relatively established nature of these methods.

Otolith shape is variable and species specific (Gaemers 1984) and can also vary significantly within species. Within species variation in otolith shape has been used successfully to discriminate among stocks (Casselman et al.1981; Bird et al. 1986; Campana and Casselman 1993). A range of factors has been proposed to affect otolith shape within a species including genetics, growth rate, diet or temperature (Gauldie 1993); however, the nature of these effects has not been investigated experimentally. A single factor or combination of factors may result in the shape of the otolith being stock specific (Campana and Casselman 1993).

Quantitative interpretation of otolith shape can be based on a range of methods including statistical analysis of a range of morphometric measurements or through shape analysis based on Fourier series. Fourier shape analysis reduces complicated shape data into a statistically useful form. A two-dimensional shape, here being an otolith outline, is described by a series of lines, or radii, originating from a single point within the outline. A Fourier transformation converts the function produced by these radii into a series of sine waves that describe the shape of the otolith. Each sine wave in the series has both an amplitude and phase angle and these sine wave characteristics can be used to compare within and among fish. These data are input into a multivariate discriminate function analysis.

Fourier shape analysis may provide information on the stock structure of Patagonian toothfish due to the broad distribution of the species and the likelihood that there may be variation in the factors that affect otoliths shape among these potentially disparate habitats. For example, Patagonian toothfish occur along the slope of Chile and the Falkland Islands shelf, areas that are likely to have oceanographic characteristics different from more oceanic habitats such as the Kerguelen Plateau, Macquarie Island and the Prince Edward Islands.

The trace element composition of otoliths can reflect the physiological and environmental history of many fish species and has been demonstrated to be an effective means of stock discrimination (e.g. Edmonds et al.1989; Kalish 1990; Thresher et al.1994, Campana et al.1994; Thorrold et al.1997; Thresher 1999). A range of analytical methods has been employed to determine otolith chemical composition including electron microprobe, proton induced x-ray emission, inductively coupled plasma atomic emission spectroscopy and inductively coupled plasma mass spectrometry and the relative advantages of these methods are discussed in Campana et al. (1997). Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) is currently among the most accurate and sensitive methods available for the measurement of trace elements in otoliths (Campana et al. 1997; Thresher 1999). This technique can rapidly sample small quantities of otolith in situ, in defined locations and at a spatial resolution of as little as 10 μm^2 and can detect isotopes at nanomolar (parts per billion) concentrations. However, LA-ICPMS does have some shortcomings including an inability to measure accurately some of the more abundant elements in otoliths such as sodium (Na), potassium (K) and iron (Fe) (Campana et al.1997). Nevertheless, LA-ICPMS is the most frequently employed method for stock discrimination studies based on otolith chemistry and recent developments of the method continue to increase its usefulness (e.g. Sinclair et al.1998).

The chemical composition of otoliths can be a useful discriminator among fish stocks due to the fact that otoliths record a permanent record of chemical variation experienced by the fish. Several factors have been suggested to affect trace element incorporation into otoliths including temperature (Radtke et al. 1990; Fowler et al.1995b), salinity (Kalish 1990; Fowler et al. 1995b), environmental concentration (Thorrold et al. 1997), rate of crystallisation (Fowler et al.1995b), ontogeny (Fowler et al.1995a), and physiology (Kalish 1989; 1991). Many of these factors are likely to vary significantly among the broadly separated toothfish habitats of the Southern Ocean. Furthermore, otoliths are not resorbed or reworked after deposition, except under conditions of extreme stress (Mugiya et al. 1991). Therefore, the chemical record in the otolith represents a history of the fish's chemical environment, albeit modified through physiological processes (Kalish 1991; Fowler et al. 1995a). Due to the temporal nature of the otolith record the earliest deposited material reflects larval and juvenile environments and the material deposited most recently at the otolith edge is a record of the period immediately prior to capture. These properties, combined with the spatial resolution of LA-ICPMS may enable stock discrimination at both the juvenile and adult stages for Patagonian toothfish.

3. Need

Data on fish age are essential to determine the production of fish stocks. Collection of data on fish age early in the history of a fishery is particularly important to determine the natural mortality rate of a population. Data relating to the age structure of populations and growth rates can also be applied to the identification of stocks. Annual estimates of population age structure are employed to identify population responses to exploitation and environmental variability and can be used to develop more data intensive methods of population assessment (eg Virtual Population Analysis). Otoliths are the structures employed most often to estimate age of fishes and validation research at the ANU indicated that toothfish otoliths were well-suited to this purpose.

A "stock" is the basic unit of management in most fisheries and identification of these units is a prerequisite to accurate assessment and effective management. There are no data on stock structure for Patagonian toothfish. As a result, we have no knowledge regarding the potential impacts of toothfish exploitation outside the AFZ on toothfish stocks within the AFZ.

4. Objectives

1. Validate otolith-based age estimates for Patagonian toothfish (*Dissostichus eleginoides*);
2. Establish routine age estimation procedures for Patagonian toothfish;
3. Determine growth rates for Patagonian toothfish from the AFZ surrounding Macquarie Island;
4. Establish an inter-agency (international) program to determine if there is variation in the interpretation of toothfish otolith sections;
5. Liaise with groups involved with modelling the dynamics of toothfish populations; and,
6. Apply otolith morphometrics and otolith chemistry to discrimination of possible toothfish stocks.

5. Methods

The research methods identified here are presented in detail in Appendices 1 and 2.

1. Fish age was estimated by interpretation of opaque and translucent zones in otolith thin sections.
2. Age estimates from otolith thin sections were validated based on the 'bomb radiocarbon chronometer'.
3. Fourier shape analysis was used to reduce complex otolith shape data into a format suitable for statistical analysis and the identification of putative stocks of Patagonian toothfish.
4. Transects of the chemical composition of toothfish otoliths were produced by analysis of otolith thin sections with a laser ablation-inductively coupled plasma-mass spectrometer. These data were analysed statistically to determine of otoliths from toothfish from different locations had distinctive chemical compositions.

6. Results/Discussion

Detailed presentation of the results and discussion appears in Appendices 1 and 2.

7. Benefits

Opportunity costs associated with inaccurate assessment and/or inappropriate management of fisheries resources can be extremely large. Over-exploitation and subsequent collapse of stocks of *Notothenia rossii* in the Southern Ocean has resulted in lost opportunity costs of tens of millions of dollars. Although, accurate estimates of lost revenue are difficult to determine in the case of the *N. rossii* fishery and extrapolation to the Patagonian toothfish fishery is difficult, the outlays required to provide an accurate assessment of the resource based on timely research would constitute an extremely small fraction of the potential long-term value of the fishery.

Direct benefits would be realised in the following areas:

1. Benefits to industry:
   a) reduced uncertainty in estimates of production for toothfish;
   b) greater predictability of long-term sustainable yields for toothfish;
   c) minimisation of expenditure for age estimation and stock structure research.
2. Benefits to management:
   a) increased confidence in population models and overall reduction of uncertainty in management regimes.
3. Benefits to the community:
   a) optimum management of fisheries resources;
   b) both short- and long-term reductions in expenditures on fisheries research;
4. Benefits to the scientific community:
   a) development and refinement of new methods for fisheries research;
   b) development of co-operative research on Southern Ocean resources.

8. Further Development

The research results presented here have been disseminated broadly to the relevant bodies and agencies involved in research and management of Patagonian toothfish including state, Commonwealth and regional fisheries agencies and scientific organisations. This has been achieved through direct collaboration with scientists at the British Antarctic Survey, Muséum National d'Histoire Naturelle (Paris), National Institute for Water and Atmospheric Research (New Zealand), Old Dominion University (U.S.A.), Australian Antarctic Division, CSIRO Marine Laboratories, Department of Natural Resources and Environment (Victoria) and at CCAMLR. Outcomes of this research were presented at a meeting of the Subantarctic Fisheries Assessment Group (SAFAG) and, in addition to scientists present at the meeting, there were both industry and AFMA representatives. As a result, the outputs from this research are subject to further development by other individuals and groups.

Scientists from the Fisheries and Forestry Sciences Division, Bureau of Rural Sciences and the Division of Botany and Zoology, Australian National University have continued their involvement in research on Patagonian toothfish and continue to assist in research to refine age estimates from toothfish otoliths and establish uniform protocols for otolith reading.

9. Conclusion

All objectives of the project, as listed below were met.

1. Validate otolith-based age estimates for Patagonian toothfish (*Dissostichus eleginoides*)
2. Establish routine age estimation procedures for Patagonian toothfish;
3. Determine growth rates for Patagonian toothfish from the AFZ surrounding Macquarie Island;
4. Establish an inter-agency (international) program to determine if there is variation in the interpretation of toothfish otolith sections;
5. Liaise with groups involved with modelling the dynamics of toothfish populations; and,

6. Apply otolith morphometrics and otolith chemistry to discrimination of possible toothfish stocks.

The key outcome of this research program has been improvement of the stock assessment process for toothfish and development of further research to build on the findings. Notably, parameterisation of growth models has been adjusted to comply with data provided by this program and management measures that take account of the multiple stocks of toothfish in the Southern Ocean have been highlighted in further discussions. Manuscripts (see Appendices) detailing the results of this research program have been presented to both the Subantarctic Fisheries Assessment Group (SAFAG) of the Australian Fisheries Management Authority and to the Working Group on Fisheries Stock Assessment (WG-FSA) of the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR). These results have been used for further stock assessment research including refinement of model parameters, development of length at age matrices and more detailed stock separation aimed at finding possible stocks at even finer scales than those identified in this study.

10. References

References are presented in Appendices 1 and 2.

11. Intellectual Property

No commercial intellectual property arose from this work.

12. Staff List

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Janet Pritchard (Division of Botany and Zoology, Australian National University)
Justine Johnston (Division of Botany and Zoology, Australian National University)

13. Appendix 1

Validation and direct estimation of age and growth of Patagonian toothfish *Dissostichus eleginoides* based on otoliths

FRDC Project 97/123

Validation and direct estimation of age and growth of Patagonian toothfish *Dissostichus eleginoides* based on otoliths

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Abstract

Age estimates for Patagonian toothfish *Dissostichus eleginoides* based on thin sections of otoliths were validated based on measurements of radiocarbon in cores isolated from whole otoliths. A total of 994 otoliths collected from the trawl fishery targeting toothfish in the Australian Fishing Zone surrounding Macquarie Island during the 1995/96 and 1996/97 were used to determine length at age and growth rates. The majority of the fish in the sample were estimated to be less than 15 years old, far younger than the maximum age in excess of 40 years estimated in the radiocarbon validation study. Von Bertalanffy growth functions (VBGF) were fitted to the length and age data by year and sex; however, uncertainties for parameter estimates and the relatively poor fit of the VBGF were affected by the limited size range of fish in the sample. Estimates of K and L_∞ for the different years and sexes ranged from 0.005-0.116 (95% confidence intervals) and 1,087 to 10,405 (95% confidence intervals), respectively. Subsamples of otolith sections prepared from toothfish collected at Macquarie Island were read by four independent readers working at different laboratories. There was evidence of systematic bias between some of the readers, although the small confidence intervals for differences estimated at each age indicated that individual readers were consistent in their interpretation of presumed annual increments.

Introduction

Catches of Patagonian toothfish, *Dissostichus eleginoides*, in the southern oceans have increased dramatically during the past decade and, with the inclusion of illegal, unregulated and unreported fishing may exceed 70,000 tonnes in some years (CCAMLR 1998). The impact of current catches on toothfish stocks is unknown due to the lack of information on the biology of this species. Effective management of this species will require significant research in a range of areas including age and growth, life history, migration and movements, stock discrimination and others.

Patagonian toothfish is a member of the family Nototheniidae, a group endemic to Antarctic and subantarctic waters and characterised by lack of a swim bladder and the presence of glycoproteins in their blood, which act to prevent these fish from freezing in water temperatures below 0°C (Kock 1992). Collections of larval toothfish are extremely rare, but they are believed to be pelagic and settle to demersal habitats as juveniles at a length of approximately 10 cm (Evseenko et al. 1995). As adults, Patagonian toothfish are demersal and distributed in slope waters around subantarctic islands and seamounts of the Southern Ocean and their distribution centres on the Subantarctic Convergence Zone in the area between 45°S and 55°S latitude. Toothfish are also caught in the slope waters off Argentina and Chile south of about 35°S latitude.

Validated estimates of age and growth for Patagonian toothfish have not been reported, but are critical to the sustainable management of this species and urgently required in order to determine basic life history characteristics including longevity, growth rates, age at maturity and mortality rates. Ultimately, these data are essential to estimate the productivity of Patagonian toothfish and to establish sustainable rates of fishing.

Few data are available on age and growth of toothfish and those studies that are available provide age estimates based on unvalidated methods. Yukhov (1971) and Frolkina (1977) estimated ages based on whole otoliths and Hureau and Ozouf-Costaz (1980) used scales. Furthermore, the largest toothfish used in these studies were less than 100 cm SL, although toothfish of more than 200 cm SL are captured regularly. On the basis of the interpretation of annuli in scales Hureau and Ozouf-Costaz (1980) estimated toothfish from the Kerguelen Plateau region to be about 6 years and 18 years of age at 30 cm SL and 70 cm SL, respectively. The oldest age estimate they produced for a toothfish was 21 years for a 85 cm SL individual. In addition, Hureau and Ozouf-Costaz (1980) indicated that a workshop on age determination of Antarctic fish produced good agreement between age estimates based on scales and broken and burnt otoliths.

Age estimates from otolith thin sections for Patagonian toothfish from South Georgia were reported by Cassia (1998) and compared with age estimates from scales. There was good agreement between scale and otolith estimates; however, it was concluded that scales were more appropriate for age estimation of toothfish as they were easier to read, whereas otolith sections were frequently totally opaque. The maximum age found in the study was 24 years for a 223cm (total length) Patagonian toothfish. Age of Antarctic toothfish, *Dissostichus mawsoni*, a species closely related to Patagonian toothfish, was estimated from thin sections of otoliths (Burchett et al. 1984). The apparent success of the otolith thin section method provides additional support for further investigation of the method and its application to studies of Patagonian toothfish age and growth.

Validation is a critical component of the age estimation procedure, however, there are no reported studies of age validation for any nototheniid or Antarctic fish species. Validation is a process whereby the accuracy of an age estimation method is determined and, in the first instance, can be linked to confirming the temporal significance of zones that are counted when estimating age (Kalish et al. 1995). Age validation is a difficult process, even for temperate and tropical species. The remoteness of the southern oceans, the biology of the targeted fish species and the nature of the fishing and processing operations further complicates the validation process. Potentially, these issues make a method based on mark and recapture a poor candidate for age validation of nototheniid fishes. Methods based on the chemical composition of otoliths, including the radiometric method (Campana et al. 1990, Fenton and Short 1992) and the bomb radiocarbon chronometer (Kalish 1993, Kalish 1995a, Kalish et al. 1996) are more suitable as they do not entail marking and recapturing of fish; they simply require otolith samples.

The aim of this study is to validate age estimates for Patagonian toothfish based on thin sections from otoliths, provide an indication of length at age and growth rates for the population of toothfish at Macquarie Island and determine variability in age estimates for toothfish made by different laboratories.

Materials and Methods

Radiocarbon analysis

Patagonian toothfish otoliths were obtained from a range of sources in order to ensure broad temporal and spatial coverage. Due to limited information on the separation of toothfish stocks it was not known if fish sampled later in life would have moved significant distances, potentially complicating interpretation of radiocarbon data. Furthermore, toothfish fisheries are biased in terms of the size, and presumably age, of fish sampled; trawl fisheries capture smaller (younger) fish and longline fisheries capture larger (older) fish. The need to sample fish with birth dates spanning the period from about 1955 to 1985 for effective use of the bomb radiocarbon chronometer necessitated the collection of samples from a range of sources with different collection dates (Table 1). Samples were obtained from Macquarie Island, Heard and Macdonald Islands, Kerguelen Island, Prince Edward Islands, Falkland Islands, South Georgia and Chile (between 46° S and Cape Horn; 1996) (Fig. 1). These samples were obtained through the cooperation of numerous individuals and agencies (see acknowledgments).

Otoliths were weighed dry and then prepared for radiocarbon and stable carbon isotope analysis. In previous studies (e.g. Kalish 1993, Kalish et al. 1996) the earliest formed regions of individual otoliths were isolated with a fine, high speed drill and it was possible to 'sculpt' from the adult otolith, an otolith that was representative of a juvenile fish. This process was initially attempted in the study of toothfish otoliths, however, it was found that these samples were not amenable to sculpting. During attempts at sculpting toothfish otolith cores, the sample would fracture in an unpredictable and irregular manner. In general, it appeared that these samples were too brittle and could not be ground using our established methods. Alternate methods for the isolation of toothfish otolith cores were investigated. The most suitable technique tested was based on the use of an 'ultrasonic disc cutter'. Whole otoliths, sulcus acusticus side up, were affixed to glass slides with Crystalbond. Otolith cores were isolated in the transverse plane with disc cutters of either 1.5 mm or 3.0 mm diameter (Fig. 2 and 3). These initial cores eliminated older otolith material deposited in the sagittal plane, but still contained material deposited throughout the life of the fish in the transverse otolith plane. Therefore, further sample preparation was required to eliminate older otolith material. The brittle nature and small size of these otolith cores, between 1.0 mm and 2.5 mm in diameter, made standard grinding methods impractical. We used a hand operated mechanical grinding jig for further grinding to remove excess otolith material in the transverse plane. The mechanical grinding jig allows samples to be ground on standard wet/dry papers, but makes it possible to control the amount of material removed in each grinding stage. Otolith cores prepared with the ultrasonic disc cutter were affixed to a 25 mm diameter (10 mm thick) stainless steel disc with Crystalbond. The disc, with the otolith core attached, is inserted into the mechanical grinding jig. The jig was adjusted in 2 μm steps, thereby exposing only 2 μm of the otolith core at any one time for grinding. The core was ground on both the dorsal and ventral surfaces to ensure that the final sample contained material from only the earliest formed regions of the otolith. The position of 'landmarks', particularly distinct opaque zones formed in early life, were monitored frequently. This ensured that the final otolith sample contained material deposited only during what was presumed to be the first year of the fish's life which is spent in the surface mixed layer of the Southern Ocean. The final product was a single piece of otolith aragonite (Fig. 3). Sample weights ranged from about 2.1 to 10.9 mg. The lightest samples (< 4.0 mg) were produced with the ultrasonic disc cutter with a diameter of 1.5 mm; heavier samples were produced with the disc cutter with a bore of 3.0 mm.

Accelerator mass spectrometry was used to measure radiocarbon in samples of otolith cores. Otolith carbonate was converted to CO_2 by reaction *in vacuo* with 100% phosphoric acid. An aliquot of the CO_2 was used to determine $\delta^{13}C$ for each sample and the remaining CO_2 was converted to graphite for analysis of radiocarbon. Radiocarbon levels in each sample were determined by AMS at one of three laboratories: the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratories (Livermore, California), the U.S. National Science Foundation Accelerator Mass Spectrometry Facility at the University of Arizona (Tuscon, Arizona) and the Radiocarbon Accelerator Unit, Oxford University (Oxford, England). Details of specific analytical procedures used can be obtained from each laboratory. Radiocarbon values are reported as $\Delta^{14}C$, which is the age- and fractionation-corrected per mil deviation from the activity of nineteenth century wood (Stuiver and Polach 1977). Reported errors for the radiocarbon data are 1 standard deviation. Radiocarbon errors include both counting errors and laboratory random errors.

Age and birth date estimates for toothfish were based on interpretation of opaque and translucent zones in otolith thin sections. Standard procedures were employed for thin section preparation and these are detailed in the description of methods used for determination of age and growth. In most cases, otolith pairs were available and the right otolith was used for radiocarbon analysis with the left otolith retained for sectioning and age estimation. In some cases, only one otolith was available for analysis and the single otolith was used for both radiocarbon measurement and sectioning. The otolith coring procedure described above resulted in an intact otolith with only the core removed (Fig. 3). These 'coreless' otoliths were still suitable for sectioning with standard procedures, however, it was necessary to estimate the number of presumed annual increments removed in the coring procedure. In most cases, this was estimated as one year based on the size of the core and comparisons of cored sections with intact sections. Age estimates from otolith sections were made before radiocarbon data were available to ensure independence of the data sets.

Age and growth

Otoliths samples were obtained from Macquarie Island in the 1995/96 and 1996/97 austral summers. Fish were captured in demersal trawls by the Austral Leader from within the Australian Fishing Zone (AFZ) surrounding Macquarie Island. An Australian fisheries observer removed left and right sagittal otoliths from fish while at sea and recorded fish weight, length and sex. Each otolith pair was stored in an individual envelope and a sub-sample of the total collection was used in this study. This sub-sample was a combination of randomly selected otoliths and systematically chosen length classes to ensure that a wide range of lengths were uniformly sampled for subsequent development of an age-length key.

Left and right otoliths from each fish were cleaned and individually weighed on an electronic balance to 0.1 mg. The length, width and depth of each otolith were measured to the nearest 0.1 mm, using vernier calipers.

Right otoliths were prepared for estimation of age. The approximate position of the core was determined and marked on the sample in pencil. Otoliths were placed, four per row, on a thin, cured layer of polyester resin and then fully embedded in a layer of polyester resin. Resin blocks were sectioned using a low speed saw with 14 cm diameter by 500 μm thick diamond embedded blade. Four serial transverse sections of 600μm each were taken in the region of the otolith core. Serial sections were affixed to glass slides and some otolith material was removed using a lapping wheel and 240 or 400 wet and dry carbide paper. Optical density of the sections was checked frequently, under a compound microscope, during the grinding process to ensure that the sections were prepared to a thickness that was optimal for subsequent reading. After grinding was completed, the sections were polished using 1200, 2400, 4000 wet and dry carbide paper to give a smooth finish.

Otolith Reading

Four sections were available for reading from each otolith. The section chosen for age estimation was selected on the basis of clarity of increments and the proximity of the section to the otolith core. In some cases, ages were estimated from more than one section from an individual otolith in order to corroborate age estimates obtained from a particular section. Annual increments formed in the first three to five years are complex structures and individual annual increments appear to consist of multiple opaque and translucent zones. After three to five years, annual increments are typically bipartite structures consisting of one opaque and one translucent zone, when viewed with a compound microscope at magnifications of up to 400x. However, 'splitting' of opaque zones does occur in some outer increments and can complicate interpretation of presumed annual increments.

Several regions of each otolith section were commonly viewed to estimate the age of an individual, due to the fact that, in most cases, no single region provided a record of clear annual increments throughout the life of a fish. Otolith sections were viewed at 40, 100, 200 or 400x magnification, although the first annual increment was often identified under 40x magnification. In most cases, 100x magnification was used to identify subsequent opaque and translucent zones for age estimation. The presumed first increment was distinguished as a continuous translucent zone around the transverse otolith section (Figs. 4, 5 and 6). In sections that incorporated the otolith core, the sulcus acusticus usually penetrated to the edge of what was presumed to be the first annual increment, and this 'marker' was frequently used as a guide for identification of the first increment. After identification of the first increment, subsequent opaque zones were counted from the region of the otolith core towards the otolith edge to provide an estimate of fish age. Estimation of age based on counts of presumed annual increments was often complicated by the presence of presumed non-annual marks or checks that were both regular and irregular in appearance. In order to overcome many of the complicating factors associated with age estimation, 'keys' were developed that provided estimates of the width of particular otolith regions in relation to age. For example, the first three to five annual increments were characterised as extremely opaque and wider, relative to other annual increments. The next seven to nine increments were slightly thinner and less opaque.

Outer increments in the otoliths of fish with presumed ages in excess of about 14 years of age were relatively thin and uniformly spaced (Fig. 6). However, no single set of rules applied in all cases, and, where possible, several regions from a single section, and, ideally, counts from different sections were compared in order to provide a final age estimate for an individual toothfish.

Scanning electron microscopy (SEM) was used to provide some verification of the position of the first annual increment by quantification of presumed daily increments. Selected thin sections prepared for the reading of annual increments were finished further before acid etching by polishing with 0.25 μm aluminium oxide and 0.25 μm diamond paste. Samples were washed in distilled water in an ultrasonic bath and then etched with 0.1 M HCl for 2 min. Etched thin sections were coated with gold and observed in a Cambridge Stereoscan SEM.

One hundred otoliths were re-read to establish the precision of within-reader age estimation. Between-reader variability was estimated initially by independent readers who read 34 toothfish otoliths collected from throughout the distribution of Patagonian toothfish and used in the bomb radiocarbon validation study. Precision of age estimates was tested using the coefficient of variation as set out in Chang (1982) for both inter and intra-reader variability.

