# Use of the bomb radiocarbon chronometer to validate fish age

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FISHERIES RESEARCH & DEVELOPMENT CORPORATION

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Cover illustration: The cover background shows the distribution of radiocarbon in the south central and south western Pacific Ocean from 170° E to 120° W along a section at 32° S. The depth range shown is from 50 m to 1100 m where red is the highest $\Delta^{14}C$ (~150‰) and blue is the lowest $\Delta^{14}C$ (~ -150‰). The water samples were collected between 30 May and 30 July 1992 by the R/V Knorr as part of the World Ocean Circulation Experiment (WOCE). The analyses of radiocarbon in seawater dissolved inorganic carbon were carried out by the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) at the Woods Hole Oceanographic Institution. The figure was prepared by NOSAMS and is used with their permission.

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## 1993/109 Use of the bomb radiocarbon chronometer to validate fish age

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**Objectives:**

1. Validate ages of southern bluefin tuna (*Thunnus maccoyii*);
2. Determine suitability of the radiocarbon chronometer for age validation of oreo species;
3. Validate ages of redfish (*Centroberyx affinis*);
4. Validate ages of blue grenadier (*Macruronus novaezelandiae*);
5. Validate ages of school shark (*Galeorhinus galeus*), and;
6. Determine other species suitable for age validation via the bomb radiocarbon chronometer in collaboration with other Australian researchers and fisheries managers.

**Non-Technical Summary**

**Outcomes Achieved**

The outputs from this research will reduce uncertainty in stock assessments for more than 20 commercially important fish species. In some cases, notably southern bluefin tuna, the findings of this study have resulted in significant changes in estimates of stock productivity and sustainable yields. Therefore, the outputs improve stock assessments and provide greater certainty for the sustainable management of key fisheries resources. The project measured radiocarbon in 28 commercially important fish species including species from the waters of each State and the Northern Territory and has relevance to many of Australia's most valuable finfish fisheries. Furthermore, the results provide conclusive support for comments made in a recent review of age determination and age validation methods which stated that '…bomb derived radiocarbon from nuclear testing provides one of the best validation approaches available for long-lived fishes'. Due to the effectiveness of the method, age validation has been achieved for a broad range of species at a cost to stakeholders far below that of 'traditional' validation techniques. The results also demonstrate the broad application of the method and highlight the value of measurements of radiocarbon to investigations of fish biology and ecology with results providing new insight into the ecology of deep-sea and coastal fish species. In addition to the direct benefits to the management of Australian fish species, this study has also increased the profile of Australian fisheries science internationally and resulted in new collaborations between Australian and overseas scientists.

Estimates of fish age are necessary to determine growth rates, mortality rates, age at first maturity, and other basic parameters relevant to understanding the biology of exploited fish populations. Furthermore, many fisheries resources are managed on the basis of age structured population models. Therefore, it is extremely important to determine the accuracy of fish age estimates used in the assessment of fisheries resources. Otoliths, calcium carbonate gravity and auditory receptors in the inner ear of teleost fish, are the primary source of information on the age and growth of fishes. However, the estimation of fish age from otoliths is not always a simple process. This is because the structures used most frequently to estimate fish age, opaque and translucent zones in thin sections of otoliths, are not always formed annually. A critical aspect of studies of fish age is to validate an age estimation method, where validation is the process used to determine the accuracy of a method used to estimate fish age, usually by independent confirmation of the frequency of formation of the opaque and translucent zones. A new method of age validation, used extensively for the first time in this study, is based on the measurement of radiocarbon in fish otoliths.

The ability to measure radiocarbon levels in the environment and the successful interpretation of these data are two of the great scientific findings of the twentieth century, and, in 1960 Willard Libby was awarded the Nobel Prize in Chemistry for the development of the radiocarbon dating method. Today, the measurement of radiocarbon has numerous applications in the physical, biological, and social sciences. However, for investigations of fish biology, the measurement and application of radiocarbon data has, until now, lagged behind the technological advances making it possible to carry out high precision analyses on small samples. The revolutionary advance of radiocarbon measurement by accelerator mass spectrometry (AMS), first developed about 20 years ago, makes it possible to analyse very small samples (< 1 mg) for radiocarbon, and was first applied as a 'bomb radiocarbon chronometer' to the validation of fish age by Kalish (1993). Recent reviews on fish age and growth (e.g. Campana 2001) have acknowledged that the bomb radiocarbon chronometer is the most effective method available to validate methods to estimate the ages of fish.

Human activities have had a major impact on radiocarbon levels in both the atmosphere and oceans with three activities being most significant: 1) burning of fossil fuels; 2) atmospheric detonation of nuclear bombs; and, 3) generation of power by nuclear fission. The second activity, although a profoundly negative event, does have a 'silver lining'. Atmospheric testing of nuclear weapons in the 1950s and 1960s resulted in a drastic increase in radiocarbon levels in both the atmosphere and the ocean, and some of this radiocarbon was incorporated into living organisms. The increase in ocean radiocarbon during the 1960s was so rapid that the changing levels of radiocarbon during those years can be used as a de facto time scale. Otoliths have been shown to retain a chemical record that may be interpretable as a complex function of variations in a fish's physiology and environment over time. Among the constituents of otoliths, radiocarbon has been shown to be a good proxy for radiocarbon levels in seawater, indicating that these structures can be used as 'clocks' over recent decades. Using AMS, it is possible to measure with high precision, levels of radiocarbon in samples that contain less than 0.5 mg of carbon. Calcium carbonate otoliths are 12% carbon making it feasible to analyse radiocarbon levels in otolith samples that weigh less than 5 mg. For many commercially important fish species, the amount of otolith calcium carbonate deposited in the first year of life exceeds 5 mg. This makes it possible to analyse radiocarbon levels in selected regions of a fish's otolith, including that portion that was deposited within 1 year or less of birth. By selecting otoliths from fish with presumed birth dates during the bomb-related increase in ocean radiocarbon and analysing segments of the otolith formed soon after birth, the fish's true age can be estimated. Ultimately, these data can be used to validate a method of age estimation. Furthermore, this validation procedure provides an estimate of the age of a fish that is independent of the counting of opaque and translucent zones in otolith thin sections, the method used most often to estimate the age of fish.

This project measured radiocarbon in calcified tissues of 28 commercially important fish species and over 300 high precision accelerator mass spectrometry measurements of radiocarbon were completed. The majority of measurements were made on otolith carbonate although vertebral collagen was analysed in two species (school shark, broadbill swordfish) and vertebral carbonate in one species (broadbill swordfish). The technique of radiocarbon dating was used for age validation of one species, orange roughy, as opposed to the bomb radiocarbon chronometer used for the others. There are degrees of success in any age validation study and four levels of 'validation' are identified for the species in the table. Three asterisks (***) indicates a successful validation for a species across a broad range of fish ages and evidence that age estimates based on the method of choice, typically interpretation of zones in otolith thin sections, are accurate. Two asterisks (**) indicates that the age estimation method provides reasonable estimates of fish age, but further work may be required to improve understanding of the accuracy of the method. One asterisk (*) indicates that the study of age validation identified potentially serious faults in the age estimation method and further research is required to ensure accurate age estimates are available for assessment of the species. No asterisks beside a species indicates that inadequate data were collected to draw a conclusion regarding the accuracy of an age estimation procedure for the species.

| | | | |
|---|---|---|---|
| school shark* | black oreo** | ruby snapper** | spangled emperor*** |
| blue grenadier*** | warty oreo** | crimson seaperch** | banded morwong*** |
| pink ling*** | spikey oreo** | fingermark seaperch** | jackass morwong** |
| orange roughy** | smooth oreo** | saddletail seaperch** | Patagonian toothfish*** |
| redfish*** | eight barred rockcod*** | red emperor** | southern bluefin tuna*** |
| bight redfish** | silver trevally** | goldband snapper*** | swordfish |
| king dory*** | jack mackerel*** | sharptooth snapper** | blue-eye trevalla* |

This study demonstrates the effectiveness of the bomb radiocarbon chronometer for studies of age validation and supports further use of this method. The research also provides valuable information on the habitats and migration of several fish species. For example, radiocarbon measurements in otoliths demonstrated the polar distribution of smooth oreo juveniles and the estuarine habitats of juvenile tropical seaperchs. Finally, radiocarbon data from otoliths of marine fishes can provide powerful constraints on both carbon cycle models and ocean general circulation models, data that are essential to research on climate and global change.

Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of fish biology* 59: 197-242.

Kalish, J. M. 1993. Pre- and post-bomb radiocarbon in fish otoliths. *Earth and Planetary Science Letters* 114: 549-554.

KEYWORDS: radiocarbon, otoliths, age and growth, age validation, Australian fisheries, carbon flux

# Chapter 2 Background

Data on fish age are essential for determining the production of fish stocks. Although age data are routinely collected by a range of methods, it is infrequent that an age estimation method is proven to be accurate. Several methods are used to validate fish ages; however, these possess many shortcomings. For example, capture-mark-recapture studies are extremely expensive, labour intensive, often have a very negative effect on the growth of the tagged fish while at liberty, and are not feasible for many species including deep-sea species. Furthermore, estimation of the growth increment on an otolith or vertebra, between the time of the initial capture, marking and subsequent recapture constitutes the most rudimentary validation of age. This is due to the fact that it is only possible to determine the time elapsed between marking and recapture; the number of years prior to capture of the individual remains unknown. Therefore, the true age of the fish can not be determined only the validity of the most recently deposited increments. Radiometric age estimation using various activity ratios (e.g. $^{210}Pb/^{226}Ra$) can, in theory, overcome many of the shortcomings of capture-mark-recapture experiments; however, several unique problems are introduced by the use of radiometric methods. Several key assumptions must be made to apply the radiometric method including: constant uptake of radioisotopes relative to calcium during the life of the fish; constant rate of otolith mass growth or a known rate(s) of otolith mass growth, and; the otolith must be a "closed system" with respect to the radioisotopes under consideration. Even without violation of any of these assumptions the radiometric method is problematic due to the need for relatively large sample sizes ($\geq$1 gram) and the relatively low precision of the method resulting from extremely low levels of radioactivity relative to background for the radioisotopes typically considered. Given the extreme importance of age estimation studies and the problems with currently established methods of age validation, there is tremendous scope for the development and application of new methods.

A recently developed method of age validation, the bomb radiocarbon chronomter, overcomes many of the shortcomings of other methods. Kalish (1993) described the new method and demonstrated its efficacy with the long-lived teleost *Pagrus auratus*. The bomb radiocarbon chronometer method is based on the occurrence of an anthropogenic chemical marker in otoliths, specifically the sharp increase in radiocarbon (reported as $\Delta^{14}C$, the age- and fractionation-corrected per mil deviation from the radiocarbon activity of nineteenth century wood) in the 1950s and 1960s that resulted from extensive atmospheric testing of nuclear weapons by the United States, Soviet Union and Great Britain. This spike of radiocarbon would be evident in the otoliths of fish species that inhabit at least the upper 200 m or mixed layer of the water column. Kalish (1993) has shown that fish otoliths are an excellent proxy for determining levels of radiocarbon in seawater dissolved inorganic carbon (DIC). Because otoliths are not resorbed during the life of the fish, these structures retain a permanent record of ocean radiocarbon. The atmospheric testing of nuclear weapons in the 1950s and 1960s has had a tremendous effect on the levels of radiocarbon in both the atmosphere and oceans (Broecker et al., 1985). Because these tests had both a relatively abrupt start and end, a temporally discrete pulse of radiocarbon was injected into the ecosphere. This marker, or transient tracer, is one of the most effective tools at our disposal for investigations of carbon flux and ocean circulation. Fortuitously, this anthropogenic input of radiocarbon has also provided a readily detectable mark on fish otoliths and, for that matter, most living organisms. Similar to the injection of oxytetracycline hydrochloride (OTC) into fish during capture-mark-recapture experiments, radiocarbon provides a mark on otoliths that can be associated with a point in time. Kalish (1993) demonstrates this property of the radiocarbon mark and shows that maximal rates of increase for ocean radiocarbon, measured via the otolith radiocarbon proxy, are associated with the peak atmospheric testing of nuclear bombs. Radiocarbon increased rapidly in the surface ocean from about 1958 until a peak around the mid-1970s although this varies with ocean and latitude.

To use the bomb radiocarbon chronometer for the validation of age, it is only necessary to measure radiocarbon in the earliest formed portions of the otolith. Because of the small sample size requirements of accelerator mass spectrometry (AMS) (now as little as 0.2 mg of carbon), the otolith material formed in the first year, or less, of life is generally adequate for high precision analysis. Measurements of radiocarbon in the earliest formed otolith material can be related to the known temporal variation of bomb radiocarbon in the environment. An age "calibration curve" based on otolith $\Delta^{14}C$ in known age fish (or other organisms e.g. hermatypic corals), such as *P. auratus* (Kalish 1993) could be produced for an ocean region and $\Delta^{14}C$ determinations from other species could be applied to the relationship. Alternatively, reliable results could be obtained by producing a sigmoid $\Delta^{14}C$ versus presumed annual increment curve and, ultimately, assigning ages to the fish.

In many cases, age validation via the measurement of $^{14}C$ in otoliths would be more effective than mark-recapture methods. Mark-recapture studies only provide direct evidence of the frequency of annual increment formation between the time of marking and recapture, whereas determination of $^{14}C$ levels in fish otoliths can be used to determine the absolute age of individual fish.

Otolith chemistry, including measurements of radiocarbon, can be used to investigate a range of other issues on the ecology and biology of fish that are relevant to the assessment and sustainable development of marine resources. The distribution of radiocarbon is already defined on spatial and temporal scales that make it possible to use these data to develop a further understanding of stock structure, migration and movement.

Major spatial and temporal gaps in the distribution of $CO_2$ in the South Pacific and Southern Oceans have hindered attempts to assess the importance of these waters as a sink for anthropogenic $CO_2$ (Tans et al., 1990) produced by the combustion of fossil fuels. A major source of data on the flux of $CO_2$ between the atmosphere and oceans has been measurements of $^{14}C$ made in hermatypic corals from tropical and subtropical waters; however, the major limitation of corals as recorders of these data is that suitable corals are restricted to the upper 50 meters of tropical and subtropical oceans. Because measurements of $^{14}C$ in fish otoliths are a good proxy for $^{14}C$ in seawater DIC and teleost fishes are present in all marine environments; radiocarbon measurements from fish otoliths will make it feasible to model more accurately the global flux of carbon and understand the fate of atmospheric $CO_2$, the principal greenhouse gas. Furthermore, the longevity of many fish species, combined with our ability to accurately determine the age of some species, makes it possible to extend the ocean $\Delta^{14}C$ record to pre-bomb periods in a wide range of environments. The existence of otolith archives at fisheries laboratories throughout the world ensures the accessibility of this data source.

Although not the major impetus for the proposed research, $\Delta^{14}C$ data collected from fish otoliths will provide additional inputs to models of global carbon flux and determine the roll of the oceans as a sink for $CO_2$.

## Chapter 3 Need

Accurate estimates of fish age are among the most important biological parameters required to develop sound population dynamics models and assessments of stock status. These assessments are essential for the development of sound management strategies for individual species and ecosystems. The degree of uncertainty associated with age estimation can have a dramatic influence on the overall uncertainty of a stock assessment. Minimisation of this source of uncertainty can be achieved, in part, by developing reliable, accurate and validated methods.

Australian fisheries exploit species with a broad range of life history strategies from short–lived highly fecund species such as skipjack tuna to extremely long–lived moderately fecund species like orange roughy. There is a clear need to accurately identify the place of each species within this spectrum and to validate or determine the level of accuracy of methods employed for routine age estimation of the fish catch.

Routine methods of age validation are poorly suited to many of the fish species exploited in Australian waters. In addition, for those species where routine validation may be suitable, an effective validation would require many years, if not decades of research. This time frame is inadequate for many species that are already heavily exploited (e.g. southern bluefin tuna, blue grenadier, redfish, school shark and others) and an alternative and rapid method such as the bomb radiocarbon chronometer should be applied to those species that would be amenable to this approach.

# Chapter 4 Objectives

1. Validate ages of southern bluefin tuna (*Thunnus maccoyii*);
2. Determine suitability of the radiocarbon chronometer for age validation of oreo species;
3. Validate ages of redfish (*Centroberyx affinis*);
4. Validate ages of blue grenadier (*Macruronus novaezelandiae*);
5. Validate ages of school shark (*Galeorhinus galeus*); and,
6. Determine other species suitable for age validation via the bomb radiocarbon chronometer in collaboration with other Australian researchers and fisheries managers.

# Chapter 5 Radiocarbon and fish biology

John Kalish

**Summary**

Measurements of radiocarbon are extremely valuable in many fields of scientific research, but application of these data to studies of fish biology have been rare. Recent advances in the techniques used to quantify radiocarbon, particularly accelerator mass spectrometry, have increased the feasibility of applying these data to a range of problems. Background information relevant to the use of radiocarbon in studies of fish biology is presented and the basis for a new method of age validation using the "bomb radiocarbon chronometer" is discussed. The sharp increase in radiocarbon in seawater dissolved inorganic carbon resulting from the atmospheric testing of nuclear bombs in the 1950's and 1960's provides a discrete chemical signature that can be identified in fish otoliths, and this signature can be related to specific points in time. Age validation using the bomb radiocarbon chronometer eliminates the need to carry out expensive and time consuming tagging experiments for many fish species. Reduced somatic growth associated with tagging studies and stress related to capture, handling, marker injection and tagging are eliminated. Furthermore, capture-mark-recapture experiments only validate the period of time between the marking of a fish and the time of recapture, whereas the bomb radiocarbon chronometer provides an estimate of the actual fish age, not just the time period during which the fish is at liberty. Radiocarbon data from the otoliths of a deep-sea teleost, the smooth oreo (*Pseuodocyttus maculatus*) demonstrate the utility of these data in determining the juvenile habitat of this species. Results obtained from measuring radiocarbon in otoliths support the broad application of these techniques in both age validation and life-history studies.

**Introduction**

The ability to measure radiocarbon levels in the environment and the successful interpretation of these data are two of the great scientific findings of the twentieth century, and, in 1960 Willard Libby was awarded the Nobel Prize in Chemistry for the development of the radiocarbon dating method. Today, the measurement of radiocarbon has numerous applications in the physical, biological, and social sciences (Taylor et al. 1992). However, for investigations of fish biology, the measurement and application of radiocarbon data has lagged behind the technological advances making it possible to carry out high precision analyses on small samples. The revolutionary advance of radiocarbon measurement by accelerator mass spectrometry (AMS) is little more than a decade old (Bennett et al. 1977; Nelson et al. 1977) and was only recently applied as a "bomb radiocarbon chronometer" to the validation of fish age (Kalish 1993). Because both radiocarbon methods and data are unfamiliar to many, this paper presents background information that is essential to an understanding of the field. It then highlights the major applications of radiocarbon data to studies involving fish biology with an emphasis on otoliths and areas for further investigation. Radiocarbon data from the otoliths of a deep-sea teleost, *Pseudocyttus maculatus*, are presented and provide an indication of the utility of these data in life history studies.

**Origin of radiocarbon in the environment**

Three isotopes of carbon occur in nature; two isotopes of stable carbon ($^{12}C$, $^{13}C$) and radiocarbon ($^{14}C$). The lighter stable isotope $^{12}C$ accounts for about 98.89% of all carbon and the remainder is primarily $^{13}C$. Radiocarbon is far less abundant with about 1 atom of $^{14}C$ for every $10^{12}$ atoms of $^{12}C$. Radiocarbon is produced naturally by the interaction of cosmic rays with atmospheric atoms. Cosmic rays enter the earth's atmosphere, collide with atmospheric atoms and, in some instances, these collisions result in the ejection of a neutron from the atmospheric atom. If the neutrons interact with atoms of nitrogen ($^{14}N$), a proton can be replaced by the neutron and the resulting atom becomes radiocarbon. The radiocarbon configuration of 6 protons and 8 neutrons is unstable. The mean lifetime of a $^{14}C$ atom is 8267 years at which time the atom undergoes the transformation back to $^{14}N$ by the ejection of an electron, or beta particle ($\beta^-$) and an antineutrino ($\nu^-$), which results in the conversion of one of the neutrons to a proton. On the basis of the logarithmic decay pattern the half-life of $^{14}C$ is 5730 years (Lederer et al., 1967).

**Reporting of radiocarbon data**

Accepted standards for the reporting of radiocarbon values are discussed in Stuiver and Polach (1977) and essential points are summarized here. All radiocarbon results must be reported relative to the NBS (National Bureau of Standards) oxalic acid standard or another accepted standard, such as the ANU (Australian National University) sucrose standard which has been intercalibrated with the NBS standard. The absolute international standard activity (AISA) is 95% of the activity (count rate) in the year 1950 AD normalized to a stable 13C/12C value ($\delta^{13}C$) of -19‰ relative to PDB (peedee belemnite stable carbon and oxygen isotope standard) (Craig 1957). The inclusion of the year 1950 in the definition of the standard fixes the activity of the standard despite the fact that it is decaying over time. Normalization relative to a fixed value for $\delta^{13}C$ accounts for isotopic fractionation of the standard that may occur in the course of laboratory procedures. The radiocarbon activities estimated from the oxalic acid standard are similar to those estimated for the atmosphere, via the tree ring proxy (discussed below), prior to the industrial age (pre-1850 AD).

Radiocarbon levels can be reported in specific forms for different research fields. In archaelogical, anthropological, and geological research, the age of the sample in years is most important and should be reported as a conventional radiocarbon age or radiometric age in years BP (before present, where the present is standardized as the year 1950). The use of this form of age notation implies adherence to specific conventions regarding the reporting of ages (Stuiver and Polach 1977).

Researchers in oceanography, climatology, geochemistry, dendrochronology, and the like, generally report radiocarbon data as $\Delta^{14}C$, and a similar convention is recommended for fish biology. Radiocarbon measurements reported as $\Delta^{14}C$ represent the per mil (‰) deviation of the sample from the activity of 19th century wood, after corrections for isotopic fractionation to a $\delta^{13}C$ of -25‰ (relative to the PDB standard) and corrections for sample age (decay until 1950 AD) (Stuiver and Polach 1977):

$$\Delta^{14}C=\delta^{14}C-2(\delta^{13}C+25)(1+\delta^{14}C/1000)$$

where

$$\delta^{14}C=\left(\frac{14C/Csample - 14C/Cstandard}{14C/Cstandard}\right)1000$$

Although $\Delta^{14}C$ should be used only when samples have been age-corrected, this correction is not always applied. For example, oceanographers generally use $\Delta^{14}C$ when presenting data on radiocarbon in seawater DIC (dissolved inorganic carbon), DOC (dissolved organic carbon), or POC (particulate organic carbon) despite the fact that age corrections are not possible for these data.

Given the known half-life of radiocarbon, three basic assumptions are central to the application of the radiocarbon dating method: 1) the distribution of radiocarbon in the biosphere is uniform in time and space; 2) the radiocarbon being measured in a sample derives from a living organism and, during life, the organism derived its complement of carbon from the biosphere, and; 3) upon death, the organism or relevant portion of the organism being measured for radiocarbon ceased to exchange carbon with the environment. In many cases, these assumptions are not valid and these potential problems are discussed below.

**Radiocarbon pathways**

Of particular relevance to fish biologists is the pathway of radiocarbon from the atmosphere to aquatic environments. Radiocarbon produced by cosmic rays entering the atmosphere becomes part of the atmospheric reservoir as it is oxidized to form $^{14}CO_2$. A portion of the atmospheric $^{14}CO_2$ ultimately becomes incorporated into the ocean at a rate dependent on several factors including: 1) the difference between the concentrations of CO2 in the air and the ocean, and; 2) the rate of gas transfer between the air and ocean.

Depending on the concentration of CO2 in the air and sea, the net transfer of gas maybe into or out of the water. The rate of CO2 transfer across the air-sea interface in a region is generally calculated on the basis of wind speed, although there is no consensus on the relationship between wind speed and the rate of gas exchange (Liss and Merlivat 1986).

Comparison of radiocarbon time series determined from tree rings and corals produced prior to anthropogenic inputs indicates that there was some form of balance between the input of $^{14}CO$

to the ocean and the production of radiocarbon in the atmosphere (Fig. 5.1) (Druffel and Suess 1983). Under these steady state conditions, the atmospheric levels of radiocarbon were 40 to 50‰ greater than those measured in the ocean; a true equilibrium not being achieved within the atmosphere-ocean system. In addition to exchange with the atmosphere, the surface mixed layer of the ocean is constantly loosing radiocarbon, in the form of DIC, to other pools through several processes including: mixing with deeper water; incorporation of DIC into organic carbon pools (DOC, POC, and biota), and; indirectly, through incorporation of POC in sediments. There is a significant flux of bomb radiocarbon labelled POC to the deep ocean and this radiocarbon-laden POC is ultimately derived from DIC in surface waters (Druffel and Williams 1990; Druffel et al. 1992). Bomb-labelled DOC is also produced by organisms as they process DIC. On the basis of proxy records of radiocarbon in seawater DIC that have been obtained from corals, it is apparent that these losses of radiocarbon from surface ocean DIC were relatively constant and balanced by the production and incorporation of cosmogenically produced $^{14}C$ prior to the industrial age (Druffel and Suess 1983).

**Anthropogenic effects on radiocarbon levels**

Human activities have had a major impact on radiocarbon levels in both the atmosphere and oceans with 3 activities being most significant: 1) burning of fossil fuels; 2) atmospheric detonation of nuclear bombs, and; 3) generation of power by nuclear fission. The combustion of fossil fuels (e.g. coal, oil) has accelerated dramatically since the mid-19th century and a current estimate of the atmospheric input of carbon derived from fossil fuels is about 5.3 Gt.yr-1 (gigatons per year) (Tans et al. 1990). Because fossil fuels are the products of animals and plants that died millions of years ago, they no longer contain detectable quantities of radiocarbon. Release of this $^{14}C$-free CO2 into the atmosphere has resulted in dilution of the cosmogenically produced radiocarbon. This decrease in atmospheric and ocean radiocarbon levels between about 1850 and the present is called the Suess effect (Se) and it is easily recognized in radiocarbon records obtained from the tree ring and coral proxies (Fig. 1). More recently, it has become obscured by the $^{14}C$ inputs from other anthropogenic sources.

Atmospheric tests of nuclear bombs between 1952 and 1963 increased atmospheric levels of $^{14}C$ by almost 100% (Fig. 5.2) (Nydal and Lovseth 1983; Levin et al. 1985). This increase in radiocarbon levels has been measured directly in the atmosphere and via the tree ring proxy. Although the increase in ocean radiocarbon due to nuclear testing was not as large as that in the atmosphere, it is, by far, the most significant event in any time series of ocean radiocarbon levels (Fig. 5.3) (Druffel and Linick 1978; Nozaki et al. 1978; Broecker et al. 1985; Kalish 1993).

Radiocarbon is produced in several phases of nuclear energy production. Recent estimates suggest that the mean annual production of $^{14}C$ from the nuclear energy cycle over the next 10 years will be approximately one half of the annual natural production of radiocarbon (Briggs and Hart 1988). Radiocarbon produced during the nuclear energy cycle is unlikely to be detected in aquatic organisms unless they are in close proximity to the source of $^{14}C$.

**Methods of radiocarbon measurement**

Three techniques are commonly used for the measurement of radiocarbon including: gas proportional counting (GP) of CO2, acetylene (C2H2), and methane (CH4); liquid scintillation counting (LS) (encompassing liquid scintillation spectrometry), and; accelerator mass spectrometry (AMS). Each technique has specific advantages or limitations; however, all are in a constant state of development and improvement. The earliest measurements of radiocarbon were made using the GP method and modern systems of this type are capable of providing the highest level of precision for radiocarbon determinations.

Although GP methods are highly precise, they require relatively large sample sizes and/or long counting times (Kromer and Munnich 1992; Polach 1992). Standard GP analyses require several grams of carbon making the technique impractical for analyses involving fish otoliths (calcium carbonate is 12% carbon). High precision is attainable with many AMS systems within 1 h for smaller amounts of sample, albeit at a considerably greater cost.

The AMS technique is based on the counting of atoms of a radioisotope rather than quantification of the isotope's decay as in the GP and LS techniques. Several factors make it difficult to directly quantify the number of radiocarbon atoms in a sample. In a modern sample of carbon, derived perhaps from the bones of a recently killed fish, there are about 1012 stable carbon atoms ($^{12}C$ and $^{13}C$) for every atom of $^{14}C$. If the fish bones had been obtained from a prehistoric midden that was 30,000 years old, then the number of $^{14}C$ atoms would have been reduced by more than a factor of 10. This occurs because of the radioactive decay of the $^{14}C$ in the sample and the fact that, under normal circumstances, substantial amounts of new $^{14}C$ are not incorporated after the sample has died. The presence of atoms of $^{14}N$ and molecules of $^{12}CH2$ and $^{13}CH$ with the same mass as $^{14}C$ is an additional complication. The magnitude of this problem can be appreciated when it is realized that the atmosphere is about 78% nitrogen and these atoms are not eliminated even from the finest vacuum systems. Bennett et al. (1977) discussed the method for the elimination $^{14}N$ atoms from the ion beam of the AMS system; a key to the success of this method for the measurement of radiocarbon.

Accelerator mass spectrometry measurements are generally made on samples that have been converted to graphite. Several methods are available for the prepartion of graphite targets and AMS laboratories must maintain strict control over the preparation of these targets to maintain sample purity, density, and other properties (Jull et al. 1986; Vogel et al. 1987). The graphite target is placed in a vacuum chamber with an ion source, typically cesium. The target is bombarded with cesium ions and the carbon is sputtered from the graphite target. Negatively charged C ions are accelerated through a series of focusing and mass selecting devices and attracted to the positive terminal of the accelerator which can be charged with up to +3 million volts. In the center of the accelerator, the C- ions pass through a stripper cell containing gas or foils. Collisions between the material in the stripper cell and the C- ions result in the removal of up to 4 or 5 electrons and the formation of C3+ or C4+ ions (the production of different charge states is dependent on the energy of the accelerator, e.g. 2 MV or 6 MV). The ions are now repelled, accelerating them further and they are selected on the basis of energy and charge by a series of electrostatic deflectors and focusing magnets, resulting in the measurement of carbon isotopes by gas or solid state ionization detectors.

Accelerator mass spectrometry has two important advantages over other techniques of radiocarbon measurement: 1)the ability to deal routinely with samples containing 1 mg of carbon (analyses have been carried out with 20 μg of carbon, but with reduced precision (Vogel et al. 1989)) and; 2) the ability to make a high precision (1% error) measurement in about one hour. The major drawbacks of AMS are the high cost, which is typically twice as expensive as measurements made by GP or LS techniques, and slightly lower precision. The high cost of AMS is balanced, to some extent, by the short amount of time it takes to analyze a sample. Furthermore, it is likely that precision of AMS measurements of radiocarbon will increase in the future (Beukens 1992).

**Application of radiocarbon measurements to fish biology**

Measurements of radiocarbon in aquatic organisms have been relatively rare. On the other hand, there are numerous measures of radiocarbon made directly on seawater DIC or through proxies, particularly hermatypic corals. These measurements of radiocarbon in seawater can provide data that are relevant to several processes including the flux of CO2 between the atmosphere and oceans and the horizontal and vertical mixing of water masses. A combination of factors make these applications of radiocarbon data feasible. For measurements of seawater radiocarbon prior to extensive testing of nuclear bombs in the late 1950's, the long half-life and slow mixing times of the ocean are most critical. This combination of factors makes it possible to recognize, for example, deep water that has been isolated from the atmosphere for hundreds, if not thousands of years. As a result, upwelling events, where cold, nutrient-rich water is brought to the surface, can be recognized due to the presence of low radiocarbon values in surface waters. Radiocarbon is also a good indicator of upwelling because of its relatively steep vertical gradient from the surface waters to about 800 m depth and the negligible effect of biological processes on radiocarbon levels, thus simplifying data interpretation. Perhaps the most significant application of seawater radiocarbon data involves the use of these data in the calibration of GCM's (general circulation models) (Oeschger et al. 1975; Sieganthaler 1983; Toggweiler et al. 1989a, 1989b; Sarmiento et al. 1992). There are several papers which can be consulted to provide further information on the application of radiocarbon data to studies of ocean circulation and CO2 flux (Druffel 1981, 1987, 1989; Broecker et al. 1985; Toggweiler et al. 1991; and others). In freshwater systems, the nature of radiocarbon variation is somewhat different from that of the oceans. In most cases, the turnover rate in freshwater habitats is adequately rapid that measurements of radiocarbon will reflect atmospheric values. Exceptions to this are those systems that are feed largely by groundwater that may have been isolated from the atmosphere for hundreds or thousands of years.

The main aim of most studies that have measured radiocarbon in aquatic organisms has been to determine the flow of carbon through the environment and these have usually dealt with the flow of carbon to the deep-sea (Williams et al. 1970; Williams et al. 1978; Pearcy and Stuiver 1983). This can be accomplished most simply by determining $\Delta^{14}C$ in animal tissues. These analyses could be used to determine, for example, if a deep-sea fish was consuming significant quantities of carbon that were derived from surface waters and can provide insight into the behavior of fishes although the interpretation of the results may be uncertain. For example, Pearcy and Stuiver (1983) found that muscle tissue from the abyssal macrourid Coryphaenoides armatus had $\Delta^{14}C$ values higher than would be expected on the basis of their depth of capture. This was determined to be due to a combination of factors including a diet consisting of pelagic cephalopods and fishes

(Pearcy and Ambler 1974) and the fact that this species does occur in midwater, far above the sea floor (Pearcy 1976).

Interpretation of $\Delta^{14}C$ values obtained from fish tissues are further complicated by the lack of understanding regarding the flux of particulate and dissolved organic carbon (POC and DOC) in the ocean (Druffel and Williams 1990; Druffel et al. 1992); a topic beyond the scope of this paper. Unlike the otoliths, the isotopic composition of fish muscle tissue would be largely determined by the isotopic composition of the diet (Gearing et al. 1984). As a result, POC and DOC would play a much greater role in determining the radiocarbon levels measured in tissues other than otoliths. Measurements of radiocarbon in ocean POC indicate that bomb produced radiocarbon is distributed throughout the water column, whereas there is no evidence of bomb radiocarbon being present in DOC (Druffel et al. 1992).

Measurements of fish otolith $\Delta^{14}C$ provide results that are similar to those made for $\Delta^{14}C$ in seawater DIC (Kalish 1993). Studies of stable carbon isotopes (Kalish 1991a; 1991b) suggested that 70% of the carbon deposited in otoliths was derived directly from seawater DIC. This carbon would be incorporated into otoliths after uptake from seawater by the gills. The endolymph that surrounds the otoliths is in close contact with capillaries and its composition is related to that of the blood plasma (Kalish 1991c). Under these conditions, some carbon derived from ingested food would also become incorporated in the otoliths and this appears to be the source for the remaining 30% of otolith carbon. In most fish species, the fact that about 30% of otolith carbon might be derived from food would have little effect on $\Delta^{14}C$ measured in the otolith because most fish consume prey that spend the majority of their time within a similar depth range. In some species, however, it is possible that diet would have a significant effect on $\Delta^{14}C$ measured in otoliths (Kalish 1993). Further research on the sources of otolith carbon and their potential variation within and among species would be useful for the interpretation of carbon isotope data obtained from otoliths.

Because the carbon of otolith $CaCO_3$ is deposited throughout the life of a fish and it is not reworked after deposition it can provide a record of $\Delta^{14}C$ in seawater DIC (Kalish 1993). These data can be used in many ways including investigations of ocean circulation, carbon flux, and fish biology. The most straightforward application of otolith $\Delta^{14}C$ data involves using these measurements as a proxy for $\Delta^{14}C$ in seawater DIC. Otoliths from archives maintained by most fisheries laboratories can provide a time series of $\Delta^{14}C$ variations in a particular region and these can be applied to oceanographic and atmospheric studies as discussed above. Hermatypic corals have been used as proxies for $\Delta^{14}C$, but corals are restricted to shallow tropical and subtropical seas (Druffel and Linick 1978; Nozaki et al. 1978; Druffel 1987; Toggweiler et al. 1991). A recent study demonstrated the feasibility of extracting $\Delta^{14}C$ time series from a temperate bivalve species, Arctica islandica (Weidman and Jones in press) and like fish otoliths, these structures show great promise for obtaining further records of ocean radiocarbon in both mid- and high-latitudes.

Radiocarbon data obtained from corals collected from 3 locations (Druffel 1980, 1981; Landman et al. 1988) and from fish otoliths (Kalish 1993) are compared in Fig. 5.3. The data show variations in radiocarbon from the early 20th century to the present. For 3 locations $\Delta^{14}C$ is about -50‰ until 1955, whereas the data from the Galapagos coral indicate that radiocarbon levels were somewhat lower in that region. This is due to the presence of relatively old, upwelled water around the Galapagos Islands. All records show an increase in $\Delta^{14}C$ starting around 1955 to 1960 which is related to the atmospheric testing of nuclear bombs. The highest levels of radiocarbon were measured in the corals from Belize/Florida and Heron Island, Australia due to their relatively close proximity to the source of radiocarbon in the subtropical north Pacific Ocean. The increase in radiocarbon in the New Zealand fish otoliths and the Heron Island coral lags about 1 year behind the Florida coral. This is due to the relatively rapid meridional transport of radiocarbon in many regions, when compared with the latitudinal transport. There is a further delay at the Galapagos due to the origin of the water masses in that region. Also, the lower peak values for $\Delta^{14}C$ in the Galapagos coral are related to the upwelling in that region. Features such as these can be used to develop an understanding of the flux of radiocarbon and, ultimately, $CO_2$ between the atmosphere and ocean.

Because some data on seawater $\Delta^{14}C$ already exist at most latitudes and depths (Broecker et al. 1985), we can use these data to help us estimate the habitat of fish species or particular life history stages that have remained elusive. A good example of this application is provided by recent measurements of $^{14}C$ made in the otoliths of the smooth oreo (Oreosomatidae: *Pseudocyttus maculatus*) (Kalish unpublished data). Smooth oreo is a commercially important deepwater trawl species in both New Zealand and Australia and it is known to also occur off of South Africa and Kerguelen Island. The adults of the species appear to be restricted to latitudes between 25°S and 52°S (James et al. 1988). In New Zealand approximately 20,000 tons of smooth oreo are trawled each year from depths of about 800 to 1200 m. Despite the abundance of adults (>135 mm SL), the juveniles are extremely rare and appear to have a distribution that is quite different from that of the adults. The majority of observed juvenile smooth oreos (32 specimens) (<135 mm SL) have been collected in association with krill or in the stomachs of fin whales and were captured between 60°S and 68°S latitude (Abe 1957; Svetlov 1978; Abe and Suzuki 1981). Three other juveniles have been collected: two at about 44°S latitude near New Zealand and one at about 48°S latitude near Patagonia. Furthermore, the adults are apparently adapted to deepwater by their bluish grey to greyish brown color and very large eyes; juveniles seem better adapted to surface waters because they are silvery with an irregular pattern of bluish blotches and small eyes. From the results of the few juvenile collections and their physical appearance, it has been assumed that these fish occur in surface waters.

Based on the limited biological data on smooth oreos, it was hypothesized that juveniles occurred in the Southern Ocean south of 60°S. I performed an initial test of this hypothesis by determining $\Delta^{14}C$ in the juvenile segments of smooth oreo otoliths. Three groups of smooth oreo otoliths were collected: 1) 15 sagittae from females (>40 cm TL) with estimated ages in excess of 40 years (pre-bomb hatch date) based on counts of presumed annual increments made on otolith thin sections; 2) 15 sagittae from females (<18 cm TL) with estimated ages of less than 8 years (post-bomb hatch date), and; 3) outer edges of sagittae from an additional 15 females (>40 cm TL) to represent calcium carbonate deposited in the adult habitat. All fish were collected by demersal trawling at depths of about 1000 m on the South Chatham Rise east of New Zealand in 1987. For the first 2 groups, otolith material deposited in about the first 2 years of life was isolated from each otolith and the material from the 2 groups of fish was pooled separately. Calcium carbonate from the older fish was presumed to have been deposited pre-bomb, whereas CaCO3 from the younger fish was presumed to have been deposited post-bomb.

Relatively large negative values for $\Delta^{14}C$ from the 2 smooth oreo samples (Table 5.1) indicate that it is extremely unlikely that the adult smooth oreos spent their juvenile lives in surface waters at mid-latitudes. In fact, comparison with data on $\Delta^{14}C$ measured in seawater DIC (Figs. 4 and 5) provides support for 2 hypotheses: 1) adult smooth oreos collected in New Zealand waters use waters south of 60'S latitude as a juvenile nursery ground and; 2) juvenile smooth oreos collected at mid-latitudes have strayed from the normal nursery ground or they are at the northern edge of their range. If the younger smooth oreos had been in about the top 200 m of the water column as juveniles, a $\Delta^{14}C$ value of about +100‰ (Broecker et al. 1985; Kalish 1993) would have been expected (Figs. 4 and 5). For the older smooth oreos, with a presumed "pre-bomb" hatching date before 1950, a $\Delta^{14}C$ value of about -55‰ (Bien et al. 1960; Rafter and O'Brien 1970; Kalish 1993) was anticipated. Because $\Delta^{14}C$ values for both samples appear to be significantly lower than any pre-bomb values in low and mid latitude surface waters (Broecker et al. 1985) (Fig. 5.4), it must be concluded that the juvenile smooth oreos either live well below the surface mixed layer or they occur at high latitudes in the Southern Ocean. Measurements of $\Delta^{14}C$ in seawater in the region of the Chatham Rise (east of New Zealand), the major trawling ground for adult smooth oreo, indicated that it would be necessary for the post-bomb group of juvenile oreos to be at depths of about 800 m and in midwater (2000 m bottom depth) to encounter water with a $\Delta^{14}C$ value of about -85‰ (Sparks et al. 1989) (Fig. 5.5). The $\Delta^{14}C$ value of -95‰ for the pre-bomb juvenile would be reasonable for a fish living at around 800 m in Southern Hemisphere mid-latitudes prior to 1950 (Ostlund and Stuiver 1980). Two factors make this deep-water distribution for smooth oreos unlikely: 1) the disruptive color pattern and small eye indicative of a fish living in surface waters and; 2) the occurence of the copepod Neocalanus tonsus in the stomachs of the 2 smooth oreo juveniles caught in New Zealand waters. Furthermore, the radiocarbon levels measured in the otoliths are consistent with those expected for a fish living in the surface mixed layer at about 60°S latitude, in agreement with the capture location for 32 of 35 collections of juvenile smooth oreo.

**Age validation using the bomb radiocarbon chronometer**

To the fish biologist, the validation of fish age is another valuable application of radiocarbon measurements made in otoliths. Kalish (1993) described this age validation technique and applied it to the long lived teleost *Pagrus auratus*. This technique has little to do with the conventional method of radiocarbon dating; instead it takes advantage of the significant anthropogenic influence on environmental radiocarbon levels, specifically the atmospheric testing of nuclear bombs.

The bomb related increase in ocean radiocarbon can be used for age validation because of the relatively discrete nature of the radiocarbon pulse due to the bomb tests (Fig. 5.3). Measurements of radiocarbon in seawater DIC in the late 1950's and the early 1970's provided 2 data points which effectively define both the pre-bomb minimum and post-bomb maximum radiocarbon levels. These data provided evidence for the nature of the rise in ocean radiocarbon levels, however, the rate of increase has been more clearly defined by measurements of radiocarbon in the skeletons of hermatypic corals. These measurements of radiocarbon derived from seawater and corals provide a clear indication of radiocarbon levels at different points in time and in certain locations. Variations in radiocarbon levels at other latitudes can be further estimated from known-age fish otoliths (Kalish 1993) and bivalves (Weidman and Jones 1993). Because levels of radiocarbon in the ocean can be related to a specific point in time; it is feasible to use bomb derived radiocarbon as a chronometer.

Validation of fish age using the bomb radiocarbon chronometer involves AMS measurements of radiocarbon in otolith material deposited in the early part of a fish's life. In fact, this validation technique would not be practical without the development of AMS which makes it possible to carry out precise measurements of radiocarbon in samples of otolith $CaCO_3$ weighing less than 25 mg. Otolith material deposited in the first year of a fish's life will contain radiocarbon indicative of that time period. High levels of radiocarbon (>-20‰) are a clear indication that a fish was hatched after the atmospheric testing of nuclear weapons; low levels (<-30‰) provide evidence that a fish had hatched prior to 1958. Selection of otoliths from fish with presumed hatching dates between about 1960 and 1970 provides the greatest resolution for age determination using the radiocarbon chronometer. In the mid-1960s it is feasible to estimate the date of hatching to within 6 months or less.

This validation technique is most useful for fishes living in the surface mixed layer of the ocean. For fish living their early juvenile lives below about 300 meters, it becomes more difficult to determine the rate of flux of radiocarbon to those depths. Because there is considerable uncertainty regarding the penetration of radiocarbon into depths greater than 800 meters, the technique has little immediate value for validating the age of those species that spend their entire lives at these depths (see Fig. 5.5). However, the validation technique is practical for those deep-sea species that have a juvenile stage that occurs in the surface mixed layer. There are many species that fit this description including Sebastes spp., Anoplopoma fimbria, several species in the Oreosomatidae, and others.

Otoliths from fish with presumed hatching dates between about 1960 and 1970 should be used for age validation based on the bomb radiocarbon chronometer. For many species, obtaining otoliths of this age is not difficult due to the extensive otolith material held in the archives of many fisheries laboratories. Also, because many species of fish live for more than 30 years, it is still feasible to collect material that can be used for radiocarbon age validation.

Although the period from 1960 to 1970 is considered to be most effective for age validation, it is feasible to use later years including 1970 to the present. A gradual decrease in ocean radiocarbon can be seen after the peak around 1974 (Fig. 5.3); however, data from the oceans are too sparse to draw conclusions regarding the rate of decrease. Further measurements of $\Delta^{14}C$ in otoliths of known age fish (Kalish 1993) or other radiocarbon proxies can be used to define this decrease and determine the utility of this time period in age validation studies. The decrease in radiocarbon in seawater DIC results from losses to other pools or advection of radiocarbon to other areas. In some regions the reduction in radiocarbon levels may be adequately rapid to provide resolution suitable for age validation studies. The ability to use data from these years is dependent on 3 factors including: 1) relatively rapid loss of radiocarbon to other pools or regions; 2) high precision (<1% error) estimates of radiocarbon and; 3) ability to discriminate between similar radiocarbon levels associated with the increase in bomb $^{14}C$ or the subsequent decrease in $^{14}C$. Examination of atmospheric radiocarbon data collected from several locations over the last 2 decades (Levin et al. 1992) provides an indication of the complexity of this problem and the potential differences that might occur in different ocean regions.

Age validation using the bomb radiocarbon chronometer has numerous advantages over other methods of age validation. The need to carry out expensive and time consuming tagging experiments is no longer necessary for validating the age of many fish species. Reduced somatic growth associated with tagging studies and stress related to capture, handling, marker (e.g. oxytetracycline hydrochloride, SrCl) injection, and tagging are eliminated (McFarlane and Beamish 1987, 1990; Saunders et al. 1990). Furthermore, capture-mark-recapture experiments only validate the period of time between the marking of a fish and the time of recapture, whereas the bomb radiocarbon chronometer provides an estimate of the actual fish age, not just the time period during which the fish is at liberty. Finally, AMS analyses of radiocarbon can be carried out with small samples of otolith material (<25mg). Radiometric age estimation using various activity ratios (e.g. 210Pb/226Ra) can, in theory, overcome many of the shortcomings of capture-mark-recapture experiments including mortality and injury due to tagging and reduced growth rates due to stress from both handling and the immune response resulting from tag related injuries. However, several unique problems are introduced by the use of radiometric methods. Several key assumptions must be made to apply the radiometric method including: constant uptake of radioisotopes relative to calcium during the life of the fish; constant rate of otolith mass growth or a known rate(s) of otolith mass growth, and; the otolith must be a "closed system" with respect to the radioisotopes under consideration. Although the analysis of otolith cores may reduce some of the complications associated with the above assumptions, it would not overcome these problems (Campana et al. 1990; Fenton and Short 1992). Even without violation of any of these assumptions the radiometric method is problematic due to the need for relatively large sample sizes (≥1 gram) and the relatively low precision of the method resulting from extremely low levels of radioactivity relative to background. The bomb radiocarbon chronometer offers considerable advantages over both tagging and radiometric age determination.

It may be feasible to validate age using radiocarbon decay for very long-lived fishes. A sample from the central portion of an otolith, similar to that suggested for the application of the bomb chronometer, undergoes natural radioactive decay acccording to the logarithmic decay law. In most cases, this decay would not be detectable due to the relatively long half-live of $C^{14}$ (5730 years) relative to the "typical" age of a fish, and the precision of AMS. However, fish species such as orange roughy (*Hoplostethus atlanticus*), with maximum ages estimated to be in excess of 150 years (Fenton et al. 1991), may be amenable to radiocarbon dating techniques. The first consideration would be to achieve the highest precision practical. For AMS, this is currently between 5‰ and 10‰ for small samples, or about 40 to 80 years. Ideally, a series of radiocarbon measurements should be made from the earliest formed portion of the otolith, to the otolith edge in order to follow the decrease in age (increase in radiocarbon level) of otolith material. Alternatively, a radiocarbon measurement might be made at the otolith edge only; this value being taken as the initial radiocarbon level in the center of the otolith prior to any significant radioactive decay. Of course, it is assumed that radiocarbon levels have been constant during the life of the fish, an assumption that may be reasonable for deep-sea species.

Further development of radiocarbon methods that are applicable to fish biology will depend on 3 major factors including: 1) development of the AMS technique, particularly relating to increased precision and further reductions in sample size requirements; 2) increased understanding of the flux of carbon as DIC, DOC, and POC through the oceans, and; 3) increased understanding of the sources of carbon for otoliths and other tissues. Because of the importance of age determination and age validation, measurements of radiocarbon in fish otoliths will almost certainly play an important roll in studies of fish biology and in fisheries management. These radiocarbon data can also be incorporated into databases that are an important part of investigations into global change.

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Table 5.1. $\Delta^{14}C$ values measured in juvenile and adult portions of the otoliths of three groups of adult smooth oreo (*Pseudocyttus maculatus*) collected on the South Chatham Rise off the east coast of New Zealand. $\Delta^{14}C$ data from *Pagrus auratus* otoliths collected in New Zealand waters are presented for comparison. The location category refers to the presumed source of radiocarbon measured in the otolith sample. These data are to be compared with the measurements of $\Delta^{14}C$ in seawater DIC shown in Figs. 4 and 5. Errors are one standard deviation.

| Sample | $\Delta^{14}C$ (‰) | Location | Source |
|---|---|---|---|
| Pre-1950 P. maculatus Juvenile section | -94.2±8.1 | | This study |
| Post-1980 P. maculatus Juvenile section | -84.6±7.5 | | This study |
| Post-1980 P. maculatus adult section | -54.8±7. | 950 m (44°S,178°E) | This study |
| 1943 *Pagrus auratus* | -51.1±7.0 | surface (39°S,179°E) | Kalish 1993 |
| 1980 *Pagrus auratus* | +98.4±8.0 | surface (39°S,179°E) | Kalish 1993 |

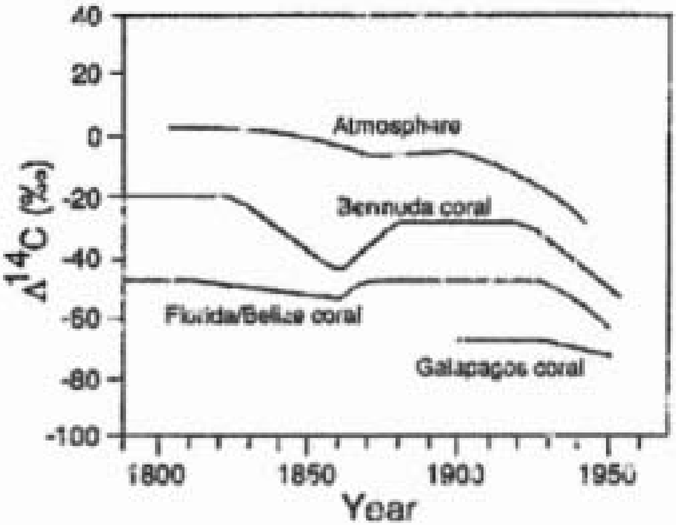


Figure 5.1. Radiocarbon levels in the atmosphere and ocean between the years 1800-1952, as determined by the tree ring and coral proxies. Atmospheric $\Delta^{14}C$ is consistently greater than $\Delta^{14}C$ measured in the oceans. The decrease in atmospheric $\Delta^{14}C$, beginning around 1900, is due to the burning of fossil fuels, which results in the release of $^{14}C$-free CO2 and the dilution of atmospheric radiocarbon. The amount of this decrease is called the Suess effect. The Suess effect is also evident in the ocean $\Delta^{14}C$ records after about 1920; however, the magnitude of the effect is not as great as in the atmosphere. (Figure modified from Druffel and Suess 1983).

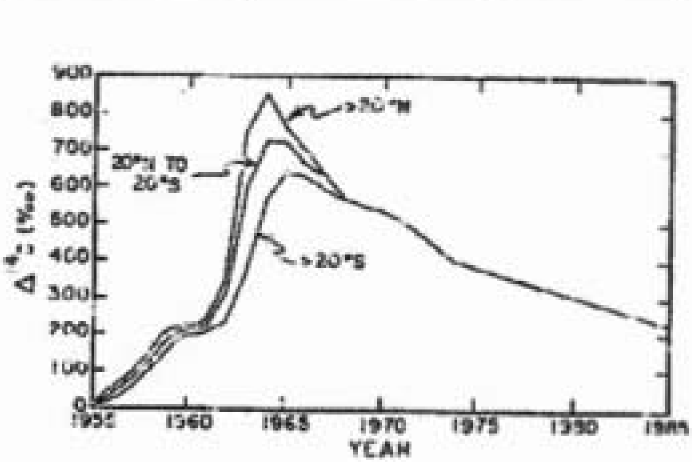


Figure 5.2. Variations in atmospheric $\Delta^{14}C$ versus time at three latitudes since the beginning of atmospheric testing of nuclear weapons. The Limited Test Ban Treaty curtailing atmospheric testing was signed in 1963. The increase in atmospheric radiocarbon due to atmospheric testing of nuclear weapons was about 100% and this represented about a 5% increase in terrestrial radiocarbon. The atmospheric radiocarbon data are from Nydal and Lövesetb (1983) and Levin et al. (1985) and the figure is from Broecker et al. (1985).