Inter-laboratory comparison of age estimates

Independent agencies estimated age from a sub-sample of the otolith sections produced from toothfish collected at Macquarie Island. The agencies involved included the Australian National University (ANU), Central Ageing Facility (Australia) (CAF), National Institute for Water and Atmospheric Research (New Zealand) (NIWA) and Old Dominion University (USA) (ODU). Direct comparisons were only made between the ANU age estimates and those from the other laboratories. The number of samples read by different laboratories varied, but was at least 100. In order to compare data and detect any systematic bias between readers, age estimates from each pair of readers and the 95% confidence interval about the mean age assigned by one reader for fish assigned a given age by a second reader were plotted against one another as described in Campana et al. (1995). Linear regression lines were fitted and tested to determine if there were significant differences from a slope of one and an intercept of zero. Coefficients of variance (CV), as described in Chang (1982), were calculated to provide a statistical evaluation of the repeatability of age estimates.

Analysis of age and length data

Von Bertalanffy growth functions were fitted to length at age data and significant differences between fishing seasons and sexes tested. Data were fit separately for each season and sex using the maximum likelihood procedure in the JMP statistical package and the model

$$l_t = L_\infty \left[1 - e^{-K(t-t_0)}\right]$$

where L_∞ is the theoretical asymptotic length, t_0 is the hypothetical age when L is zero and K is the growth coefficient that indicates the rate at which the length of the fish approaches L_∞.

Due to the non-linear formulation of the VBGF, analysis of covariance can not be used for comparisons of parameter estimates. Analysis of the residual sum of squares was used to test for significant differences in parameter estimates of the VBGF as described in Chen *et al.* (1992)

$$F = \frac{\dfrac{RSS_p - RSS_s}{DF_{RSS_p} - DF_{RSS_s}}}{\dfrac{RSS_S}{DF_{RSS_S}}} = \frac{\dfrac{RSS_p - RSS_S}{3 \times (K-1)}}{\dfrac{RSS_S}{N - 3 \times K}}$$

where RSS_P = residual sum of squares of the VBGF fitted by pooled growth data, RSS_S=sum of the residual sum of squares of the VBGF fitted to growth data for each individual sample, N = total sample size, and K = number of samples in the comparison.

Two outliers in the 1996/97 season had a large effect on the calculation of VBGF parameter estimates, causing unrealistic estimates of L_∞. However, there was no statistical reason to exclude these fish from the data, therefore, analyses for the 1996/97 season were conducted both with and without these two points.

Results

Age validation

Radiocarbon (reported as $\Delta^{14}C$) data from Patagonian toothfish (Table 1) are plotted versus birth date estimated from otolith thin sections in Figure 7. Standard deviations for the radiocarbon data are included in the graph, however, as these errors are all below 1%, they do not extend beyond the data points. The results indicate pre-bomb radiocarbon levels for toothfish of less than -110‰, in agreement with independent estimates of $\Delta^{14}C$ in surface seawater dissolved inorganic carbon from similar latitudes (Broecker et al. 1985, Gordon and Harkness 1992, Berkman and Forman 1996). There is a trend of increasing radiocarbon starting around 1960; the rate of increase in Southern Ocean radiocarbon around the 1960s and 1970s, as measured in toothfish otolith cores, is consistent with the known effect of atmospheric testing of atomic weapons. These results validate that opaque and translucent zones visible in transverse sections of otoliths from Patagonian toothfish are formed annually.

Radiocarbon and birth date data from toothfish otoliths collected at Kerguelen Island demonstrate coherence in $\Delta^{14}C$ from samples collected over a time period of more than 15 years (Fig. 7). Otoliths were sampled from moderate-sized toothfish (standard length <128 cm) collected by French trawlers operating around Kerguelen Island in 1979. These toothfish had estimated ages between 19 and 11 years and associated birth dates ranged between 1960 and 1968. These samples yielded $\Delta^{14}C$ ranging from –77.0‰ to –38.5‰ (Table 1). Toothfish collected from different locations at least 15 years later, but with similar $\Delta^{14}C$ measured in their otolith core, had birth date estimates from otolith thin sections ranging from 1957 to 1969. This result provides further strength to the validation by demonstrating the likely annual nature of opaque and translucent zones in thin sections of otoliths from younger toothfish and the coherence of these age estimates and $\Delta^{14}C$ from older individuals.

Comparison of toothfish birth date estimates from two independent readers demonstrated that interpretation of opaque and translucent zones in the otoliths was consistent between readers. Birth date estimates from the two readers were plotted versus $\Delta^{14}C$ (Fig. 8) and both sets of birth dates define the increase in radiocarbon associated with atomic testing. Direct comparison of age estimates made by Reader 1 and Reader 2 indicated that the difference between age estimates was less than four years for most otoliths (Figure 9), however, this was equivalent to a percentage error of up to 33%. Although reader bias was not evident in the comparison of the two readers, further investigation of potential reader bias and inter-laboratory calibration is warranted before the age estimation method is established or used to provide data for stock assessment methods such as cohort analysis.

Southern Ocean radiocarbon levels are far below those measured in temperate waters. Figure 10 includes data from both subantarctic Patagonian toothfish and temperate snapper *Pagrus auratus* otoliths (Kalish 1993) and indicates the relative depletion in radiocarbon between these regions. Despite the differences in radiocarbon levels relative trends associated with the input of bomb radicarbon to the ocean are similar among the ocean sectors with the exception of the Falkland Islands data.

Age and growth

Collections from both seasons contained more females than males (283:204 for 95/96, 256:171 for 96/97) and this bias in sex ratio was signficant ($p<0.0001$). In addition, female fish in both samples were significantly longer than male fish ($p< 0.0001$). This resulted from the lack of large male fish with only two males >1100 mm for both collections. The 1996/97 season contained more very large and very small fish to ensure that a broad length range of toothfish was sampled. The relationship between fish total length and fish weight was similar for collections from 1995/1996 and 1996/1997 with both seasons combined yielding an exponential relationship of weight = $3.06 \times 10^{-8} \times$ total length$^{2.856}$ (Fig 11). There was a strong linear relationship between fish weight and otolith weight ($r^2=0.96$, $p<0.0001$) and highly significant correlations between all otolith morphometrics (weight, length, width and depth) and both otolith section age and fish length (Figs. 12 and 13). Only relationships with otolith weight and depth were linear, with otolith length, width and depth reaching an asymptote as fish growth slowed.

Von Bertalanffy growth functions (VBGF) were fitted to each season and sex separately. There were significant differences between fishing seasons, caused mainly by the two largest fish from the 96/97 season (Table 2, Fig. 14). The 1996/97 season also contained many more large fish, which yielded greater ages based on the otolith sections. Data from the two largest fish were removed from the data set as they caused the parameter estimation procedure to produce unrealistic estimates of L_∞ (Table 2). After removal of these points from the 1996/97 data, VBGF parameter estimates for both fishing seasons were similar. The values estimated for t_0 were adequately small (Table 2) for both seasons to suggest a reasonable fit of the VBGF for smaller fish. Mean values estimated for L_∞ ranged from 1384 to 2999, close the maximum size recorded for Patagonian toothfish, however, the 95% confidence intervals were large (Table 2). Estimates of K were generally low and ranged from 0.022 to 0.72, indicating that Patagonian toothfish are slow growing, but, again, the 95% confidence intervals were large (Table 2). There was little evidence of an asymptote in the relationship between fish length and age; this was almost certainly due to the lack of large fish from both seasons (Fig. 14). Significant differences between male and female VBGF parameters for the 1996/97 season were found ($F_{(3,492)} = 14.6$, $P< 0.05$) and are probably due to the lack of large males. No significant difference between the sexes was found for the 1995/96 season ($F_{(3,496)} = 2.4$, $P = 0.1$).

Precision of age estimates

Differences between repeat readings by a single reader were normally distributed with a single mode of 1 year (Fig. 15). Fifty six percent of readings were within 1 year, and 94% within 3 years with a single outlier of 10 years. No systematic bias with age was evident (Fig. 15). Differences between repeat age estimates were slightly larger in older fish, though the percentage error is smaller (Fig. 15). The coefficient of variation was calculated to be 14.9, and was affected by several large outliers.

Between reader precision for the sample from predominantly large toothfish from a range of locations used for the radiocarbon validation study showed no systematic bias between two independent readers (Table 1). Thirty eight percent of readings were within 1 year of each other and 74% were within 3 years of each other. Coefficient of variation was 22.5, and again substantially affected by several outliers with large differences between the two readers. Some of the differences may be partly attributable to the poor section quality of otoliths which had their cores removed for radiocarbon analysis; interpretation of the inner opaque and translucent zones was more difficult in these samples. Birth dates calculated from the otolith section age estimates, when plotted against $\Delta^{14}C$ (Fig 8) demonstrated the magnitude of age estimation errors for these larger fish in relation to the radiocarbon validation.

Ages were estimated for 30 fish from each of 7 fisheries as part of a study of toothfish stock discrimination based on otolith morphometrics and otolith chemistry (Timmiss and Kalish submitted). Samples were from Macquarie Island, Heard and MacDonald Islands, Kerguelen Island, Prince Edward Islands, Falkland Islands, South Georgia and Chile (between 46° S and Cape Horn). These fish ranged in age from 9 to 35 years and thin sections demonstrated opaque and translucent zones that were similar in structure to those quantified in sections from toothfish collected in the AFZ around Macquarie Island.

Inter-laboratory comparison of age estimates

The inter-laboratory comparison of age estimates for Patagonian toothfish demonstrated that each laboratory was identifying similar structures as annuli. There was evidence of bias between some readers and these differences in age estimates may have resulted from different interpretation of specific otolith regions. There was no evidence of systematic bias between readers from ANU and NIWA over the range of ages (Fig. 16 and 17). The intercept of the regression line was not significantly different from 0 (intercept = -0.265), nor was the slope of the line significantly different from 1 (slope = 1.033). The coefficient of variation for age estimates from these two readers was 13.63. Systematic bias was evident between and ANU and CAF with the reader from ANU providing slightly greater ages over most of the age range (Fig. 16 and 17). Differences at ages 3 and 4 suggest that readers were interpreting the first and second increments differently, although the small confidence intervals indicated readers were internally consistent. The regression line indicated that the intercept was not significantly different from 0 (intercept = -0.287), but the slope was significantly different from 1 (slope = 0.898, $p<0.05$). The coefficient of variation was 21.20, however, this is significantly affected by the systematic bias (Campana et al. 1995) and a better indication of precision can be obtained from the standard errors plotted in Fig. 16.

The greatest bias occurred between age estimates from the ANU and ODU (Fig. 16 and 17). Differences in age estimates for ages 3 and 4 were opposite to that found between ANU and CAF. Whereas CAF obtained older ages than ANU for these young fish, ODU obtained younger ages. Mean age estimates by the ODU reader were 1 to 2 years younger than the ANU estimates and the coefficient of variation was 28.3.

Scanning electron microscopy demonstrated the existence of otolith microstructure, generally interpreted as daily increments, in the Patagonian toothfish otoliths (Fig. 18 and 19). Although the temporal nature of the increments was not validated, these presumed daily increments could be used to provide some corroboration of the position of the first annual increment. Counts of presumed daily increments from the otolith core to the approximate outer edge of the first annulus (as estimated by the CAF) in three otoliths ranged from 134-185. Similar counts to the first annulus (as estimated by the ANU) ranged from 265-321.

Discussion

Age validation

The world's oceans are not homogenous in relation to the distribution of radiocarbon. The fact that radiocarbon varies spatially and temporally through the oceans is the basis for the value of this radioisotope as a tracer of ocean circulation and carbon flux between the ocean and atmosphere (e.g. Broecker et al. 1985). Although these properties are extremely important for studies of ocean dynamics, they increase the difficulty associated with the interpretation of radiocarbon data in fish otoliths in relation to age. For some fish species the distribution of various life history stages and the interpretation of otoliths in relation to fish age is well understood, for example, New Zealand snapper (*Pagrus auratus*) and Arcto-Norwegian cod (*Gadus morhua*). Analyses of radiocarbon from otoliths of these species can be used to determine the temporal variation of radiocarbon in particular locations (Kalish 1993), data that are important to research on ocean and atmosphere dynamics.

Due to the potentially broad distribution of juvenile Patagonian toothfish and the geographic extent of sample collections for this study across all sectors of the Southern Ocean, it was not feasible to construct a calibration curve of radiocarbon variation.

Furthermore, it is clear that there are differences in radiocarbon flux across sectors of the southern oceans. For example, waters that flow to the east as the Antarctic Circumpolar Current and then move north as the Peru Current would have signatures characterised by relatively low $\Delta^{14}C$. The Brazil Current, a western boundary current with relativey high $\Delta^{14}C$, mixes with the Falkland Current, predominated by subantarctic water, and may yield higher $\Delta^{14}C$ in the Falkland Island region. For the most part, these differences are manifestations of the atmospheric flux of radiocarbon and vertical and horizontal transport in the ocean.

Some Falkland Islands data diverge from the overall trend and have relatively high $\Delta^{14}C$ compared with other toothfish. The limited Falkland Islands data indicate a possible link between temperate and subantarctic radiocarbon in the region and this would be expected as suggested above. Furthermore, temporal variation in mixing may result in significant interannual variation in ocean $\Delta^{14}C$ superimposed on the bomb radiocarbon increase.

It was not feasible to determine statistically, deviations of $\Delta^{14}C$ measured in toothfish otolith cores from an estimated bomb radiocarbon curve. In a previous study (Kalish 1995b), an age estimation error was considered likely if a datum fell outside the 95% confidence interval for the curve describing the relationship between year and $\Delta^{14}C$. For this study on Patagonian toothfish, it was only feasible to provide a relatively crude validation of the age estimation method, essentially that bipartite structures made up of at least one opaque and one translucent zone are likely to be formed annually. This is due to the fact that birth dates could only be classified as pre-bomb or post-bomb and, more generally, as occurring during the rapid increase in ocean radiocarbon during the 1960s. It is difficult to translate this information into detectable age estimation errors, however it appears suited to recognition of age estimation errors on the order of 5 to 10 years.

Estimation of age and growth

Results presented here indicate that it is possible to determine the age of Patagonian toothfish with accuracy and precision that is adequate for some forms of stock assessment. Within and between reader variability of age estimates were relatively high, but within the range reported in other studies (Kimura and Lyons 1991, Campana et al. 1995). High coefficients of variation were due to a combination of the relatively old age of toothfish and difficulty in the interpretation of some increments. Within-reader variability had a mode of one (Fig. 15) indicating ages were higher on the second reading, though this was not significantly different from 0. This error was probably due to the interpretation of the first 3-5 annuli, and in particular the first annulus. The inner region of toothfish otoliths is more opaque than other segments, and is difficult to interpret in many sections. In general, the interpretation of the inner annuli was based on the presumption that they contain several opaque and translucent zones. Variability in the structure of these inner increments and the subjective nature of their interpretation undoubtedly causes some level of imprecision. Unfortunately, the validation based on the bomb radiocarbon chronometer was unable to resolve potential errors of this small magnitude in this species, particularly due to the lack of data on radiocarbon variability in the southern oceans and the nature of the broad geographic distribution of toothfish.

Further age validation research on this species is warranted to provide a better indication of the accuracy of the age estimates determined from otolith sections. Differences in age estimates for younger toothfish (< 5 years) among three laboratories indicated that there was a difference in the interpretation of the earliest formed opaque and translucent zones. A problem that frequently occurs in age estimation studies based on otoliths is the inability to define the first increment. Unfortunately, standard capture, mark and recapture studies on trawl or longline caught toothfish are unable to resolve this problem. A definitive validation for the first annual increment requires marking of individuals known to be less than one year of age and these are unlikely to be captured based on the capture methods typically employed in the subantarctic fisheries.

Alternatively, daily increments can be used to confirm the position of the first annual increment. This study has demonstrated that presumed daily increments formed during the larval and early juvenile periods could be discerned in otoliths collected from adult Patagonian toothfish. Collection of a series of small toothfish and the quantification of daily increments can be valuable tool in identifying earlier annuli. Several studies have previously identified daily increments in Antarctic fishes (e.g. Radtke et al. 1989, Ruzicka and Radtke 1995). In this study, consistent patterns of presumed daily increments were observed in Patagonian toothfish otoliths based on electron microscopy and these data were useful in estimating the position of the first annual increment. The results from a small sample of otoliths supported the use of the first annual increment as identified by the ANU and NIWA readers. However, the temporal nature of the 'daily' increments has not been validated and further investigation of these structures is warranted to validate and support toothfish age estimates from otoliths.

No large systematic biases were evident between otolith readers, however, further collaboration is required to define annuli more clearly and provide more precise estimates of toothfish age. Additional inter-laboratory comparisons, particularly on otoliths from fish that have been marked with some form of calciphilic marker (e.g. oxytetracyline hydrochloride or strontium chloride) are essential to provide a better indication of the accuracy of age estimates and to ensure that interpretation is consistent among agencies. This is of critical importance due to the fact that numerous agencies are involved in management of toothfish and these organisations may carry out independent studies of toothfish age and apply these data to stock assessments. Although mark-recapture studies are unlikely to be suitable for validation of perhaps the first and second increments, it would provide validation of annuli for later increments. Interpretation of otoliths from marked fish after several years at liberty would be particularly important.

Otolith collections that are more representative of the toothfish population from a particular location are needed for accurate modelling of fish growth. To determine population age and growth, this study used two otoliths collections obtained from toothfish captured around Macquarie Island by demersal trawling. Trawls for Patagonian toothfish typically operate at depths of less than 1000m, not as deep as the average capture depth for toothfish fished by longlines (e.g. Des Clers et al. 1996). As toothfish inhabit deeper depths with greater fish size (Duhamel 1993) larger fish were excluded from the Macquarie Island collections. Based on the known maximum size of toothfish collected at numerous locations, the Macquarie Island samples used in this study are not representative of the Macquarie Island population; they are representative only of the currently exploited population.

There was a significant difference between both the number and length of each sex in both seasons which is either due to a bias in the capture method or reflective of a lack of large males in the population. Significant differences in length between sexes have been recorded from Patagonian toothfish collected from the Falkland Islands and South Georgia fisheries (Des Clers et al. 1996). They found that male fish were typically less than 1400 mm, although one large male was 1700 mm. However, there was no significant difference in the number of each sex in the Falkland Islands (Des Clers et al. 1996). Therefore, the difference in lengths observed at Macquarie Island and the Falkland Islands may indicate that male fish do not grow as large as female. This could be linked to the toothfish life history strategy, if it is such that there is no benefit to male fish growing large. Alternatively, male toothfish may inhabit different habitats to females, and either escape capture or suffer increased mortality, preventing them from reaching large size. Also, as the sample investigated here was stratified on the basis of length and not a random sample of the total catch it may not be representative of the sex ratio of whole population.

There were significant differences between VBGF parameters for male and female toothfish collected in the 1996/97 season, but these results were inconclusive. This was largely due to the lack of large males, which resulted in lower estimates of L_∞ and higher estimates of K for males. Male and female toothfish appear to grow at similar rates in the first 10-15 years, after which the lack of male fish is responsible for this significant difference. Furthermore, this was exaggerated by the higher number of large female fish in the 1996/97 season compared to the 1995/96 season. Further research is required to determine possible differences in growth rates for male and female toothfish.

Conclusions

Toothfish otoliths analysed for radiocarbon yielded otolith section ages from 2 to 43 years and associated birth dates between 1940 and 1988. The resultant relationship between birth date estimated from the otolith thin sections and $\Delta^{14}C$ measured in the otolith cores showed that $\Delta^{14}C$ was consistent with the expected variation in Southern Ocean $\Delta^{14}C$ and thereby provided a validation for the age estimates. There were significant differences in otolith core $\Delta^{14}C$ for the toothfish from different localities, consistent with the broad geographic separation of the collections and indicative of stock separation for toothfish in the southern oceans. Based on a sample of 994 otoliths from Macquarie Island, toothfish are slow growing with estimates of K and L_∞ for the different years and sexes ranging from 0.005-0.116 (95% confidence intervals) and 1,087 to 10,405 (95% confidence intervals), respectively. Effective modelling of toothfish growth will require samples that are more representative of the population and should include fish captured by trawling and, from greater depths, by longlining. A subsample of toothfish otolith sections read by four laboratories demonstrated some bias in age estimates, but this was considered small given the apparent maximum age of toothfish of around 45 years. Further validation of toothfish age is required to confirm the nature of the first annulus and quantify the precision of age estimates from otolith thin sections.

Acknowledgments

Samples were obtained due to cooperation among several groups including Austral Fisheries (Murray France and crew of the Austral Leader), Australian Fisheries Management Authority (Anthony De Fries, Stephanie Kalish) Australian Antarctic Division (Dick Williams), British Antarctic Survey (Julian Ashford, Andrew North), South African Sea Fisheries Research Institute (David Japp), The Falkland Islands Fisheries Department (Conor Nolan) and the Museum National d'histoire Naturelle (Paris). We thank Julian Ashford (Old Dominion University), Peter Horn (National Institute for Water and Atmospheric Research), Kyne Krusic-Golub and Corey Green (Central Ageing Facility) for their cooperation in the reading of toothfish otoliths and Shoko Okada (Australian National University) for scanning electron microscopy. This research was supported by funds from the Antarctic Sciences Advisory Committee and the Fisheries Research and Development Corporation.

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Figure Captions

Figure 1. Antarctica and the Southern Ocean region with the locations of Patagonian toothfish *Dissostichus eleginoides* fisheries sampled for the validation and age and growth study.

Figure 2. Patagonian toothfish otolith affixed to a glass microscope slide with Crystalbond and shown after isolation of core region with the ultrasonic disc cutter. The diameter of the core is 3.0 mm.

Figure 3. Patagonian toothfish otolith after removal from the glass slide. Crystalbond was removed by dissolving in acetone. The core, isolated with the ultrasonic disc cutter, comes free after removal of the Crystalbond and is now ready for further grinding with the mechanical jig described in the text. The diameter of the core is 3.0 mm.

Figure 4. Transverse section of an otolith from a Patagonian toothfish estimated to be 14 years of age. The broad and narrow arrows indicate the first continuous translucent zone, estimated to be the completion of the first annual increment by the ANU and CAF, respectively.

Figure 5. Transverse section of an otolith from a Patagonian toothfish estimated to be 15 years of age. The broad and narrow arrows indicate the first continuous translucent zone,

estimated to be the completion of the first annual increment by the ANU and CAF, respectively.

Figure 6. Transverse section of an otolith from a Patagonian toothfish estimated to be 25 years of age. The broad and narrow arrows indicate the first continuous translucent zone, estimated to be the completion of the first annual increment by the ANU and CAF, respectively.

Figure 7. $\Delta^{14}C$ of Patagonian toothfish otolith cores plotted against the birth date determined by Reader 2 (see Table 1). Errors plotted are 1 standard deviation, but most are too small to be seen due to the size of the data points.

Figure 8. $\Delta^{14}C$ of Patagonian toothfish otolith cores plotted against the birth dates determined by Reader 1 and Reader 2 (see Table 1). Age has been clearly underestimated for at least two of the Falkland Islands toothfish by Reader 1. Errors plotted are 1 standard deviation, but most are too small to be seen due to the size of the data points.

Figure 9. Differences (years) between age estimates determined for the validation set of otoliths (Table 1) by Reader 1 and Reader 2.

Figure 10. $\Delta^{14}C$ of Patagonian toothfish otolith cores plotted against the birth date determined by Reader 1 (see Table 1). $\Delta^{14}C$ data from New Zealand snapper *Pagrus auratus* otolith cores are plotted against the true birth dates (Kalish 1993) and

demonstrate the difference in $\Delta^{14}C$ between temperate and subantarctic waters, but also demonstrate similar trends in $\Delta^{14}C$ due to bomb radiocarbon. Errors plotted are 1 standard deviation, but most are too small to be seen due to the size of the data points.

Figure 11. Length plotted against weight for Patagonian toothfish captured at Macquarie Island in the austral summers of 1995/96 and 1996/97. The exponential relationship between these data is weight = $3.06 \times 10^{-8} \times$ total length$^{2.856}$. Otolith samples from these fish were used in the study of age and growth.

Figure 12. Relationships between Patagonian toothfish age estimated from otolith thin sections and otolith weight and otolith morphometrics.

Figure 13. Relationships between Patagonian toothfish total length and otolith weight and otolith morphometrics.

Figure 14. Age estimated from otolith thin sections plotted against total length and von Bertalanffy growth functions for Patagonian toothfish captured at Macquarie Island in the austral summers of 1995/96 (upper) and 1996/97 (lower). Parameter estimates for the von Bertalanffy growth functions are presented in Table 2.

Figure 15. Age bias graph (upper) for a pair-wise comparison of repeat age estimates by Reader 1. Error bars represent the 95% confidence interval about the mean age assigned by Reader 1 (2nd reading) for all fish assigned a given age by Reader 1 (1st reading).

Distribution of age differences between otolith readings (lower). Differences were calculated by subtracting the first age estimate from the second.