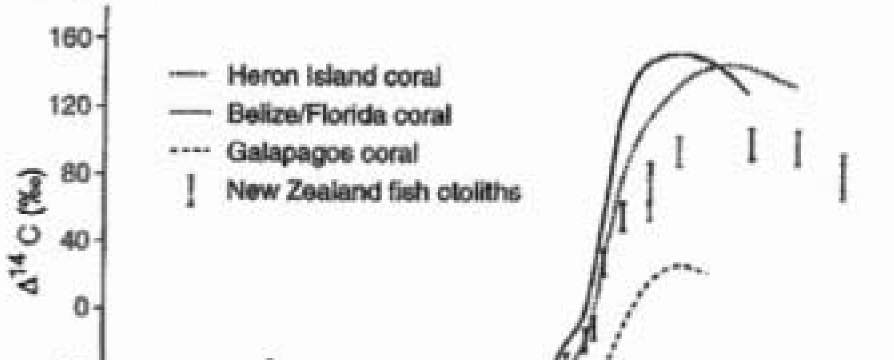


Figure 5.3. Variations in ocean $\Delta^{14}C$ versus time in the ocean at four locations. The differences in peak $\Delta^{14}C$ among sites are related to location relative to the sources of bomb radiocarbon. Decreases in $\Delta^{14}C$ after peak values vary based on the redistribution of radiocarbon due to ocean circulation and the loss of radiocarbon in seawater DIC to other pools such as DOC and POC. Low $\Delta^{14}C$ values in Galapagos coral are related to the upwelling of water low in $\Delta^{14}C$. Belize/Florida coral data are from Druffel (1980) and Druffel and Linick (1978); Galapagos coral data from Druffel (1981); Heron Island coral data from Landman et al. (1988); fish otolith data from Kalish (1993).

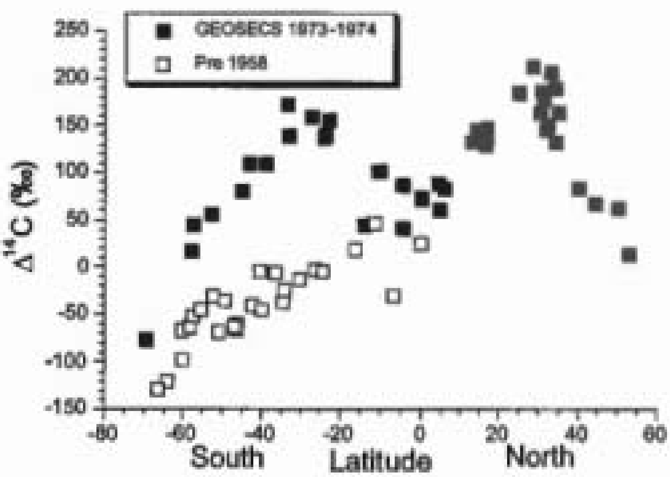


Figure 5.4. Latitudinal variation in $\Delta^{14}C$ in Pacific Ocean surface waters both before and after significant inputs due to the atmospheric testing of nuclear weapons. From Broecker and Peng 1982.

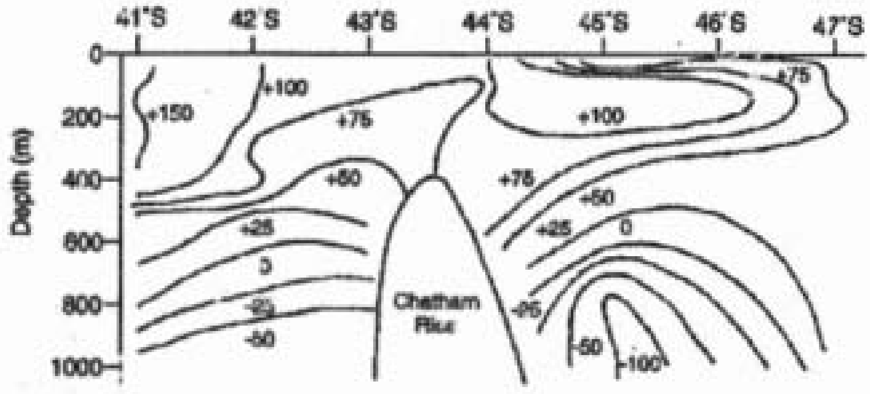


Figure 5.5. Plot of variation in $\Delta^{14}C$ along 179°E in the south Pacific Ocean east of New Zealand. Figure modified from Sparks et al. 1989.

# Chapter 6 Investigating global change and fish biology with fish otolith radiocarbon

John Kalish

**Summary**

Fish otoliths, calcium carbonate gravity and auditory receptors in the membranous labyrinths of teleost fish, can provide radiocarbon data that are valuable to a wide range of disciplines. For example, the first pre- and post-bomb time series of radiocarbon levels from northern or southern hemisphere temperate oceans was obtained by carrying out accelerator mass spectrometry analyses on selected regions of fish otoliths. These data can provide powerful constraints on both carbon cycle models and ocean general circulation models. Because fish otoliths can serve as a proxy of radiocarbon in seawater dissolved inorganic carbon in all oceans and at most depths, there is considerable scope for further investigations of otolith radiocarbon in relation to both oceanography and global change. In addition to applications relevant to global change, fish otoliths are also valuable sources of information on the age, growth, and ecology of fishes with age being among the most important parameters in population modeling and fisheries management. Use of the bomb radiocarbon chronometer to validate fish age determination methods offers considerable advantages over traditional forms of age validation and promises to become a standard tool in fish biology and fisheries management. Radiocarbon data from otoliths can also provide valuable information on the ecology of fishes and has already provided surprising information relevant to the ecology of some deep-sea fishes.

## Introduction

Measurements of radiocarbon in seawater dissolved inorganic carbon can be used to investigate both ocean circulation and the flux of carbon between the atmosphere and ocean. Radiocarbon studies of ocean circulation are possible because of the long half-life of radiocarbon and the slow mixing times of the ocean. These data can be applied to investigations of specific mesoscale circulation patterns such as upwelling or to studies of global circulation patterns. One of the most important applications of seawater radiocarbon data relevant to ocean circulation and carbon flux involves the use of these data in the calibration of GCM's (general circulation models) (Oeschger et al. 1975, Siegenthaler 1983, Toggweiler et al 1989a, Toggweiler et al 1989b, Sarmiento et al 1992).

Anthropogenic inputs of carbon in the last century have perturbed the natural equilibrium of $^{14}C$ in the atmosphere and oceans. The dilution of cosmogenically produced radiocarbon by the burning of $^{14}C$-free CO2, the release of this material into the atmosphere, and its subsequent incorporation into the oceans can be used to estimate the rate of fossil fuel CO2 input into the oceans (Broecker et al 1985). The atmospheric tests of nuclear bombs between 1952 and 1963 have had a dramatic effect on atmospheric levels of $^{14}C$, increasing them by almost 100% (Nydal and Lovseth 1983, Levin et al 1985) and bomb-produced radiocarbon serves as one of the main tracers for studying the flow of carbon through the atmosphere and ocean.

Time series of radiocarbon levels in the ocean and atmosphere are necessary to develop the greatest understanding of carbon flux through the environment. Retrospective determinations of $^{14}C$ in the oceans are typically obtained by carrying out radiocarbon analyses on carbon obtained from dated bands of hermatypic corals (Druffel and Linick 1978, Nozaki et al 1978). Radiocarbon data obtained from corals has been very effective at reconstructing histories of radiocarbon levels in shallow water tropical and subtropical environments (Toggweiler et al 1991). However, measurements of radiocarbon in hermatypic corals cannot provide time series of radiocarbon levels for temperate, high latitude, and deeper waters. If such data were obtainable, there is the potential for a considerable improvement in the radiocarbon calibration of GCM's and in estimates of the flux of CO2 through the environment based on the bomb $^{14}C$ tracer.

Data presented in ref. (Kalish 1993) indicated that fish otoliths were suitable as a proxy for $^{14}C$ in seawater DIC and that these structures could provide time series that extended from the late 19th century to the present. Because fish occur in almost all aquatic environments and at all depths, fish otoliths have the potential to provide $^{14}C$ records from regions not covered by hermatypic corals and they can act as an adjunct to data derived from corals in subtropical and tropical environments.

**Fish otoliths**

Fishes are a very diverse group of animals with about 25,000 known species. They can be found in environments ranging from hypersaline desert springs and limestone aquifers to the deepest regions of the ocean. Among the fishes, the teleosts, a modern group in an evolutionary sense, are by far the most speciose and diversified with almost 20,000 species. One character that is common among teleosts is the presence of 3 pairs of otoliths ("ear stones") within the paired membranous labyrinths: the sagitta, the lapillus, and the asteriscus. Each otolith is in contact with sensory hair cells protruding from a sensory macula and these "otolith organs" act as both gravity and auditory receptors. In most fishes, one pair of otoliths, the sagittae, is by far the largest (Fig. 6.1). Sagittae are aragonitic structures that are formed through the daily deposition of aragonite-dominant and organic matrix-dominant zones. A sagitta from an adult fish typically contains less than 1% organic material, although the quantity of organic matrix deposited in the otolith can be higher in the early life of a fish. Over the course of a year in a fish's life, the structure and composition of the daily increments that are deposited will vary. Variations in the width, organic content, and trace element composition of increments have been investigated. The reasons for this variation are not well understood; however, cumulative differences in otolith ultrastructure make it possible to resolve changes in otolith structure over the course of a year. The resulting annual increments are believed to be a manifestation of several processes resulting in changes in otolith structure. In their simplest form, annual increments are comprised of an opaque zone and a translucent zone (Fig. 6.2) and, in many ways, they are similar to the annual structures called tree rings.

Both daily increments and annual increments have been studied intensively because of their importance in estimating the age and growth of fishes (Summerfelt and Hall 1987). Investigations of fish otolith structure and composition indicate that after deposition, otolith aragonite is not resorbed (Simkiss 1974), a requirement for these structures to be appropriate for the estimation of age and growth. Most importantly, the age of many species of fish can be determined with great accuracy (Smith 1993). Because the otoliths are not resorbed during the life of the fish, they also contain a permanent chemical record that can be related to the biology of the fish and changes in its environment (Kalish 1989, Kalish 1990, Kalish 1991a, Kalish 1991b). There are numerous pitfalls in relating the chemistry of fish otoliths to the environment particularly due to the fish's ability to physiologically discriminate against certain substances (Kalish 1991b, Kalish 1992). Despite these difficulties, it appears that otoliths can provide proxy records of certain properties of the fish's environment, most notably temperature, via □18O thermometry (Kalish 1991a, Kalish 1991c) and $^{14}C$ in seawater DIC (Kalish 1993).

Otoliths are the primary source of information on the age and growth of fishes. Because age and growth data are among the most important parameters in modeling the population dynamics of commercially exploited fish stocks, there are large archival collections of otoliths maintained at fisheries and oceanographic laboratories throughout the world. For some fish species, otolith collections began in the early 20th century. The existence of these collections, coupled with the fact that many fish species live to 100 years or more (Bennett et al. 1982, Campana et al 1990, Fenton et al 1991) makes it feasible to obtain data from otoliths that can be related to environments as early as the 19th century and before anthropogenic inputs had a significant impact on atmospheric radiocarbon levels. Collections of fishes in museums may provide additional data from as early as the 18th century. The presence of otoliths in sediments and at archaeological sites makes these structures valuable to investigations of palaeoenvironments.

Sagitta shape is species specific, however, in almost all cases individual sagitta from adult fish weigh less than 1 g and frequently less than 250 mg. Because of their small size, high precision analysis of otolith radiocarbon by gas proportional counting or liquid scintillation methods is impractical. Sample weights obtainable from portions of fish otoliths are easily analysed with high precision ($\leq 1\%$) by accelerator mass spectrometry (AMS).

**Obtaining $\Delta^{14}C$ data from fish otoliths**

Even if radiocarbon analyses are done using AMS, most otoliths are too small to remove successive annual layers and carry out a high precision analysis. Several options are available to overcome this problem including: 1) pooling of layers of otolith material formed over several years; 2) pooling otolith material extracted from similar portions of otoliths from several fish, and; 3) selective removal of only the earliest formed portion of the otolith (e.g. material deposited during the first year or less of life). In each instance, the collection date and age of the fish must be known and, for many species of fish this is not a problem (Kalish 1993). Pooling of otolith layers from an individual otolith, while providing adequate carbon for an analysis, reduces the temporal resolution of the data. Pooling otolith material from several fish is impractical because of the difficulty in finding adequate numbers of fish from the same year-class and errors that may result from assigning incorrect ages to individual annual increments. The third option is the most practical. Otolith growth decreases as the fish gets older; therefore the first annual increment has the greatest mass. For many fish species, the otolith material deposited in the first year of life will weigh more than 25 mg. Selection of a fish species and otolith samples that can be aged with a high level of reliability is essential. Subsequent selection of otoliths from fish with a range of ages (or birth dates) is a simple matter. This strategy was employed in ref. (Kalish 1993).

**Example of $\Delta^{14}C$ data from fish otoliths**

Radiocarbon data collected from otoliths of *Pagrus auratus* collected off the east coast of the North Island of New Zealand were compared with similar data collected from hermatypic corals (Fig. 6.3). Ref. (Kalish 1993) describes the sample processing and analysis in detail. Prior to the atmospheric testing of nuclear bombs, the radiocarbon record is similar to that measured in corals from Belize and Florida. As expected, these records diverge when bomb radiocarbon inputs become a factor, and the New Zealand otolith record lags behind the Belize/Florida record by about a year. Data from Heron Island corals are very similar to the New Zealand data during the late 1950's and early 1960's. These relationships and others between the coral and otolith data sets provide an indication of the rates of atmospheric and oceanic transport of radiocarbon to different regions.

Although there appears to be a slight decrease in $\Delta^{14}C$ measured in *Pagrus auratus* otoliths between 1918 and 1950, this decrease is not significant and, therefore, does not provide evidence for the Suess effect in temperate waters of the Southern Hemisphere. These results are in agreement with radiocarbon data obtained from corals collected from the southwestern Great Barrier Reef, Australia where no evidence for a Suess effect was found (Druffel and Griffin 1993). Despite the agreement between these 2 data sets, further results are probably required to confirm that a Suess effect was not evident in Southern Hemisphere temperate and high latitude waters.

The measurement of radiocarbon in fish otoliths also has applications to studies of fish biology. The validation of methods of fish age estimation is probably the most important. Many fisheries resources are managed on the basis of age-structured population models. Furthermore, fish ages are necessary to determine growth rates, mortality rates, age at first maturity, and other basic parameters relevant to understanding the biology of exploited populations. As discussed previously, otoliths are the principle source of information on fish age; however, the estimation of ages from otoliths is not always a simple process. This is because the opaque and translucent zones used to estimate fish age are not always formed annually. In many cases, an experienced otolith "reader" can distinguish between "false" and "true" annual increments, but this process is not always straightforward.

Due to uncertainties in standard age estimation procedures, validation of age estimation methods has become an essential aspect of fisheries research (Beamish and McFarlane 1983). Unfortunately, standard validation procedures such as capture-mark-recapture studies often have a negative effect on the growth and survival of tagged fish (McFarlane and Beamish 1987, McFarlane and Beamish 1990, Saunders et al 1990). Furthermore, these studies are expensive and time consuming. An alternative validation procedure involves measuring the radiocarbon levels in the earliest formed portions of fish otoliths isolated from fish with presumed birth dates prior to the atmospheric bomb tests of the late

1950's and during the period of rapid increase in ocean radiocarbon levels from about 1960 to 1970 (Kalish 1993). Because of the extensive collections of otoliths in archives, this validation procedure is feasible for fish with even a moderately long lifespan. Also, studies of fish age and growth in recent years indicate that there are many fish species with maximum ages in excess of 50 years.

Radiocarbon data from fish otoliths may also provide information that is useful in determining the distribution of fish species or life-history stages of fish that are rarely encountered. Ref. (Kalish 1994) presented radiocarbon data from the otoliths of smooth oreo (*Pseudocyttus maculatus*), a deepwater (800 to 1200 m) mid-latitude species that suggests that the juvenile stages of this species live in surface waters of the Southern Ocean south of 60°S latitude. These findings are supported by several collections of extremely rare juvenile smooth oreos south of 60°S latitude (Abe 1957, Svetlov 1978, Abe and Suzuki 1981).

Otoliths can be an important component in the sediments and at archaeological sites. For example, in Australia, hundreds of otoliths have been found at an ancient aboriginal site at Lake Mungo, now a dry lake basin. Radiocarbon dating of charcoal from this site indicates that it was inhabited over 20,000 years ago and that fish constituted a major portion of the diet. Radiocarbon dating of otoliths from the site and subsequent □18O analyses of seasonally deposited layers could provide an indication of seasonal temperature cycles during the Pleistocene.

**Conclusions**

Measurements of radiocarbon levels in fish otoliths by AMS have a broad range of potential applications and these data can be used to investigate areas as diverse as ocean circulation, carbon flux, fish age determination, and palaeoclimatology. The importance of fish otoliths to fish age estimation, and ultimately to fisheries management, ensures the availability of this data source. Consultation with fisheries agencies in various regions or specialists in otolith research should provide adequate information to ascertain the availability of otoliths for specific investigations.

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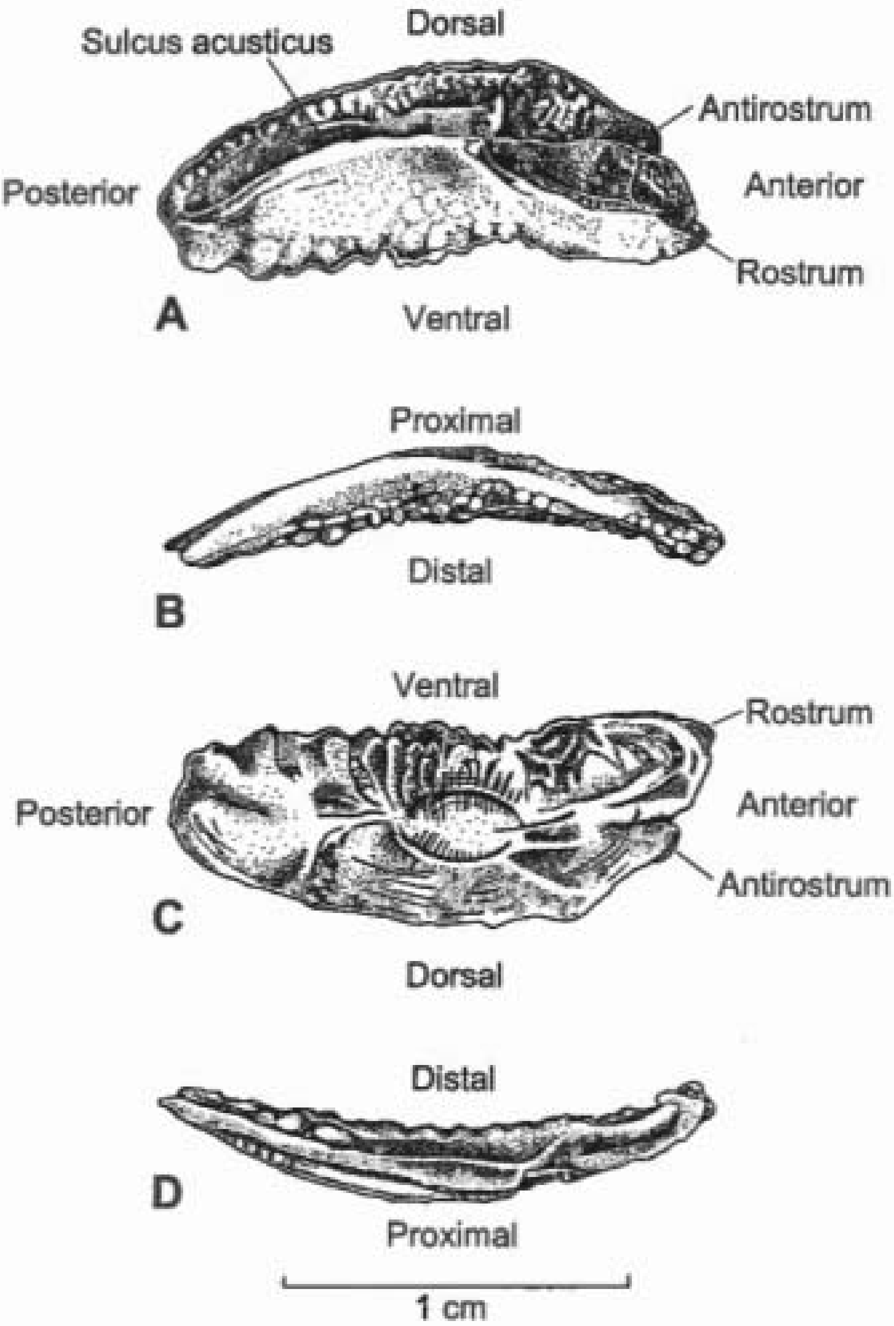


Figure 6.1. Several views of a left sagitta from a teleost fish (Arripis trutta) illustrating both shape and orientation. Drawing by Darren Stevens, New Zealand NIWA.

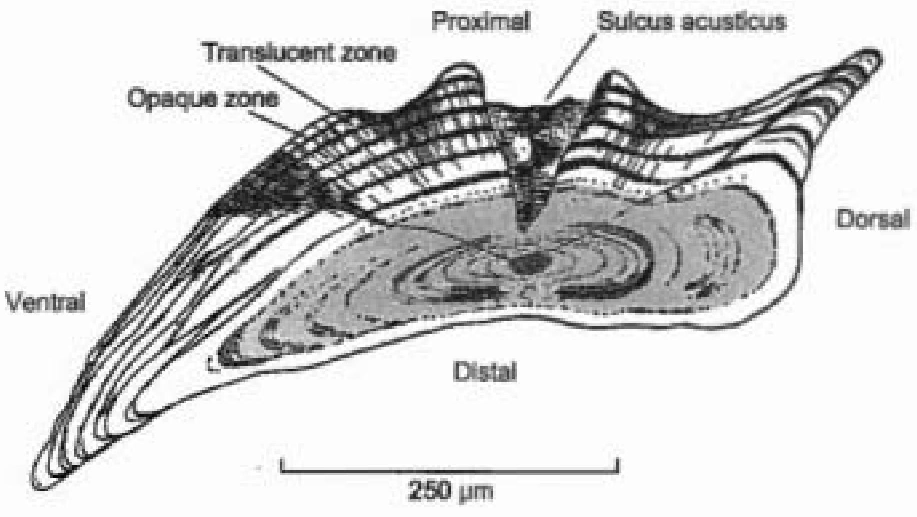


Figure 6.2. Transverse section of a sagitta from a teleost fish (Arripis trutta). The age of the fish estimated from this otolith was 8 years on the basis of counts of opaque and translucent zones from the center of the otolith section to its proximal edge. The stippled region at the center of the otolith represents approximately one year of growth. Drawing by Darren Stevens, New Zealand NIWA.

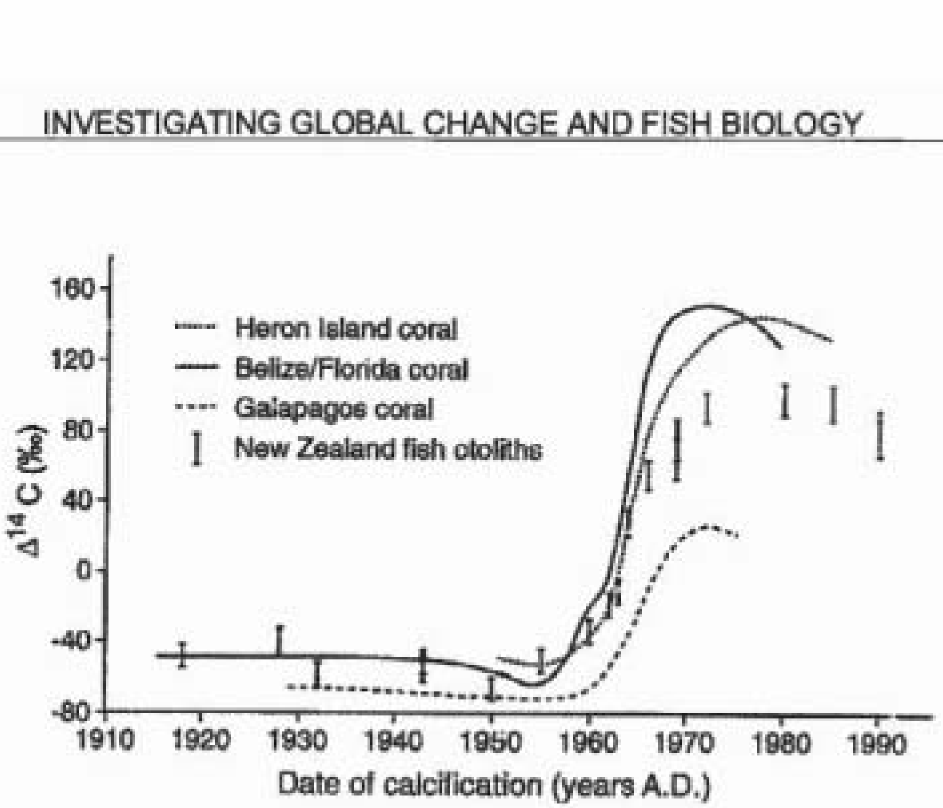


Figure 6.3. Time series of $\Delta^{14}C$ from corals and fish otoliths. Belize/Florida coral data are from Druffel (1980) and Druffel and Linick (1978); Galapagos coral data from Druffel (1981). Heron Island coral data from Landman et al. (1988); fish otolith (*Pagrus auratus*) data from Kalish (1993). Error bars on otolith data are ± 1 sd.

# Chapter 7 Use of the Bomb Radiocarbon Chronometer to Determine Age of Southern Bluefin Tuna (*Thunnus maccoyii*)

John Kalish, Justine Johnston, John Gunn and Naomi Clear

**Summary**

The growing otoliths of fish incorporate radiocarbon in concentrations that are equivalent to that found in ambient seawater dissolved inorganic carbon. Therefore, pulses of anthropogenic radiocarbon produced by the atmospheric detonation of nuclear weapons can ultimately be detected in otoliths. This study estimates the age of large southern bluefin tuna *Thunnus maccoyii* using an age estimation procedure based on the determination of levels of bomb-derived radiocarbon in otoliths. Radiocarbon data from selected regions of southern bluefin tuna otoliths indicate that this species may reach ages in excess of 30 years. Furthermore, individuals that approach the asymptotic length are likely to be 20 years of age or older. The data agree generally with accepted models of southern bluefin growth, but show that these fish live longer than was believed previously. Comparisons between otolith section and bomb radiocarbon age estimates indicate that reading otolith sections is an effective method to estimate the age of larger southern bluefin. The presence of a significant number of individuals greater than 20 years of age in the southern bluefin population may alter estimates of natural mortality rates currently used in Virtual Population Analysis models for stock assessment of this species.

**Introduction**

Southern bluefin tuna (*Thunnus maccoyii*) is a highly migratory species that is found throughout the Southern Ocean north of about 60°S latitude. Southern bluefin is exploited by fishers from Australia, Japan, New Zealand, Indonesia, Taiwan, and Korea and it is the most valuable commercial finfish species in Australia with the annual Australian catch valued at more than $80 million (Kailola et al. 1993). Catch rates for southern bluefin have decreased dramatically in the last decade, presumably due to increased fishing pressure on the resource, associated with its increased value (Caton et al. 1990). International management of southern bluefin has been placed under the jurisdiction of the Convention for the Conservation of Southern Bluefin Tuna (CCSBT), a trilateral convention that includes Australia, Japan, and New Zealand as its members. The CCSBT has indicated that age estimation of southern bluefin is a high priority research area (Anon. 1994). Furthermore, the lack of validated catch-at-age data for the exploited population has introduced significant uncertainty into the Virtual Population Analysis that is the primary stock assessment tool used by the CCSBT.

Age estimation for the larger tunas including southern bluefin and northern bluefin (Thunnus thynnus) is problematic due to difficulties involved in the interpretation of marks on hard parts, such as otoliths and vertebrae (Hurley and Isles 1983, Prince and Pulos 1983, Prince 1985, Thorogood 1987). Furthermore, logistical problems associated with the mobility and longevity of these species make tagging studies and, concomitantly, the validation (sensu Kalish et al. 1995) of a "preferred" age estimation procedure, both difficult and costly. Despite the complications associated with age estimation and the related validation for the larger Thunnus spp., these areas of research have a high priority in many nations that have significant fisheries for these species. In Australia research related to age estimation for southern bluefin includes a mark-recapture study using strontium chloride to mark calcified tissues (Clear et al. In preparation), investigation of conventional age estimation methods based on otoliths and vertebrae (Gunn et al. 1995), and the application of bomb radiocarbon measured in otoliths to age determination and validation (this study).

Although obvious zones are present in the otoliths and vertebrae of southern bluefin of all sizes (Gunn et al. 1995), and annual bands have been validated in the otoliths of fish up to 6+ years (133 cm LCF) (Clear et al. In preparation), there are still problems with the interpretation of zones from calcified tissues of larger and presumably older fish. In larger southern bluefin, discrepancies in the number of presumed annual increments counted in otoliths and vertebrae have lead to uncertainty over which, if either, provides a true estimate of age for mature fish. An additional complication is the lack of known age individuals and the rarity of tagged and returned fish with long periods at liberty (>10 years). Similar problems exist for age estimation of Atlantic bluefin tuna (Thunnus thynnus) (Lee & Prince 1995). Until recently, stock assessment of southern bluefin was carried out under the assumption that these fish can be up to 20 years of age (Collette & Nauen 1983, Majkowski

& Hampton 1983, Caton et al. 1990); however, the only direct evidence for this came from one tag recovery 20 years after release of a one year old bluefin (CSIRO unpublished data).

Determination of southern bluefin longevity and validation of age estimation methods for larger individuals is required in order to increase the reliability of stock assessments for this species. Zone counts in otoliths (Gunn et al. 1995) have suggested ages well in excess of the previously accepted maximum age of 20 years, but in the absence of tag-recapture data, there has been little chance of validating these estimates.

The bomb radiocarbon chronometer (Kalish 1993, 1995a, 1995b) provides an alternative method to tag-recapture studies. The bomb radiocarbon chronometer can be used to estimate the age of individual fish and the technique is well-suited to estimating the age of southern bluefin tuna. Because there is no accepted "routine" method for age estimation of larger southern bluefin tuna, direct estimation of age on the basis of the bomb radiocarbon chronometer presents a viable alternative to test assumptions regarding southern bluefin longevity. Southern bluefin are suited to age estimation using bomb radiocarbon due to the presumed longevity of the species and the likelihood that individuals in the present population were spawned during the bomb-related increase in radiocarbon in the atmosphere and ocean (Kalish 1993).

**Materials and methods**

Southern bluefin otoliths (sagittae) were selected from otolith archives maintained at the CSIRO Marine Laboratories (Hobart, Tasmania). A single otolith from each pair was selected for analysis with the other otolith, when available, being retained for studies of routine age estimation procedures. The majority of otoliths selected were from large individuals as these fish are likely to be more suitable for age estimation on the basis of bomb radiocarbon due to their presumed birth date. Otoliths were obtained from large fish caught off southeast Tasmania and in the Java Sea between 1988 and 1994. Otoliths from two southern bluefin that were one year of age (55 cm FL) were also selected to assist with calibration and to provide an indication of the decrease in ocean radiocarbon since 1980. The small fish were caught off the southwest coast of Western Australia in 1985 and 1993.

In this study, calibration refers to the process of establishing a relationship between surface ocean radiocarbon levels in a region and time (Kalish 1995b).

Otoliths were weighed dry and then prepared for radiocarbon and stable carbon isotope analysis. The earliest formed portions of individual otoliths was isolated with a fine, high speed drill. This was achieved by "sculpting" from the larger otolith, an otolith that was representative of a southern bluefin less than 1 year of age. During the sculpting process the position of "landmarks" such as the otolith core and zones associated with the presumed first annual increment were monitored frequently. This ensured that the sculpted otolith contained material only deposited during the early life of the fish. The final product was a single piece of otolith aragonite (Fig. 7.1). Sample weights ranged from about 13 to 24 mg. Otolith carbonate was converted to CO2 by reaction in vacuo with 100% phosphoric acid. An aliquot of the CO2 was used to determine $\delta^{13}C$ for each sample and the remaining CO2 was converted to graphite (Lowe and Judd 1987) for analysis of radiocarbon. Radiocarbon levels in each sample were determined by accelerator mass spectrometry (AMS) at the Institute of Geological and Nuclear Sciences, Lower Hutt, New Zealand (Wallace et al. 1987). Radiocarbon values are reported as $\Delta^{14}C$, which is the age- and fractionation-corrected permil deviation from the activity of nineteenth century wood (Stuiver and Polach 1977). Radiocarbon determinations were made via the NBS oxalic acid standard in conjunction with the ANU sucrose standard. Reported errors for the radiocarbon data are 1 standard deviation. Radiocarbon errors include both counting errors and laboratory random errors.

There are few radiocarbon data available that are suitable for the temporal calibration of radiocarbon data obtained from the earliest formed portions of southern bluefin tuna otoliths. This portion of the otolith is presumed to be deposited in the tropical and subtropical Indian Ocean, specifically in the region of the Java Sea and southeast Indian Ocean. Radiocarbon data were selected from both the pre- and post-nuclear testing GEOSECS (Geochemical Ocean Section Study) expeditions to the Indian Ocean (Bien et al. 1965, Stuiver and Ostlund 1983), studies of corals from Cocos-Keeling Island (Toggweiler et al. 1991) (Table 7.1), and the otoliths of two 55 cm southern bluefin tuna that were one year (Table 7.2) of age based on previous studies of southern bluefin age and growth (Gunn et al. 1995). These data were used to model the bomb-related increase in Indian Ocean radiocarbon during the 1960s and 70s.

For zone counts, otoliths were embedded in polyester resin and sectioned with a low-speed diamond saw. Three parallel replicate sections were taken in the dorso-ventral plane (transverse), one anterior to the primordium, one through the primordium and one posterior to it. Each section was then mounted on glass rounds and polished to thicknesses of 0.4-0.6 mm using 600 grade wet-dry paper.

Age was estimated from otolith sections by a reader that had read more than 1000 southern bluefin otolith sections (NPC). These age estimates were compared with age estimates derived from otolith radiocarbon data.

**Results**

Birth date estimates for individual tuna were determined directly from the otolith radiocarbon data. A second order polynomial function that describes the bomb-related increase in $\Delta^{14}C$ in the eastern Indian Ocean was established from a combination of the GEOSECS data, the Cocos-Keeling coral date, and the two small southern bluefin of known age (birth dates 1984 and 1992). Pre-bomb $\Delta^{14}C$ measured from seawater dissolved inorganic carbon (DIC) and corals, both representative of the year 1953, were not used in the estimation of the function. Radiocarbon data are not available in the relevant area between about 1963 and 1970. The function determined from these data (Fig. 7.2) describes a time series of $\Delta^{14}C$ that is similar to that modelled for $\Delta^{14}C$ in the southern hemisphere tropical Indian Ocean by Broecker et al. (1985). The resulting function was used to estimate the birth dates of the large southern bluefin tuna on the basis of the $\Delta^{14}C$ measured in the earliest formed regions of the individual otoliths. $\Delta^{14}C$ data measured in the sagittae of 22 southern bluefin tuna are plotted with $\Delta^{14}C$ determined in surface seawater DIC from the GEOSECS expeditions (Bien et al. 1965, Stuiver and Ostlund 1983) and from known age segments of hermatypic corals from Cocos-Keeling Island in the Indian Ocean (Toggweiler et al 1991) (Fig. 7.3).

Radiocarbon-based estimates of birth dates for the 20 large southern bluefin tuna range from 1958 to 1973 (Table 7.2). The birth date estimated for the oldest tuna in this study provides an indication of the latest reasonable birth date (i.e. the fish could be older). Because this individual was spawned prior to significant atmospheric testing of nuclear weapons, the portions of the otolith that were analysed do not contain any detectable bomb-derived radiocarbon. Radiocarbon-based birth dates from southern bluefin were also plotted with otolith radiocarbon data from *Pagrus auratus* and *Centroberyx affinis* (Fig. 7.4). The data from *Pagrus auratus* describe an established calibration curve for increases in radiocarbon in the temperate South Pacific Ocean off New Zealand (Kalish 1993). The data from C. affinis are based on age estimates derived from the reading of otolith sections combined with radiocarbon analyses and are representative of changes in ocean radiocarbon off the east coast of Australia at temperate latitudes (Kalish 1995). The tuna birth dates and corresponding $\Delta^{14}C$ values are coincident with both the P. auratus and C. affinis data until the 1970s when tuna otolith $\Delta^{14}C$ reaches higher levels than the data from P. auratus. In later years the tuna data agree more closely with the C. affinis data.

Age estimates for southern bluefin tuna were calculated on the basis of the radiocarbon-based birth date estimates and known collection dates for individual fish (Table 7.2) and were compared with age estimates from the counting of presumed annual increments on transverse sections of otoliths. Because pairs of otoliths were not obtained from all fish only 15 of the 22 samples analysed for radiocarbon had corresponding otolith sections. An age difference plot, where the difference between the age estimated from the otolith sections and $\Delta^{14}C$ is plotted as a function of the "bomb radiocarbon age", was used to compare age estimates (Fig. 7.5). Pairwise age comparisions between the section and bomb radiocarbon estimates suggest that there may be significant differences among the two methods of age estimation (two-tail t-test; df=14; P=0.051). If the bomb radiocarbon age estimates are taken as the standard, then there appears to be evidence that section ages of several of the youngest fish may be overestimates for this small sample.

When $\Delta^{14}C$ measured in southern bluefin otoliths is plotted as a function of the birth dates estimated from the reading of otolith sections several data points diverge significantly from the bomb radiocarbon curve derived from *Pagrus auratus* otoliths (Fig. 7.6). Specifically, three southern bluefin section ages (Sample Nos. 203, 564, 598) fall outside the curves defined by the 95% confidence limits determined for individual predicted values from the P. auratus radiocarbon and birth date data. The ages (birth dates) of these presumably younger fish, with greater quantities of bomb radiocarbon in their otoliths, appear to be overestimated as they fall to the left of the bomb radiocarbon curve defined by P. auratus.

**Discussion**

Objective estimates of southern bluefin tuna age are possible on the basis of radiocarbon analyses made in the earliest formed segments of otoliths. The age estimates were based on the assumption that most of the observed variation in otolith $\Delta^{14}C$ was related to differences in the birth date of individual southern bluefin. The primary support for this assumption is derived from the fact that there were rapid increases in radiocarbon in tropical and temperate oceans during the 1960s and 70s and that otolith radiocarbon is a good proxy of radiocarbon in seawater DIC (Kalish 1993).

Radiocarbon data from selected regions of southern bluefin tuna indicate that this species can reach ages in excess of 30 years. Furthermore, individuals that approach the asymptotic length are likely to be 20 years of age or older. The data agree generally with accepted models of southern bluefin tuna growth derived from tagging data and with growth curves based on zone counts in otoliths across the size range of the species (Gunn et al. 1995). A detailed comparison with current growth models was not possible because of the size range of individuals used. The estimates of length at age suggest that the estimate of $L\infty$ is good and, furthermore, that it is not feasible to estimate age from length for southern bluefin tuna greater than about 180 cm LCF.

Peak radiocarbon levels measured in southern bluefin tuna otoliths are higher than those measured in *Pagrus auratus* otoliths from off the east coast of New Zealand, but similar to those measured in *Centroberyx affinis* otoliths from the Tasman Sea off southeast Australia. The higher level of radiocarbon in the tuna otoliths would be expected on the basis of the relative proximity of the northeastern Indian Ocean and the temperate central South Pacific Ocean to northern hemisphere atmospheric testing. Rapid transport of seawater through the Pacific-Indian Oceans throughflow region (Fieux et al. 1994) would result in effective transport of radiocarbon from the Pacific to the Indian Ocean and, ultimately, would also result in relatively high radiocarbon levels in the tropical and subtropical Indian Ocean. The ocean off southeast Australia would also be expected to receive greater quantities of bomb derived radiocarbon due to ocean transport via the East Australian Current.

There are several possible sources of error that may affect the strict interpretation of radiocarbon in southern bluefin tuna otoliths in terms of birth dates/age. Firstly, the material isolated for radiocarbon analyses is sculpted from the whole otolith. Incomplete removal of otolith calcium carbonate deposited later in life (i.e. younger otolith material) would affect the level of radiocarbon measured in that sample. For example, many of the tuna otoliths that had relatively high radiocarbon levels (>50‰), that is relatively young fish, were estimated to be older on the basis of reading otolith sections. If sculpting failed to remove all otolith material deposited after the first year of life for fish spawned in the late 1950s or 1960s, then younger otolith material with higher radiocarbon levels would "contaminate" the sample. This would result in a radiocarbon-based age estimate that was younger than the true value. The majority of discrepancies between section age and bomb radiocarbon age indicate that the radiocarbon age is younger than the section age. On this basis, contamination of the samples with calcium carbonate deposited after the first year of life and/or a slight bias to overestimate age from otolith sections must be considered as possibilities.

The inclusion of younger otolith material in a sample believed to be representative of the first year of growth may occur due to inaccurate sculpting, however, the degree of contamination required to explain the larger age discrepancies observed is not likely. A mass balance model was used to illustrate the effect of different levels of contamination on $\Delta^{14}C$ for the two samples that showed the greatest difference between bomb radiocarbon age and section age. Sample No. 203 was estimated to be 16 years of age (birth date of 1972) on the basis of $\Delta^{14}C$ (116.2±9.4‰), whereas the otolith section age was 24 years (birth date of 1964). $\Delta^{14}C$ in the eastern Indian Ocean was estimated to be about 40‰ in 1964 (see Fig. 7.2), well below the value measured in Sample No. 203 (Table 7.2). If this fish was, in fact, spawned in 1964 and the sample was sculpted without contamination, then we would expect the sample of about 20 mg to have a $\Delta^{14}C$ of 40‰. Consider the possibility that, after sample sculpting is complete, the sample contains 18 mg of material with a $\Delta^{14}C$ of 40‰ and 2 mg of material deposited after the first year of life and with a $\Delta^{14}C$ of 50‰. In this case, the sample would have resulted in a $\Delta^{14}C$ of 40.5‰. Assume each additional 2 mg of younger calcium carbonate, that replaces 2 mg of calcium carbonate deposited during the first year of life, results in a 10‰ increase in mean $\Delta^{14}C$ of the inappropriately included material. Under this scenario, it would be necessary for the sculpted Sample No. 203 to contain 2 mg of calcium carbonate with a $\Delta^{14}C$ of 40‰ deposited during the first year of life and 18 mg with a $\Delta^{14}C$ of 130‰ to obtain a $\Delta^{14}C$ of 116‰ for a southern bluefin spawned in 1964. Given the degree of care taken with the sculpting process this extent of contamination is extremely unlikely. Furthermore, this model overestimates the probable $\Delta^{14}C$ for the contaminant and it is likely to be much lower in those instances where the sample sculpting process was imprecise. A similar model was applied to the results from Sample No. 564 where bomb radiocarbon and otolith section age estimates differed by 7 years (Table 7.2). In order for the correct birthdate to be 1960 ($\Delta^{14}C$ of about -18‰) for Sample No. 564, it would have been necessary for the 18.2 mg sample to contain 15.5 mg of "contaminant" with a $\Delta^{14}C$ of about 90‰. These arguments assume that, although spatial variation in surface ocean $\Delta^{14}C$ can be significant over the possible range of 1 year old southern bluefin (see below), this variation is small when compared with temporal variation in $\Delta^{14}C$ during the 1960s and early 1970s. The relatively small variation in $\Delta^{14}C$ over large (primarily meridional) spatial scales is evident in Fig. 7.4 where data derived from the east coasts of New Zealand (*Pagrus auratus*) and New South Wales (*Centroberyx affinis*), and the eastern Indian Ocean are compared.

The likelihood of sample contamination by otolith material deposited later in life could be reduced by sculpting smaller samples. In this study, otolith sample weights for radiocarbon analyses were maintained between 13.1 and 24.4 mg (1.6 to 2.9 mg of carbon) based on the requirement for a minimum quantity of carbon to achieve a specified level of analytical precision at the AMS facility that did the analyses. Analysis of radiocarbon by AMS has developed to the point where it is possible, at some facilities, to obtain high precision analyses on samples that contain less than 0.5 mg of carbon (4 mg of $CaCO_3$). Because the ability to analyse small samples varies among laboratories, it is important to consult with the operators of AMS facilities before submitting samples for radiocarbon analysis. It is recommended that the smallest sample size that can be analyzed with high precision ($<\pm10$‰) be used for AMS analysis in those cases where the species under investigation has relatively small otoliths.

Southern bluefin tuna is a highly migratory species and appears to travel great distances during the first year of life (Shingu 1978, CSIRO unpublished data). The exact nature of these movements is not well-defined and some individuals appear to migrate south along the western coast of Australia, whereas other young southern bluefin may move in a south-westerly direction towards the coast of South Africa. Data on southern bluefin movements indicate that one year old fish can move significant distances from the spawning grounds in the tropical Indian Ocean south of Java, between about 10°S and 15°S latitude. Southern bluefin 25 cm LCF (90-120 days old) have been collected between Northwest Cape and Freemantle, Western Australia and fish between 50-55 cm LCF (one year old) have been captured off New South Wales, Tasmania, Victoria, South Australia, Western Australia and South Africa (CSIRO unpublished data). These movements would expose individual southern bluefin to varying levels of radiocarbon while the earliest formed portions of the otolith were being deposited. In addition, the rapid increase in ocean radiocarbon levels between about 1960 and 1975 would expose individual fish to temporally varying radiocarbon.

Radiocarbon data in Stuiver and Ostlund (1983) show significant latitudinal and longitudinal variation during the 1977-78 Indian Ocean GEOSECS expedition. These data can be used as a basis for understanding the distribution of bomb carbon in earlier years. The highest concentration of bomb carbon was found in the central gyre of the Indian Ocean between about 10° S and 35°S. In the eastern section of the gyre $\Delta^{14}C$ values were up to about 140‰, but were slightly lower in the western portion of the gyre. Far greater variation in $\Delta^{14}C$ values was found with latitude. In 1977-78, maximum $\Delta^{14}C$ values of around 140‰ in southern tropical latitudes of the Indian Ocean decreased to about 28‰ (eastern Indian Ocean) and -14‰ (western Indian Ocean) at around 50°S latitude, the southern extent of the range of adult southern bluefin. The range of $\Delta^{14}C$ values was much less at the southernmost extent of the distribution of 1 year old southern bluefin at around 35°S latitude. In 1977-78, $\Delta^{14}C$ levels were about 98‰ at 35°S latitude in both the eastern and western Indian Ocean.

As juvenile southern bluefin migrate south or southwest from the tropical Indian Ocean the growing otolith will integrate radiocarbon levels over a broad temporal (months) and spatial (1000s of km) scales. Because both the path taken by individual tuna and the temporal and spatial variation in surface ocean radiocarbon over time will vary, individual otoliths will incorporate different levels of radiocarbon, regardless of the birth date of individual fish. The effect of this variation can be reduced by sculpting smaller otolith samples for radiocarbon analysis, thereby limiting the range of possible movements by young fish as they move from the spawning grounds.

Fish species incorporate different levels of carbon isotopes into the CaCO3 of the otoliths and these differences may be linked to metabolic rate effects (Kalish 1991). Southern bluefin tuna otoliths have been shown to be relatively depleted in $^{13}C$ compared with otoliths from other non-scrombrid fishes. This fractionation of carbon isotopes in the otoliths is likely to be reflected in the incorporation of $^{14}C$, however, these effects have been considered in this study as the calculation of $\Delta^{14}C$ accounts for fractionation (Stuiver and Polach 1977).

Southern bluefin tuna otoliths for this study were selected at random from large fish sampled by CSIRO researchers and collaborators. The sample is not adequate to estimate the range of ages present in the population of southern bluefin tuna, but it does suggest that a large percentage of the fish greater than 180 cm FL are at least 20 years of age and that southern bluefin can live to ages in excess of 30 years. Furthermore, the results show that reading otolith sections is an effective method to estimate the age of larger southern bluefin. The analysis of additional samples could provide data relevant to defining the age structure of larger southern bluefin tuna and more precisely define the accuracy of age estimation by the reading of otolith sections.

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Table 7.1. Measurements of $\Delta^{14}C$ made on tropical Indian Ocean surface water samples or corals from latitudes comparable to locations of juvenile *Thunnus maccoyii* otolith deposition. DIC is dissolved inorganic carbon.

| Latitude | Longitude | Sample type | Sample date | $\Delta^{14}C$ (‰) | Reference |
|---|---|---|---|---|---|
| 12°S | 97°E | hermatypic coral | July 1953 | -30 | Toggweiler et al 1991 |
| 12°S | 97°E | hermatypic coral | July 1970 | 118 | Toggweiler et al 1991 |
| 12°S | 97°E | hermatypic coral | July 1972 | 129 | Toggweiler et al 1991 |
| 12°S | 97°E | hermatypic coral | July 1973 | 130 | Toggweiler et al 1991 |
| 12°S | 97°E | hermatypic coral | July 1974 | 134 | Toggweiler et al 1991 |
| 12°S | 97°E | hermatypic coral | July 1976 | 121 | Toggweiler et al 1991 |
| 10°31'S | 105°34'E | seawater DIC | 19 Oct 1960 | -16 | Bien et al. 1965 |
| 18°49'S | 88°33'E | seawater DIC | 27 Nov 1960 | -7 | Bien et al. 1965 |
| 36°18'S | 98°41'E | seawater DIC | 29 Dec 1960 | -25 | Bien et al. 1965 |
| 33°14'S | 108°45'E | seawater DIC | 1 Jan 1961 | -15 | Bien et al. 1965 |
| 34°11'S | 105°49'E | seawater DIC | 25 Nov 1962 | 20 | Bien et al. 1965 |
| 29°15'S | 109°58'E | seawater DIC | 8 Mar 1978 | 140 | Stuiver and Ostlund 1983 |

Table 7.2. Southern bluefin tuna (*Thunnus maccoyii*) fish and otolith data. Sample weights indicate the weight of otolith material separated for individual analyses of stable carbon and radiocarbon and are representative of less than the first year of otolith growth for an individual fish. The birthdate was determined as discussed in the text. The age is the date caught minus the birthdate determined from the radiocarbon data.

| Sample No. | Date caught | Fork length (cm) | Otolith wt. (g) | Sample wt. (mg) | $\delta^{13}C$ (‰,PDB) | $\Delta^{14}C$(‰) | Birth date (years, A.D.) | Age (years) | Otolith section age (birth date) |
|---|---|---|---|---|---|---|---|---|---|
| 190 | 26/11/8 | 195 | 0.1768 | 19.7 | -6.49 | 29.3±8.7 | 1963 | 25 | 25 (1963) |
| 195 | 2/12/88 | 190 | 0.1802 | 17.6 | -8.38 | 119.7±9.6 | 1972 | 16 | |
| 203 | 2/12/88 | 185 | 0.1578 | 19.2 | -6.70 | 116.2±9.4 | 1972 | 16 | 24 (1964) |
| 529 | 12/7/89 | 180 | 0.1465 | 19.1 | -7.84 | 41.2±9.7 | 1964 | 25 | 26 (1963) |
| 552 | 25/6/89 | 180 | 0.1523 | 19.8 | -7.16 | 68.1±9.2 | 1966 | 23 | 26 (1963) |
| 564 | 4/7/89 | 195 | 0.1695 | 18.2 | -7.32 | 78.5±10.1 | 1967 | 22 | 29 (1960) |
| 584 | 9/7/89 | 195 | 0.1851 | 19.5 | -8.42 | 69.6±8.5 | 1966 | 23 | |
| 598 | 15/7/89 | 182 | 0.1861 | 12.3 | -7.26 | 75.2±10.7 | 1967 | 22 | 27 (1962) |
| 642 | 29/6/89 | 186 | 0.2353 | 24.4 | -8.18 | -38.8±7.1 | 1958 | 31 | |
| 753 | 16/6/89 | 190 | 0.1863 | 18.6 | -7.26 | 55.2±9.9 | 1965 | 24 | |
| 2278 | 24/2/93 | 55 | 0.0135 | 13.8 | 8.53 | 80.0±8.6 | 1992 | 1 | |
| 4670 | 11/11/9 | 184 | 0.1550 | 13.1 | -6.59 | 101.0±11.0 | 1970 | 23 | 24 (1969) |
| 4682 | 18/11/9 | 180 | 0.1774 | 15.6 | -6.79 | 88.2±10.8 | 1968 | 25 | 23 (1970) |
| 4693 | 18/11/9 | 185 | 0.1684 | 19.6 | -7.33 | 73.8±10.4 | 1967 | 26 | 27 (1966) |
| | 3 | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 4698 | 14/11/9 | 201 | 0.1719 | 15.8 | -7.58 | 50.1±9.4 | 1965 | 28 | |
| 4732 | 18/4/85 | 55 | 0.0140 | 14.2 | -9.51 | 115.1±9.2 | 1984 | 1 | |
| 6288 | 6/11/94 | 190 | 0.219 | 16.8 | -6.8 | 71.1±10.2 | 1967 | 27 | 30 (1964) |
| 6289 | 3/12/94 | 194 | 0.2046 | 19.8 | -6.8 | 37.2±11.9 | 1964 | 30 | 26 (1968) |
| 6290 | 28/12/9 | 189 | 0.2835 | 19.8 | -7.1 | 85.7±10.7 | 1968 | 26 | 28 (1966) |
| 6291 | 6/12/94 | 196 | 0.2773 | 19.2 | -7 | -18.1±8.8 | 1960 | 34 | 34 (1960) |
| 6292 | 6/12/94 | 196 | 0.2402 | 19.8 | -7.8 | 73±8.6 | 1967 | 27 | 28 (1966) |
| 6293 | 26/12/9 | 199 | 0.2093 | 19.9 | -7.4 | 54.2±8.6 | 1965 | 29 | 29 (1965) |

4

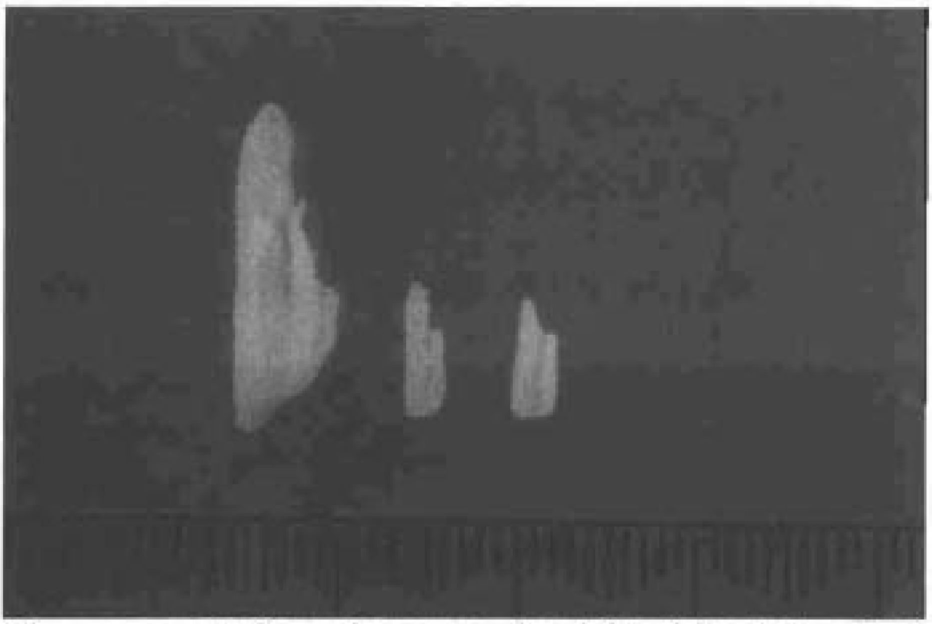

Figure 7.1. *Thunnus maccoyii.* Comparison among, from left to right: whole sagitta (219 mg) from 190 cm southern bluefin, sculpted sagitta (18.6 mg) from 190 cm southern bluefin , whole sagitta (14.2 mg) from 55 cm southern bluefin.