Figure 16. Age bias graphs for pair-wise comparisons of repeat age estimates by Reader 1 (Australian National University), Reader 2 (Central Ageing Facility) (upper), Reader 3 (National Institute for Water and Atmospheric Research) (centre) and Reader 4 (Old Dominion University) (lower). Error bars represent the 95% confidence interval about the mean age assigned by Reader 1 for all fish assigned a given age by a second reader. Age estimate comparisons were based on randomly selected samples from Macquarie Island. Samples were prepared by Reader 1.

Figure 17. Distribution of age differences between otolith readings based on pair-wise comparisons of age estimates by Reader 1 (Australian National University), Reader 2 (Central Ageing Facility) (upper), Reader 3 (National Institute for Water and Atmospheric Research) (centre) and Reader 4 (Old Dominion University) (lower). Age estimate comparisons were based on randomly selected samples from Macquarie Island. Samples were prepared by Reader 1.

Figure 18. Scanning electron micrograph of an acid etched thin section of an otolith from a Patagonian toothfish estimated to be 16 years of age. The arrows labelled ANU and CAF identify the estimated position of the outer edge of the first annual increment as determined by those two groups. Counts of presumed daily increments were made from the otolith core to these positions.

Figure 19. Scanning electron micrograph of an acid etched thin section of an otolith from a Patagonian toothfish estimated to be 16 years of age. The presumed daily increments shown here are dorsal to the otolith core.

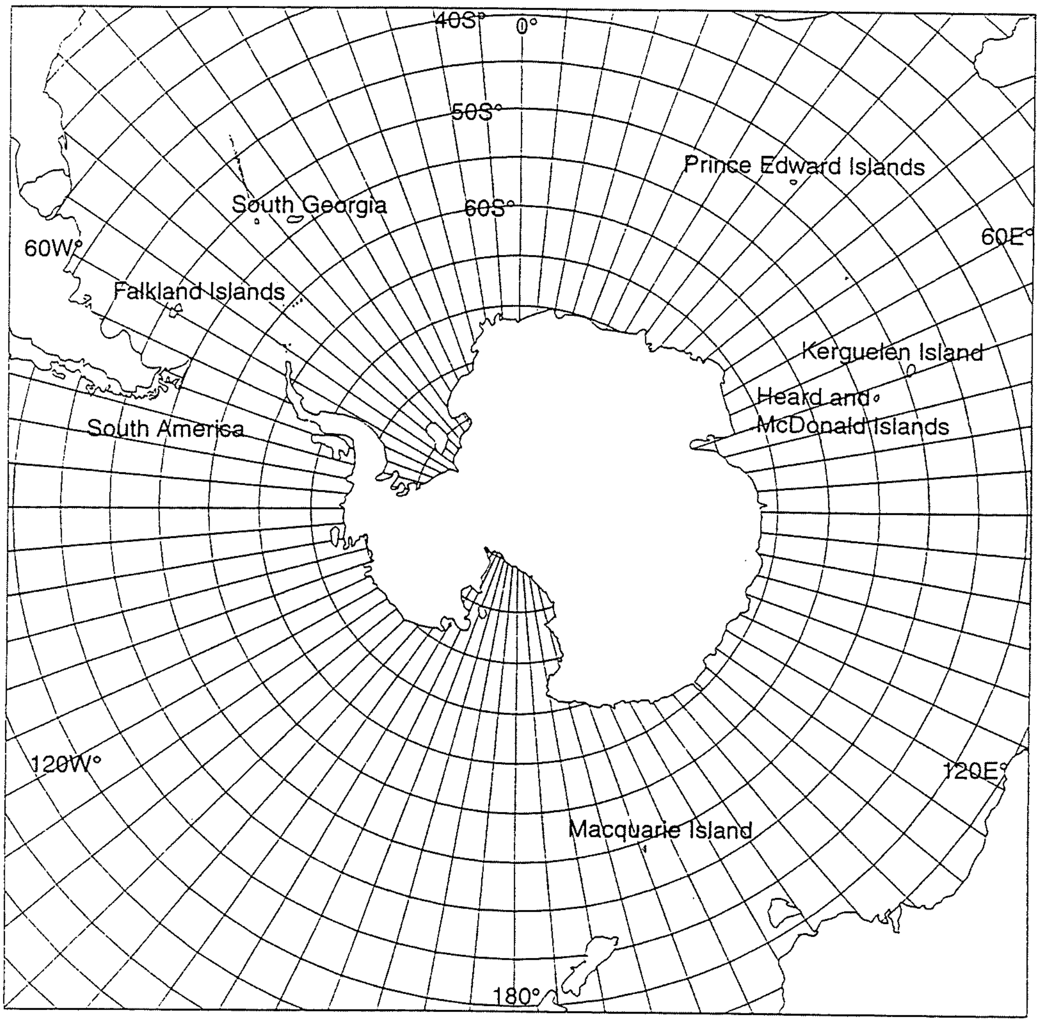
Table 1. Fish, otolith, and radiocarbon data for Patagonian toothfish *Dissostichus eleginoides* collected from several sites. Errors are standard deviations.

Sample No.	Collection location	Collection date	Fish length (cm)	Otolith weight (g)	Sample weight (mg)	$\Delta^{14}C$ (‰)	Otolith section age (years) Reader 2	Birth date (year A.D.)	Otolith section age (years) Reader 1	Birth date (year A.D.)
DTN 2	South Georgia	23 Aug 1980	204	-	10.2	-132.6±7.3	40	1940	40	1940
DTN 10	South Georgia	23 Aug 1980	126	-	9.8	-96.3±7.9	14	1966	14	1966
DTN 13	South Georgia	23 Aug 1980	165	-	9.3	-128.3±7.9	18	1962	18	1962
PT 1	Macquarie I	27 Jan 96	187	0.6827	2.3	-110±5.7	41	1957	40	1958
PT 15	Falkland I	Apr 94	152	0.425	2.2	-11.2±8.7	26	1968	28	1966
PT 24	Falkland I	Apr 94	158	0.3843	7.5	56.5±6.3	25	1969	28	1966
PT41	Falkland I	Apr 94	165	0.56	9.5	-66.4±9.1	29	1965	30	1964
PT 46	Falkland I	Apr 94	170	0.4908	2.1	4.1±9.1	27	1967	22	1973
DEF 1	Falkland I	1995	-	0.3468	7.5	-40.3±6.3	33	1962	31	1964
DEF 2	Falkland I	1995	-	0.2087	7.9	-78.9±6.8	15	1980	16	1979
DEF 3	Falkland I	1995	-	0.4501	9.6	-66.2±5.4	35	1960	38	1957
DEF 4	Falkland I	1995	-	0.3755	9.9	-35.7±5.4	31	1964	28	1967
DEF 5	Falkland I	1995	-	0.3169	9.5	-30.2±4.6	28	1967	37	1958
DEF 14	Falkland I	1995	-	0.3012	9.5	25.3±6.8	25	1964	25	1970
DEF 18	Falkland I	1995	-	0.3990	9.6	-91.1±5.4	37	1958	38	1957
DEF 26	Falkland I	1995	-	0.2581	8.8	-29±5.8	20	1975	22	1973
DEF 34	Falkland I	1995	-	0.1916	9.9	30.6±6.1	10	1985	8	1987
DEF 66	South Georgia	1995	-	0.3936	7.8	-101.2±5.9	34	1961	40	1955
H90 0280	Heard I	25 May 90	84	0.1512	7.6	-0.1±6.1	14	1976	12	1978
H90 0279	Heard I	25 May 90	92	0.1665	7.7	-31.5±6.3	18	1972	14	1976
H90 3035	Heard I	17 Jun 90	21	0.0094	9.4	-43.9±5.8	2	1988	2	1988
DE KI 4	Kerguelen I	21 Dec 79	114	0.2255	4.6	-77.0±6.0	15	1964	19	1960
DE KI 5	Kerguelen I	20 Jan 79	112	0.1863	7	-58.9±6.1	15	1964	16	1963
DE KI 6	Kerguelen I	20 Dec 79	128	0.3006	3.6	-75.8±7.3	15	1964	17	1962
DE KI 7	Kerguelen I	20 Dec 79	99	0.2018	5	-38.5±5.7	11	1968	15	1964
DE 10	Prince Edward I	19 Nov 96	140	0.432	7	-115.2±5.0	39	1957	43	1953
DE 27	Prince Edward I	6 Nov 96	136	0.4822	8.1	-11.6±5.6	26	1970	26	1970
DE 35	Prince Edward I	10 Nov 96	167	0.5363	8.1	-122.1±5.1	38	1958	34	1962
DE 85	Prince Edward I	20 Sep 96	172	0.4752	6.3	-126.8±5.0	40	1956	42	1954
DE89	Prince Edward I	20 Sep 96	138	0.4306	6.1	-94.4±5.3	29	1967	32	1964
DE 95	Prince Edward I	20 Sep 96	146	0.3331	10.5	-108.2±5.0	29	1967	29	1967
DESAM 15	Chile	31 Dec 95	190	0.6614	9.2	6.7±5.2	23	1972	24	1971
DESAM 20	Chile	22 Dec 95	217	0.5896	6.5	-83±4.8	34	1961	28	1967
DESAM 21	Chile	28 Dec 95	215	0.5716	7.3	-58.5±5.3	27	1968	26	1969
DESAM 31	Chile	25 Jan 96	174	0.4867	10.9	-93.4±5.6	35	1961	35	1961
DESAM 34	Chile	5 Jan 96	168	0.5064	9.7	-55±4.9	31	1965	28	1968
DESAM 63	Chile	15 Feb 96	174	0.7231	9.4	-93.2±6.0	36	1960	39	1957

Table 2. Estimated parameters and 95% confidence intervals of the von Bertalanffy growth function (VBGF) for *Dissostichus eleginoides* collected from Macquarie Island during the 1995/1996 and 1996/1997 fishing seasons. Data are presented by year and sex. The VBGF from 1996/1997 was calculated with all fish (N=498) and less the two largest fish (N=496) due to their dramatic effect on parameter estimates for the VBGF. Comparisons between VBGF parameters between years and between sexes within a year showed a significant difference between males and females in 1996/1997 ($F_{(3,492)}=14.6$, $P<0.05$). The VBGF parameters were not signficantly different between sexes in 1995/1996 ($F_{(3,496)}=2.4$, $P<0.1$).

Season	Sex	N	L_∞			t_0			K		
			Estimate	Lower CI	Upper CI	Estimate	Lower CI	Upper CI	Estimate	Lower CI	Upper CI
95/96	combined	500	1892	1458	3737	-3.53	-5.89	-1.95	0.040	0.015	0.066
	male	204	1392	1087	3795	-3.00	-7.08	-0.9	0.063	0.013	0.116
	female	283	1674	1337	3055	-2.51	-5.27	-0.79	0.053	0.020	0.087
96/97 (all)	combined	498	2999	2248	5154	-2.42	-3.44	-1.59	0.025	0.012	0.038
	male	171	1384	1126	2114	-1.37	-3.01	-0.27	0.072	0.035	0.114
	female	246	3298	2210	10405	-2.74	-4.52	-1.42	0.022	0.005	0.041
96/97 (-2)	combined	496	2217	1818	3094	-1.77	-2.7	-1.03	0.039	0.024	0.054
	male	171	1384	1126	2114.	-1.37	-3.01	-0.27	0.072	0.035	0.114
	female	245	2053	1640	3300	-1.54	-3.09	-0.45	0.045	0.022	0.070

Figure 1



40S°
0°
50S°
60S°
Prince Edward Islands
South Georgia
60W°
60E°
Falkland Islands
Kerguelen Island
Heard and
McDonald Islands
South America
120W°
120E°
Macquarie Island
180°

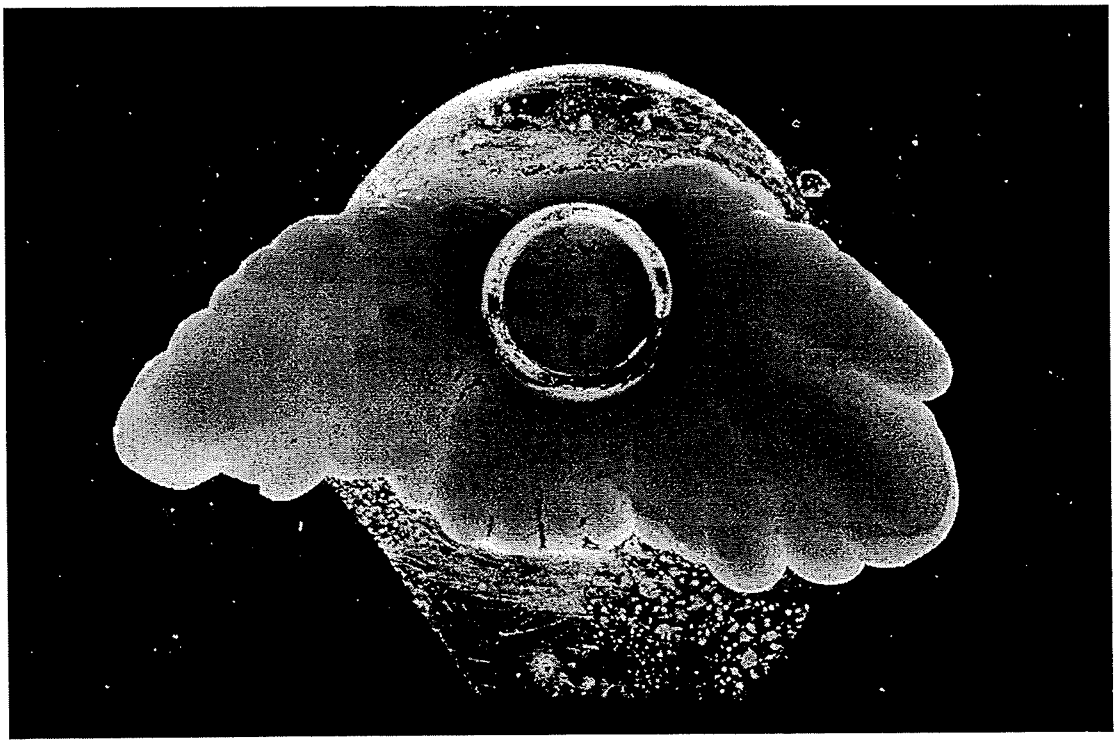
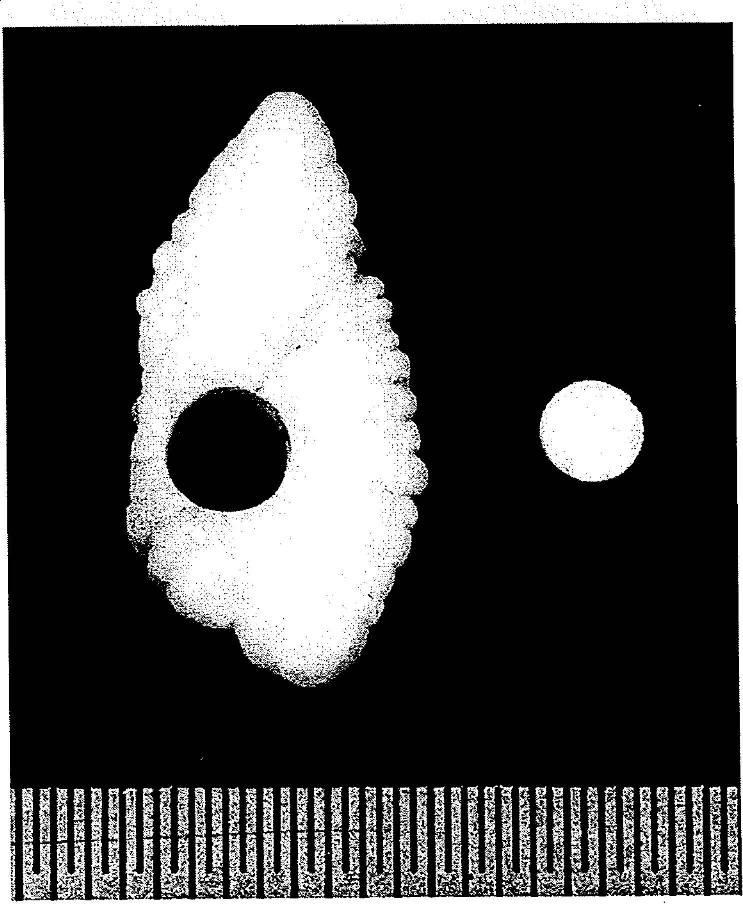


Figure 2



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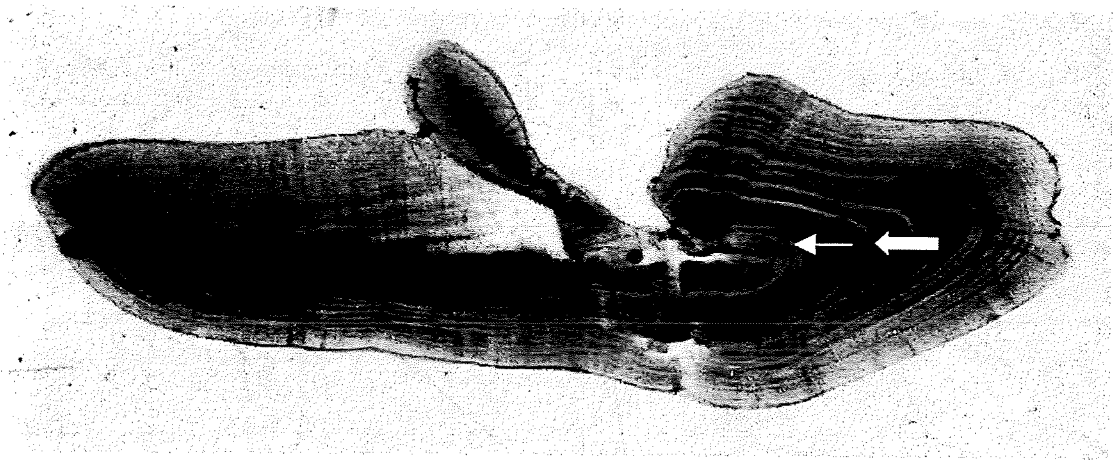


Figure 4

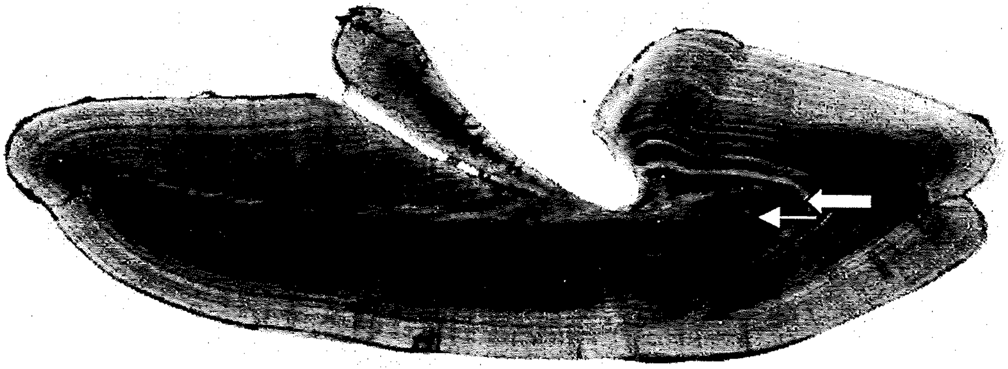


Figure 5

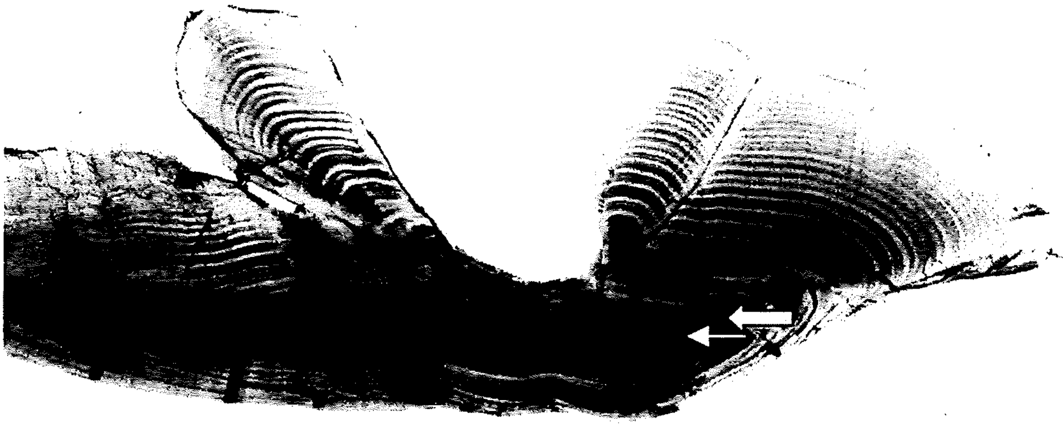
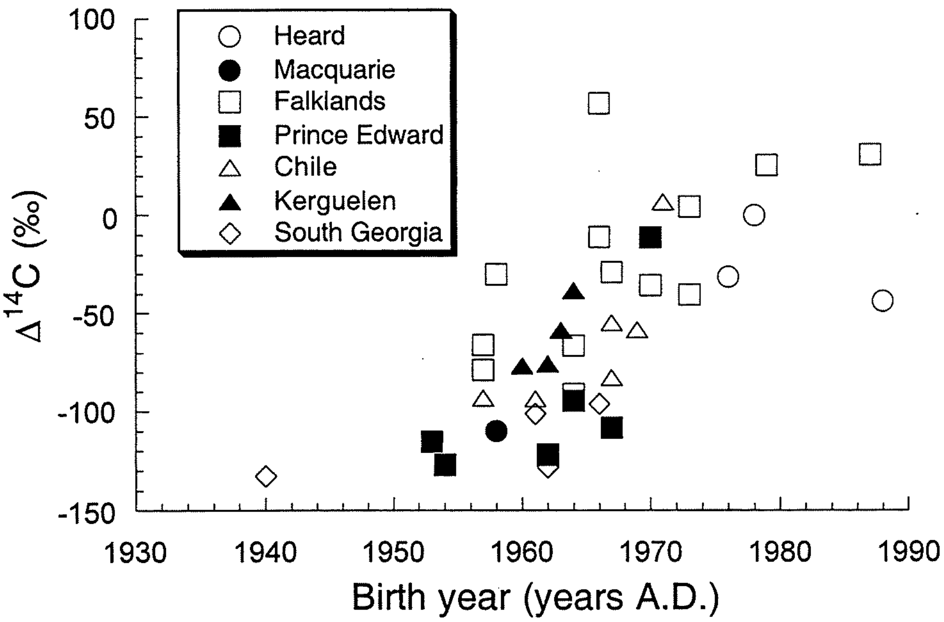


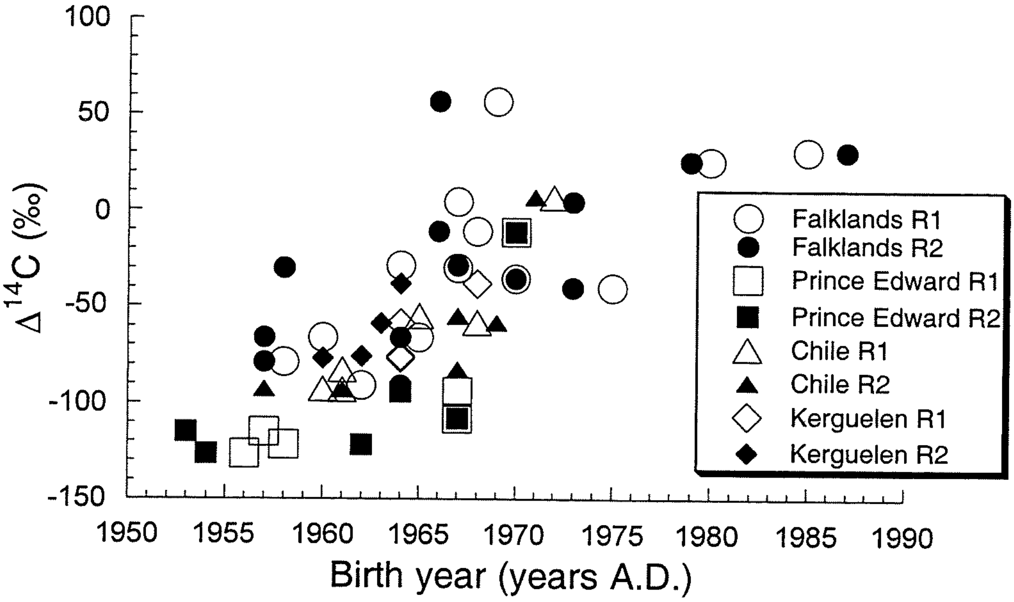
Figure 6

Figure 7



○ Heard
● Macquarie
□ Falklands
■ Prince Edward
△ Chile
▲ Kerguelen
◇ South Georgia
$\Delta^{14}C$ (‰)
100
50
0
-50
-100
-150
1930
1940
1950
1960
1970
1980
1990
Birth year (years A.D.)

Figure 8



100
50
0
-50
-100
-150
Δ¹⁴C (‰)
1950
1955
1960
1965
1970
1975
1980
1985
1990
Birth year (years A.D.)
○ Falklands R1
● Falklands R2
□ Prince Edward R1
■ Prince Edward R2
△ Chile R1
▲ Chile R2
◇ Kerguelen R1
◆ Kerguelen R2

Figure 9

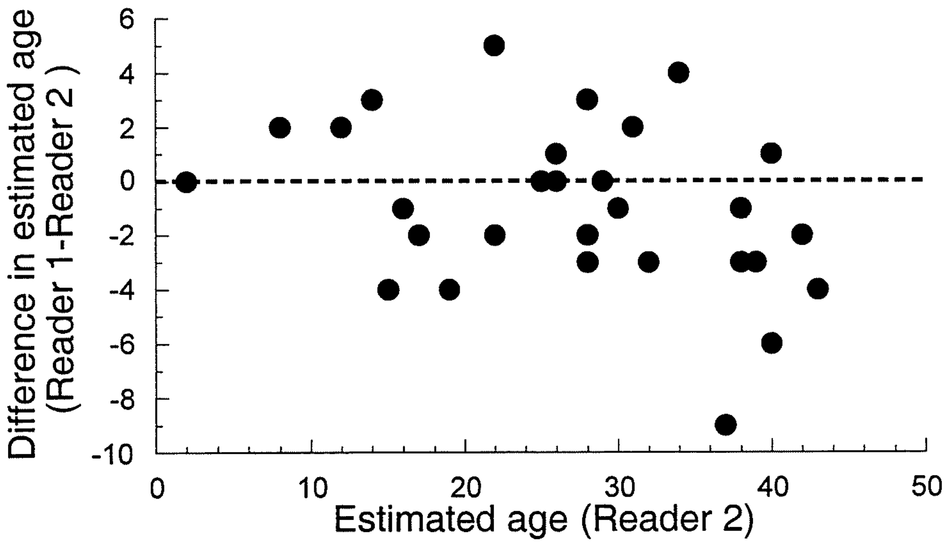
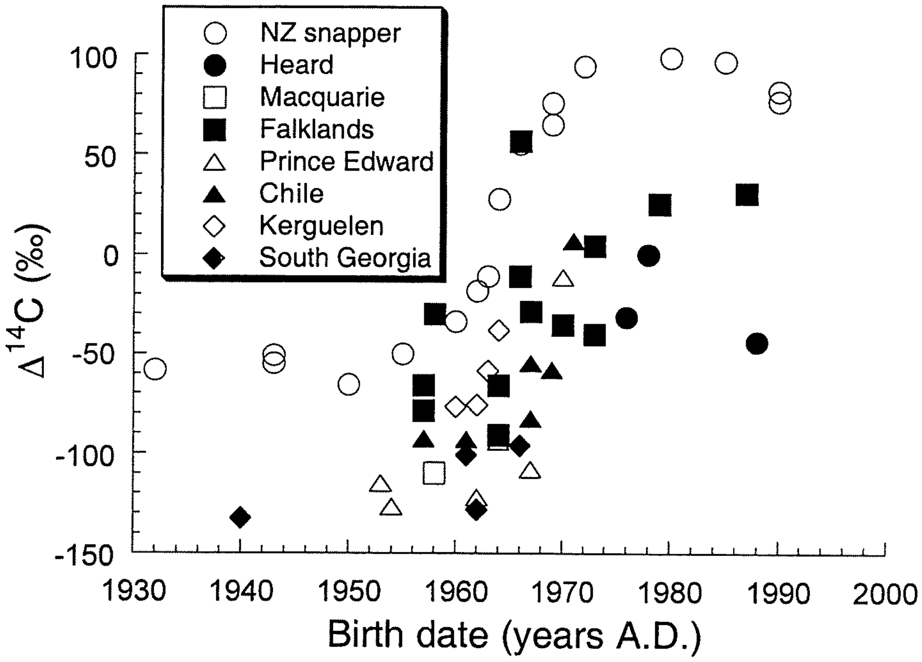

Difference in estimated age
(Reader 1-Reader 2)
6
4
2
0
-2
-4
-6
-8
-10
0
10
20
30
40
50
Estimated age (Reader 2)

Figure 10



○ NZ snapper
● Heard
□ Macquarie
■ Falklands
△ Prince Edward
▲ Chile
◇ Kerguelen
◆ South Georgia
100
50
0
-50
-100
-150
$\Delta^{14}C$ (‰)
1930
1940
1950
1960
1970
1980
1990
2000
Birth date (years A.D.)

Figure 11

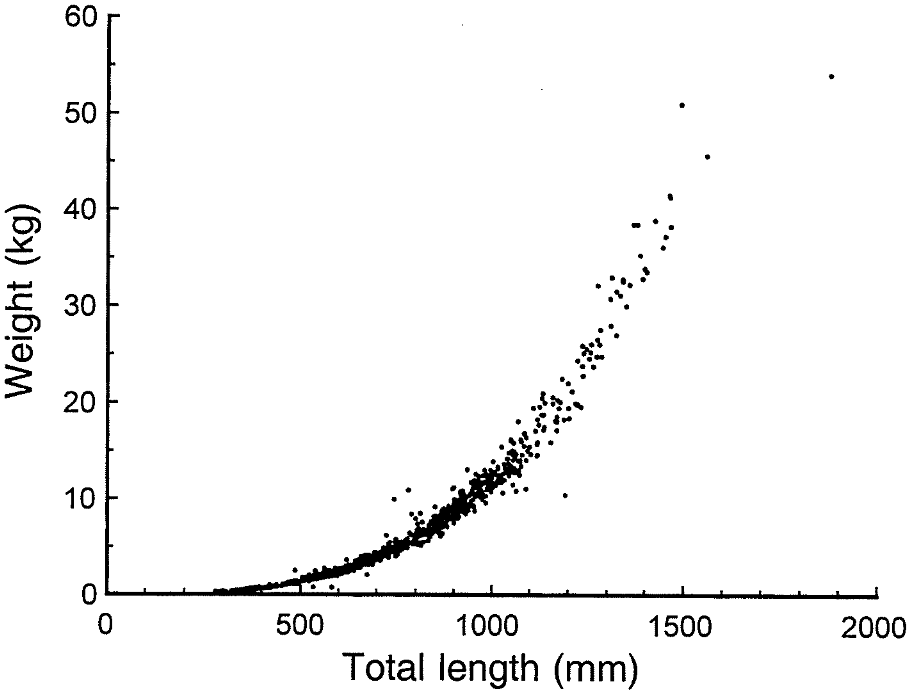

60
50
40
30
20
10
0
Weight (kg)
0
500
1000
1500
2000
Total length (mm)

Figure 12

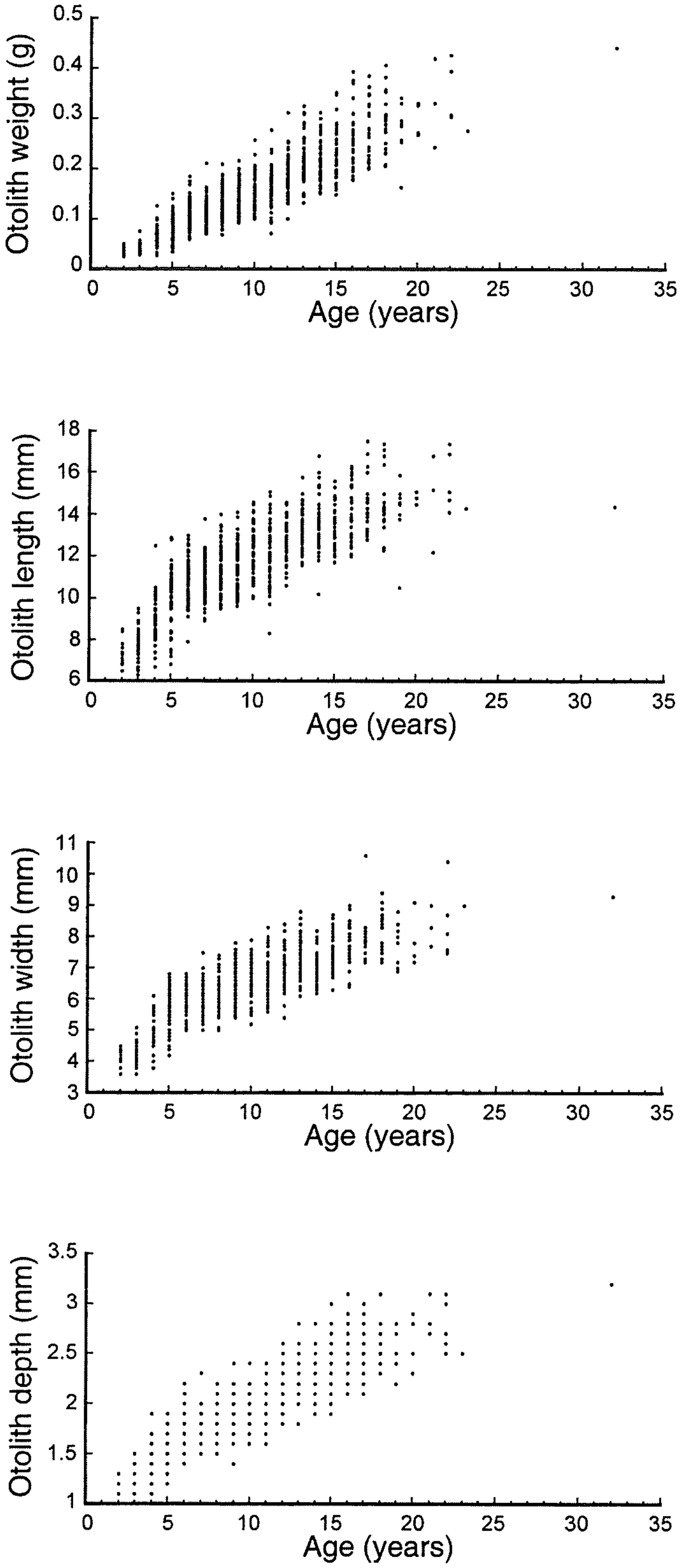
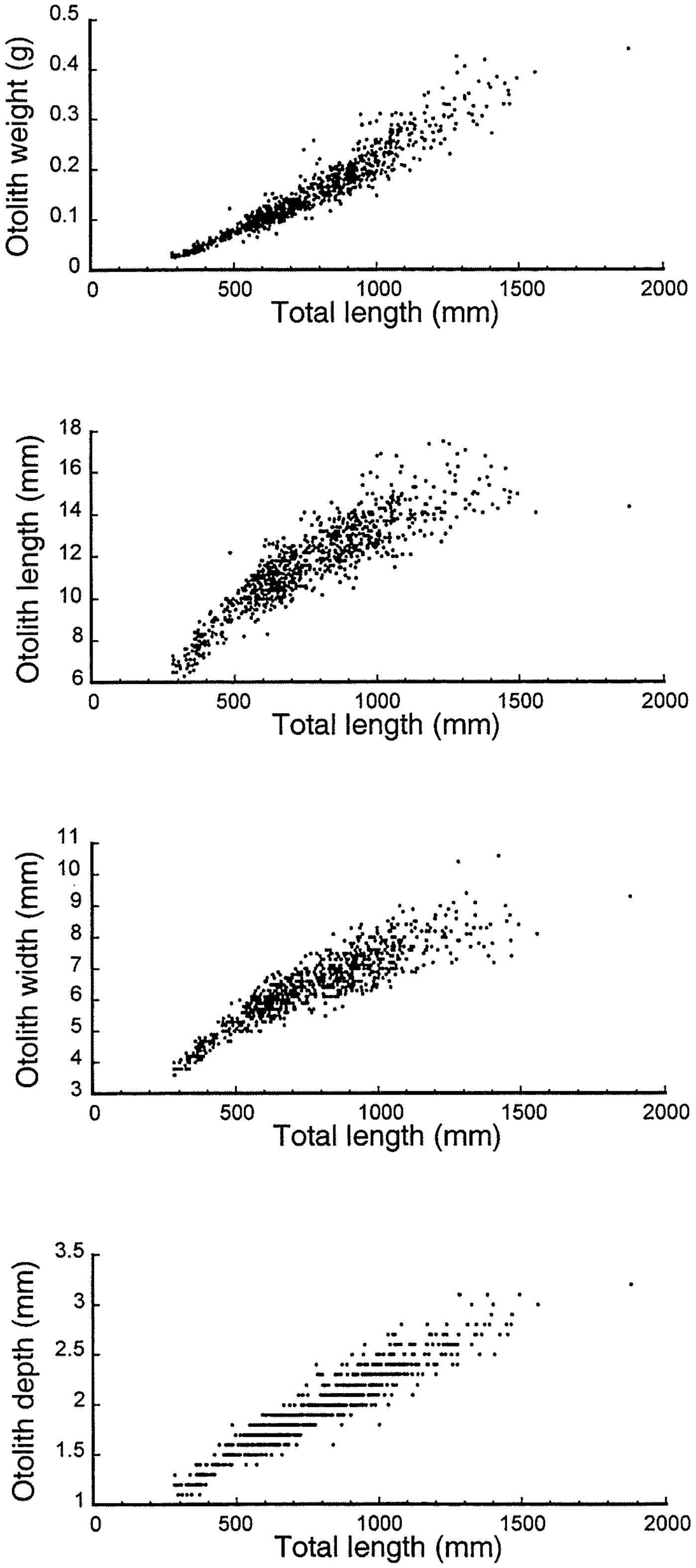
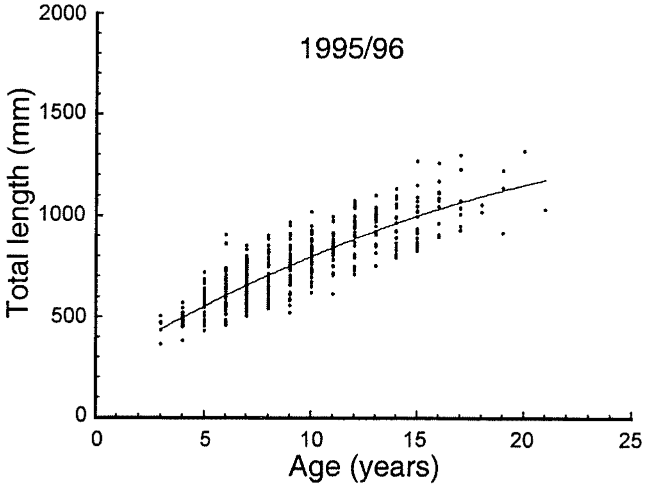

Otolith weight (g)
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
30
35
Age (years)
Otolith length (mm)
18
16
14
12
10
8
6
0
5
10
15
20
25
30
35
Age (years)
Otolith width (mm)
11
10
9
8
7
6
5
4
3
0
5
10
15
20
25
30
35
Age (years)
Otolith depth (mm)
3.5
3
2.5
2
1.5
1
0
5
10
15
20
25
30
35
Age (years)

Figure 13



Otolith weight (g)
0.5
0.4
0.3
0.2
0.1
0
0
500
1000
1500
2000
Total length (mm)
Otolith length (mm)
18
16
14
12
10
8
6
0
500
1000
1500
2000
Total length (mm)
Otolith width (mm)
11
10
9
8
7
6
5
4
3
0
500
1000
1500
2000
Total length (mm)
Otolith depth (mm)
3.5
3
2.5
2
1.5
1
0
500
1000
1500
2000
Total length (mm)

Figure 14


1995/96
Total length (mm)
2000
1500
1000
500
0
0
5
10
15
20
25
Age (years)

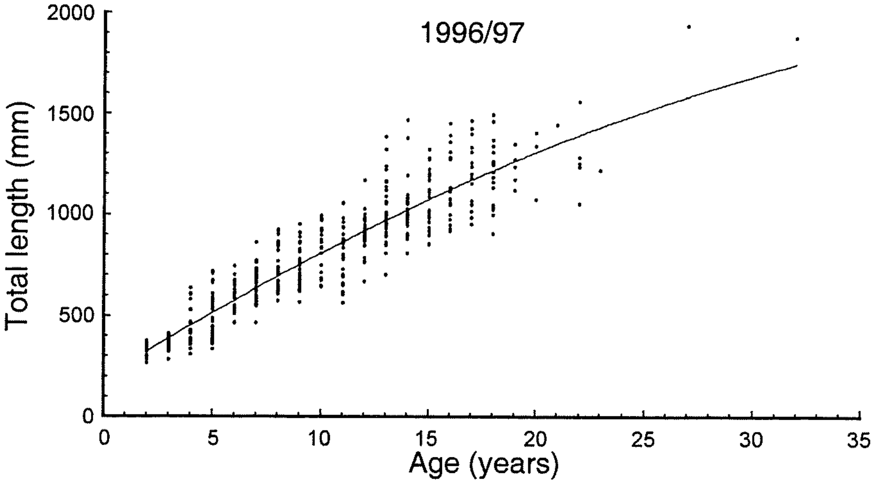

1996/97
Total length (mm)
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
Age (years)

Figure 15

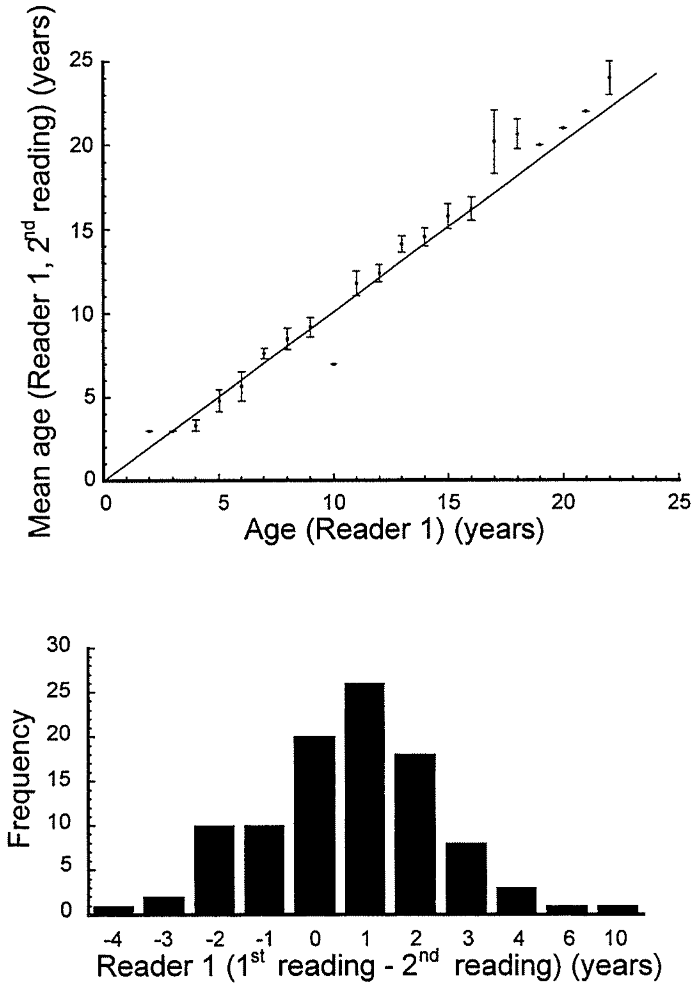
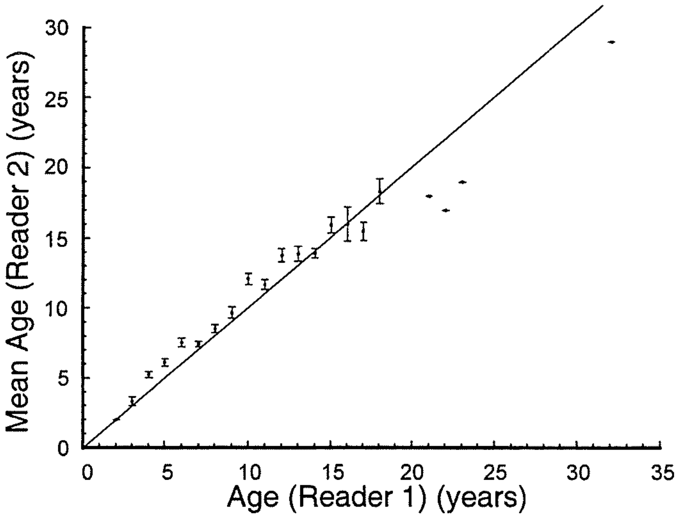
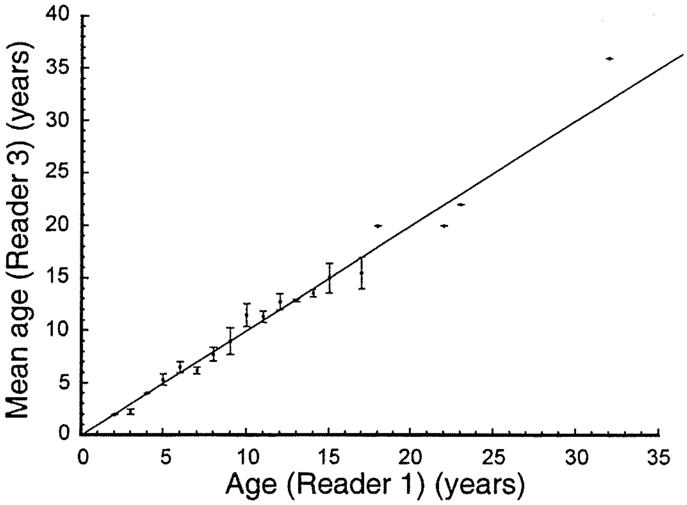

Mean age (Reader 1, 2nd reading) (years)
0
5
10
15
20
25
Age (Reader 1) (years)
0
5
10
15
20
25
Frequency
0
5
10
15
20
25
30
-4
-3
-2
-1
0
1
2
3
4
6
10
Reader 1 (1st reading - 2nd reading) (years)

Figure 16



Mean Age (Reader 2) (years)
0
5
10
15
20
25
30
Age (Reader 1) (years)
0
5
10
15
20
25
30
35



Mean age (Reader 3) (years)
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10
15
20
25
30
35
40
Age (Reader 1) (years)
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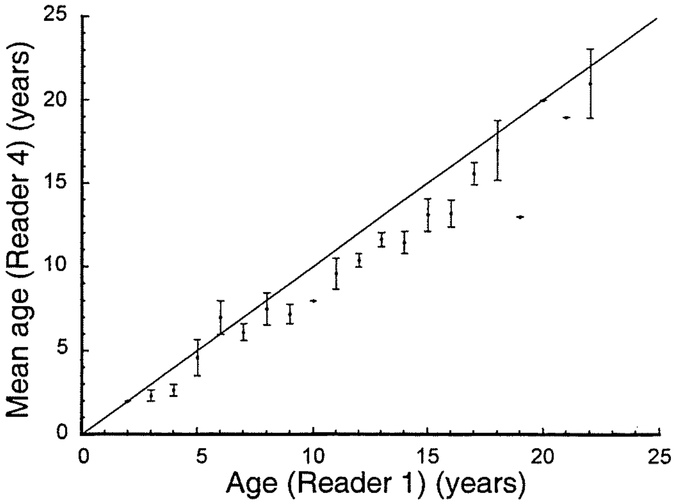
5
10
15
20
25
30
35



Mean age (Reader 4) (years)
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5
10
15
20
25
Age (Reader 1) (years)
0
5
10
15
20
25

Figure 17

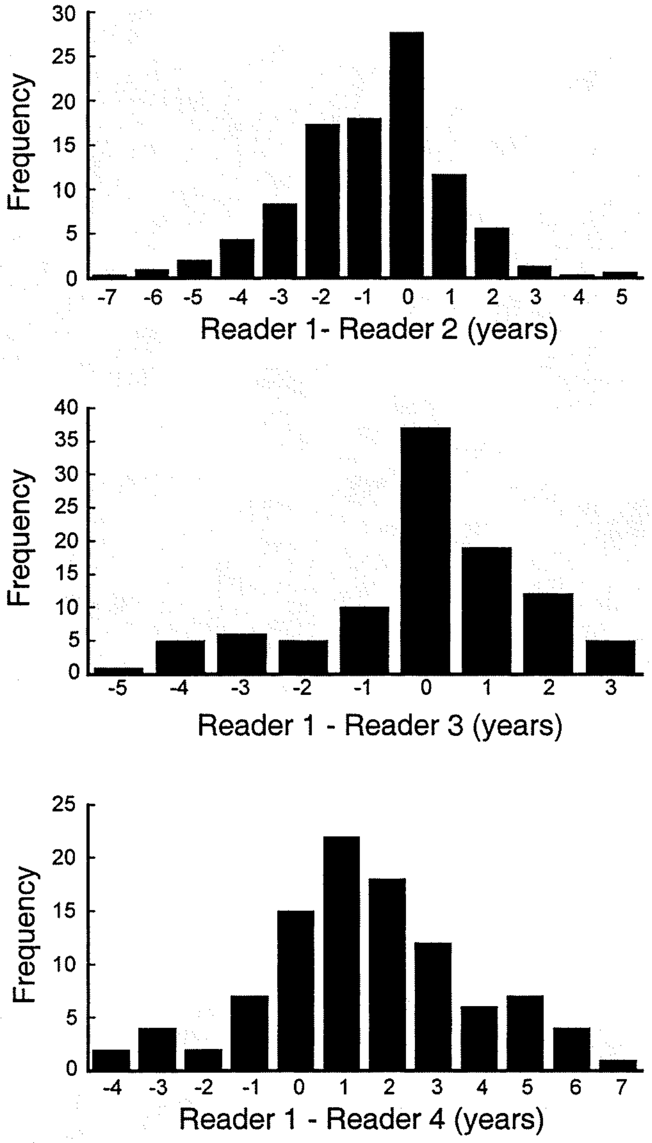

Frequency
30
25
20
15
10
5
0
-7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5
Reader 1- Reader 2 (years)
Frequency
40
35
30
25
20
15
10
5
0
-5 -4 -3 -2 -1 0 1 2 3
Reader 1 - Reader 3 (years)
Frequency
25
20
15
10
5
0
-4 -3 -2 -1 0 1 2 3 4 5 6 7
Reader 1 - Reader 4 (years)