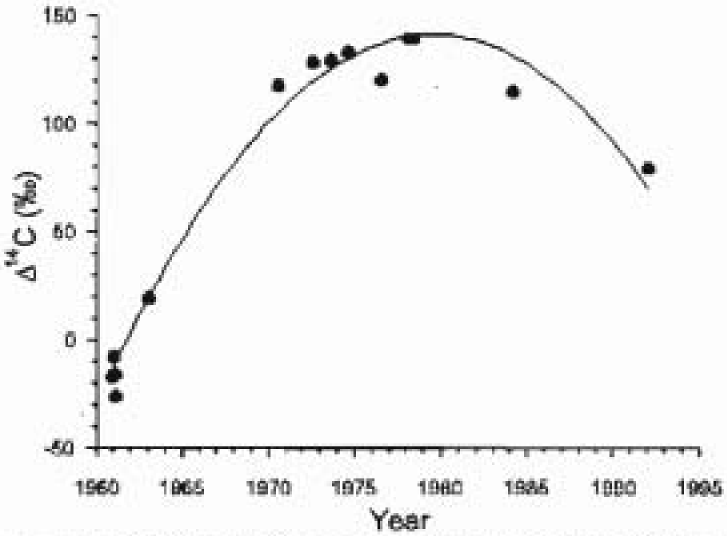


Figure 7.2. *Thunnus maccoyii.* Data and function used to provide a calibration for Indian Ocean $\Delta^{14}C$ values during the increase in bomb-derived radiocarbon. Data points prior to 1980 were derived from measurements of dissolved inorganic carbon in surface seawater (Bien et al. 1965, Stuiver & Ostlund 1983, Toggweiler et al 1991). Data points from 1984 and 1992 were determined from measurements of radiocarbon in whole otoliths of 1 year old southern bluefin tuna. The polynomial function is: $\Delta^{14}C = -1759667 + 1778$ (year) - 0.45 (year2).

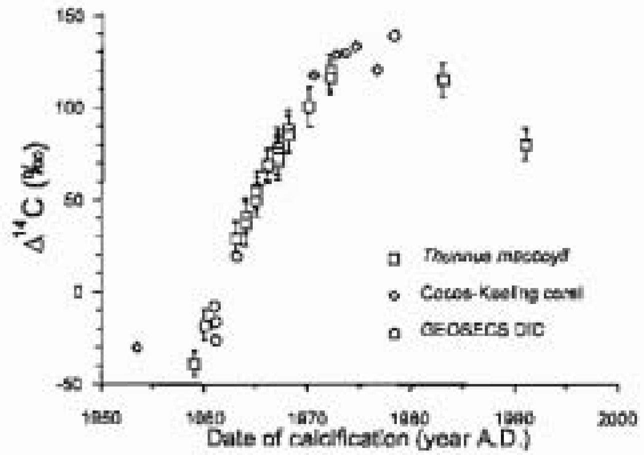


Figure 7.3. *Thunnus maccoyii.* Radiocarbon data from southern bluefin tuna otoliths, Cocos-Keeling Island hermatypic coral (Toggweiler et al. 1991), and GEOSECS (Geochemical Ocean Section Study) DIC (dissolved inorganic carbon) (Bien et al. 1965, Stuiver and Ostlund 1983). The date of calcification for the southern bluefin tuna data was determined on the basis of a second order polynomial function described by the post-bomb coral and GEOSECS radiocarbon data, as well as 2 small southern bluefin tuna with birthdates of 1983 and 1991.

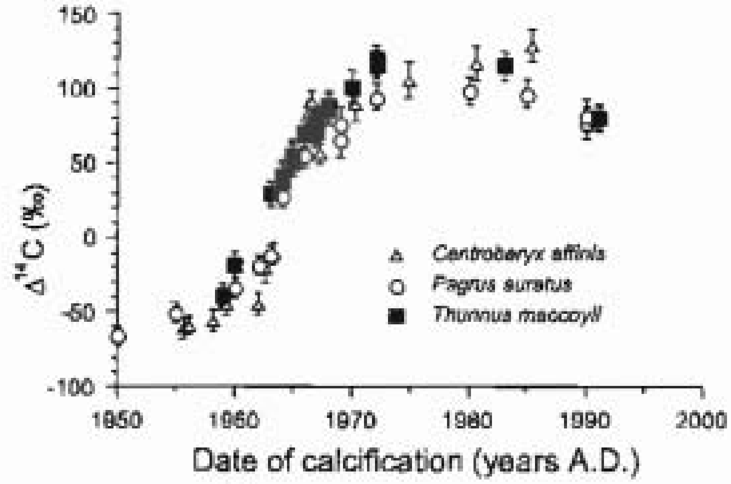


Figure 7.4. *Thunnus maccoyii.* $\Delta^{14}C$ of southern bluefin tuna otolith cores plotted against the birth date determined from radiocarbon levels. $\Delta^{14}C$ data from *Pagrus auratus* otolith cores are plotted against the true birth date (Kalish 1993) and $\Delta^{14}C$ values from Centroberyx affins (Kalish 1995b) are plotted against birth dates determined from reading otolith sections. For southern bluefin tuna, *Pagrus auratus*, and *Centroberyx affinis* $\Delta^{14}C$ values are based on otolith material deposited over a time period equivalent to about the first year of life. Errors are ±1 sd.

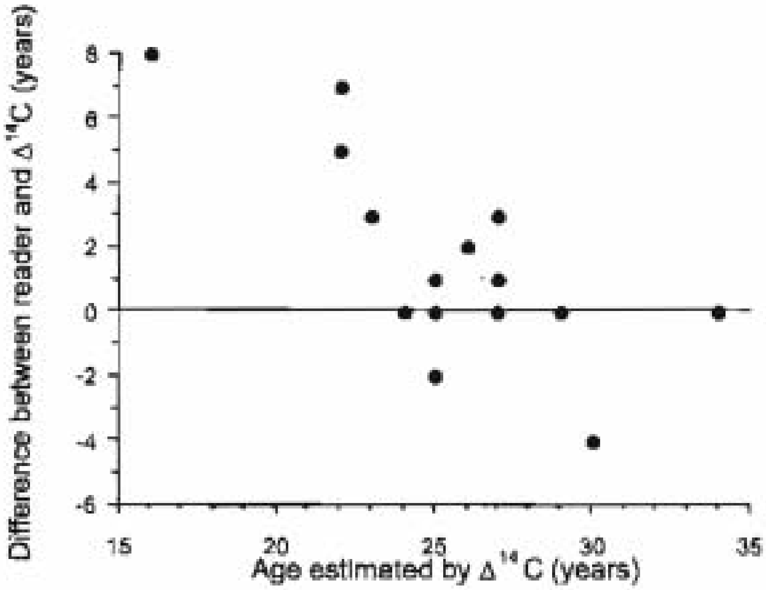


Figure 7.5. *Thunnus maccoyii.* Differences (years) between age estimates determined from radiocarbon data and reading otolith thin sections.

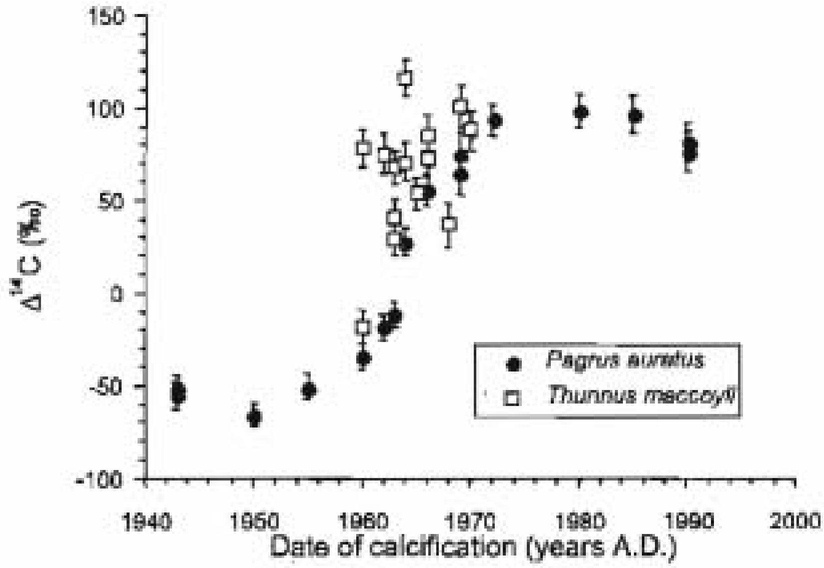


Figure 7.6. *Thunnus maccoyii.* $\Delta^{14}C$ values versus birth date estimates for southern bluefin tuna from otolith readers plotted with $\Delta^{14}C$ data versus birth date data for *Pagrus auratus*.

# Chapter 8 Application of the Bomb Radiocarbon Chronometer to the Validation of Redfish *Centroberyx affinis* Age

John Kalish


**Summary**

Validation of methods used to estimate fish age is a critical element of the fish stock assessment process. Despite the importance of validation, few procedures are available that provide unbiased estimates of true fish age and those methods that are available are seldom used. The majority of these methods are unlikely to provide an indication of the true age of individual fish, data that are best suited to the validation process. Accelerator mass spectrometry analyses of radiocarbon in selected regions of *Centroberyx affinis* otoliths, were used to validate the age estimation method for this species. Radiocarbon data from the otoliths of *Centroberyx affinis* with presumed birthdates between 1955 and 1985 described the increase in ocean radiocarbon attributable to the atmospheric detonation of nuclear weapons in the 1950s and 1960s. The results confirm the longevity of *Centroberyx affinis* and demonstrate the effectiveness of the bomb radiocarbon chronometer for the validation of age estimation methods.


**Introduction**

Several procedures are used for age estimation of teleosts; however, the counting of zones on otoliths appears to be used most frequently . Numerous validation studies for a range of fish species support the application of otolith zone counts for the routine estimation of fish age (see Summerfelt and Hall 1987; Smith 1992; Secor et al. 1995) , where validation refers to the process of estimating the accuracy of the age estimation method (Kalish et al. 1995).

Validation is considered to be an essential component of age estimation studies; however, validation is not always attempted or it is not completed satisfactorily (Beamish and McFarlane 1987). The most frequently employed methods for validation include: 1) mark-recapture studies in conjunction with the injection of a substance (e.g. oxytetracycline hydrochloride or strontium chloride) capable of marking calcified tissues; 2) analysis of length frequency modes; 3) monitoring of strong year classes; 4) marginal increment analysis, and; 5) radiometric age estimation. Beamish and McFarlane (1987) suggested "…that validation of ages of older fish requires either a mark-recapture study or the identification of known-age fish in the population."

Because many of these validation techniques are only applied to a restricted number of age groups, the validation may only be partial. In some instances, a combination of methods is employed. For example, age validation of orange roughy (*Hoplostethus atlanticus*) has employed analysis of length frequency modes to validate zones on the otoliths of small individuals (Mace et al. 1990) and radiometric age estimation to validate extreme longevity in larger individuals (Fenton et al. 1991). However, an approach that combines several methods cannot always provide conclusive validation of an age estimation procedure. In addition, problems with the precision of the age validation procedure may be significant.

Among the methods identified above, only mark-recapture studies and radiometric age estimation are suitable to the validation of age for longer lived fish with maximum ages in excess of about 20 years. Tagging studies, the most commonly employed method of validation, are also impractical for many species due to low rates of recapture, softness of tissues, depth of occurrence, and post-harvest processing strategies. Furthermore, most tagging studies only provide an indication of the time elapsed between tagging and recapture and they do not provide a measure of true fish age.

Radiometric age estimation based on the activity ratios of various naturally occurring radionuclides (e.g. 210Pb/226Ra) is gaining acceptance as an age validation technique (Bennett et al. 1982; Camapana et al. 1990; Fenton et al. 1991). Although radiometric age estimation is capable of overcoming many of the shortcomings of previously mentioned validation techniques; it introduces several unique problems associated with key assumptions (Fenton and Short, 1992) and the relatively low precision of the method. In addition, radiometric age estimation requires sample sizes of about 1 g, making it necessary to pool samples from several otoliths for each analysis. This precludes the ability to detect age estimation errors resulting from incorrect interpretation of otolith zones; however, the technique is well-suited as a general indication of fish age.

A new technique of age validation, based on the "bomb radiocarbon chronometer" (Kalish

1993, Kalish 1995) overcomes some problems associated with other age validation procedures and makes it possible to estimate the absolute age of individual fish. The method is based on the quantification of anthropogenic radiocarbon in selected regions of otoliths. Atmospheric testing of nuclear weapons in the 1950s and 1960s resulted in a drastic increase in radiocarbon levels in both the atmosphere and the ocean, and some of this radiocarbon was incorporated into living organisms. The increase in ocean radiocarbon during the 1960s was so rapid that the changing levels of radiocarbon during those years can be used as a de facto time scale. Otoliths have been shown to retain a chemical record that may be interpretable as a complex function of variations in a fish's physiology and environment over time (Kalish 1990, 1991a). Among the constituents of otoliths, radiocarbon has been shown to be a good proxy for radiocarbon levels in seawater dissolved inorganic carbon (Kalish 1993). Using accelerator mass sprectrometry, it is possible to measure with high precision (errors <1%), levels of radiocarbon in samples that contain less than 0.5 mg of carbon (e.g., Vogel et al. 1989). Aragonitic otoliths are 12% carbon making it feasible to analyse radiocarbon levels in otolith samples that weigh less than 5 mg. For many comercially important fish species, the amount of otolith calcium carbonate deposited in the first year of life exceeds 5 mg. This makes it possible to analyse radiocarbon levels in selected regions of a fish's otolith, including that portion that was deposited within 1 year or less of birth. By selecting otoliths from fish with presumed birth dates during the bomb-related increase in ocean radiocarbon and analysing segments of the otolith formed soon after birth, the fish's true age can be estimated. Ultimately, these data can be used to validate a method of age estimation. Furthermore, this validation procedures provides an estimate of the age of a fish that is independent of the counting of otolith zones.

This study presents results from an investigation into the application of the bomb radiocarbon chronometer to the validation of the age estimation method for redfish *Centroberyx affinis*. Redfish are an important commercial species in the trawl fishery off southeast Australia; however, little information is available on their biology (Kailola et al. 1993). Juveniles are collected by trawling in shallow coastal waters and the adults are found on the continental shelf and slope down to depths of at least 450 m (May and Maxwell 1986). In Australian waters, redfish reach a maximum size of about 40 cm FL and are believed to be slow growing. Previous estimates of age based on the number of zones visible in whole otoliths produced maximum ages of 16 years (Diplock 1984), whereas recent age estimates based on zones in otolith thin sections indicate maximum ages in excess of 40 years (Smith and

Robertson 1992). Because of their depth of occurrence and presumed age range, redfish are an ideal candidate for age validation based on the radiocarbon chronometer.

**Materials and methods**

Sagittal otoliths from redfish, *Centroberyx affinis*, were selected from archives at the Marine Science Laboratories, Queenscliff, Victoria, Australia. The samples selected were from redfish collected by commercial trawlers operating out of Uladulla (35°S, 151°E), New South Wales, Australia in March 1993. One sagitta from each pair was embedded in polyester resin and 4 thin sections were taken in the transverse plane within the proximity of the otolith core. These sections were mounted on glass slides and the ages of fish were estimated by counting the number of presumed annual increments present. The otoliths were read by 5 independent readers to provide estimates of fish age. These age estimates were used as a guide to select otoliths for radiocarbon analysis. The greatest age resolution of the bomb radiocarbon chronometer is between about 1960 and 1974, the period when the rate of increase of radiocarbon in the ocean is greatest. For this reason, the majority of otoliths selected were from fish with presumed birthdates during those years. Otoliths from fish that were presumed to have been spawned prior to significant atmospheric testing of nuclear weapons were also selected to provide an indication of pre-bomb radiocarbon levels in the ocean off southeast Australia and to provide an indication of the maximum fish age. Additional samples were also selected from redfish with presumed ages of less than 10 years to determine the peak in ocean radiocarbon in the region and to estimate the rate of loss of radiocarbon to other pools. Samples selected for analysis and age estimates based on thin sections are shown in Table 8.1.

Sample preparation procedures were similar to those described in Kalish (1993) for snapper, *Pagrus auratus*. Otolith aragonite deposited during a period presumed to be less than the first 8 months of life was isolated from one sagitta from each fish. This was accomplished by cutting and grinding the otolith with a hand-held high speed drill to remove material deposited after what was presumed to be the first annual increment. Further grinding of the remaining portion of the otolith was used to "sculpt" the material into a structure with the shape and dimensions of the earliest formed portions of a redfish sagitta. Sagittae from very small redfish and zones visible on the otolith being sculpted were used as a guide. Counts of presumed daily increments in otolith thin sections suggested that the aragonite isolated for radiocarbon analyses may have been deposited in less than 8 months. Sample weights ranged from about 17 to 25 mg. Otolith carbonate was converted to CO2 by reaction in vacuo with 100% phosphoric acid. An aliquot of the CO2 was used to determine $\delta^{13}C$ for each sample and the remaining CO2 was converted to graphite using excess H2 and an iron catalyst (Lowe and Judd 1987). The graphatised samples were analysed for radiocarbon. Radiocarbon levels in each sample were determined by accelerator mass spectrometry (AMS) at the Institute of Geological and Nuclear Sciences, Lower Hutt, New Zealand (Wallace et al. 1987). Radiocarbon values are reported as $\Delta^{14}C$, which is the age- and fractionation-corrected deviation (parts per thousand) from the activity of nineteenth century wood (Stuiver and Polach 1977). Age corrections are based on the mean estimate of age determined from reading of otolith sections. Radiocarbon determinations were made via the NBS oxalic acid standard in conjunction with the ANU sucrose standard. Reported errors are 1 standard deviation for both radiocarbon data and age estimates based on the reading of otolith sections. Radiocarbon errors include both counting errors and laboratory random errors.

**Results**

The radiocarbon data obtained from redfish otoliths were plotted against the presumed birth date estimated from the otolith sections from the same fish (Fig. 8.1). These data were plotted with bomb radiocarbon calibration data for temperate latitudes in the South Pacific determined from snapper otoliths (Kalish 1993) (Fig. 8.1). The radiocarbon data from snapper are used as a calibration between surface ocean radiocarbon levels measured at temperate latitudes in the southern hemisphere and time, as the snapper data are representative of the true variation in radiocarbon (Kalish 1993). On this basis, variations between radiocarbon levels for years/birth dates estimated from sections of redfish otoliths and radiocarbon levels determined from snapper otoliths are representative of possible errors in the estimation of birth date for individual redfish.

The change in radiocarbon level over time, as estimated from the redfish otoliths, describes a curve that is representative of the pre- and post-bomb flux of radiocarbon in the ocean and is consistent with the data from snapper. Radiocarbon levels increased in the early 1960s because of the flux of bomb radiocarbon from the atmosphere to the ocean and these levels peaked after about 1975. In later years, the bomb-derived radiocarbon became redistributed to other carbon pools including organisms, sediments, dissolved organic carbon, and particulate organic carbon.

The majority of the redfish data fall within the curves defined by the upper and lower 95% confidence limits determined for individual predicted values from the snapper data. Redfish and snapper data from the period spanning the rapid increae of radiocarbon (1955-1980) were modeled separately with a cubic polynomial (Table 8.2). The cubic polynomial was selected a priori because it was most likely to represent the two inflections in radiocarbon levels due to anthropogenic inputs from atomic testing and the subsequent decrease in radiocarbon resulting from loss to other pools. All but three of the redfish data points fell within the 95% confidence limits determined from the snapper data and those three points were on, or just beyond, the curves defined by the confidence limits. The snapper confidence limits are fully enclosed by the confidence limits defined by the redfish data.

**Discussion**

Radiocarbon analyses of redfish otoliths indicate that the counting of annual increments in otolith thin sections is a valid method for estimating redfish age. The oldest fish in the sample were at least 33 years old based on pre-bomb radiocarbon levels measured in several otoliths and estimates of age based on counts of annual increments suggest that the oldest fish may have been over 37 years of age (Table 8.1). Using the bomb radiocarbon chronometer, it is not possible to provide an unequivocal estimate of the maximum redfish age; it is possible only to determine that an individual fish was spawned prior to the flux of bomb radiocarbon into the temperate South Pacific Ocean around 1960. If later collections of redfish otoliths are made, for example in the year 2000, then the maximum age that could be estimated would be 40 years.

Three possible explanations for deviations between the snapper and redfish data include: (1) errors in redfish age estimates determined from otolith thin sections; (2) differences in the uptake rates of carbon and, concomitantly, radiocarbon from the environment for the two species; and (3) differences in oceanographic and atmospheric circulation resulting in a different flux of radiocarbon in the snapper and redfish habitats. There may be some errors in the estimates of redfish age, but if errors doe exist, they cannot be detected on the basis of the radiocarbon data presented in this study. There were three instances, however, where the section age birth date for redfish did not fall within the 95% confidence limits defined by the polynomial regression of the snapper data between 1955 and 1980, (sample Nos. 18, 24, and 44). In addition, the redfish datum from 1985 (sample No. 30) appeared to deviate from the radiocarbon calibration curve; a possible explanation for this is discussed later.

One assumption that was made in the interpretation of the radiocarbon data obtained from fish otoliths was that there were no significant differences in the uptake rates of carbon containing ions between fish species. The potential sources of carbon to the $CaCO3$ of the otolith incude dissolved inorganic carbon from the seawater and organic carbon derived from food. Data are limited, however, there is evidence that a large percentage (>60%) of otolith carbon is derived directly from dissolved inorganic carbon in seawater (Kalish 1991b, 1991c). Regardless of the sources of otolith carbon, studies indicate that uptake rates for ions can vary among species (see Hoar and Randall 1984). The radiocarbon data, however, are unlikely to be adequate to resolve this variation.

There are distinct differences in ocean circulation between the habitats of the snapper and redfish used in this study and these differences are likely to result in detectable differences in the temporal variation in ocean radiocarbon. The snapper were from the eat coast of the North Island of New Zealand and the redfish were collected off southeastern Australia, a separation of over 2000 km. Redfish live in a region of southeastern Australia that is influenced by the East Australian Current, a major current that has its origin in tropical oceans and would be a rich source of bomb-derived radiocarbon. The snapper were collected in a region of New Zealand that is not characterised by strong currents, but it does derive some water from the region of the Tasman Front via the East Auckland Current (Heath 1985). These differences suggest that greater quantities of bomb radiocarbon may reach southeast Australia via ocean circulation.

Other studies on the flux of radiocarbon to the South Pacific Ocean indicated that a peak in surface ocean radiocarbon occurred around 1974 (Landman et al. 1988; Toggweiler et al. 1991; Kalish 1993). These studies also showed a gradual decline, albeit at different rates, in ocean radiocarbon after 1974 as the radiocarbon became incorporated into other inorganic and organic carbon pools. The redfish radiocarbon data suggest that radiocarbon levels off the coast of New South Wales were still increasing up to 1985. Furthermore, the maximum

$\Delta^{14}C$ value of 128.6±10.7‰ from 1985 is significantly higher than the maximum value of 98.4±8.8‰ measured for *Pagrus auratus* from a similar latitude off New Zealand (Kalish 1993), but lower than peak $\Delta^{14}C$ values obtained from corals from Fiji (138‰) (Toggweiler et al. 1991) and Heron Island (145‰) (Landman et al. 1988). This result may be an indication of the relative transport of dissolved inorganic carbon from the Coral Sea to the coast of New South Wales via the East Australian Current. Nevertheless, atmospheric transport is likely to be the predominant influence on the present distribution of radiocarbon in the surface ocean. Other features of the radiocarbon time series based on the redfish otoliths might be explained on the basis of regional oceanography; however, given the fact that the majority of differences between the redfish and snapper data are not significant, these distinctions would not be well supported.

Application of the bomb radiocarbon chronometer to the estimation of fish age requires the existence of a calibration, such as the snapper data, so that temporal variations in radiocarbon for a particular region can be approximated. For many regions tehse data are lacking and the snapper data presented in Kalish (1993) represented the first pre- and post-bomb time series of radiocarbon at temperate latitudes of the northern or southern hemisphere. A similar time series for northern temperate latitudes was produced by Weidman and Jones (1993) on the basis of radiocarbon data obtained from the shells of the bivalve Arctica islandica. Several radiocarbon time series obtained from tropical and subtropical hermatypic corals are available (e.g., Druffel and Linick 1978; Nozaki et al. 1978; Toggweiler et al. 1991). The available radiocarbon data, however, are not always adequate to provide an accurate estimate of radiocarbon in a particular region and, in some instances, the best data that are available are the single measurements of pre- and post-bomb radiocarbon made directly on seawater as part of programs such as the GEOSECS expeditions (e.g., Broecker et al. 1985). Despite the lack of calibration data in some regions, the bomb radiocarbon chronometer can still be extremely effective because of our basic understanding of the flux of radiocarbon in the marine environment. For example, the presence of very low levels of radiocarbon (i.e., less than –40‰) would indicate that the fish was born prior to 1960. Alternatively, a rapid increase in radiocarbon over time would provide a validation for a series of otoliths presumed to have birth dates between about 1960 and 1970, even in the absence of any calibration data. Of course, calibration curves can be developed for other regions using radiocarbon data obtained from otoliths where an independent validation of age is available; this was the strategy employed in developing the calibration based on snapper otolith radiocarbon (Kalish

1993).

The major limitations to the application of the bomb radiocarbon chronometer for age estimation include the requirement for otoliths from fish with birth dates between about 1960 and 1975 and complications in determining the environmental variation in radiocarbon experienced during early life for some deepwater or highly migratory species. Given the apparent range of ages for many commercially important species the requirement for samples from fish with birth dates between 1960 and 1975 is not always a problem. Furthermore, many fisheries laboratories maintain extensive otolith archives and this can make it possible to use the method for species with relatively short life spans. Although the method cannot determine accurately the age of fish spawned prior to 1960, the absence of bomb radiocarbon in a sample provides an indication that a fish is relatively long lived. In addition, increased precision of radiocarbon estimates may make it possible to utilise the decrease in bomb radiocarbon, after about 1980, as a chronometer; however, this chronometer would be unlikely to provide the temporal resolution achievable during the 1960s and early 1970s.

It may be difficult or impossible to apply the bomb radiocarbon chronometer to some deep-sea or highly migratory fish species. The flux of bomb-derived radiocarbon to the deep sea may be very slow or, in some cases, undetectable. Some deepwater fish do have larval and juvenile stages that are present in surface waters and these species should be amenable to age estimation on the basis of bomb radiocarbon levels (Kalish 1995). Highly migratory species may be problematic because of difficulties in determining the region where the early growth stages of the fish took place.

The bomb radiocarbon chronometer is suitable for determining the absolute age of a range of fish species. Despite its utility, however, cost may prevent the application of the method in some situations. Typical costs for accelerator mass spectrometry analysis in a single otolith sample are about AUS$800 (2001). This cost includes conversion of the sample to CO2 and then graphite. Other procedures, including otolith sculpting, can be accomplished easily in most laboratories. The number of samples to be analysed is dependent on the degree of validation that is desired; achieving a very precise estimate of the accuracy of an age estimation method would, undoubtedly, be more costly. On the other hand, confirming the temporal meaning of the zones being counted is a critical part of the validation procedure that could be achieved with a single, carefully selected, radiocarbon analysis. Given the costs and time involved in completing a tagging study, the bomb radiocarbon chronometer represents a viable and cost-effective means of validation.

This study indicates the suitability of reading otolith sections to estimate the age of redfish and demonstrates the efficacy of age validation using the bomb radiocarbon chronometer. The procedure is rapid and relatively low cost and provides data suitable for a complete validation of age estimation procedures. The radiocarbon data presented here can also be applied to investigations of carbon flux and ocean circulation.

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Table 8.1a. Fish, otolith and radiocarbon data from *Centroberyx affinis* collected in March 1993 off Ulladulla, New South Wales, Australia. Values of $\Delta^{14}C$ are reported with ±1 standard deviation. Sample weights indicate the weight of otolith material separated for individual analyses of stable carbon and radiocarbon and are representative of less than the first year of otolith growth for an individual fish.

| Sample No. | Laboratory accession no. | Sex | Fish length (cm) | Fish wt. (g) | Otolith wt. (mg) | Sample wt. (mg) | $\delta^{13}C$ (‰,PDB) | $\Delta^{14}C$(‰) | Mean age (years) | Mean Birthdate (years, A.D.) |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 | RRL NZA1234 | M | 27 | 500 | 955 | 18.5 | -1.29 | -60.7± 6.7 | 37.3±1.3 | 1955.7±1.3 |
| 9 | RRL NZA1234 | M | 29 | 571 | 1025 | 21.6 | -1.85 | -59.4± 6.7 | 36.9±3.0 | 1956.1±3.0 |
| 4 | RRL NZA1234 | M | 26 | 471 | 970 | 21.5 | -1.35 | -56.1± 7.7 | 34.9±2.6 | 1958.1±2.6 |
| 23 | RRL NZA1234 | F | 29 | 651 | 1096 | 23.3 | -1.24 | -44.7± 7.6 | 33.7±1.1 | 1959.3±1.1 |
| 18 | RRL NZA1234 | F | 32 | 853 | 1080 | 17.4 | -0.94 | -44.7± 7.4 | 31.0±1.0 | 1962.0±1.0 |
| 35 | RRL NZA1234 | M | 25 | 432 | 817 | 24.3 | -1.37 | -21.3±8.6 | 30.4±0.8 | 1962.6±0.8 |
| 10 | RRL NZA1234 | M | 29 | 598 | 879 | 18.2 | -1.48 | -10.5± 7.4 | 29.7±1.0 | 1963.3±1.0 |
| 15 | RRL NZA1234 | M | 28 | 645 | 913 | 25.5 | -1.55 | 55.9± 7.3 | 25.7±2.1 | 1967.3±2.1 |
| 26 | RRL NZA1234 | F | 28 | 559 | 912 | 15.4 | -1.3 | 72.1±7.8 | 26.7±0.5 | 1966.3±0.5 |
| 44 | RRL NZA1234 | M | 27 | 497 | 775 | 21.8 | -2.43 | 83.2± 7.8 | 26.6±0.8 | 1966.4±0.8 |
| 24 | RRL NZA1234 | F | 33 | 971 | 1015 | 18.5 | -2.68 | 90.9±7.7 | 26.4±0.8 | 1966.6±0.8 |
| 11 | RRL NZA1234 | F | 28 | 586 | 691 | 23.5 | -1.6 | 88.8± 11.1 | 22.7±0.8 | 1970.3±0.8 |
| 1 | RRL NZA1234 | F | 25 | 442 | 637 | 19.6 | -0.63 | 105.9± 11.9 | 18.1±0.7 | 1974.8±0.7 |
| 8 | RRL NZA1234 | F | 27 | 457 | 563 | 22.1 | -1.58 | 117± 11.3 | 12.4±0.5 | 1980.5±0.5 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 30 | RRL NZA1234 | F | 27 | 454 | 584 | 23.4 | -1.5 | 128.6± 10.7 | 7.6±0.5 | 1985.4±0.5 |
| 2 | RRL NZA1234 | Imm | 9.3 | - | 92 | 21.5 | -1.34 | 80.8±8.3 | 2±0 | 1991.0±0 |

Table 8.1b. continued Age estimation data from *Centroberyx affinis* otolith sections collected off Ulladulla, New South Wales, Australia in March 1993. Fish ages were estimated by 5 readers, with readers 4 and 5 each providing 2 estimates of age. All age and birthdate estimates presented in the table are determined from counts of presumed annual increments in otolith thin sections. Values of mean age and mean birthdate are reported with ±1 standard deviation.

| Sample No. | Reader (Age, Birth date) | | | | | | | Mean age (years) | Mean Birth date (years, A.D.) |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4/1 | 4/2 | 5/1 | 5/2 | | |
| 12 | 37 (1956) | 39 (1954) | 35 (1958) | 37 (1956) | 37 (1956) | 38(1955) | 38 (1955) | 37.3±1.3 | 1955.7±1.3 |
| 9 | 34 (1959) | 35 (1958) | 37 (1956) | 37 (1956) | 37 (1956) | 35 (1958) | 43 (1950) | 36.9±3.0 | 1956.1±3.0 |
| 4 | 34 (1959) | 33 (1960) | 32 (1961) | 34 (1959) | 38 (1955) | 34 (1959) | 39 (1954) | 34.9±2.6 | 1958.1±2.6 |
| 23 | 33 (1960) | 33 (1960) | 34 (1959) | 33 (1960) | 34 (1959) | 33 (1960) | 36 (1957) | 33.7±1.1 | 1959.3±1.1 |
| 18 | 32 (1961) | 31 (1962) | 29 (1964) | 31 (1962) | 31 (1962) | 31 (1962) | 32 (1961) | 31.0±1.0 | 1962.0±1.0 |
| 35 | 30 (1963) | 30 (1963) | 30 (1963) | 30 (1963) | 32 (1961) | 30 (1963) | 31 (1962) | 30.4±0.8 | 1962.6±0.8 |
| 10 | 29 (1964) | 30 (1963) | 29 (1964) | 29 (1964) | 31 (1962) | 29 (1964) | 31 (1962) | 29.7±1.0 | 1963.3±1.0 |
| 15 | 27 (1966) | 27 (1966) | 26 (1967) | 27 (1966) | 27 (1966) | 27 (1966) | 25 (1968) | 25.7±2.1 | 1967.3±2.1 |
| 26 | 27 (1966) | 26 (1967) | 26 (1967) | 27 (1966) | 27 (1966) | 27 (1966) | 27 (1966) | 26.7±0.5 | 1966.3±0.5 |
| 44 | 25 (1968) | 25 (1968) | 25 (1968) | 24 (1969) | 27 (1966) | 24 (1969) | 30 (1963) | 26.6±0.8 | 1966.4±0.8 |
| 24 | 26 (1967) | 26 (1967) | 26 (1967) | 26 (1967) | 28 (1965) | 26 (1967) | 27 (1966) | 26.4±0.8 | 1966.6±0.8 |
| 11 | 23 (1970) | 23 (1970) | 21 (1972) | 23 (1970) | 23 (1970) | 23 (1970) | 23 (1970) | 22.7±0.8 | 1970.3±0.8 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 17 (1976) | 19 (1974) | 18 (1975) | 18 (1975) | 19 (1974) | 18 (1975) | 18 (1975) | 18.1±0.7 | 1974.8±0.7 |
| 8 | 12 (1981) | 13 (1980) | 13 (1980) | 12 (1981) | 12 (1981) | 12 (1981) | 13 (1980) | 12.4±0.5 | 1980.5±0.5 |
| 30 | 7 (1986) | 7 (1986) | 7 (1986) | 8 (1985) | 8 (1985) | 8 (1985) | 8 (1985) | 7.6±0.5 | 1985.4±0.5 |
| 2 | 2 (1991) | 2 (1991) | 2 (1991) | 2 (1991) | 2 (1991) | 2 (1991) | 2 (1991) | 2±0 | 1991.0±0 |

Table 8.2. Curvilinear regression results for radiocarbon versus birthdate (year) data from snapper (*Pagrus auratus*) and redfish (*Centroberyx affinis*) between the years 1955 and 1980.

| | r2 | intercept | x | x2 | x3 | SE(x) | SE(x2) | SE(x3) |
|---|---|---|---|---|---|---|---|---|
| *Pagrus auratus* (n=10) | 0.97 | 2.82 x 108 | -4.30 x 105 | 218.9 | -0.037 | 1.22 x 105 | 62.1 | 0.010 |
| *Centroberyx affinis* (n=14) | 0.91 | 3.04 x 108 | -4.63 x 105 | 235.9 | -0.040 | 2.33 x 105 | 118.4 | 0.020 |

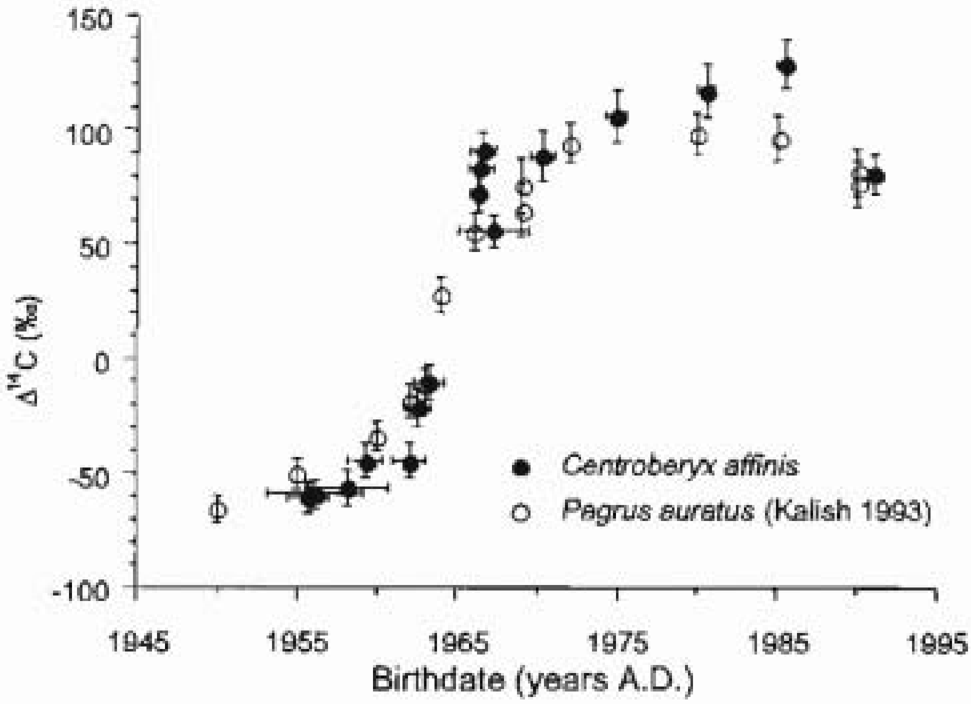


Figure 8.1. $\Delta^{14}C$ of *Centroberyx affinis* otolith cores plotted against presumed birthdate. $\Delta^{14}C$ data from *Pagrus auratus* otolith cores are plotted against the true birthdate and the data are from Kalish (1993). Birthdates were estimated from otolith sections read by multiple readers. For both C. affinis and P. auratus, $\Delta^{14}C$ values are based on otolith material deposited over a time period less than the first year of life. Errors are ±1 sd.

# Chapter 9 Application of the Bomb Radiocarbon Chronometer to the Validation of Blue Grenadier *Macruronus novaezelandiae* Age

John Kalish, Justine Johnston, David Smith, Sandy Morison and Simon Robertson

**Summary**

Accelerator mass spectrometry was used to measure radiocarbon in the earliest formed portions of selected blue grenadier *Macruronus novaezelandiae* otoliths to provide a validation of fish age estimates based on the quantification of opaque and translucent zones in otolith thin sections. $\Delta^{14}C$ data from blue grenadier otoliths were compared with previous estimates of $\Delta^{14}C$ in seawater dissolved inorganic carbon at similar latitutes, longitudes, and depths to link variation in otolith $\Delta^{14}C$ to time. Minimum otolith $\Delta^{14}C$ was -76.9±7.7‰, indicative of pre-bomb radiocarbon levels below the surfaced mixed layer at latitudes where juvenile blue grenadier are found. When plotted versus fish age estimated from otolith sections, the majority of the $\Delta^{14}C$ data combined to define a curve indicative of the increase in bomb radiocarbon in temperate oceans of the Southern Hemisphere and indicates that age estimation procedures based on otolith thin sections are satisfactory for blue grenadier age. If otolith section age estimates were correct, peak otolith $\Delta^{14}C$ of 106.8±7.9‰ occurred during the late 1960s, earlier than expected. This may be a manifestation of an increase in mixed-layer depth associated with increased frequency of zonal westerly winds at this time.

**Introduction**

Blue grenadier (Merlucciidae: *Macruronus novaezelandiae*) is a major component of the demersal trawl fisheries in both Australia and New Zealand. In New Zealand, where this species is commonly referred to as hoki, trawl catches exceed 200,000 tons per annum, and it is the largest fishery by weight and value (Annala 1994). About 2,500 tons of blue grenadier are caught off southeast Australia each year where it represents an important and increasing element of the trawl catch (Smith 1994). Merlucciids are very important commercial species in both southern and northern temperate oceans and many stocks have been studied intensively. Fish age is a critical aspect of these studies; validation of age estimation procedures, however, is rarely achieved because of the difficulties in tagging these species and the lack of known age individuals.

Development of age validation methods based on the chemical composition of fish otoliths makes it possible to estimate fish age without the complications of mark-recapture studies. For soft-bodied, deep water species that are virtually impossible to tag successfully, chemical methods are essential. Application of radiometric age estimation, based on radioisotopic disequilibria (e.g. 210Pb/226Ra activity ratios) to several deep-water species has proven to be successful (Bennett et al. 1982, Campana et al. 1991, Fenton et al. 1991), and the method has been applied recently to estimation of blue grenadier age (Fenton and Short 1995). Fenton and Short (1995) stated that radiometric age estimates were in "approximate agreement" with a study of blue grenadier growth (Kenchington and Augustine 1987) that was based on presumed annual increments in whole otoliths and otolith thin sections. Errors associated with radiometric age estimation, however, were relatively large and the level of agreement with the study of Kenchington and Augustine (1987) indicates that further research is required to validate age estimation methods for blue grenadier. Furthermore, sample sizes required for analysis of low-level radioisotopes by □-spectrometry are large and it was necessary to pool 6 otolith cores (Fenton and Short 1995) for each analysis. This makes it difficult to apply radiometric age estimation data to validation (sensu Kalish et al. 1995) of the "routine" age estimation procedure that, for blue grenadier, is based on interpretation of presumed annual increments in otolith thin sections.

Recent developments in the estimation of fish age based on the radiocarbon levels in otoliths can provide estimates of the absolute age of individual fish (Kalish 1993, Kalish 1995a, 1995b, Kalish et al. 1996). Atmospheric testing of nuclear weapons in the 1950s and 1960s resulted in a dramatic increase in both atmospheric and ocean radiocarbon. During the peak rate of increase in ocean radiocarbon between about 1960 and 1970, estimates of levels of bomb radiocarbon in the ocean, via the otolith proxy, become an effective chronometer for the determination of fish age. In addition, the age of fish can be broadly classified as pre-bomb or post-bomb, where earliest formed regions of otoliths from pre-bomb fish have $\Delta^{14}C$ values that are typically below -40‰, a value that is representative of the surface ocean prior to significant atomic testing (Broecker et al 1985). The relationship between time and $\Delta^{14}C$ is straightforward in relation to fish living all or, at least the early stages (e.g. first 6 months) of their lives in the surface mixed layer of the ocean. An additional variable, for those species that live at greater depths, relates to the time required for radiocarbon, in the form of dissolved inorganic carbon (DIC) and particulate organic carbon (POC), to penetrate to those depths. Several studies have considered the rate at which radiocarbon mixes into the deep-sea (e.g. Broecker et al. 1985, Sarmiento 1986, Toggweiler et al. 1989, Druffel et al. 1992, Jain et al. 1995) and this variable can be readily estimated at moderate depths by comparing depth profiles of radiocarbon made before and after atomic testing. Confirmation of the penetration of bomb-produced radiocarbon to particular depths could have also been achieved by measuring levels of tritium at depth; tritium is only present in the environment as a product of atomic testing and related anthropogenic sources.

Blue grenadier are found at depths ranging from about 200 to 700 m with young juveniles occurring predominantly at depths of 200 to 400 m (Kuo and Tanaka 1984). Radiocarbon analyses of the earliest formed regions of blue grenadier otoliths deposited in the first year of life or less would be a proxy for measurements of radiocarbon in seawater DIC at depths of about 200 to 400 m and would also serve to validate methods of age estimation used for blue grenadier. Using the bomb radiocarbon chronometer, this study seeks to validate the otolith thin section method of age estimation for blue grenadier and to provide data on the flux of radiocarbon in temperate southern hemisphere oceans.

**Materials and methods**

Sagittal otoliths from blue grenadier, *Macruronus novaezelandiae* were selected from archived material from the Tasmanian Department of Sea Fisheries, Australia. Sample preparation procedures were similar to those described in Kalish (1993, 1995b) for snapper, *Pagrus auratus* and redfish, *Centroberyx affinis*. The majority of samples selected were from blue grenadier collected by commercial trawlers fishing along the west coast of Tasmania in 1979 and 1980 (Table 9.1). These archived otolith collections were used because the presumed maximum age of blue grenadier is approximately 25 years. If these estimates were correct, more recent collections would be unlikely to include fish with birth dates prior to extensive atmospheric testing and during the maximum rate of increase in ocean radiocarbon between about 1960 and 1970; the period during which the bomb radiocarbon chronometer provides the finest age resolution. Additional otoliths collected in 1992 and 1993 were analysed to determine ocean radiocarbon levels between 1980 and the present.

One sagitta from each pair was embedded in polyester resin and thin sections were taken in the transverse plane within the proximity of the otolith core. These sections were mounted on glass slides and the ages of fish were estimated by counting the number of presumed annual increments present. Most otoliths were read by four independent readers (three from the Central Ageing Facility, Queenscliff, Victoria and one from Australian National University) to provide estimates of fish age. Age estimates were used as a guide in selection of otoliths for radiocarbon analysis. Samples selected for analysis and age estimates based on thin sections are shown in Table 9.1.

Otolith aragonite deposited during a period presumed to be less than the first 6 months of life was isolated from one sagitta from each fish. The earliest formed portion of individual otoliths was isolated with a fine, high speed drill. This was achieved by "sculpting" from the larger otolith, an otolith that was representative of a blue grenadier of about 6 months of age. The final product was a single piece of otolith aragonite. Sample weights ranged from about 17 to 26 mg (Table 9.1). Otolith carbonate was converted to CO2 by reaction in vacuo with 100% phosphoric acid. An aliquot of the CO2 was used to determine $\delta^{13}C$ for each sample and the remaining CO2 was graphitised for analysis of radiocarbon. Radiocarbon levels in each sample were determined by accelerator mass spectrometry (AMS) at the Institute of Geological and Nuclear Sciences, Lower Hutt, New Zealand (Wallace et al. 1987). Radiocarbon values are reported as Δ14C, which is the age- and fractionation-corrected permil deviation from the activity of nineteenth century wood (Stuiver and Polach 1977). Age corrections are based on the mean estimate of age determined from reading of otolith sections. Radiocarbon determinations were made via the NBS oxalic acid standard in conjunction with the ANU sucrose standard. Reported errors are 1 standard deviation for both radiocarbon data and age estimates based on the reading of otolith sections. Radiocarbon errors include both counting errors and laboratory random errors.

**Results**

Radiocarbon data obtained from blue grenadier, snapper (*Pagrus auratus*) (Kalish 1993), and redfish (*Centroberyx affinis*) (Kalish 1995b) were plotted against the presumed birth date estimated from otolith thin sections (Fig. 9.1). The New Zealand snapper data are used to provide a standard curve that is indicative of the temporal changes in surface ocean radiocarbon in the temperate South Pacific Ocean. Radiocarbon data from redfish collected off New South Wales were included because these fish occur in closer proximity to the blue grenadier used in this study. The majority of the blue grenadier data fall near a curve defined by the snapper and redfish data; however, several data points (Table 9.1) deviate significantly from the curve defined by snapper and redfish. There are three groupings of blue grenadier data points that appear to deviate from the standard curve (Fig. 9.1). These groups include: 1) sample numbers 72 and 196 that both yielded pre-bomb radiocarbon levels (i.e. <-40‰); 2) sample numbers 87, 185, and 408 that had highest levels of bomb radiocarbon, and; 3) sample numbers 8 and 31 that were estimated to have been spawned during the decline in bomb radiocarbon between about 1980 and 1985.

Despite the small discrepancies with the standard curve, the radiocarbon data from blue grenadier otoliths indicate that quantification of presumed annual increments in thin sections of otoliths is a good estimator of blue grenadier age. Furthermore, it is reasonable to conclude that some deviation from the standard curve, in blue grenadier $\Delta^{14}C$, is an indication of subtle differences in radiocarbon transport between different geographic regions and depths.

Age estimates for individual blue grenadier were calculated on the basis of the ocean radiocarbon calibration defined by $\Delta^{14}C$ in snapper otoliths. This was accomplished by modelling the change in snapper $\Delta^{14}C$ using a cubic spline and using the resulting relationship to estimate blue grenadier birth date based on $\Delta^{14}C$ measured in the otolith. Before converting blue grenadier $\Delta^{14}C$ to birth date and age an additional "correction factor" was applied to account for the depth of occurrence of juvenile blue grenadier as detailed below.

The depth distribution of juvenile blue grenadier in the ocean off southeastern Australia is not well described, although trawl surveys suggest that its depth distribution is similar to that determined in studies of blue grenadier off New Zealand. Exploratory trawl surveys off New Zealand indicated that juvenile blue grenadier less than 1 year old are most abundant at depths of between 200 and 400 m (Kuo and Tanaka 1984). At these depths, the radiocarbon content of seawater DIC is likely to be less than that measured in the surface mixed layer. Furthermore, the penetration of bomb-produced radiocarbon below the mixed layer is likely to be delayed. The lowest level of radiocarbon measured in the blue grenadier otoliths was -76.9±7.7‰ for a fish that was estimated to have been spawned in 1958. This is lower than the few pre-bomb (between about -40 and -60‰) measurements of radiocarbon made in Indian and Pacific Ocean surface waters at temperate latitudes (e.g. Bien et al. 1965, Broecker et al. 1985). Estimates of vertical profiles of ocean radiocarbon prior to atomic testing in the late 1950s (Broecker and Peng 1982, Broecker et al. 1985) suggest that the difference between surface mixed layer radiocarbon and that measured in the otolith of the blue grenadier spawned in 1958 would be consistent with a depth of occurrence just below the surface mixed layer.

Trawl survey data and the limited pre-bomb radiocarbon data, indicate that small juvenile blue grenadier are present below the surface mixed layer making it necessary to consider the rate of mixing of bomb-produced radiocarbon for those fish that were spawned after significant atomic testing. Information from depth profiles collected at two times at a similar location in the Indian Ocean can be used to estimate the rate of penetration of bomb radiocarbon: once in December 1960 at 36°18'S latitude 98°41'E longitude (Bien et al. 1965) and, again in February 1978 at 39°57'S latitude 109°58'E longitude (Stuiver and Ostlund 1983). Data from these 2 profiles are plotted together in Fig. 9.2. By 1978 bomb radiocarbon had mixed to a depth of about 1050 m and decreased linearly with depth from the surface. If it is assumed that there is a rapidly mixed surface layer of about 200 m in this region, then the bomb radiocarbon must have moved to deeper water at a rate of about 47 m per year from 1960 to 1978. Using this rate of penetration and an average depth of 300 m for juvenile blue grenadier, about 2 years would be required for the bomb radiocarbon to penetrate to blue grenadier depths. On this basis a "correction factor" of 2 years was added to estimates of blue grenadier birth dates determined on the basis of otolith $\Delta^{14}C$. Data on corrected $\Delta^{14}C$ birth dates that were close to the dates of the Indian Ocean vertical profiles (1960 and 1978) of radiocarbon are plotted in Fig. 9.2 and suggest good agreement between the two sources of radiocarbon measurement.