Figure 18

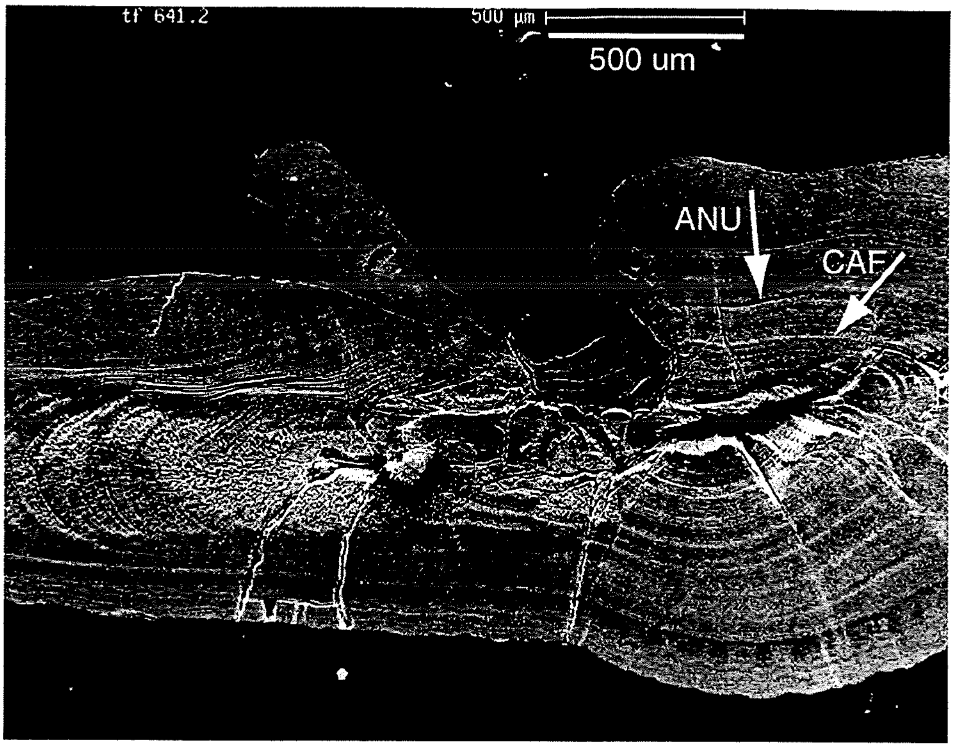
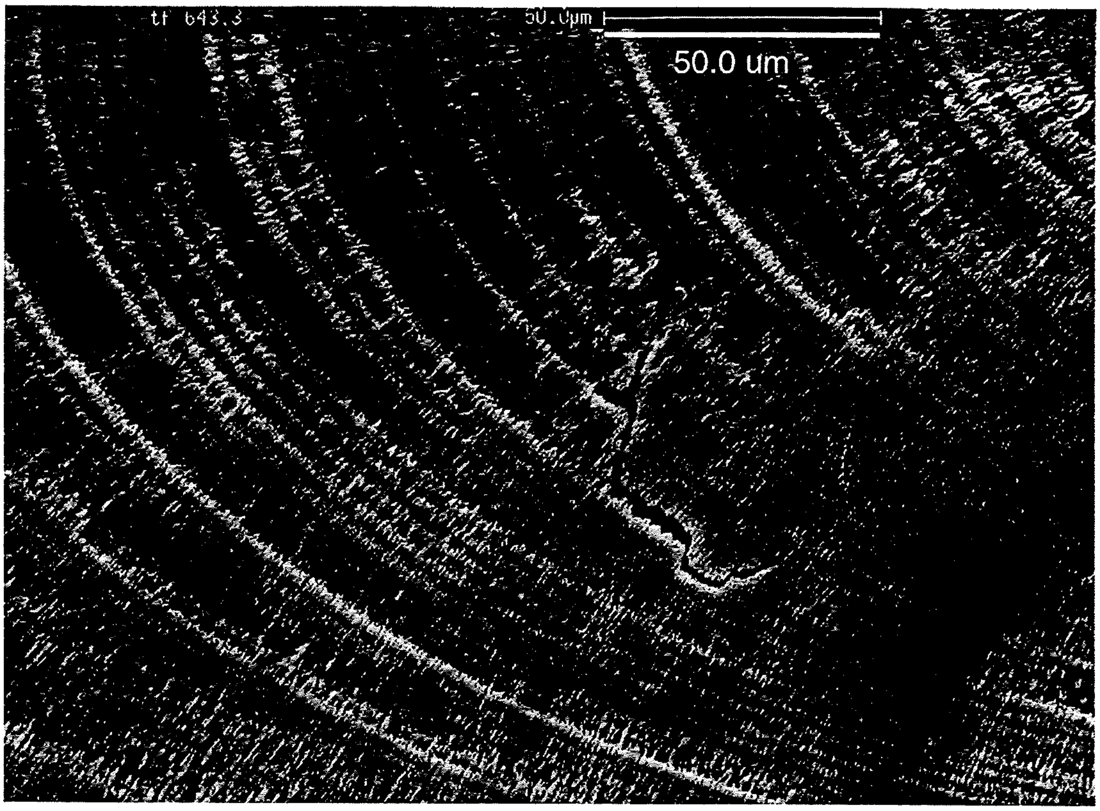


Figure 19



14. Appendix 2

Discrimination of Patagonian toothfish *Dissostichus eleginoides* stocks based on otolith composition and morphometrics

FRDC Project 97/123

Discrimination of Patagonian toothfish *Dissostichus eleginoides* stocks based on otolith composition and otolith morphometrics

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Abstract

Otolith morphometrics and otolith chemistry were used to discriminate among putative stocks of Patagonian toothfish *Dissostichus eleginoides* in the Southern Ocean region. Otolith samples were collected from fishing vessels engaged in longlining or trawling activities in toothfish fisheries around Chile, Falkland Islands, Heard and McDonald Islands, Kerguelen Island, Macquarie Island, Prince Edward Islands and South Georgia. Otolith shapes were described with Fourier shape descriptors and otolith chemistry was determined with laser ablation-inductively coupled plasma mass spectrometry. Jackknifed multivariate canonical analyses were used to classify samples to location. Overall classification success was 73% based on the chemical composition of the otolith cores. Otolith chemistry was far more effective than otolith morphometrics for the identification of stocks although both methods provided evidence for stock separation. Analysis of chemical data from the cores of toothfish otoliths collected in several fisheries suggests that there are at least four stocks in the Southern Ocean. These stocks include a South American group, Falkland Islands group, Macquarie Island group and an Indian Ocean group comprised of toothfish collected from islands in the Indian Ocean sector of the Southern Ocean (Prince Edward Islands, Kerguelen Island and Heard and McDonald Islands). Stock differences were determined on the basis of the composition of otolith cores and this suggest that there are at least four distinct spawning areas or nursery grounds for Patagonian toothfish and minimal dispersal among these areas. The lack of separation of toothfish from the Indian Ocean group may be due to movement of larval or adult toothfish between these islands or the inability of the otolith chemistry method employed to separate these potential stocks.

Introduction

The biology and wide distribution of Patagonian toothfish among subantarctic islands and seamounts suggests that several stocks may be present. In many cases, known regions of high toothfish abundance are separated by large distances (>1000 km) (Fig. 1). This broad geographic separation between most toothfish fisheries and the fact that many of the suitable subantarctic island habitats are under control of different soverign nations has resulted in the treatment of many toothfish populations as separate stocks, despite the lack of scientific data to support this assumption. Studies directed at the identification of potential stocks of Patagonian toothfish are essential to ensure sustainable development of this valuable Southern Ocean resource.

The identification of unique management units or stocks is central to the effective regulation of commercial fisheries (e.g., Ihssen et al. 1981, Begg et al. 1999). The identification of stocks is, however, problematic due to the difficulty in uniformly defining these units and the lack of universal methods suitable for the investigation of stock-related issues. Established methods for the identification of fish stocks include methods based on capture-mark-recapture studies, molecular biology (e.g. allozyme electrophoresis, mtDNA analysis, DNA sequencing), meristics, morphometrics, parasites, and the trace element chemistry of calcified tissues. Stock identification based on allozyme electrophoresis, meristics, and morphometrics are all believed to provide some indication of the degree of relatedness among individuals on the basis of phenotypic characteristics. Capture-mark-recapture and parasite studies may provide an indication of the degree of mixing and are largely based on the geographical isolation of populations. These methods are, however, less definitive if extensive mixing has occurred during the egg, larval, or early juvenile stages.

This study aims to determine if separate stocks of Patagonian toothfish are present in the Southern Ocean. Two otolith based methods, otolith morphometrics and otolith chemistry, will be employed due to the availability of otolith samples from a broad range of sites and the relatively established nature of these methods.

Otolith morphometrics

Otolith shape is variable and species specific (Gaemers 1984) and can also vary significantly within species. Within species variation in otolith shape has been used successfully to discriminate among stocks (Casselman et al. 1981; Bird et al. 1986; Campana and Casselman 1993). A range of factors has been proposed to affect otolith shape within a species including genetics, growth rate, diet or temperature (Gauldie 1993); however the nature of these effects has not been investigated experimentally. A single factor or combination of factors may result in the shape of the otolith being stock specific (Campana and Casselman 1993).

Quantitative interpretation of otolith shape can be based on a range of methods including statistical analysis of a range of morphometrics or through shape analysis based on Fourier series. Fourier shape analysis reduces complicated shape data into a statistically useful form. A two-dimensional shape, here being an otolith outline, is described by a series of lines, or radii, originating from a single point within the outline. A Fourier transformation converts the function produced by these radii into a series of sine waves that describe the shape of the otolith (Fig. 2). Each sine wave in the series has both an amplitude and phase angle and these sine wave characteristics can be used to compare within and among fish. These data are input into a multivariate discriminate function analysis.

Fourier shape analysis may provide information on the stock structure of Patagonian toothfish due to the broad distribution of the species and the likelihood that there may be variation in the factors that affect otoliths shape among these potentially disparate habitats. For example, Patagonian toothfish occur along the slope of Chile and the Falkland Islands shelf, areas that are likely to have oceanographic characteristics different from more oceanic habitats such as the Kerguelen Plateau, Macquarie Island and the Prince Edward Islands.

Otolith chemistry

The trace element composition of otoliths can reflect the physiological and environmental history of many fish species and has been demonstrated to be an effective means of stock discrimination (e.g. Edmonds et al. 1989; Kalish 1990; Thresher et al. 1994, Campana et al. 1994; Thorrold et al. 1997; Thresher 1999). A range of analytical methods has been employed to determine otolith chemical composition including electron microprobe, proton induced x-ray emission, inductively coupled plasma atomic emission spectroscopy and inductively coupled plasma mass spectrometry and the relative advantages of these methods are discussed in Campana et al. (1997). Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) is currently among the most accurate and sensitive methods available for the measurement of trace elements in otoliths (Campana *et al.* 1997). This technique can rapidly sample small quantities of otolith *in situ*, in defined locations and at a spatial resolution of as little as 10 μm^2 and can detect isotopes at nanomolar (parts per billion) concentrations. However, LA-ICPMS does have some shortcomings including an inability to measure accurately some of the more abundant elements in otoliths such as sodium (Na), potassium (K) and iron (Fe) (Campana *et al.* 1997). Nevertheless, LA-ICPMS is the most frequently employed method for stock discrimination studies based on otolith chemistry and recent developments of the method continue to increase its usefulness (e.g. Sinclair et al. 1998).

The chemical composition of otoliths can be a useful discriminator among fish stocks due to the fact that otoliths record a permanent record of chemical variation experienced by the fish. Several factors have been suggested to affect trace element incorporation into otoliths including temperature (Radtke *et al.* 1990; Fowler *et al.* 1995b), salinity (Kalish 1990; Fowler et al. 1995b), environmental concentration (Thorrold et al. 1997), rate of crystallisation (Fowler et al. 1995b), ontogeny (Fowler et al. 1995a), and physiology (Kalish 1989;1991). A recent review of the use of otolith chemistry in studies of stock discrimination is provided by Thresher (1999). Many of these factors are likely to vary significantly among the broadly separated toothfish habitats of the Southern Ocean. Furthermore, otoliths are not resorbed or reworked after deposition, except under conditions of extreme stress (Mugiya et al. 1991). Therefore, the chemical record in the otolith represents a history of the fish's chemical environment, albeit modified through physiological processes (Kalish 1991; Fowler et al. 1995a). Due to the temporal nature of the otolith record the earliest deposited material reflects larval and juvenile environments and the material deposited most recently at the otolith edge is a record of the period immediately prior to capture. These properties, combined with the spatial resolution of LA-ICPMS may enable stock discrimination at both the juvenile and adult stages for Patagonian toothfish.

Methods

Sample collection

Otoliths collections were obtained from Macquarie Island (Mac) (1995/97), Heard and Macdonald Islands (HIMI) (1998), Kerguelen Island (Kerg) (1996), Prince Edward Islands (PEI) (1995/96), Falkland Islands (Falk) (1996), South Georgia (SOG) (1996) and Chile (SAM), between 46° S and Cape Horn (1996). Otoliths were removed from fish, by the respective government organisations' fisheries observers and fish length, weight and sex recorded. Samples used for morphometric and chemical analysis were sub-samples of larger collections selected from specific size classes that, for the most part, encompassed fish >100 cm total length (TL) (Table 1). Macquarie Island otoliths from the 80-90 cm size class with an equal sex ratio were used in shape analysis to test sex differences. A separate collection of larger Macquarie Island fish was used for chemical analysis. Fish from Macquarie Is, and HIMI were caught by trawl, those from the Falkland Is, South Georgia, Chile and Kerguelen Is were caught by longline and samples from Prince Edward Is fish were captured by both methods.

Shape description and Fourier transformation

Otolith images were captured, digitised and analysed using several computer programs. Left otolith images were captured using NIH Image v1.49 using a Kodak Megaplus digital video camera attached to a stereo dissecting microscope at 8x magnification. Otoliths were placed sulcus acusticus side up, with the rostrum - anti-rostrum axis positioned horizontally on the computer screen. The otolith outline was traced and area, perimeter, and x-y center of each otolith were determined with the appropriate functions in NIH Image.

The 2-dimensional otolith shape was then converted to a 1-dimensional function using the x-y centre as the starting point. 128 distances from the x-y center to the otolith edge were measured at equal angles (Fig. 2). A Fast Fourier Transformation was conducted on the 1-dimensional function produced by the radial points. The calculated Fourier transformation described the function produced from the radii and, consequently, otolith shape using a series of 128 sine functions (i.e. the number of radii). The first harmonic has a wave length of 128 (units are arbitrary and are equal to the number of points), the second harmonic has a wave length of 64 (128/2) and the nth harmonic has a wavelength of 128/n. All harmonics have a phase angle and an amplitude; the phase angle positions the curve, and the amplitude represents the magnitude of the effect of a particular harmonic on the final description of otolith shape.

Statistical shape analysis

Univariate and multivariate analyses were conducted to determine geographic effects on otolith shape and, consequently, the amount of structuring among sites. As in Campana and Casselman (1993) phase angles were ignored because their circular nature caused a bimodal distribution, which could not be overcome by any statistical transformation. Amplitudes were divided by the square of the first amplitude to remove the effects of otolith size. The log of each standardised amplitude was taken to normalise distributions. Univariate analysis of variance (ANOVA) for geographic location were calculated for each of the first 10 amplitudes. Sex effects were also tested, though were limited to Macquarie I samples due to the lack of sex data and the female bias of most other collections. South Georgia was excluded from the analyses involving sex as no data were available, but all collections were included for the geographic tests. Several points were considered to be outliers and excluded from each analysis due to large residuals.

Multivariate statistics were conducted on amplitudes 2-9. Canonical variates were calculated using only females to avoid any sex differences with South Georgia excluded due to the lack of sex data. Classification success was calculated by allocating each point to the nearest fishery mean using canonical variate data. This process was jackknifed so that the point being classified was excluded from the calculation of the fishery mean. Mahalanobis distances were calculated between each pair of fisheries, and geographic distances between fisheries were estimated from the island or coast of one fishery to the other. Distances between distant fisheries were estimated via intermediate sites (e.g. distance from Prince Edward Is to Macquarie Is was estimated via the Kerguelen Plateau). Mahalanobis distances were plotted against geographic distances, but no statistical tests could be conducted as fisheries were compared pair-wise and points are not independent.

Laser ablation ICPMS

The approximate position of the primordium was marked and otoliths were set in polyester resin. Using a low speed saw with a diamond blade, a 700 μm section was removed as close to the primordium as possible, and sections were affixed with polyester resin to 50 mm x 30 mm glass slides. Each slide contained 3 rows of 4 otolith sections, a total of 12 sections from different otoliths, and was ground and polished using 400 and 1200 wet and dry carbide paper in distilled water to remove excess polyester resin and minimise surface contamination. All otoliths were ultrasonically cleaned in MilliQ water, covered and stored in an airtight dry cabinet with dessicant until analysis.

Otoliths were sampled in order of preparation, which was randomised with respect to fishery of origin. A silicate glass standard (NBS 12) was used for calibration, as an aragonite standard was not available. Three calibration and background readings of 1 minute duration were taken for each slide, with one sample each before, midway through and after all otoliths were sampled.

The laser was a LambdaPhysik LPX 120i ArF EXCIMER laser with an ultraviolet wavelength of 193 nm, 80 mJ energy and 40 watts power. More detailed technical data on the laser and sample cell can be found in Sinclair et al. (1998) and Eggins et al. (1998). Sampling was conducted with a laser pulse frequency of 0.2 s and a spot diameter of 65 μm. Samples were placed in a vacuum sealed stage and ablated otolith material was advected from the stage to the ICPMS by 1100 cc/min of argon gas and 300 cc/min helium. A Hewlett Packard 4500 series ICPMS measured each isotope for 20 ms, with a 10 ms period to switch to the next isotope.

Transects from the core to the ventral edge of the otolith were conducted by moving the sample stage at a constant speed with DC motor drive (see Sinclair et al. 1998 for details). Pre-ablation with the laser was conducted to remove a small amount of material and reduce possible surface contamination from the chosen path.

Interpretation of chemical analyses

Laser ablation ICPMS data can drift in time and with the amount of calcium carbonate material ablated, therefore, a standardisation procedure was necessary to interpret results

(Campana et al. 1994). Fifteen isotopes representing 12 elements (Li, Mg, Ca, Ti, Ni, Co, Cu, Zn, Rb, Sr, Ba and Pb) were chosen for the analysis based on the results of a pilot study using five otoliths. ^{59}Cobalt was measured to act as a tracer of resin contamination, as it was not detectable at significant levels in the otolith, and was at concentrations of 2000 µmol/molCa in the polyester resin. Samples at the otolith periphery or at cracks with high ^{59}Co values were therefore excluded from further analysis.

All measurements were corrected for background levels and drift was corrected within each slide using the relevant calibrations from the silicate standard with each isotope treated independently. Each measurement was multiplied by the relevant drift factor, standardising to the middle calibration for each slide. Absolute abundances were calculated using the known concentrations in the NBS 12 silicate standard and expressed in terms of µmol of isotope x per mole of Ca as shown in Equation 1.

$$\left(\frac{I_x/I_{Ca}}{C_x/C_{Ca}}\right)\times \text{analyte} = \left(\frac{I_x/I_{Ca}}{C_x/C_{Ca}}\right)\times \text{standard}$$

$$(C_x/C_{Ca})\times \text{analyte} = \frac{(\mathrm{I}_x/I_{Ca})\times \text{analyte}}{\left(\frac{I_x/I_{Ca}}{C_x/C_{Ca}}\right)\times \text{standard}} \qquad \text{Equation 1}$$

where I = intensity measured, C= concentration and X = element measured.

^{43}Calcium was chosen to standardise all other isotopes as the ICPMS measures Ca accurately with few interferences. The standardisation to Ca and relative abundance also corrected drift between slides. Corrected distributions of each isotope were calculated and means were tested for differences from background levels.

Two isotopes each of Ca (^{43}Ca and ^{44}Ca), Zn (^{66}Zn and ^{68}Zn) and Ba (^{137}Ba and ^{138}Ba), were measured and standardised to estimate elemental abundances. Estimated elemental abundances of Zn and Ba derived from each isotope were plotted against each other to determine the reliability of the measurements over the range of concentrations encountered. Linear correlation coefficients (r^2) and regression models were calculated for each element treating otolith core and otolith edge data separately. All measurements were standardised to Ca as measured by ^{43}Ca. Estimates of Ca abundance using ^{44}Ca were subtracted from estimates using ^{43}Ca (1,000,000 μmol/mol Ca). The distribution of residuals was used to determine the precision of Ca measurements.

Twenty measurements of each isotope were averaged as close to the edge as possible, with a maximum ^{59}Co count of 5μmol/mol Ca. Similarly, using notes from the time of sampling 20 measurements from the core were averaged for each isotope. These 20 measurements represent a seven second time span, and a distance of approximately 140μm. Hereafter, these will be referred to as points representing adult and juvenile chemistry respectively.

Analysis of variance (ANOVA) was conducted between core and edge points for each isotope. Isotope concentrations were transformed by taking the natural log for statistical analysis. This was done to normalise distributions and is statistically necessary as measurements are actually ratios of Ca. There were unequal variances for cores and edges in several isotopes; therefore, Welch's ANOVA was used to test these elements as it does not assume equal variance. For isotopes with non-normal distributions, the non-parametric Kruskal-Wallis test was used to test for significant differences between positions. Otolith cores and edges were tested separately for possible differences associated with the fishery and the data were transformed by taking the natural log. One way ANOVA, using pooled estimates of variance, was used to test for significant location (fishery) effects for all elements except Li. Welch's ANOVA was used to test Li data as there were major differences in variance among sites. Plots of location against elemental abundance were created and several outliers were identified and removed from the multivariate analysis, but were not removed from the univariate analysis. Bivariate plots of all combinations of elements, separated by location, were created to help identify possible differences among fisheries.

Multivariate analysis

Canonical variates were calculated for otolith edges and cores separately, using Li, Mg, Sr, Ba, Cu, Zn and Rb which were log transformed before analysis. Loadings were standardised using the sum of squares for each element separately. The mean of each location for the first and second variates was plotted with 95% confidence intervals. Each otolith was classified to the nearest location mean and classification success determined. Classifications were jack-knifed, that is each point was excluded from the analysis when it was being classified.

Transect analysis

Ontogenetic variation in otolith composition was visualised through plots of selected otolith constituents graphed versus otolith position (a proxy for time). ICPMS data were transformed and reduced to concentrations relative to Ca. Transect paths left by the laser during the ablation process were viewed with a reflected light microscope and the length of each transect was measured. In addition, a single otolith was used to produce a 2-dimensional map of selected trace elements measured in the otolith. Contours of elemental concentrations were based on 24 transects measured in the lateral-medial plane across the transverse section. Data reduction was similar to that used for individual transects.

Results

Otolith Morphometrics

The effectiveness of standardisation procedures in removing otolith size from the data was tested on amplitudes 2 to 5. There was a significant linear relationship between unstandardised amplitudes 2 to 5 and otolith area ($r^2 = 0.16, 0.48, 0.13, 0.37$, respectively $n = 373$, $p < 0.001$ for all) and, therefore, it was important to account for otolith size in the analysis. All amplitudes were divided by the square of the first amplitude to remove size effects, as the first amplitude was correlated with both fish length ($r^2 = 0.74$, $n = 374$, $p < 0.0001$) and otolith area ($r^2 = 0.98$ $n = 374$ $p < 0.0001$). Length distributions from each location were different (Table 1) and any effect of fish size could create artificial location effects from sampling artefacts. Standardised amplitudes were not significantly correlated with fish length with the exception of amplitude 3 ($r^2 = 0.05$ $n = 374$, $p = 0.01$).