The agreement between age estimates based on otolith sections and otolith $\Delta^{14}C$ is good, however, there are several instances where significant differences are evident (Sample nos. 87, 185, 408). No attempt was made to model $\Delta^{14}C$ age for three samples (Sample nos. 8, 31, and 196) because of their position on the radiocarbon curve. Both samples 8 and 31 had near peak values of $\Delta^{14}C$ and it would be difficult to estimate their age due to the relatively broad peak in $\Delta^{14}C$ after about 1975. A $\Delta^{14}C$ age was not estimated for sample 407 because, based on the clearly pre-bomb $\Delta^{14}C$ value measured in the otolith core, this fish was spawned prior to detectable inputs of bomb $^{14}C$ into the ocean. It is possible only to state that this fish was spawned prior to about 1958 (Kalish 1995a).

Both otolith section and $\Delta^{14}C$ ages were plotted against standard length and with von Bertalanffy growth functions for female and male blue grenadier (Kenchington and Augustine 1987, Horn and Sullivan 1996, Smith et al. 1995) (Fig. 9.3). Both estimated ages from otolith sections and otolith $\Delta^{14}C$ are in general agreement with the growth functions; however a series of $\Delta^{14}C$ ages (Sample nos. 87, 185, 408) diverge from the growth functions. Otolith section ages should agree with the von Bertalanffy growth functions described in Smith et al. (1995) as the otoliths used in the present study comprised part of the data set used to describe growth for blue grenadier from western Bass Strait.

Otolith weight and blue grenadier age estimated from otolith sections and $\Delta^{14}C$ are plotted in Fig. 9.4. The limited data suggest that otolith weight is a fair indicator of fish age for this species and provide a further indication that $\Delta^{14}C$ values obtained for sample nos. 87, 185, and 408 are divergent from the expected trend.

**Discussion**

Differences between the plots of otolith radiocarbon and year (Fig. 9.1) for blue grenadier, snapper and redfish are not large given the geographic separation between the samples used in the respective studies. Sample collection locations for the 3 species were separated geographically by up to 10° of latitude and by over 30° of longitude. Limited ocean radiocarbon data are available in southern hemisphere temperate latitudes, although useful measurements of radiocarbon in seawater DIC in the region of southeast Australia were carried out in survey work of the 1960s and 70s. Despite the lack of data, two hypotheses can be put forward to explain the divergence between the blue grenadier, snapper, and redfish data sets. These include: 1) geographic separation and concomitant differences in atmosphere and ocean flux of radiocarbon, and; 2) different depth distribution and delayed flux of radiocarbon to deeper water.

The wide geographic separation between the collection sites for the snapper and blue grenadier samples is likely to result in detectable differences in the temporal variation in ocean radiocarbon. Snapper are a relatively sedentary species and the radiocarbon data from these fish are likely to be representative of the habitat along the east coast of the North Island of New Zealand, where they were collected. Blue grenadier are relatively mobile in that they have a relatively long-distance larval drift; furthermore, the nature and extent of this drift is not fully understood (Thresher et al. 1988). Regardless of the precise habitat of the blue grenadier, the ocean around Tasmania would experience very different circulation patterns from the North Island of New Zealand and, as a result, the flux of radiocarbon would also be different. Significant sources of ocean-transported bomb radiocarbon to the Tasmanian continental slope and shelf would be the later portions of both the Leeuwin and East Australian Currents. Both of these major currents have their origins in tropical oceans that would have been rich sources for bomb-derived radiocarbon. The snapper were collected in a region of New Zealand that is not characterised by strong currents, but it does derive some water from the region of the Tasman Front via the East Auckland Current (Heath 1985). These differences suggest that greater quantities of bomb radiocarbon may reach southeast Australia via ocean circulation.

In addition to the ocean-derived differences in bomb radiocarbon, there are likely to be differences in bomb radiocarbon delivered to the two locations on the basis of atmospheric circulation. The closer proximity of the snapper collection sites to the location of atomic testing in the tropical North Pacific Ocean suggests that greater levels of bomb-derived radiocarbon would have been carried to the New Zealand site by the relatively rapid transport mechanism of the atmospheric pathways. This factor, combined with the relative depth of occurrence of snapper and blue grenadier (see Results) may explain the slightly earlier manifestation of bomb-radiocarbon in snapper and redfish otoliths (Fig. 9.1).

Three $\Delta^{14}C$ values obtained from blue grenadier otoliths appear to diverge significantly from the curve that describes the increase in radiocarbon during the 1960s and 1970s. Otolith section ages suggest that these three blue grenadier (Sample nos. 87, 185, 408) were spawned in the late 1960s, whereas the quantities of bomb radiocarbon detected in these otoliths suggest birth dates in the early to mid 1970s. Plots of both otolith weight (Fig. 9.4) and standard length (Fig. 9.3) against otolith $\Delta^{14}C$ age provide strong evidence that ages based on radiocarbon are incorrect for these three fish. These $\Delta^{14}C$ age estimates may be in error due to: 1) habitat differences between these three blue grenadier and other fish used in this study, and; 2) changes in atmospheric and/or oceanic circulation in the region during the late 1960s. The first suggestion seems least likely given the clustering of these points and the small likelihood that three fish in the small sample used in this study occurred in "unusual" habitats around the same time period. However, this possibility cannot be excluded.

Changes in atmospheric and oceanic circulation in the late 1960s may have affected the distribution of radiocarbon in juvenile blue grenadier habitat around Tasmania. Several factors might increase $\Delta^{14}C$ in this habitat including an increase in the mixed layer depth associated with increased wind stress and enhanced transport of radiocarbon from tropical and subtropical waters by the Leeuwin and East Australian Currents. Atmospheric and oceanographic data indicate that there were significant changes in mesoscale ocean features in southeast Australia during the late 1960s. These changes may be a manifestation of phenomena associated with an increase in the the number of days of zonal westerly winds over southeastern Australia. Data presented in Harris et al (1988) indicated that the greatest number of days of zonal westerly winds in a given year between 1945 and 1985 occurred in 1969. These atmospheric conditions were associated with increased ocean mixing and resulted in low maximum summer sea surface temperatures off eastern Tasmania and an increase in the frequency of "spring" bloom conditions.

Frequent zonal westerly winds in southeast Australia would increase the rate of downwelling of radiocarbon-rich surface waters and may also increase transport of radiocarbon from the tropics and subtropics. These effects might explain enhanced $\Delta^{14}C$ in some blue grenadier with otolith section age birth dates in the late 1960s. Further evidence of enhanced transport of radiocarbon to southeast Australia is provided by data on redfish (Fig. 9.1) (Kalish 1995b) where several specimens from the late 1960s show evidence of unusually high $\Delta^{14}C$. These fish were likely to occur along the continental shelf off New South Wales, Australia during their first year of life and may have been exposed to increased radiocarbon due to transport by the East Australian current. Juvenile redfish are unlikely to occur significantly below the surface mixed layer making changes in radiocarbon distribution with depth of little importance in altering $\Delta^{14}C$ in the juvenile otolith.

Numerous factors play a part in determining the temporal variation in radiocarbon levels at a given location. Estimates of radiocarbon levels in fish otoliths, in conjunction with estimates of age from those same structures, can be used to develop a more detailed understanding of carbon flux and ocean circulation. A prerequisite to the application of radiocarbon data from otoliths in studies of ocean circulation is the development of a validated age estimation procedure and the assignment of correct ages to samples. In the absence of a complete understanding of variations in ocean radiocarbon levels, however, it is still possible to determine fish age from fish otolith radiocarbon due to the dramatic alteration in ocean radiocarbon associated with atomic testing.

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Table 9.1a. *Macruronus novaezelandiae*. Fish and otolith data for blue grenadier collected off southeast Australia. Fish ages estimated from otolith sections by four readers (CAF1-3, ANU1 Central Ageing Facility and Australian National University, respectively). Values of $\Delta^{14}C$, mean age and mean birth date are given ±1 SD. $\Delta^{14}C$ birth dates and ages determined on basis of $\Delta^{14}C$ measured in blue grenadier otoliths in conjunction with calibration curve derived from *Pagrus auratus* and corrected based on depth of occurrence of juvenile M.novaezelandiae (see Results). Sample weights are weight of otolith material separated for individual analyses of stable carbon and radiocarbon and are representative of less than first year of otolith growth for an individual fish. (* indicates no attempt made to provide direct estimate of fish birth date and age on basis of $\Delta^{14}C$ alone (see Results); na not applicable; PDB Pee Dee Belemnite standard; AD year of birth)

| Sample no. | Collection date | Fish length (cm) | Otolith weight (g) | Sample weight (mg) | Age (birth date) | | | | Mean birth date (year) | Mean age (year) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | CAF1 | CAF2 | CAF3 | ANU1 | | |
| 8 | 3 Feb 93 | 85 | 0.6543 | 20.2 | 13(1980) | na | na | 11(1982) | 1981 | 12 |
| 31 | 19 Dec 92 | 74 | 0.4032 | 19.9 | 8(1984) | na | na | 8(1984) | 1984 | 8 |
| 72 | 7 May 79 | 91 | 0.7853 | 16.2 | 19(1960) | 17(1962) | 18(1961) | 17(1962) | 1961.3 | 17.75 |
| 79 | 7 May 79 | 87 | 0.5906 | 17.3 | 9(1970) | 10(1969) | 9(1970) | 11(1968) | 1969.3 | 9.75 |
| 86 | 7 May 79 | 96 | 0.7577 | 25.4 | 14(1965) | 15(1964) | 14(1965) | 14(1965) | 1964.8 | 14.25 |
| 87 | 7 May 79 | 92 | 0.6795 | 23.5 | 10(1969) | 11(1968) | 10(1969) | 9(1970) | 1969 | 10 |
| 88 | 15 Oct 93 | 51 | 0.1748 | 19.9 | 3(1990) | na | na | 3(1990) | 1990 | 3 |
| 130 | 7 May 79 | 94 | 0.7423 | 23.7 | 15(1964) | 15(1964) | 16(1963) | 14(1965) | 1964 | 15 |
| 182 | 8 Jan 80 | 93 | 0.6069 | 22 | 10(1970) | 10(1970) | 10(1970) | 11(1969) | 1969.8 | 10.3 |
| 183 | 8 Jan 80 | 89 | 0.6932 | 18.2 | 12(1968) | 13(1967) | 11(1969) | 12(1968) | 1968 | 12 |
| 185 | 8 Jan 80 | 95 | 0.6846 | 26.4 | 15(1965) | 15(1965) | 16(1964) | 12(1968) | 1965.5 | 14.5 |
| 191 | 8 Jan 80 | 91 | 0.7651 | 17.1 | 14(1966) | 13(1967) | 14(1966) | 13(1967) | 1966.5 | 13.5 |
| 196 | 19 Jan 80 | 112 | 0.8564 | 19.2 | na | na | na | 22(1958) | 1958 | 22 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 407 | 7 May 79 | 94 | 0.6779 | 25.1 | 12(1967) | 13(1966) | 13(1966) | 13(1966) | 1966.3 | 12.8 |
| 408 | 7 May 79 | 100 | 0.5599 | 17 | 11(1968) | 10(1969) | 11(1968) | 12(1967) | 1968 | 11 |

Table 9.1b. (continued)

| Sample No. | Laboratory accession no. | Collection date | Sample wt (mg) | $\delta^{13}C$ (‰,PDB) | $\Delta^{14}C$(‰) | Mean birth date | Mean age | $\Delta^{14}C$ birth date (AD) | $\Delta^{14}C$ age (yr) | $\Delta^{14}C$ age-mean age (yr) |
|---|---|---|---|---|---|---|---|---|---|---|
| 8 | RRL NZA1234 | 3 Feb 93 | 20.2 | -2.77 | 71.4±8.9 | 1981 | 12 | 1981* | 12* | 0.00* |
| 31 | RRL NZA1234 | 19 Dec 92 | 19.9 | -2.94 | 74.1±8.4 | 1984 | 8 | 1984* | 8* | 0.00* |
| 72 | RRL NZA1234 | 7 May 79 | 16.2 | -2.1 | -58.8±8.2 | 1961.3 | 17.75 | 1959 | 20 | 2.25 |
| 79 | RRL NZA1234 | 7 May 79 | 17.3 | -2.95 | 60.2±8.2 | 1969.3 | 9.75 | 1969 | 10 | 0.25 |
| 86 | RRL NZA1234 | 7 May 79 | 25.4 | -3.13 | -2.2±7.9 | 1964.8 | 14.25 | 1965 | 14 | -0.25 |
| 87 | RRL NZA1234 | 7 May 79 | 23.5 | -2.56 | 106.8±7.9 | 1969 | 10 | 1974 | 3 | -7.00 |
| 88 | RRL NZA1234 | 15 Oct 93 | 19.9 | -3.53 | 74.3±8.9 | 1990 | 3 | 1992 | 2 | -1.00 |
| 130 | RRL NZA1234 | 7 May 79 | 23.7 | -2.63 | -14.4±7 | 1964 | 15 | 1964 | 15 | -0.25 |
| 182 | RRL NZA1234 | 8 Jan 80 | 22 | -3.52 | 80.6±8.3 | 1969.8 | 10.3 | 1971 | 9 | -1.25 |
| 183 | RRL NZA1234 | 8 Jan 80 | 18.2 | -1.96 | 60.2±8.3 | 1968 | 12 | 1969 | 11 | -1.00 |
| 185 | RRL NZA1234 | 8 Jan 80 | 26.4 | -2.82 | 93.3±7.9 | 1965.5 | 14.5 | 1974 | 6 | -8.50 |
| 191 | RRL NZA1234 | 8 Jan 80 | 17.1 | -3.33 | -1.8±9.2 | 1966.5 | 13.5 | 1965 | 15 | 1.50 |
| 196 | RRL NZA1234 | 19 Jan 80 | 19.2 | -2.54 | -76.9±7.7 | 1958 | 22 | 1958* | 22* | 0.00* |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 407 | RRL NZA1234 | 7 May 79 | 25.1 | -2.13 | 17.7±8.6 | 1966.3 | 12.8 | 1966 | 13 | 0.25 |
| 408 | RRL NZA1234 | 7 May 79 | 17 | -2.59 | 99.9±8.1 | 1968 | 11 | 1974 | 5 | -6.00 |

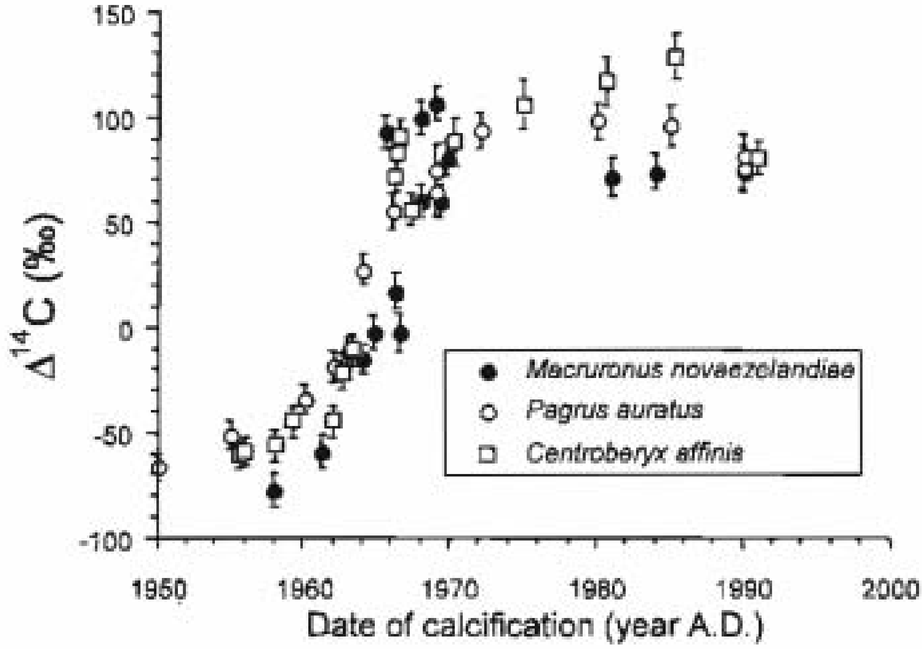


Figure 9.1. *Macruronus novaezelandiae*, *Pagrus auratus* and *Centroberyx affinis*. $\Delta^{14}C$ of otolith cores plotted against birth date determined from otolith thin-sections for M. novaezelandiae and C. affinis (Kalish 1995b) and against true birth date for P. auratus (Kalish 1993). All $\Delta^{14}C$ values based on otolith material deposited over time period less than first year of life. Means ±1 SD.

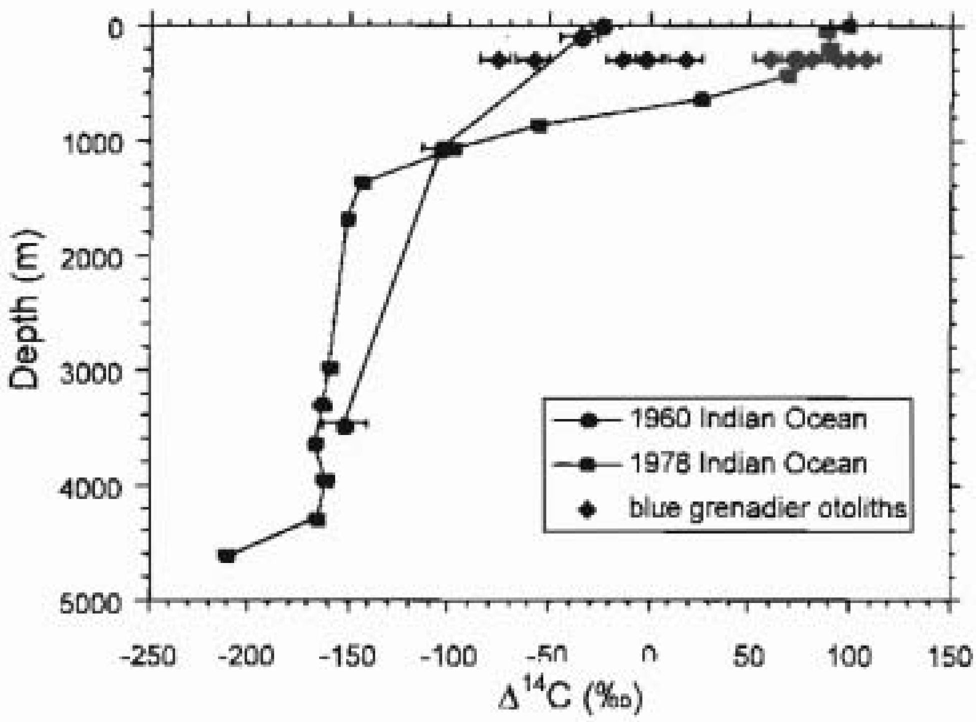


Figure 9.2. Profiles of $\Delta^{14}C$ in seawater DIC (dissolved inorganic carbon) determined in Indian Ocean on December 1960 at latitude 36°18'S longitude 98°41'E (Bien et al. 1965) and again in February 1978 at latitutde 39°57'S longitude 109°58'E (Stuiver and Ostlund 1983). Increase in $\Delta^{14}C$ between 1960 and 1978 at depths above ~1050 m is due to penetration of bomb radiocarbon. Birth dates determined on basis of $\Delta^{14}C$ measured in *Macruronus novaezelandiae* otoliths in conjunction with a calibration curve derived from *Pagrus auratus* and a correction for depth are included to show agreement between radiocarbon data derived directly from seawater DIC and otolith proxy.

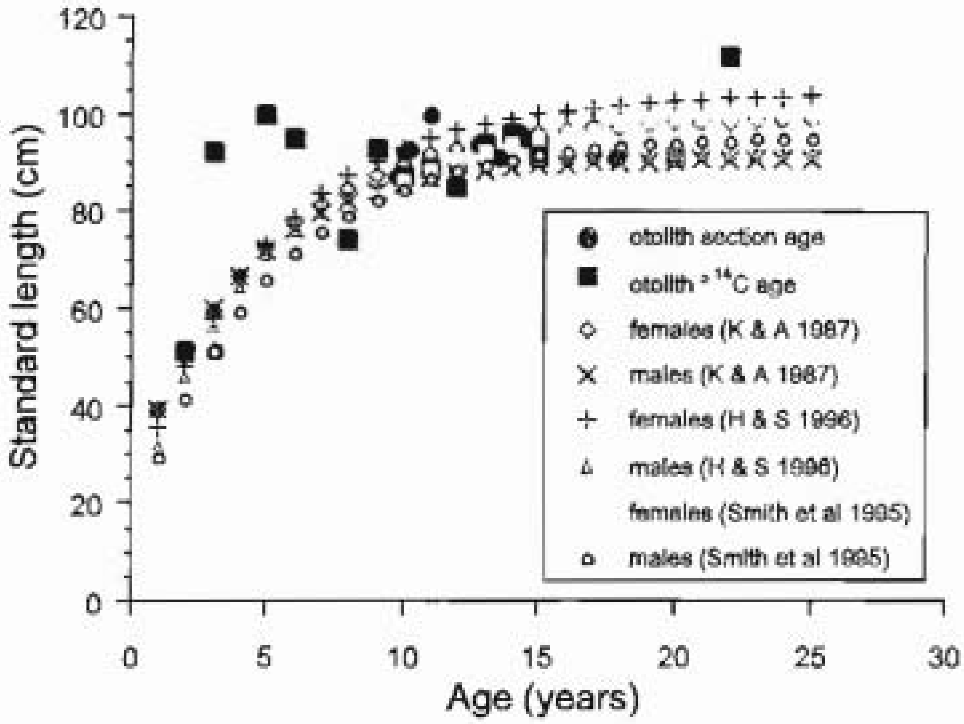


Figure 9.3. *Macruronus novaezelandiae*. Age estimates for individual blue grenadiers derived from otolith $\Delta^{14}C$ plotted with von Bertalanffy growth functions estimated for female and male M. novaezelandiae from several studies on presumed annual increments in otoliths (Kenchington and Augustine 1987; Smith et al. 1995; Horn and Sullivan 1996).

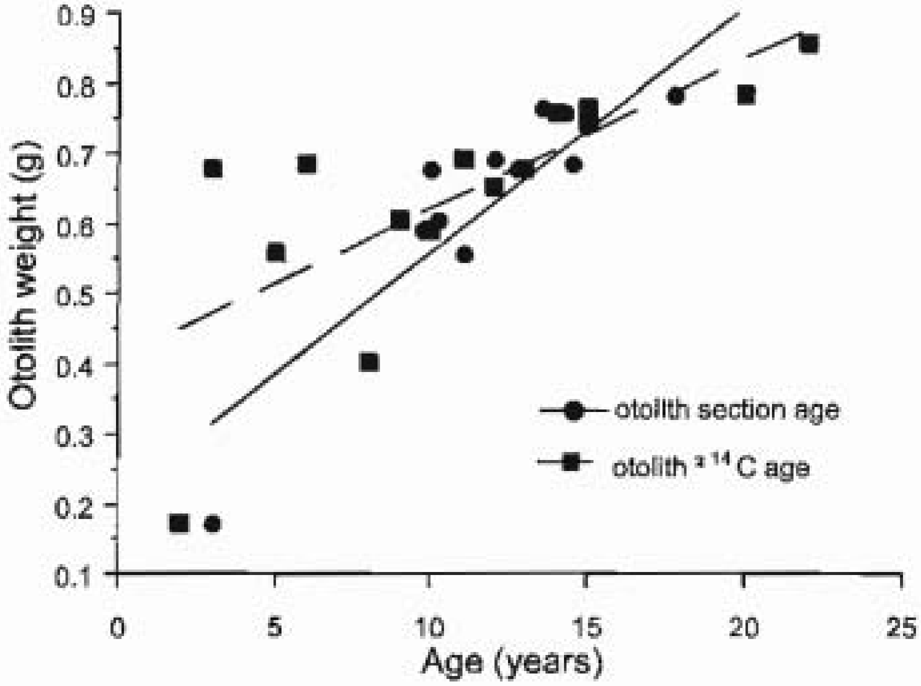


Figure 9.4. *Macruronus novaezelandiae*. Age estimates derived from otolith sections and $\Delta^{14}C$ for individual blue grenadiers plotted against otolith weight. Lines determined by linear regression indicate that otolith weight is fair proxy for age in this species. Several otolith $\Delta^{14}C$ age estimates of ~5 yr were obtained from fish with relatively heavy otoliths suggesting that these age estimates are incorrect

# Chapter 10 Determination of school shark age based on analysis of radiocarbon in vertebral collagen

John Kalish and Justine Johnston

**Summary**

Radiocarbon measured in school shark vertebrae provides strong evidence that age estimates determined from counts of presumed annual increments in stained vertebrae are gross underestimates of the true fish age. This result is reinforced by a comparison between the two independent estimates of fish age and vertebra weight. Vertebral growth is likely to be dramatically reduced or cease altogether when fish reach asymptotic length; as a result, shark vertebrae may not be well suited to estimation of age for larger and older individuals. Measurements of pre-bomb levels of radiocarbon in the earliest formed segments of school shark vertebrae provides evidence that elasmobranch vertebral tissue may be subject to limited reworking and, therefore, would be suitable for studies of temporal changes in vertebral composition as proxies for physiological and environmental changes experienced during the life of the shark.

## Introduction

The school shark (*Galeorhinus galeus*) is a demersal shark species that occurs on the continental shelves and upper continental slope to depths of 550 m of Australia, New Zealand, North and South America, Europe and Africa (Last and Stevens 1994). The Australian fishery for school shark has been in existence for more than 70 years and is an important fishery off south eastern Australia with catches exceeding 2000 t in some years. School shark has also supported fisheries off Africa, Europe, New Zealand, North America and South America.

As early as the mid-1980s the fishery for school shark was assessed as overfished. Efforts to reduce catch have been made in recent years including implementation of management arrangements based on a Total Allowable Catch (TAC) and the allocation of Individual Transferable Quotas (ITQs) (McLoughlin et al. 2000). Stock assessment of school shark status is a high priority for research and accurate estimation of age and growth is an important element of these assessments.

Age estimates for elasmobranchs are rarely validated despite the importance of age validation for accurate stock assessments (Beamish and McFarlane 1983). Validation of the routine method of age estimation for school shark has not been achieved and there is no accepted method of age estimation for this species. The objective of this study is to determine if age estimates from counts of presumed annual increments in vertebrae provide accurate estimates of school shark age. The study uses the bomb radiocarbon chronometer (Kalish 1995a) for validation and is the first application of the method to an elasmobranch species.

**Materials and methods**

School shark vertebrae were provided by the Marine and Freshwater Resources Institute (MAFRI). Vertebrae provided were all from fish caught in the Great Australian Bight. Samples included vertebrae collected from routine sampling and age estimation programs for shark during the 1990s, and three individuals that were tag-recaptures from tagging work carried out between 1973-1976 (Table 10.1). Vertebral samples collected included from 5 to 6 post cranial vertebrae and 1 or 2 of these vertebrae are typically selected for age estimation based on methods described in Walker and Moulton (1991) and Moulton et al (1992). Briefly, the age estimation method involves staining of whole vertebrae with alizarin red stain, washing and immediate viewing under a stereo microscope at low magnification using incident light. Ages are estimated based on the number of presumed annual increments with an increment comprised of one light stained band and one dark stained band.