Univariate analysis on the amplitudes of the first five harmonics was conducted separately and there were significant geographic effects for harmonics 2, 3 and 5 ($p < 0.005$ for all), but no significant location effect for the 4th amplitude ($p = 0.20$). There was a large degree of overlap among locations and no single harmonic could be used to completely separate any two locations. There was no significant sex effect for any of the first five harmonics, though sex ratios were biased. Macquarie I showed no significant sex effect for amplitudes 2, 3, 4 or 5 (n =52) with an equal sex ratio.

Canonical variates and Mahalanobis distances were calculated to investigate the location effects of geography on otolith shape. The first and second canonical variates explained 51.5% and 22.8% of the variation, respectively, for a total of 74.3% of the variation. Canonical variates were plotted and Macquarie I otoliths were clearly separated from all other locations. This may be a size effect due to the relatively small size of the fish used for the Macquarie I samples. The 95% confidence intervals for the means overlap for most population. There is some evidence for separation among locations, however, with South America and the Falkland I clustered together and both Prince Edward I fisheries clustered with HIMI and Kerguelen I (Fig. 3). Total classification success was 37%, although this is largely due to the poor classification of the 2 Prince Edward I collections with only 6 of 84 otoliths correctly assigned. Results were not improved by grouping the two Prince Edward I collections as one location. Heard and Macdonald Islands also had a low prediction success (6 from 31, Table 2) and an additional 6 were classified as Kerguelen Is otoliths. Collections of large otoliths from South America, Falkland Is and South Georgia, tended to group together with 70-80% of otoliths from each site classified to one of the three locations.

Mahalanobis distances were calculated between all sites. The general trend in the data was for statistical distance between sites to increase with geographic distance (Fig. 4). However, no statistical tests could be conducted on these data as points were not independent. These results suggest that there is stock structure among Patagonian toothfish populations and that the geographical separation among putative stocks may play a significant role in this structure.

Otolith chemistry

Of the 15 isotopes measured, 11 were significantly and consistently above background levels (^{7}Li, ^{25}Mg, ^{43}Ca, ^{44}Ca ^{63}Cu ^{66}Zn, ^{68}Zn ^{85}Rb, ^{86}Sr ^{137}Ba and ^{138}Ba; $p<0.0001$ for all, see Table 3). ^{49}Titanium, ^{60}Ni and ^{208}Pb were significantly above background levels, however, a substantial number of measurements were below background levels, and thus these isotopes were excluded from further analysis. ^{59}Cobalt was measured only as an indicator of resin contamination, and was not detectable in otoliths and also excluded from further analysis.

Calcium, Zn, and Ba, were all measured using two isotopes. While ^{66}Zn and ^{68}Zn showed a strongly significant linear relationship estimates of Zn using ^{66}Zn were generally higher than using ^{68}Zn at the otolith core and edge ($r^2 = 0.975$, slope = 0.759, $p<.0001$) (Fig. 5a). Elemental Ba concentrations were very similar based on both ^{137}Ba and ^{138}Ba, and showed a strong linear correlation with a slope not significantly different from 1 ($r^2 = 0.993$, slope = 1.004, $p< 0.0001$) (Fig. 5b). ^{43}Calcium- ^{44}Ca residuals indicate that ^{43}Ca measures significantly higher levels of Ca than ^{44}Ca (mean = 2245 ppm, sd=8495). The range of residuals is 76,552 ppm (7.6%) with 95 % of measurements within 35,000 ppm (3.5%).

Significant differences were found between the otolith core and edge for several elements. Lithium concentrations were significantly higher at the otolith core than at the edge ($p <0.0001$ Fig. 6a), whereas Sr and Ba concentrations were far higher at the edge than the core across all locations (Fig. 6b and 6c). In general, Ba showed a pattern similar to Sr and these similarities were demonstrated by LA-ICPMS life history transects made across all samples

(Fig. 7). While ^{66}Zn was significantly lower at the edge (p = 0.03), ^{68}Zn was not (p = 0.099); this is a reflection of the differences in the measured concentrations of these isotopes (Fig. 5a).

Otolith edge data showed significant geographic effects for Ba (p <0.001), Sr (p <0.001) (Fig. 8a and 8b), Cu (p=0.023), Li (p=0.015) and Mg (p<0.001). However, there is considerable overlap between locations, and no combination of two elements showed clear evidence of clustering. There was no significant location effect on otolith edges for ^{66}Zn (p=0.07), ^{68}Zn (p=0.25), or Rb (p=0.16).

Core chemistry showed significant location effects for Li (p <0.001), Mg (p <0.001) (Fig. 9a and 9b) and to a lesser extent for Sr and Ba. There was no significant location effect for Cu, ^{66}Zn, ^{68}Zn or Rb. South America and the Falkland I samples were separated from all other fisheries on a bivariate plot of Mg and Li, mainly due to low Li values (Fig. 10).

Multivariate statistics were employed to investigate further location effects using all elements that were detected consistently in toothfish otoliths. For otolith core data, the first and second canonical variates accounted for 68.2% and 25.5% of the variation, respectively. Lithium and Sr were strongly loaded on the first canonical variate, while Mg, Ba and, to a lesser extent, Cu and Zn had high loadings on the second canonical variate. South American and Falkland I fish were separated from other locations on the first canonical variate and from each other on the second canonical variate, while Kerguelen I, Prince Edward I and HIMI were all clustered together and formed an Indian Ocean group (Fig. 11). Macquarie I was slightly separated from this Indian Ocean group, though there was some misclassification between Macquarie I and the Indian Ocean group. Prediction success was 73% when HIMI, Kerguelen I, and Prince Edward I were combined to form an Indian Ocean group (Table 4). Much of the remaining misclassification was between South America and the Falkland I, and Macquarie I and the Indian Ocean group. There was very little misclassification between the toothfish otoliths collected from the 2 South American fisheries and other locations (Table 4). These results, based on the composition of otolith cores suggest that there may be multiple spawning sites or nursery areas for Patagonian toothfish in the Southern Ocean.

The first and second canonical variates accounted for 36.5% and 30.7% of variation respectively for chemical data collected from the otolith edge. Strontium, Ba, and to a lesser extent Li have high loadings for the first variate, while Sr and Mg have high loadings for the second variate. Macquarie I and HIMI are separated from the other populations on the first variate, and the Falkland I separated on the second variate (Fig. 12). Total predictive success for based on otolith edge chemistry was 62%, lower than for core chemistry (Table 5). This is likely to be an effect of capture method (i.e. longline or trawl), rather than due to location effects, as will be discussed later.

Chemical transects from the otolith core to edge generally showed rapid rises in Ba and Sr levels (Fig. 7). Lithium decreases along the transects in Indian Ocean and Macquarie Is fish, but does not change in Falkland Is and South American fish. There is no evidence for seasonal or interannual effects on otolith composition, but sampling protocols were not designed to investigate these questions. Changes in Ba and Sr concentrations track one another, although Ba concentrations are about 1/1000 those of Sr. Small increases in Sr are often closely tracked by Ba, indicating that concentrations of the two elements are highly correlated in toothfish otoliths. This is almost certainly due to the similar chemical properties of these two elements.

Two-dimensional maps of the concentrations of six elements (Li, Mg, Sr, Ba, Zn and Cu) across a transverse section of a toothfish otolith demonstrated that compositional changes measured in the direction of life history transects were indicative of the entire otolith. Furthermore, contour lines of the distribution of Sr and Ba (Fig. 13a and 13b) closely followed the orientation of annual increments in the otolith and indicated that the observed patterns were linked to ontogeny and age rather than the result of contamination or random events.

Discussion

Shape Analysis

Shape analysis found significant differences between sites, though it is difficult to identify separate stocks from these data. Despite the large variation within locations and poor classification success there was evidence for some geographic structure of toothfish stocks in the data. The 4 collections obtained from the Indian Ocean sector of the Southern Ocean (Kerguelen Is, both Prince Edward Is collections and HIMI) are more similar to each other than sites outside the Indian Ocean sector of the Southern Ocean. South America and the Falkland Is cluster together, although South America is similar to the Indian Ocean group. Macquarie Is is well separated from all other locations on the canonical variate plot, though this may be due to the size of fish. Size effects were removed in the analysis, however, it is possible that otolith shape changes with size and that these changes could affect results. The plot of geographic distance versus Mahalanobis distance also indicated that geography has some relation with otolith shape, and reasons for this are discussed below.

Otolith shape is restricted by head shape and otolith function (Gauldie 1993) and may also be affected by growth rates, temperature and genetics (Bird et al. 1986; Campana and Casselman 1993). Differences in otolith shape among stocks have been found in several species, although Campana and Casselman (1993) claim that most significant differences among stocks are caused by differences in growth rates. Growth rates from most areas are largely unknown and few data are available that would be useful in the development of hypotheses that might explain potential growth rate variation. Patagonian toothfish are commonly caught in Antarctic Intermediate Water or Circumpolar Deep Water, depending on depth and latitude (Park and Gamberoni 1997). These water masses have similar temperatures and it is likely that adult Patagonian toothfish living within these habitats will experience similar temperatures and, concomitantly, growth rates. This may result in otoliths of similar shape and poor stock discrimination based on otolith morphometrics. Also, like all nototheniid species, Patagonian toothfish have a range of adaptations to cold water environments, and these may reduce the effect of temperature on growth rates.

Chemical discrimination

Otolith chemistry data suggested the presence of as many as four Patagonian toothfish stocks including a single stock in the Indian Ocean sector of the Southern Ocean, South America, Falkland Is and Macquarie I. Geographic effects on otolith composition were most evident in portions of the otolith deposited in early life. This suggests that toothfish caught at a particular location may be recruited from the same nursery area, and that there is little migration among sites after the deposition of otolith material in the first 6 months of life. Two hypotheses may explain the clustering of Kerguelen Is, Prince Edward Is and HIMI into the Indian Ocean group. Firstly, all recruits to these fisheries may be derived from a single nursery area in the Indian Ocean sector of the Southern Ocean. Alternatively the water chemistry from the sites may be similar causing the observed clustering of locations. It is impossible to distinguish between these two hypotheses on the basis of the available otolith chemistry data.

Macquarie Is was separated from other sites though there is some misclassification with the Indian Ocean group. Most misclassification was between the Indian Ocean group and Macquarie Is (oceanic islands) and between South America and Falkland Is (South American shelf and slope). The lack of misclassification between these major groupings may result from the difference between coastal and oceanic waters. Sampling locations of South America and the Falkland Is are both under the influence of coastal habitats and would differ chemically from oceanic waters of the Southern Ocean which dominate other sample collection locations.

Separation between fisheries based on edge data is less clear and is possibly an artefact of capture method. The two sites with trawl captured fish, HIMI and Macquarie Is are separated from the other sites primarily by low Sr and Ba levels. Fish from these locations are smaller and therefore younger, thus many of these fish are likely to be too small to display the large increase in Sr and Ba levels observed at the edge of transects from the larger fish. Similarity in otolith edge chemistry between other sites may reflect the similar habitats of adults at depth, or result from the more rigid physiological control of adult fish. At the depths where most adult toothfish occur, water from all sites is likely to be Circumpolar Deep Water or Antarctic Intermediate Water (Park and Gamberoni 1997), and these water masses would have similar chemical characteristics irrespective of location. The different interpretations that result from measurments in the otolith cores and edges highlights the importance of careful selection of otolith region sampled in studies of stock discrimination (Kalish et al 1996).

Contamination

Possible contamination of otoliths after death has been the focus of recent research (e.g. Dove et al. 1996; Proctor and Thresher 1998; Milton and Chenery 1998) and must be addressed to ensure the reliability of stock discrimination studies based on otolith composition. Common sources of contamination are associated with storage, cleaning and preparation procedures. All otoliths were removed and stored in dry envelopes and this method of storage does not appear to have a significant effect on the elements used in stock discrimination of Patagonian toothfish (Dove et al. 1996; Proctor and Thresher 1998; Milton and Chenery 1998). Sample cleaning and preparation have been found to have significant effects on several elements (Delaney et al. 1996; Dove et al. 1996; Proctor and Thresher 1998; Milton and Chenery 1998), of which Cu, Zn, Li and Ba were measured in this study. Calcium, Sr and Mg are generally found to be robust, and not affected by either contamination or cleaning procedures. Preparation methods employed for the toothfish otolith samples were intended to minimise contamination. Furthermore, laser pre-ablation has been demonstrated to remove surface contamination (e.g. Sinclair et al. 1998); however, due to the porous nature of otoliths, contamination may penetrate areas beyond the ablated otolith surface. Despite the lack of empirical data on possible contamination of toothfish otoliths, the 2-dimensional map of trace elements indicated that ontogenetic patterns dominated the pattern of otolith composition, rather than a distribution that might result from external sources of contamination.

Ontogenetic and physiological effects

Large ontogenetic effects were found in otoliths for Sr and Ba, which may indicate a life-history changes, such as onset of maturity or habitat change. Strontium is the best studied minor element occurring in otoliths, mainly due to its abundance and similar chemistry to Ca. Kalish (1989;1991) proposes that Sr:Ca ratios are affected by increasing protein bound Ca, reducing free Ca in the blood plasma which is the supply for both endolymph and otolith. Therefore physiological changes may cause changes in blood-bourn Ca containing proteins, affecting otolith Sr:Ca ratios. Kalish (1991) attributes seasonal changes in protein bound Ca to the development of ovaries in female fish. Also, physiological stress has been shown to change otolith Sr:Ca ratios (Kalish 1990). A similar argument can be used to account for Ba distributions, due to its similar chemical properties to Sr. Barium is usually considered to behave differently to Ca, but may still substitute for Ca in calcium carbonate matrix (Veizer 1983). Fowler et al. (1995) have shown that Sr and Ba behave similarly in juvenile aquarium raised fish. However, Thorrold et al. (1997) report differing patterns in fish migrating from oceanic waters to estuarine waters, though both Sr and Ba patterns were consistent with environmental concentrations. Both Sr and Ba can substitute directly for Ca in the aragonite structure, therefore the ratios in the otoliths of these two elements increase when free Ca levels in blood plasma decrease. Such increases are not seen in other elements as they rarely, if ever, substitute for Ca in the aragonite structure (Veizer 1983). The rapid change in Sr and Ba levels observed in most transects may indicate a life history event that affects physiology (e.g. habitat change or onset of maturity).

Otolith chemistry results provided evidence for the existence of four distinct stocks and, potentially nursery areas for Patagonian toothfish. Kerguelen Is, Prince Edward Is and HIMI may contain fish derived from a single nursery area, although the similar water chemistry that is likely to exist at these sites may have affected the ability of otolith chemistry to distinguish among these locations. Significant and major ontogenetic effects were found for Ba and Sr and these were of much greater magnitude than geographic location effects. Further work is required to determine the cause of ontogenetic patterns in toothfish otoliths, and identify if these changes are associated with habitat shifts or associated with physiological changes (e.g. maturity).

Comparison of stock discrimination methods

Efforts to discriminate among putative Patagonian toothfish stocks based on otolith morphometrics and otolith chemistry were conducted on the same collections of toothfish otoliths; this makes it possible to compare the results achieved from the two techniques. Both methods indicated that the Falkland Is and South America are separated from all other locations. Neither method could separate locations in the Indian Ocean sector of the Southern Ocean, however, both showed increased separation with geographic distance (Fig. 4). The lack of separation among Indian Ocean sites may either indicate that neither method is adequate to separate among possible stocks that may exist or that toothfish at these locations compose a single stock.

Otolith morphometrics indicated that some separation among locations does occur though it is difficult to identify individual stocks. This method may fail to discriminate among possible stocks due to a lack of variation in otolith shape among fisheries. Changes in otolith shape as the fish grow are likely to reduce the ability to employ otolith shape for stock discrimination purposes. However, major changes in otolith shape are unlikely to occur due to the genetic control of the organic matrix that acts as a template for otolith growth and any variation that does occur is likely to result from factors that are under environmental control. For example, temperature differences across fish habitats may affect fish growth rates and, ultimately, otolith shape in such a way that it is useful for discriminating among stocks (Campana and Casselman 1993). Therefore, for otolith morphometrics to be useful for stock discrimination, it may be necessary for significant environmental variation to exist. Differences of adequate magnitude may not exist among islands in the Southern Ocean.

Juvenile chemistry clearly discriminated between sites in coastal South American waters (South America and the Falkland Is) and the Southern Ocean islands. It is probable that discrimination among locations resulted from differences in the environmental concentrations of several elements or other environmental differences that may affect fish physiology. Many studies that have successfully discriminated among stocks are based on relatively major distinctions between water masses (e.g. marine and freshwater; Kalish 1990). Otolith chemistry has discriminated between stocks from different river systems (Thorrold et al. 1997), and between several coastal regions (Edmonds et al. 1989; Campana et al. 1994) which may have distinct chemical signals, although some studies have indicated that differences in otolith composition can be associated with factors other than water composition (e.g. growth rate; Sadovy and Severin 1992, Fowler et al. 1995a: stress; Kalish 1992). It has also been used to discriminate among deep water benthic stocks from widespread geographic ranges in this study and Edmonds et al. (1991). However, otolith chemistry has never been successful in the identification of stocks of oceanic fish and failed for the southern bluefin tuna, although this may be due to the existence of a single stock (Proctor et al. 1995).

Both methods of stock discrimination failed to show clear separation in the Indian Ocean sector of the Southern Ocean. Oceanography and Patagonian toothfish biology are conducive to the hypothesis that there may be some population structuring in the Indian Ocean sector of the Southern Ocean. Kerguelen Is and HIMI are located on the Kerguelen Plateau and water between the two locations is <2000 m deep. Adult toothfish should be capable of inhabiting most demersal habitats on the Kerguelen Plateau and larval drift is likely to occur across the plateau. Therefore, Patagonian toothfish from Kerguelen Is and HIMI may form a single stock. However, deep water (>3000m) separates the Kerguelen Plateau from Prince Edward Is (Park and Gamberoni 1997) and adult Patagonian toothfish may not be capable of migrating between these locations. Furthermore, the relatively great distances between these sites may limit the likelihood of larval drift between these locations. Therefore, oceanography and toothfish biology provide a plausible basis for isolation of toothfish from the Prince Edward Islands as a stock separate from the Kerguelen Plateau. However, larval drift from west to east in the Antarctic Circumpolar Current (West Wind Drift) may occur (i.e. Prince Edward Is to Kerguelen Plateau) and this could obscure genetic differences among these putative stocks. In addition, water masses at both sites are similar with all three islands situated south of sub-Antarctic front (Park and Gambberoni 1997). Therefore, neither otolith-based technique would be expected to show substantial separation among these sites, however, the hypothesis that all three locations are a single stock can not be rejected from these data.

Otolith chemistry showed separation between South America and the Falkland Is, although there was some overlap between these two locations. The South American fishery, located on the Pacific coast of Chile, is separated from the Falkland Is by Cape Horn and approximately 800 miles. Nevertheless, the Falkland Is slope is contiguous with the Chilean slope and adult toothfish should be capable of movement between these locations. Differences in the habitat chemistry may be significant, however, with the Chilean shelf and slope exposed to northward flowing waters derived from the Antarctic Circumpolar Current, whereas the Falkland Is are bathed in southward flowing waters from the Brazil Current. Therefore, there is a real possibility of exchange of fish between this habitats, but each habitat may result in unique chemical signatures in otoliths.

Conclusions

This study provides data to support a management approach for Patagonian toothfish that is based on the existence of several distinct stocks. Otolith chemistry was more effective than otolith morphometrics for the identification of stock units. Analysis of chemical data from the cores of toothfish otoliths collected in several fisheries suggests that there are at least four stocks in the Southern Ocean. As stock differences were determined on the basis of the composition of otolith cores, this also suggests that there may be at least four distinct spawning areas or nursery grounds and that there is minimal dispersal among these areas. These stocks include a South American group, Falkland Islands group, Macquarie Island group and an Indian Ocean group comprised of toothfish collected from islands in the Indian Ocean sector of the Southern Ocean (Prince Edward Islands, Kerguelen Island and HIMI). The lack of separation of toothfish from the Indian Ocean group may be due to movement of larval or adult toothfish between these islands or the inability of the otolith chemistry method employed to separate these potential stocks.

Acknowledgments

We thank Steve Eggins (Department of Geology, Australian National University) for assistance with the LA-ICPMS analyses and otolith mapping and both Justine Johnston and Janet Pritchard (Division of Botany and Zoology, Australian National University) for general assistance. Samples were obtained due to cooperation among several groups including Austral Fisheries (Murray France and crew of the Austral Leader), Australian Fisheries Management Authority (Anthony De Fries, Stephanie Kalish) Australian Antarctic Division (Dick Williams), British Antarctic Survey (Julian Ashford), South African Sea Fisheries Research Institute (David Japp), The Falkland Islands Fisheries Department (Conor Nolan) and the Museum National d'histoire Naturelle (Paris) (Guy Duhamel).

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Figure Captions

Figure 1. Antarctica and the Southern Ocean region with the locations of Patagonian toothfish *Dissostichus eleginoides* fisheries sampled for the stock discrimination study.

Figure 2. An outline of a Patagonian toothfish *Dissostichus eleginoides* otolith (a), as used for in the Fourier shape analysis. The x-y centre of the otolith is shown with 16 radii drawn from the centre to the otolith edge. Only 16 radii are shown for clarity. Fourier shape analysis was based on128 radii which were used to define the otolith outline. In (b) the lengths of the radii are plotted against the angle to produce a function. Using a Fast Fourier Transformation, these functions were reduced to a format suitable for statistical analysis.

Figure 3. Separation of Patagonian toothfish *Dissostichus eleginoides* fisheries based on otolith morphometrics. The first and second canonical variates calculated from otolith morphometrics are plotted with 95% confidence intervals for the means. There is no separation among Kerguelen Is (Kerg), either the Prince Edward Is trawl fishery (PEDT) or the Prince Edward Is longline fishery (PEDL) or the Heard and McDonald Is fishery (HIMI). South America (SAM) toothfish otoliths appear similar to this group, but are also positioned close to the Falkland Is (Falk) samples. Macquarie Is (Mac) is clearly separated from the otolith samples from other sites, possibly due to the small size of otoliths.

Figure 4. Relationship between geographic distance between sites and canonical distance for otolith shape data (Mahal-dist) and otolith core chemistry data (core dist) from Patagonian toothfish *Dissostichus eleginoides*. Both stock discrimination methods showed increasing

statistical separation with geographic distance, but otolith chemistry showed a significantly greater degree of separation.

Figure 5. Tests of the reliability of LA-ICPMS measurements. The relationship for the 2 isotopes of Zn is linear (a), however, estimates derived from ^{66}Zn are higher than those derived from ^{68}Zn and the slope of the line fit by linear regression was signficantly less than 1.0 (slope=7.59, $p<0.0001$). Isotopic abundances of barium isotopes (b) are highly correlated and the slope of the line does not deviate from 1.0 (slope=1.004, $p<0.0001$).

Figure 6. Ontogenetic patterns of lithium, strontium and barium in Patagonian toothfish *Dissostichus eleginoides* otoliths. Lithium (a) occurred at significantly higher concentrations in the juvenile region of otoliths, particularly in fish from Kerguelen Is, Heard and McDonald Is, Prince Edward Is and Macquarie Is. Strontium (b) and barium (c) occurred at significantly higher concentrations in the adult regions of otoliths.

Figure 7. Life history transects of strontium and barium, measured by LA-ICPMS, in Patagonian toothfish *Dissostichus eleginoides* otoliths collected from six fisheries. In each case, both Sr and Ba remain relatively stable in early life, but increase rapidly in later life, after a point in time associated with maturity. The patterns of abundance are very similar for both Sr and Ba.

Figure 8. Strontium and barium concentrations in the adult region of Patagonian toothfish *Dissostichus eleginoides* otoliths by geographic location. Both Sr (a) and Ba (b) have significant geographic location effects. Typically, otoliths from smaller trawl caught toothfish

had lower edge Sr and Ba concentrations than larger longline caught toothfish. South American fish tended to have lower Sr and Ba levels despite the large size of these fish. HIMI = Heard and McDonald Islands, Ker = Kerguelen Island, PED= Prince Edward Islands, Falk = the Falkland Islands, SAM = South America, and Mac= Macquarie Island.

Figure 9. Lithium and magnesium concentrations in the juvenile region of Patagonian toothfish *Dissostichus eleginoides* otoliths by geographic location. Lithium (a) was present only at very low levels in otoliths from the Falkland Is and South America, but occurred at higher levels in fish from all other sites. Magnesium was at high concentrations in Falkland Is fish and, to a lesser degree, those from Macquarie Is. HIMI = Heard and McDonald Islands, Ker = Kerguelen Island, PED= Prince Edward Islands, Falk = the Falkland Islands, SAM = South America, and Mac= Macquarie Island.

Figure 10. Bivariate plot of lithium and magnesium measured in the juvenile region of Patagonian toothfish *Dissostichus eleginoides* otoliths. South America and the Falkland Is otoliths were separated from most other sites mainly by Li and from each other mainly by high Mg in Falkland Is otoliths and lower Mg in South American otoliths. The majority of otoliths from Macquarie Is fish cluster at the right of the figure indicating high Li and low Mg concentrations.

Figure 11. Separation of Patagonian toothfish *Dissostichus eleginoides* fisheries based on the chemistry of otolith cores. South American (SAM) toothfish and fish from the Falkland Is (Falk) were clearly separated from all other sites on the first canonical variate, and from each other by the second canonical variate. Macquarie Is (Mac) fish were separated from fish from

Kerguelen Is (Ker), Prince Edward Is (PEI) and Heard and McDonald Islands (HIMI) on both the first and second canonical variates. These results suggest that there may be at least four spawning grounds or nursery areas that result in chemically identifiable otolith cores.

Figure 12. . Separation of Patagonian toothfish *Dissostichus eleginoides* fisheries based on the chemistry of otolith edges. There was some evidence for separation among toothfish fisheries. Macquarie Is (Mac) and Heard and McDonald Islands (HIMI) toothfish otoliths were separated from other sites; however, this may have resulted from the small size, and presumably younger age, of these fish. The Falkland Is (Falk) were separated from all other and this may be linked to the nature of water masses in the Falkland Is region.

Figure 13. Two-dimensional maps of strontium and barium concentrations, produced by LA-ICPMS, in an otolith from a 203 cm (TL) toothfish collected in the Falkland Islands fishery. Twenty-four transects of otolith composition were measured across the distal-proximal plane of a transverse section. Transects were spaced at 500 µm intervals. The Sr and Ba concentration gradients (a) increased uniformly across the entire transverse section indicating that patterns of Sr and Ba concentrations were linked to ontogeny and not associated with contamination. The patterns of Sr and Ba indicated that selection of different life history transects across a transverse section of a toothfish otolith would produce similar ontogenetic patterns.

Table 1. Size range, sample size and sex distribution of Patagonian toothfish from each location used for morphometric analysis. A sub-sample of these otoliths was used for the chemical anlaysis with the exception of the Macquarie Island samples. Some sex and length data were missing from Falkland Islands and South Georgia. Size range is the smallest and largest fish in each collection N total, N female and N male represent the number of fish in each category. Random sub-samples of 30 otoliths from South America, the Falkland Is, Prince Edward LL and Kerguelen Is were used for LA-ICPMS.

Location	Size range (cm)	N total	N female	N male
South America	151-220	50	47	3
Falkland Is	151-190	52	22	5
South Georgia	Unknown	52	0	0
Prince Edward LL	130-183	42	41	1
Prince Edward Trawl	47-172	42	28	14
Kerguelen Is	83-172	51	43	8
Heard and Macdonald Is	91-149	31	29	2
Macquarie Is (chemistry)	90- 128	30	20	6
Macquarie Is (shape)	80-90	52	26	26

Table 2. Classification success of jackknifed discriminate function analysis based on otolith morphometric data. Actual locations are recorded across the top of the table and predicted locations listed vertically. Percentages are the number classified to a location divided by the total number from that location.

Predicted Location	Actual Location								
	Falk	HIMI	Kerg	Mac	Ped Longline	PedT	SAM	SOG	total
Falkland Islands	50%	13%	2%	11%	14%	17%	34%	10%	72
HIMI	2%	19%	4%	4%	10%	2%	2%	4%	19
Kerguelen Island	2%	19%	37%	15%	17%	24%	6%	17%	63
Macquarie Island	10%	6%	24%	55%	19%	12%	4%	6%	66
Prince Edward LL	2%	3%	0%	0%	10%	2%	4%	6%	12
Prince Edward T	0%	32%	8%	0%	7%	5%	6%	4%	15
South America	21%	23%	8%	4%	12%	26%	38%	0%	59
South Georgia	13%	13%	18%	11%	12%	12%	6%	54%	67
Total	52	31	51	53	42	42	50	52	373

Table 3. Median abundances of all elements included in analysis. Median and ranges are recorded for each location and element for both Juvenile and adult stages, measurements are in μmol x/mol Ca.

Geographic location	^{7}Li	^{25}Mg	^{63}Cu	^{66}Zn	^{85}Rb	^{86}Sr	^{137}Ba
South America							
juvenile	0.88(0.45-2.18)	20.3(11.5-60.2)	3.17(1.15-28.6)	1.57(0.68-12.4)	0.08(0.03-1.34)	1760(1255-2602)	1.43(0.52-4.47)
adult	0.83(0.61-3.21)	15.9(6.3-35.9)	1.54(0.78-25.1)	1.15(0.42-49.5)	0.11(0.04-0.77)	7999(5555-15839)	7.63(3.21-23.1)
Falkland Is							
juvenile	0.80 (0.28-2.97)	38.7 (11.0- 218)	2.63(0.98-13.6)	1.96(0.28-20.6)	0.10(0.05-0.35)	1727 (1443-3676)	1.67(0.74-6.97)
adult	0.86(0.48-1.51)	23.0(11.9-174)	1.77(0.86-4.73)	1.06(0.11-12.0)	0.09(0.04-0.24)	8486(4946-14092)	8.05(4.16-18.1)
Prince Edward Is							
juvenile	2.15(0.71-26.76)	20.8(10.7-53.7)	2.55(1.28-24.0)	1.26(0.33-4.42)	0.11(0.03-0.12)	2382(1749-2752)	2.79(1.34-12.2)
adult	1.00(0.00-2.45)	18.4(8.3-134)	2.4(1.05-1105)	1.15(0.14-60.7)	0.11(0.04-2.31)	8453(3993-15316)	10.17(2.46-32.4)
Kerguelen Is							
juvenile	2.31(0.42-21.42)	21.0(12.5-221)	2.63(1.11-15.1)	1.51(0.04-8.44)	0.10(0.04-0.35)	2397(693-3079)	2.18(0.05-9.9)
adult	0.97(0.45-2.55)	20.4(12.9-463)	1.82(1.09-23.7)	0.86(0.07-16.3)	0.12(0.02-0.47)	7129(369-14240)	6.6(0.25-15.6)
Macquarie Is							
juvenile	9.27(0.52-25.67)	27.1(14.5-315)	2.45(1.06-12.1)	2.17(0.01-9.09)	0.13(0.05-0.38)	2502(1480-3859)	4.02(0.61-2.79)
adult	1.02(0.70-1.45)	21.6(7.4-582)	1.94(0.88-12.9)	1.19(0.25-31.3)	0.12(0.03-1.65)	7853(6415-10662)	6.28(2.88-16.6)
HIMI							
juvenile	2.67(0.96-16.34)	18.3(14.1-30.9)	3.28(1.24-8.51)	1.71(0.28-16.2)	0.10(0.05-0.54)	2451(1636-3818)	2.13(1.48-4.61)
adult	0.97(0.43-1.93)	19.2(12.0-30.1)	2.04(1.00-5.28)	0.79(0.10-6.03)	0.10(0.02-0.49)	6705(2404-9564)	4.89(1.79-13.9)

Table 4. Classification success of discriminate function analysis based on core chemistry. Actual locations are recorded across the top and predicted locations listed vertically. Percentages, indicated below each number, are the number from each fishery allocated to a particular location. Indian Ocean is a combination of Kerguelen Is, HIMI and Prince Edward Is which occur in the Indian Ocean sector of the Southern Ocean. Predictive success was low when these locations were treated separately, with large amounts of misclassification between these sites. Total predictive success was 73% when the Indian Ocean group is combined.

Predicted location	Falk	Ind	Mac	Sam
Falkland Is	63%	2%	3%	10%
Indian Ocean	13%	76%	31%	3%
Macquarie Is	0%	14%	58%	0%
South America	23%	8%	7%	87%
Total	30	88	29	30

Table 5. Classification success of discriminate function analysis based on edge chemistry. Actual locations are recorded across the top with predicted locations listed vertically. Prediction success is expressed in percentages, listed below each number. Indian Ocean is a combination of Kerguelen Is, Prince Edward Is and Heard and Macdonald Is. Total prediction success is 63%.

Predicted location	Falk	Ind	Mac	Sam
Falkland Is	57%	7%	15%	7%
Indian Ocean	25%	65%	23%	14%
Macquarie Is	4%	14%	54%	14%
South America	14%	13%	8%	66%
Total	28	84	26	29

Figure 1

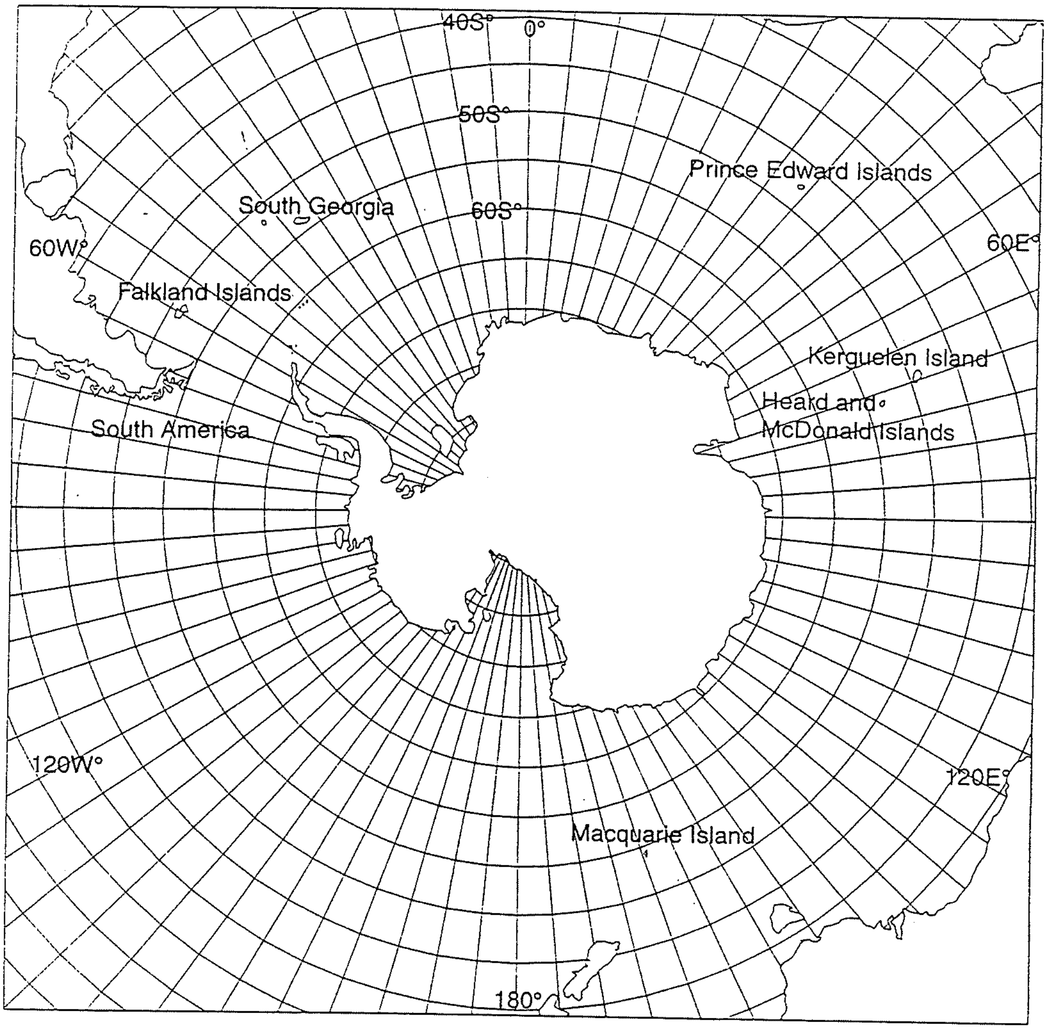
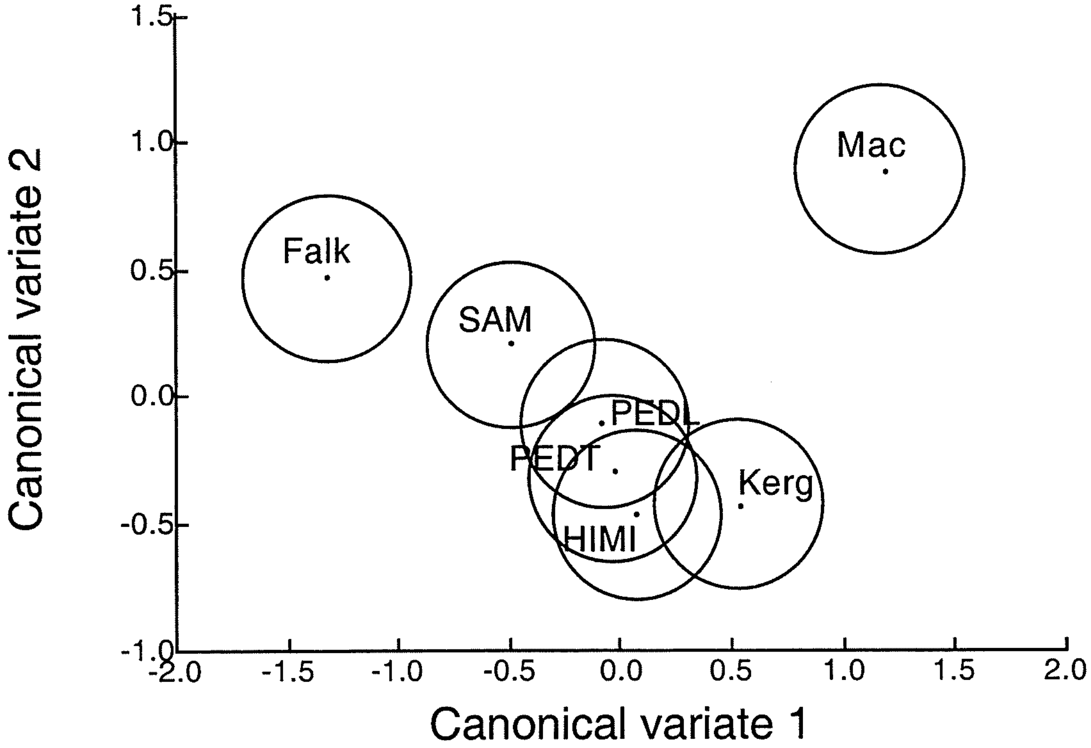
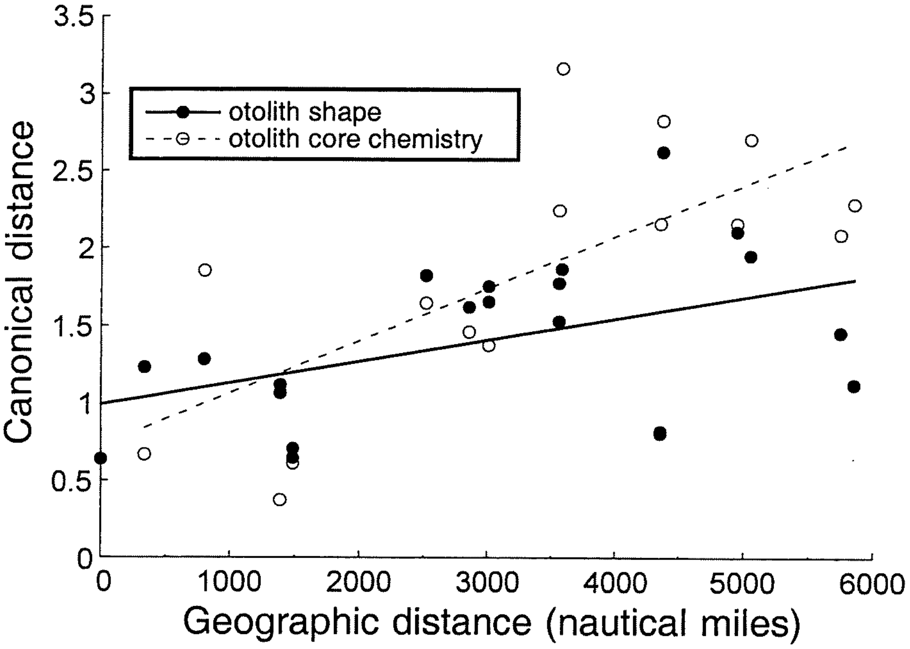

40S°
0°
50S°
60S°
Prince Edward Islands
South Georgia
60W°
60E°
Falkland Islands
Kerguelen Island
Heard and
McDonald Islands
South America
120W°
120E°
Macquarie Island
180°

Figure 3



Canonical variate 2
Canonical variate 1
1.5
1.0
0.5
0.0
-0.5
-1.0
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Mac
Falk
SAM
PEDL
PEDT
Kerg
HIMI

Figure 4



otolith shape
otolith core chemistry
Canonical distance
3.5
3
2.5
2
1.5
1
0.5
0
0
1000
2000
3000
4000
5000
6000
Geographic distance (nautical miles)

Figure 5

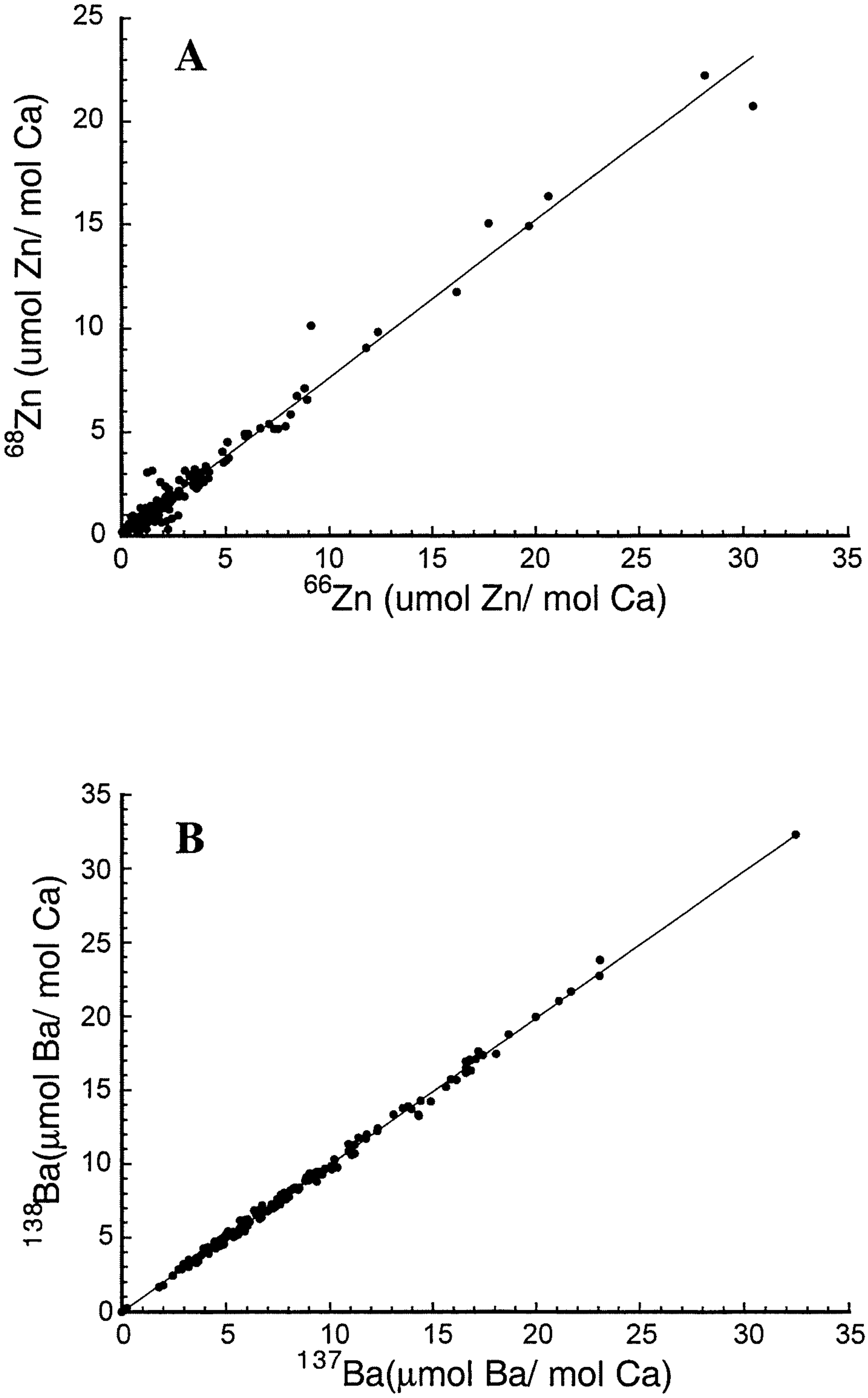

A
25
20
15
10
5
0
0
5
10
15
20
25
30
35
^{68}Zn (umol Zn/ mol Ca)
^{66}Zn (umol Zn/ mol Ca)
B
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
^{138}Ba(μmol Ba/ mol Ca)
^{137}Ba(μmol Ba/ mol Ca)

Figure 6

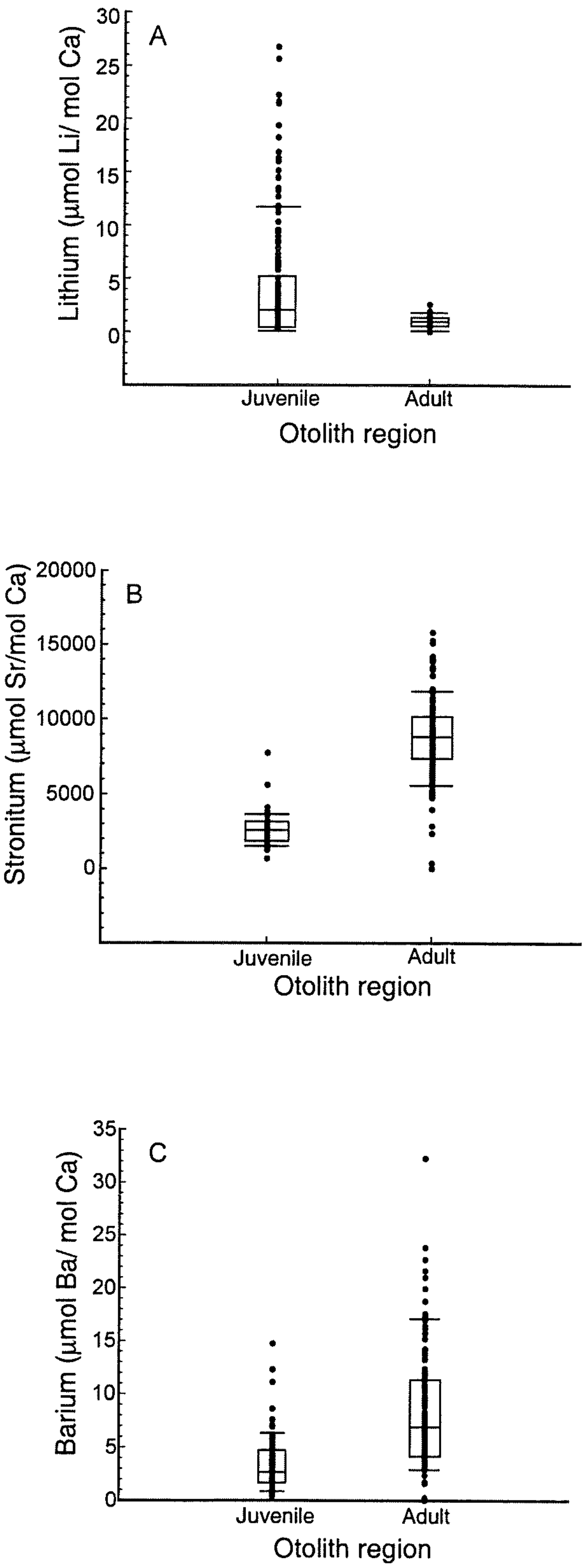

A
Lithium (µmol Li/ mol Ca)
30
25
20
15
10
5
0
Juvenile
Adult
Otolith region
B
Stronitum (µmol Sr/mol Ca)
20000
15000
10000
5000
0
Juvenile
Adult
Otolith region
C
Barium (µmol Ba/ mol Ca)
35
30
25
20
15
10
5
0
Juvenile
Adult
Otolith region

Figure 7

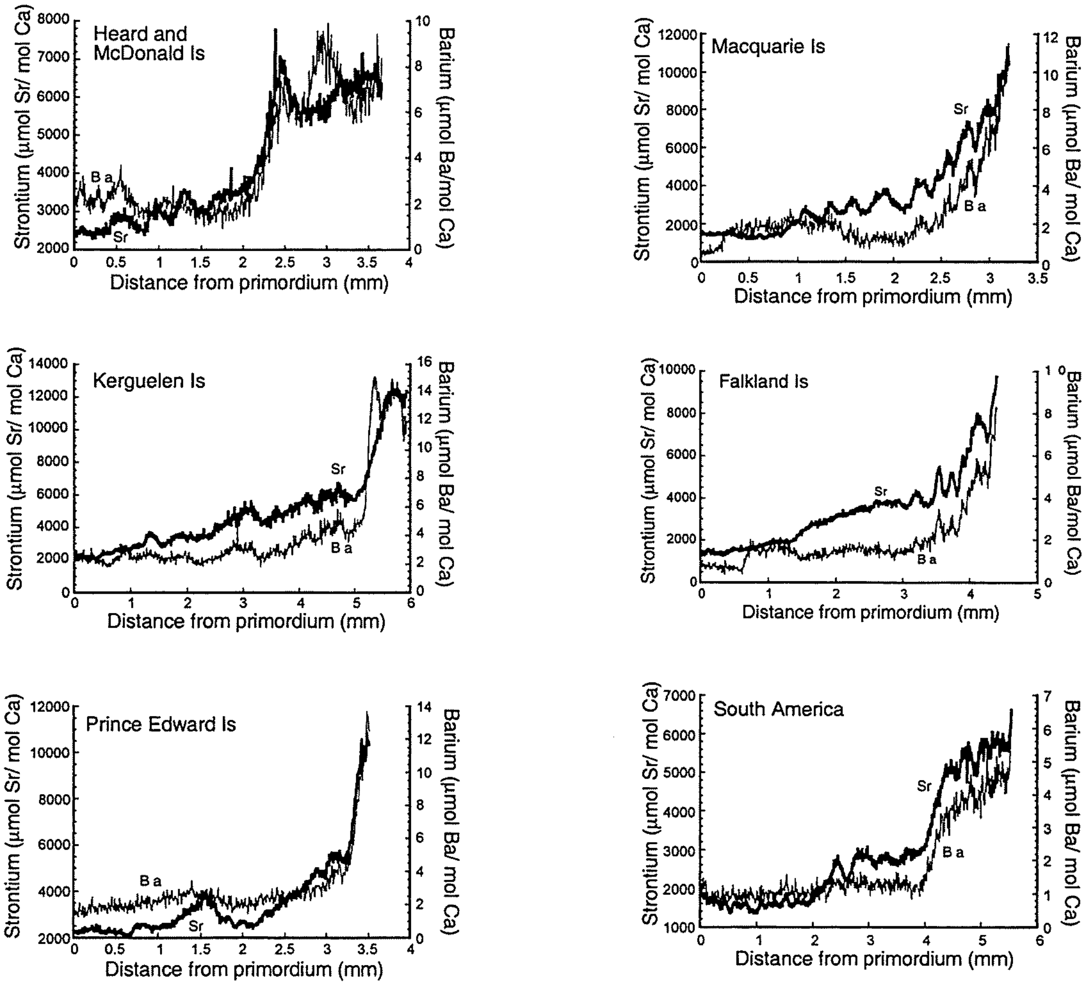
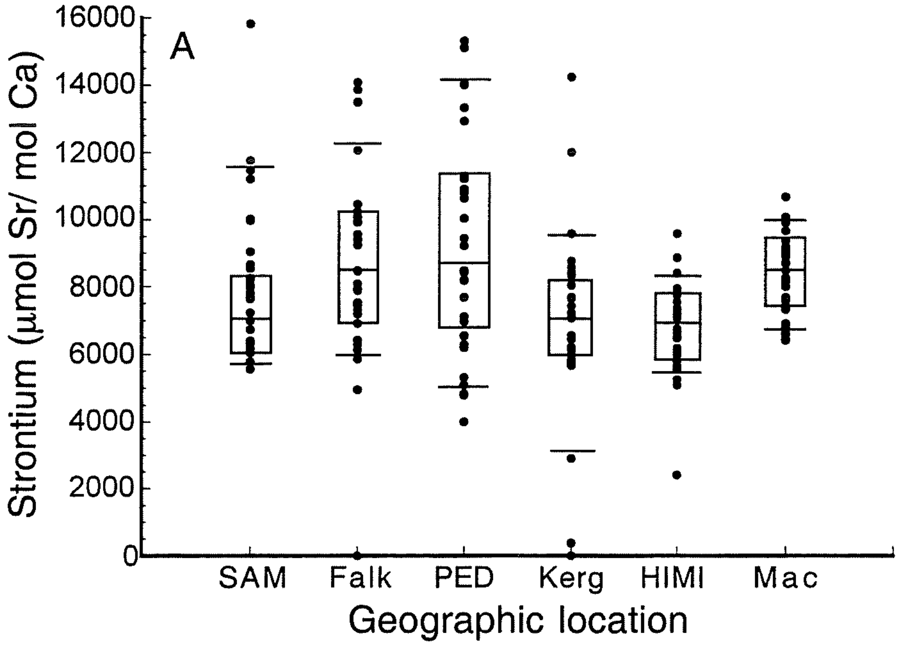
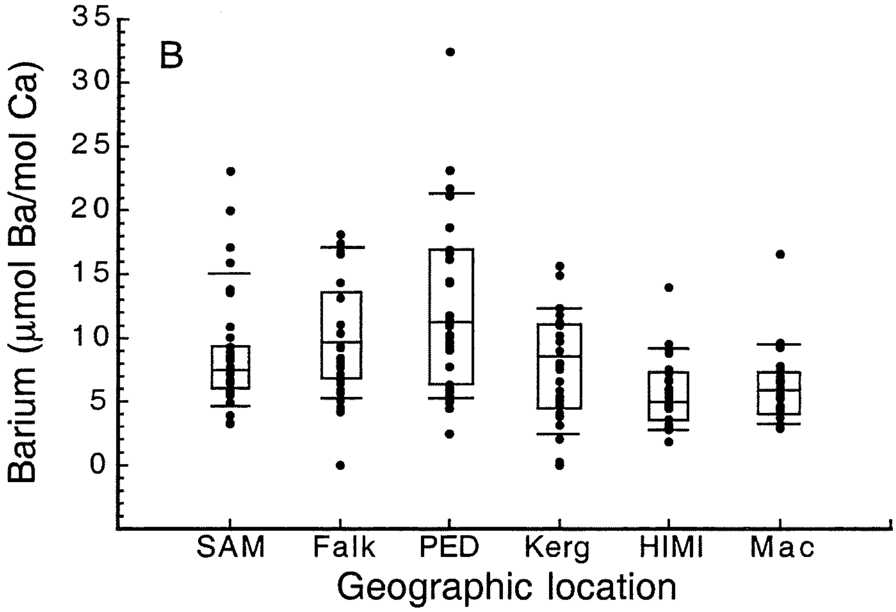

Heard and McDonald Is
Strontium (μmol Sr/ mol Ca)
Barium (μmol Ba/mol Ca)
8000
7000
6000
5000
4000
3000
2000
10
8
6
4
2
0
0
0.5
1
1.5
2
2.5
3
3.5
4
Distance from primordium (mm)
Ba
Sr
Macquarie Is
Strontium (μmol Sr/ mol Ca)
Barium (μmol Ba/ mol Ca)
12000
10000
8000
6000
4000
2000
0
12
10
8
6
4
2
0
0
0.5
1
1.5
2
2.5
3
3.5
Distance from primordium (mm)
Sr
Ba
Kerguelen Is
Strontium (μmol Sr/ mol Ca)
Barium (μmol Ba/ mol Ca)
14000
12000
10000
8000
6000
4000
2000
0
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
Distance from primordium (mm)
Sr
Ba
Falkland Is
Strontium (μmol Sr/ mol Ca)
Barium (μmol Ba/mol Ca)
10000
8000
6000
4000
2000
0
10
8
6
4
2
0
0
1
2
3
4
5
Distance from primordium (mm)
Sr
Ba
Prince Edward Is
Strontium (μmol Sr/ mol Ca)
Barium (μmol Ba/ mol Ca)
12000
10000
8000
6000
4000
2000
14
12
10
8
6
4
2
0
0
0.5
1
1.5
2
2.5
3
3.5
4
Distance from primordium (mm)
Ba
Sr
South America
Strontium (μmol Sr/ mol Ca)
Barium (μmol Ba/ mol Ca)
7000
6000
5000
4000
3000
2000
1000
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
Distance from primordium (mm)
Sr
Ba

Figure 8



A
Strontium (μmol Sr/ mol Ca)
16000
14000
12000
10000
8000
6000
4000
2000
0
SAM
Falk
PED
Kerg
HIMI
Mac
Geographic location



B
Barium (μmol Ba/mol Ca)
35
30
25
20
15
10
5
0
SAM
Falk
PED
Kerg
HIMI
Mac
Geographic location

Figure 9

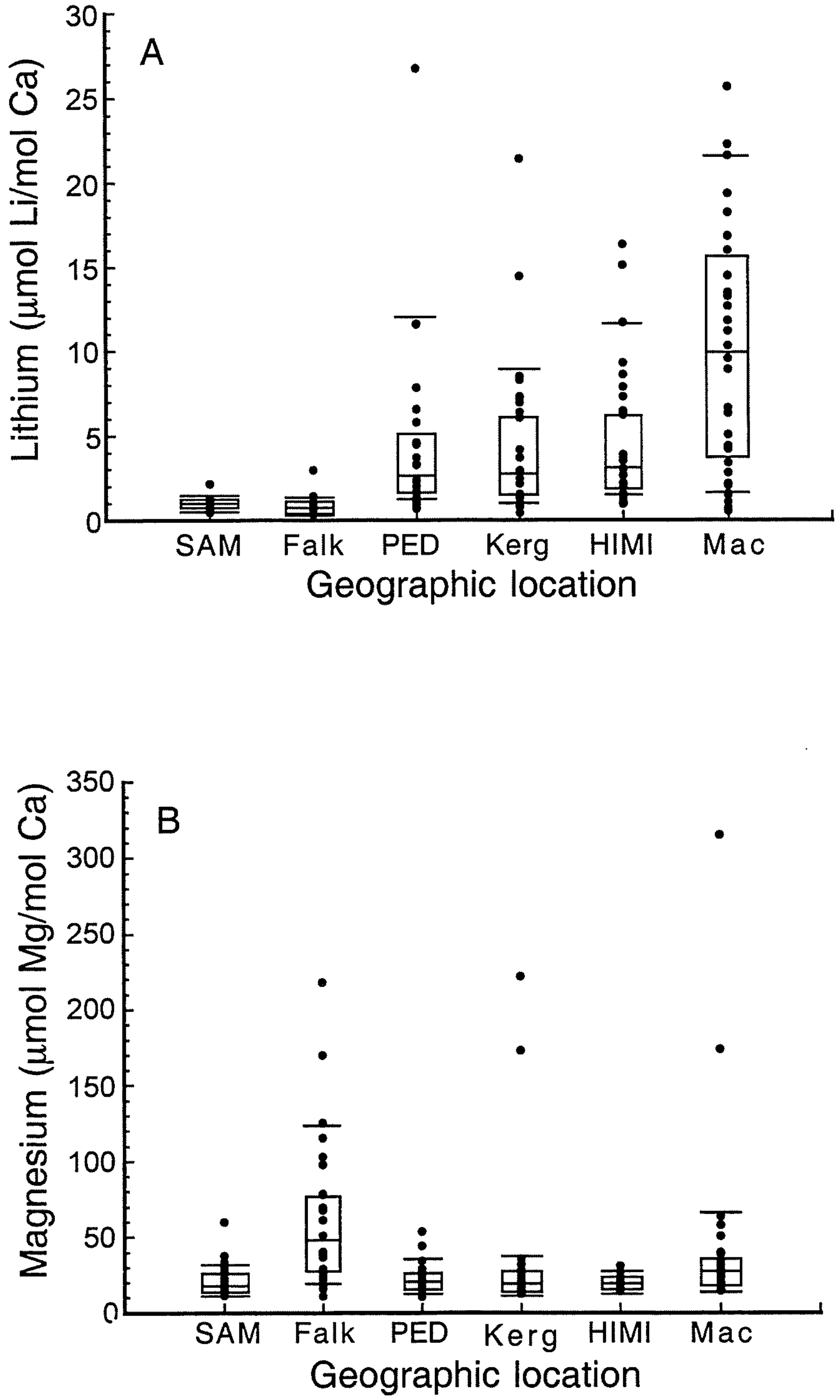

A
Lithium (µmol Li/mol Ca)
0
5
10
15
20
25
30
SAM
Falk
PED
Kerg
HIMI
Mac
Geographic location
B
Magnesium (µmol Mg/mol Ca)
0
50
100
150
200
250
300
350
SAM
Falk
PED
Kerg
HIMI
Mac
Geographic location

Figure 10

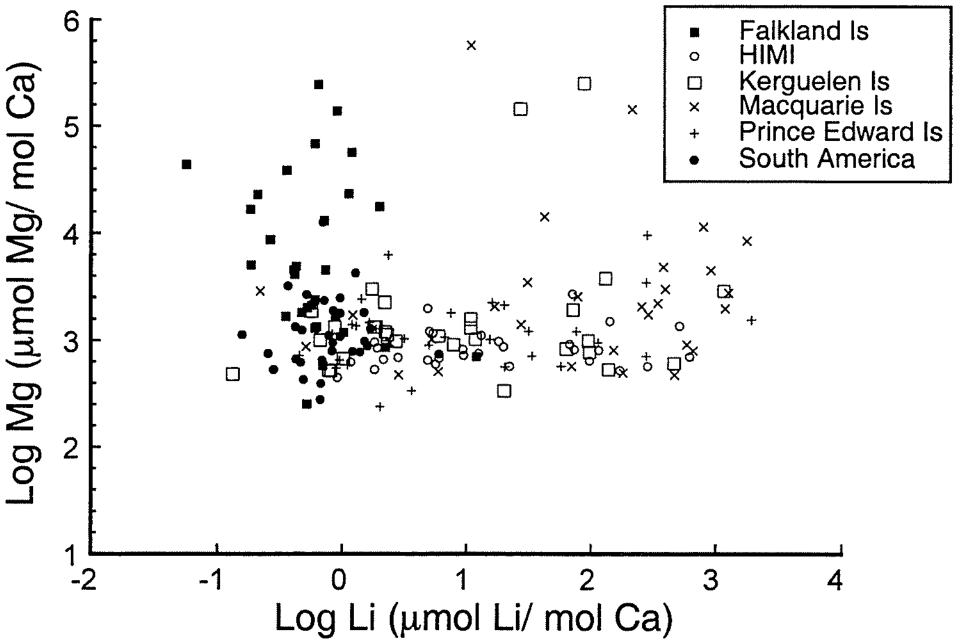

Log Mg (μmol Mg/ mol Ca)
Log Li (μmol Li/ mol Ca)
6
5
4
3
2
1
-2
-1
0
1
2
3
4
■ Falkland Is
○ HIMI
□ Kerguelen Is
× Macquarie Is
+ Prince Edward Is
● South America

Figure 11

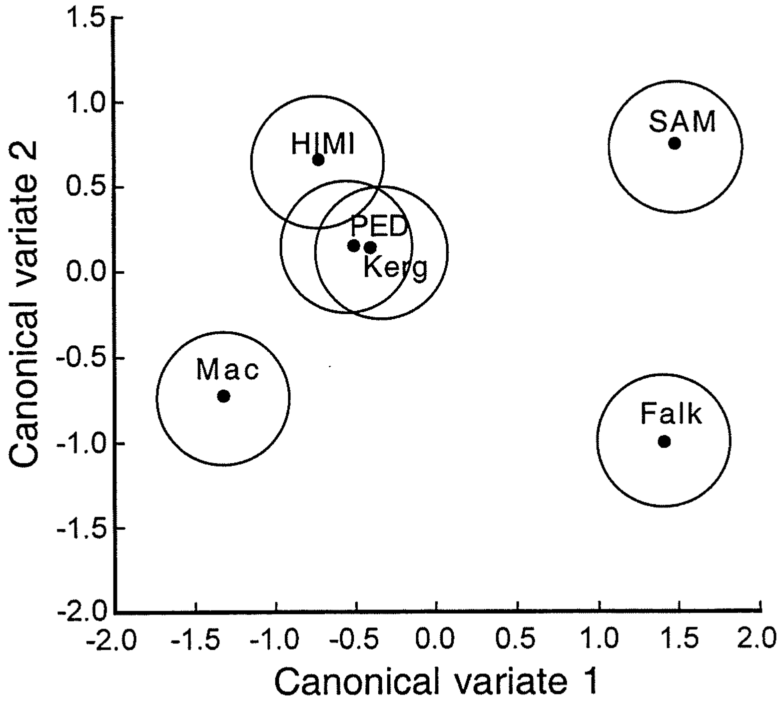

1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Canonical variate 2
Canonical variate 1
HIMI
PED
Kerg
SAM
Mac
Falk

Figure 12

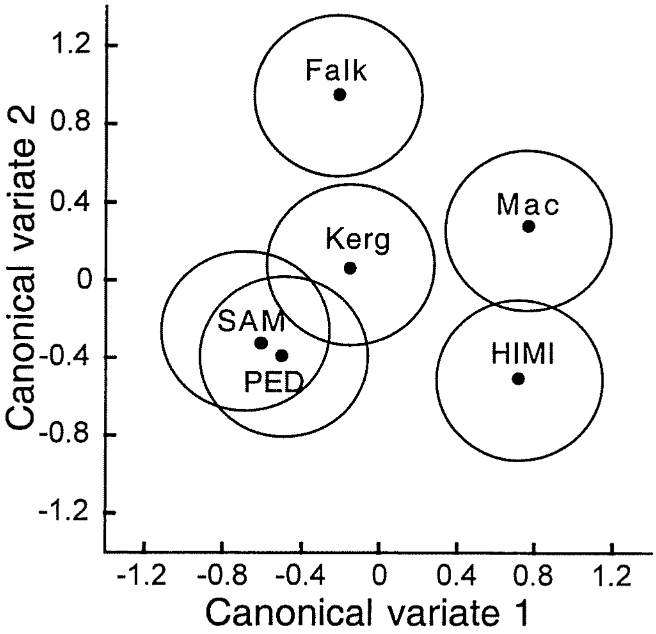

Falk
Mac
Kerg
SAM
PED
HIMI
1.2
0.8
0.4
0
-0.4
-0.8
-1.2
Canonical variate 2
-1.2
-0.8
-0.4
0
0.4
0.8
1.2
Canonical variate 1

Figure 13a

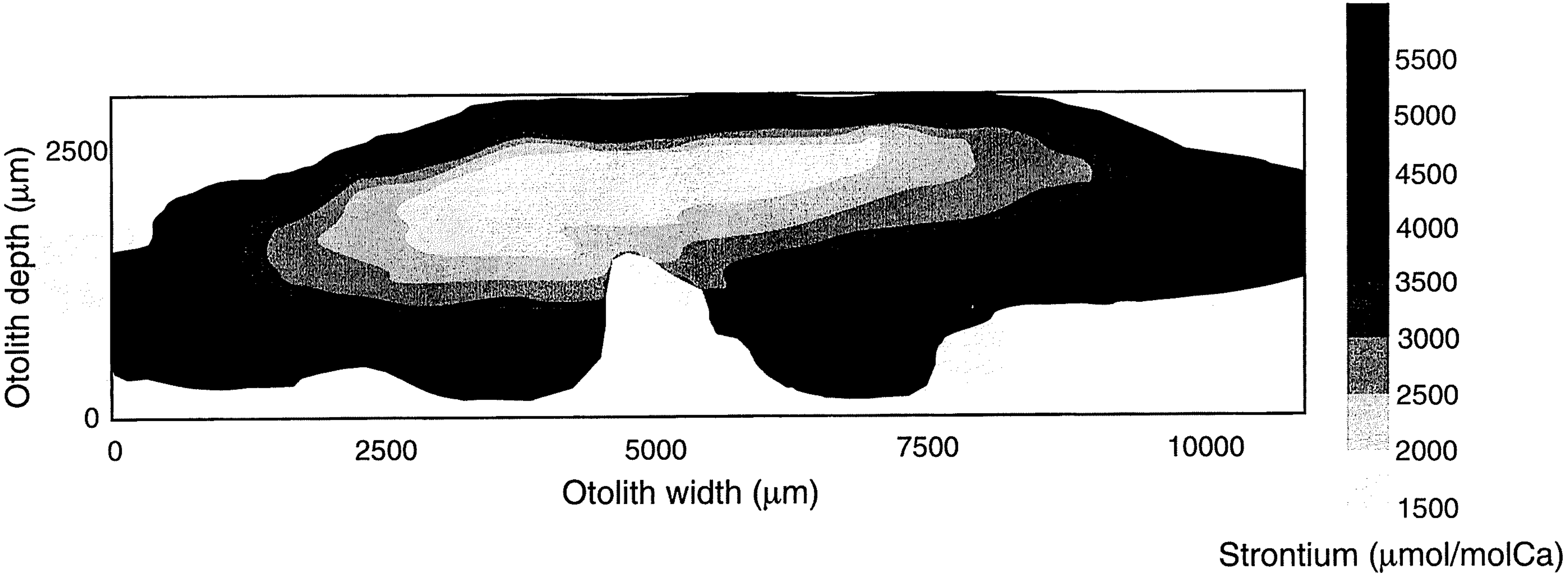
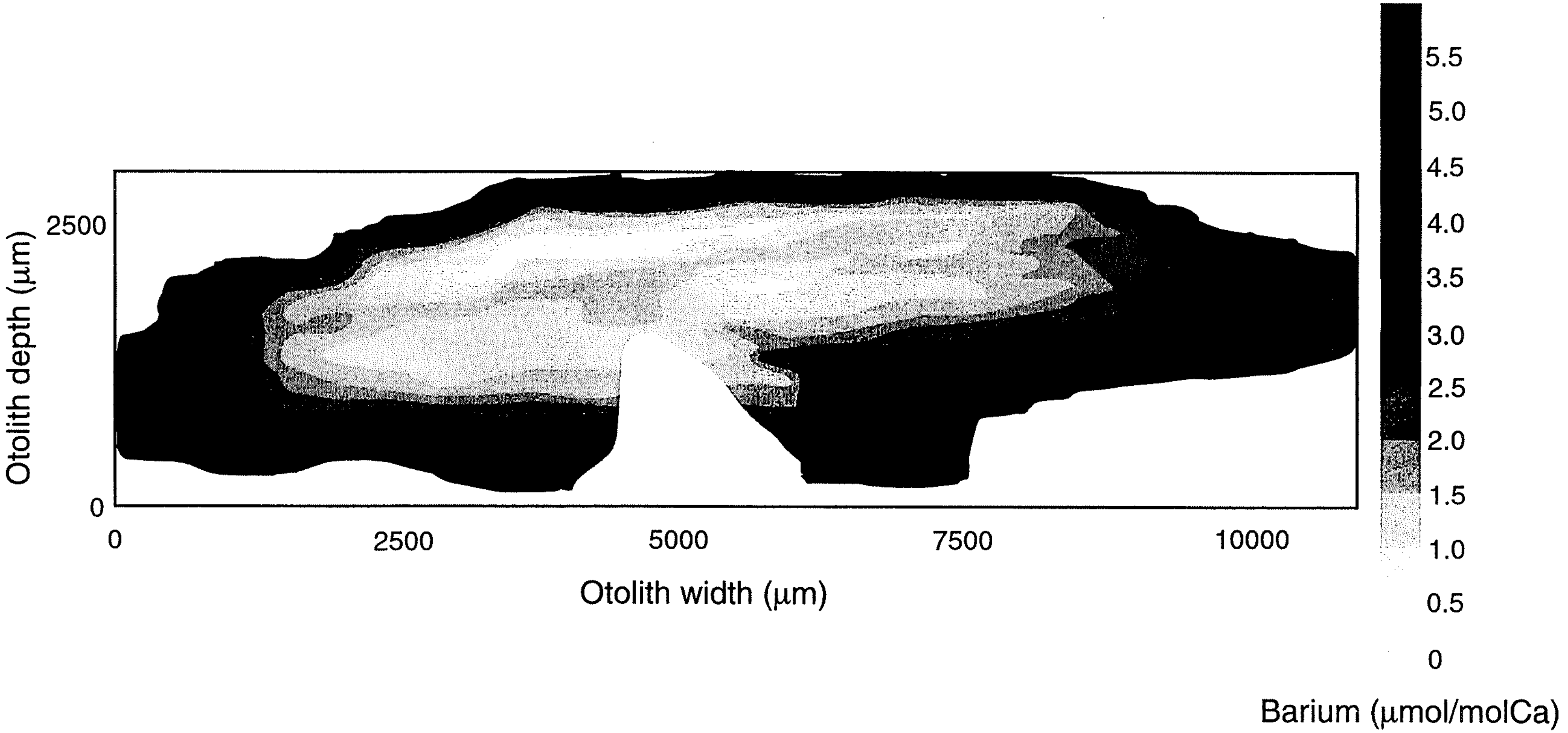

Otolith depth (μm)
2500
0
0
2500
5000
7500
10000
Otolith width (μm)
5500
5000
4500
4000
3500
3000
2500
2000
1500
Strontium (μmol/molCa)

Figure 13b



Otolith depth (μm)
2500
0
0
2500
5000
7500
10000
Otolith width (μm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Barium (μmol/molCa)