Selected regions of vertebrae from school shark were isolated by drilling and grinding using a dental-type drill. Vertebrae from newly born pups were used as a guide during the process of sample preparation. The samples were cut and ground until the material remaining was representative of the first year of life and this material was used in subsequent sample preparation.

After isolation of the selected portion of the vertebra, samples were subject to pre-treatment to isolate the collagen fraction of the bone. Unlike the research on swordfish vertebrae (Kalish, this study), which analysed both collagen and carbonate fractions of vertebrae, this research only analysed collagen fractions. Samples were soaked in 0.1M HCl to isolate the collagen fraction of the bone. The resulting gel was dissolved in 0.5M HCl, transferred to a double tube, frozen and vacuum oven dried. This material was rinsed in reverse osmosis water, frozen, vacuum oven dried and combusted. The resultant gas was reacted with catalyst to form graphite. Further preparation and handling of targets is similar to procedures described in Kalish (1995b). Collagen extractions were carried out at the Australian National University and at the Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences (New Zealand). Samples were analysed for radiocarbon by accelerator mass spectrometry at the Rafter Radiocarbon Laboratory and are reported here as $\Delta^{14}C$ (‰).

**Results**

The results of age estimates based on counts of presumed annual increments in vertebrae (MAFRI age estimates) and the radiocarbon data are presented in Table 10.1. $\Delta^{14}C$ (‰) measured in the collagen fractions from school shark vertebrae ranged from −78.9‰ to 165.7‰ for vertebrae that were assigned ages ranging from 4 to 14 years based on presumed annual increments. The MAFRI age estimates are plotted against the $\Delta^{14}C$ (‰) measured in the same vertebrae (Fig. 10.1). A radiocarbon time series, derived from New Zealand snapper (*Pagrus auratus*), and used as a calibration for radiocarbon variation in the South Western Pacific Ocean (Kalish 1993) is plotted on the same graph (Fig. 10.1). The majority of the MAFRI age estimates for school shark is far removed from the calibration time series and provide evidence for large errors in these age estimates.

Birth date estimates for individual school shark were determined directly from the vertebral radiocarbon data for comparison with the MAFRI age estimates. A third order polynomial function that describes the bomb-related increase in $\Delta^{14}C$ in the south western Pacific Ocean was established from the *Pagrus auratus* otolith data (Kalish 1993). A similar model was developed for comparison with age estimates from *Centroberyx affinis* otoliths in an earlier study (Kalish 1995b). Pre-bomb $\Delta^{14}C$ measured in *Pagrus auratus* otoliths from earlier than 1955 or after 1980 were not used in the estimation of the function. The function determined from these data (Fig. 10.2) describes a time series of $\Delta^{14}C$ that is similar to that modelled for $\Delta^{14}C$ in the southern hemisphere temperate Pacific Ocean by Broecker et al. (1985), however, this new model is likely to be a better representation of the true variation due to the nature of the data source. The resulting function was used to estimate the birth dates of the school shark on the basis of the $\Delta^{14}C$ measured in the earliest formed regions of the individual vertebrae. Birth dates were rounded to the nearest year. $\Delta^{14}C$ data measured in the vertebrae of 11 school shark are plotted with $\Delta^{14}C$ calibration for the south western Pacific Ocean in Fig. 10.3.

It was not possible to make definitive estimates of age based on vertebral $\Delta^{14}C$ for the samples that had radiocarbon levels that fell beyond the range of the model. The birth date estimate for the earliest born school shark in this study only provides an indication of the latest reasonable birth date (i.e. the fish could be older). Because one individual (sample no. 212) with a $\Delta^{14}C$ of –78.9‰ was clearly spawned prior to significant atmospheric testing of nuclear weapons, the segment of the vertebra that was analysed did not contain any detectable bomb-derived radiocarbon. Three vertebrae (sample nos. BRN3429, BRN3433, BRN5396) had $\Delta^{14}C$ levels that clearly fall above the model, however, these points are still within the 95% confidence limits of the model.

Birth dates for school shark that were calculated on the basis of vertebral radiocarbon are plotted with the age estimates from the counting of presumed annual increments in vertebrae (Fig. 10.3). A comparison of these age estimates provides evidence that the MAFRI age estimates dramatically underestimate the age of school shark by up to 18 years (Table 10.1 and Fig. 10.4). In all cases, the MAFRI age estimate is smaller than the age estimate from vertebral radiocarbon.

Vertebral weight was plotted against age estimated from radiocarbon and age based on presumed annual increments (Figures 5 and 6) due to the potential of vertebral weight to provide an independent estimate of relative age. There is no evidence of any relationship between vertebral weight and the MAFRI age estimate, whereas the radiocarbon model age estimate does show a positive correlation with vertebral weight (Fig. 10.5). This relationship is improved further by excluding those vertebral samples that clearly showed pre-bomb $\Delta^{14}C$ levels (Fig. 10.6). As indicated earlier, the age estimates for these individuals is representative of a minimum age and these fish may be older.

**Discussion**

Radiocarbon measured in school shark vertebrae provides strong evidence that age estimates determined from counts of presumed annual increments in stained vertebrae are gross underestimates of the true fish age. This result is reinforced by a comparison between the two independent estimates of fish age and vertebra weight.

Vertebral growth is likely to be dramatically reduced or cease altogether when fish reach asymptotic length; as a result, shark vertebrae may not be well suited to estimation of age for larger and older individuals. Seven out of nine age estimates based on annuli in vertebrae were between 12-15 years for sharks between 1510 – 1615 mm (TL), around the asymptotic length for school shark (Olsen 1984, Moulton et al.1992). These ages are well below the maximum known age for a school shark of about 36 years for a tagged male recaptured at a length of 1520 mm (TL) after 35.4 years at liberty (Moulton et al. 1989). Comparison of the MAFRI age estimates with radiocarbon based estimates provides evidence that the accuracy of the MAFRI estimate decreases with shark age. These results indicate that further research is required on estimates of age or, alternatively, an different method of age estimation should be developed for this shark species.

The measurements of $\delta^{13}C$ and $\Delta^{14}C$ in vertebral collagen in school shark, an elasmobranch, can be compared with those made on the vertebrae of swordfish (*Xiphias gladius*) (Kalish, this study), a teleost. In both cases, the $\delta^{13}C$ data provide a clear indication that the majority of carbon in the collagen of vertebrae is derived from metabolic sources. The measurements of $\Delta^{14}C$ in the school shark provides excellent evidence of minimal reworking of vertebral tissues due to the measurement of pre-bomb $\Delta^{14}C$ levels in fish with ages estimated to be in excess of 20 years. Relative low $\Delta^{14}C$ levels were measured in individuals with ages in excess of 25 years.

Potential reworking or remodelling of vertebral tissues is a major impediment to the use of bone in age and growth studies. Clear evidence that acellular otoliths are not reworked is one of the principal characteristics of these structures that make them suited to studies of age and growth. Similar evidence is lacking in relation to vertebrae; however, this study provides some evidence that elasmobranch vertebral tissue may be subject to limited reworking. Further research is required before a definitive conclusion can be made in relation to this issue. In contrast to these findings, the study of swordfish vertebrae provided some evidence that these structures are reworked in some way, specifically through the investment of increased quantities of mineral in the vertebrae. This increased inclusion of mineral in the vertebrae would increase the strength of the structure as the fish grows larger and exerts greater forces on the vertebrae.

**Acknowledgements**

We thank Terry Walker and Russell Hudson (MAFRI) for providing the school shark vertebrae and estimates of age based on increment counts. John Head (ANU) extracted collagen from five of the vertebral samples and provided advice on the analysis of radiocarbon in bone and we thank him for his long standing cooperation with our radiocarbon research. The staff of the Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences (New Zealand) must be thanked for the continued cooperation and the extraction, preparation and analysis of samples for radiocarbon. In particular, we thank Rodger Sparks and Dawn Chambers. This research was funded by a grant from the Fisheries Research and Development Corporation (Australia).

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Table 10.1. Shark, vertebra and radiocarbon data for school shark (*Galeorhinus galeus*). Samples collected by the Marine and Freshwater Resources Institute (MAFRI), Victoria from the Great Australian Bight.

| Sample no. | Laboratory accession no. | Collection date | Sex | Length TL (mm) | Vertebra weight (g) | Sample weight (mg) | $\delta^{13}C$ (‰, PDB) | $\Delta^{14}C$ (‰) | MAFRI age (years) | MAFRI birth date (year) | Model age (years) | Model birth date (year) | Model - MAFRI age (years) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BRN3429 | NZA8840 | 10/11/92 | F | 1540 | 1.9424 | 102.9 | -15.1 | 164.3±8.1 | 13.86 | 1978.14 | 14 | 1978 | 0.14 |
| BRN3431 | NZA8841 | 11/11/92 | F | 1510 | 1.7065 | 96.2 | -18.1 | 41.3±7.4 | 12.78 | 1979.22 | 26 | 1966 | 13.22 |
| BRN3432 | NZA8842 | 12/4/92 | F | 1600 | 2.1455 | 102.7 | -13.9 | -4.3±7 | 14.93 | 1977.07 | 30 | 1962 | 15.07 |
| BRN3433 | NZA8843 | 12/4/92 | F | 1520 | 1.5858 | 105.6 | -15.1 | 154.7±8.1 | 12.93 | 1979.07 | 15 | 1977 | 2.07 |
| BRN5396 | NZA8844 | 23/9/93 | F | 1595 | 1.436 | 74.8 | -14.5 | 165.7±8.2 | 13.01 | 1979.99 | 15 | 1978 | 1.99 |
| BRN5796 | NZA8845 | 19/9/93 | F | 1582 | 1.6688 | 92 | -14.9 | 131.3±8.7 | 12.72 | 1980.28 | 18 | 1975 | 5.28 |
| BRN5797 | NZA8863 | 1/6/93 | F | 1615 | 1.7634 | 96.3 | -14 | 97.8±7.6 | 14.73 | 1978.27 | 21 | 1972 | 6.27 |
| 212 | NZA8017 | 19/9/73 | M | 1568 | 1.87 | 213.2 | -14.2 | -78.9±7.7 | 9.71 | 1963.29 | 23 | 1950 | 13.29 |
| BRN633 | NZA8018 | 23/6/92 | M | 1141 | 1.866 | 186.6 | -17.5 | 91.5±9.1 | 3.48 | 1988.52 | 21 | 1971 | 17.52 |
| 1346 | NZA8019 | 14/11/75 | F | 1554 | 2.3 | 207.6 | -14.5 | -58.7±8.2 | no data | no data | 20 | 1955 | no data |
| B2808 | NZA8020 | 11/4/78 | F | 1575 | 1.523 | 207.9 | -17.1 | -56.9±9.2 | no data | no data | 23 | 1955 | no data |

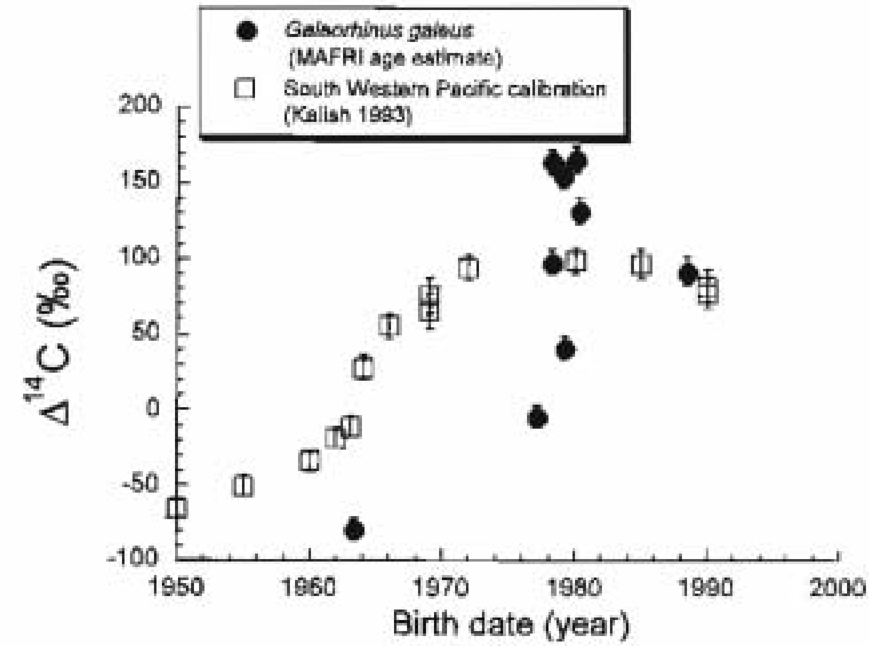


Fig. 10.1. Estimates of *Galeorhinus galeus* age determined from counts of presumed annual increments in vertebrae (MAFRI age estimates) compared with a time series of radiocarbon in the South Western Pacific Ocean determined from otolith cores of *Pagrus auratus* (Kalish 1993).

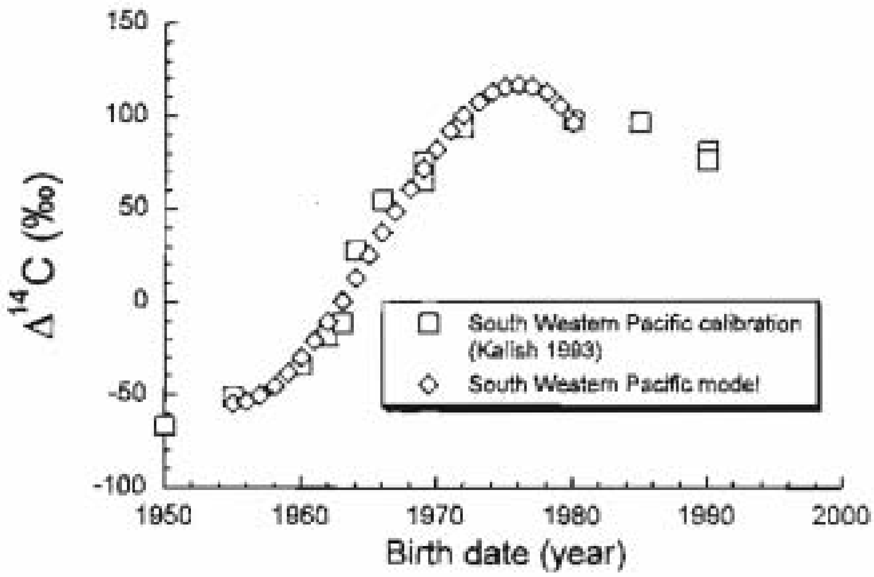


Fig. 10.2. Time series of radiocarbon in the South Western Pacific Ocean determined from otolith cores of *Pagrus auratus* (Kalish 1993) and a cubic polynomial model of the time series (South Western Pacific model). The model is truncated at the years 1955 and 1980.

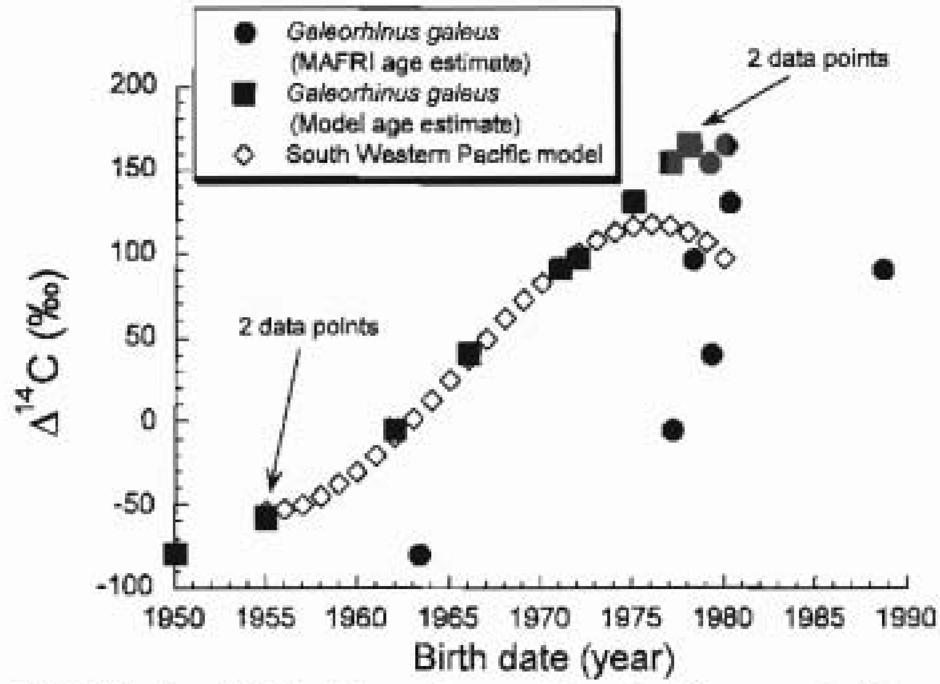


Fig. 10.3. Estimates of *Galeorhinus galeus* age determined from counts of presumed annual increments in vertebrae (MAFRI age estimate) compared with estimates of *Galeorhinus galeus* age determined from the radiocarbon measurements made in vertebra cores (Model age estimate). MAFRI age estimates were not available for several of the samples analysed for radiocarbon and only the data point showing the age estimated from vertebral radiocarbon will appear on the graph. The South Western Pacific model used to estimate these ages from the radiocarbon data is shown.

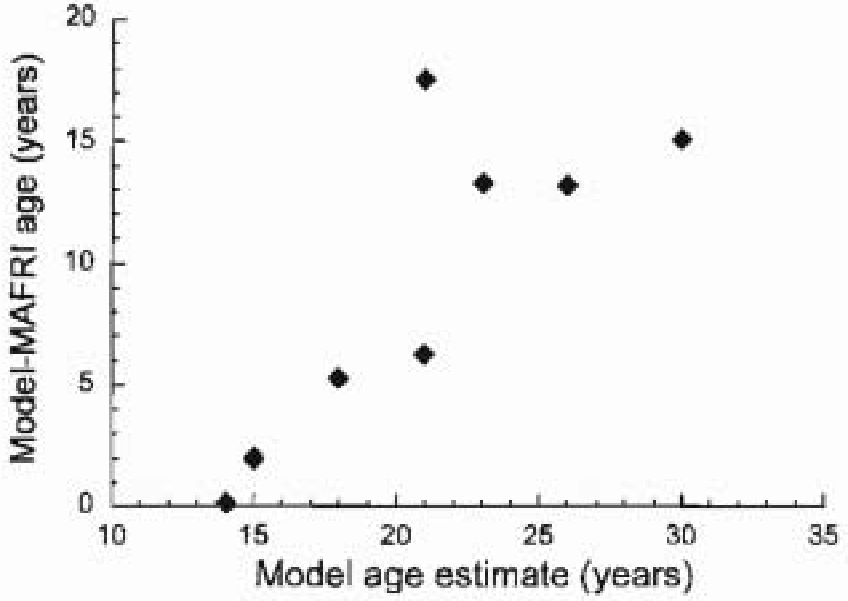


Fig. 10.4. Difference between *Galeorhinus galeus* age estimates derived from measurements of radiocarbon in vertebrae (Model age estimates) and age estimates determined from counts of presumed annual increments in vertebrae (MAFRI age estimate).

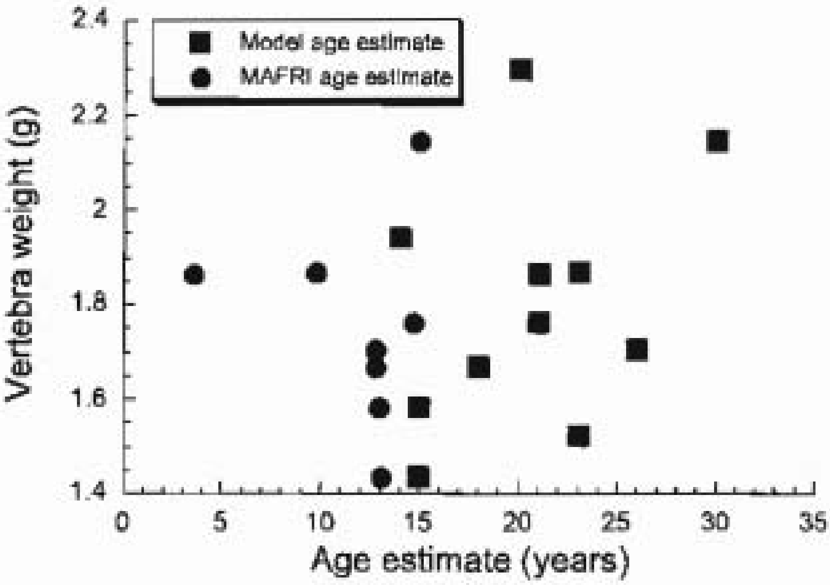


Fig. 10.5. Relationship between *Galeorhinus galeus* vertebra weight and two independent estimates of age (Model age estimate and MAFRI age estimate).

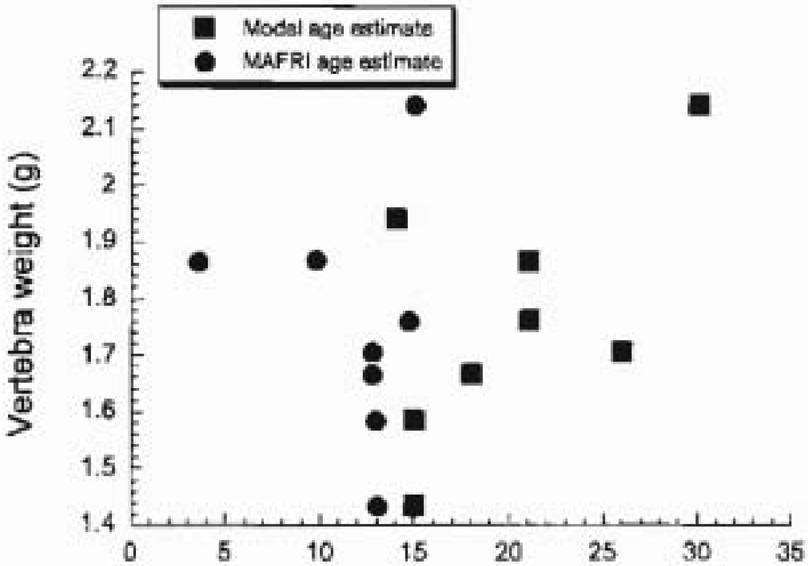


Fig. 10.6. Relationship between *Galeorhinus galeus* vertebra weight and two independent estimates of age (Model age estimate and MAFRI age estimate) with individuals excluded that had $\Delta^{14}C$ indicative of pre-bomb radiocarbon levels. Age estimates derived from radiocarbon for these fish would be minimum ages and, therefore, may bias any relationship between vertebra weight and age.

# Chapter 11 Validation of pink ling (*Genypterus blacodes*) age based on otolith radiocarbon

John Kalish, Justine Johnston, David Smith, Sandy Morison and Simon Robertson

**Summary**

Measurements of natural and bomb-produced radiocarbon in the otoliths of pink ling (*Genypterus blacodes*) indicate that estimates of age based on otolith sections are accurate. The relationships between otolith section based age estimates and radicoarbon in otolith cores, and the radiocarbon calibration curve based on *Pagrus auratus* (Kalish 1993) was excellent and indicates that otolith based estimates are likely to very accurate. Furthermore, there was good agreement between otolith readers both within and among laboratories for those samples analysed for radiocarbon. This result supports the application of the age and growth model for ling presented in Smith and Tilzey (1995). The number of radiocarbon analyses, however, was inadequate to confirm the existence of a difference in growth rate between males and females.

**Introduction**

Pink ling (*Genypterus blacodes*) occur on the continental shelf and upper slope waters of southern Australia. The species is also found off New Zealand and South America and a similar, if not identical species Genypterus capensis occurs off South Africa. Age and growth studies have been carried out on these species in Australia (Withell and Wankowski 1989, Smith and Tilzey 1995), New Zealand (Horn 1993) and South Africa (Payne 1985). Each of these age and growth studies was based on interpretation of zones in otoliths; however, only Horn (1993) included any attempt to validate the age estimation method. The marginal increment analysis presented in Horn (1993) indicated that it was often difficult to define boundaries between opaque and translucent zones and that variability within samples was high. Data in Horn (1993) indicate that results of the marginal increment analysis do not present a conclusive validation. Furthermore, marginal increment analysis cannot provide age estimates of individual fish, data that are best-suited for validation studies.

This study uses the bomb radiocarbon chronometer (Kalish 1993, 1995a, 1995b) to determine the age of individual ling and validate the otolith section method of age estimation for this species.

**Materials and Methods**

Sample preparation procedures are similar to those described in Kalish (1993, 1995b). The otoliths of pink ling are relatively large and this simplified the process of otolith sculpting. to produce a sample of about 15 mg for analysis of radiocarbon by accelerator mass spectrometry (AMS). Analyses were carried out by the Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences (New Zealand). Details of sample pre-treatment and radiocarbon analysis are as reported in previous work (Kalish 1995, Kalish et al. 1995) and, results are reported as $\Delta^{14}C$ (Stuiver and Polach 1977).

The second sagitta from each otolith pair was prepared for otolith reading. Thin sections were prepared by the Central Ageing Facility (Queenscliff, Victoria) and read by four independent readers at Australian National University (designated ANU 1 and ANU 2) and the Central Ageing Facility (designated CAF 1 and CAF 2).

**Results and Discussion**

Otolith cores from eleven pink ling were analysed for radiocarbon (Table 11.1). The results of the radiocarbon analyses were plotted versus the mean birth date estimated by four independent otolith readers and with data from snapper (*Pagrus auratus*) (Kalish 1993), redfish (*Centroberyx affinis*) (Kalish 1995b), blue grenadier (*Macruronus novaezelandiae*) (Kalish et al. 1996a), and southern bluefin tuna (*Thunnus maccoyii*) (Kalish et al. 1996b) (Fig. 11.1). On the basis of the mean birth dates estimated by the four otolith readers, the radiocarbon values from ling otoliths fall within the curve that defines the bomb radiocarbon increase in surface waters of the southwest Pacific. This indicates that mean ages estimated by the four otolith readers are close to the true fish age. Furthermore, differences in age estimates were relatively small among the readers both within and among laboratories.

The otolith section age estimates were plotted with von Bertalanffy models used to describe ling growth in Australia and New Zealand (Fig. 11.2). The Australian growth models were derived from fish captured in Bass Strait in 1984 and 1985 (Withell and Wankowski 1989) and fish captured in Western Bass Strait 1987-89 (Smith and Tilzey 1995), while the New Zealand model selected for comparison was based on samples of ling collected from the Chatham Rise between January 1991 and January 1992 (Horn 1993). The samples used for radiocarbon analysis were among those used in the study of ling age and growth included in Smith and Tilzey (1995) and, therefore, are representative of age estimates obtained in that study.

The relationship between otolith weight and fish age indicates that otolith weight is a fair estimator of age for the samples analysed for radiocarbon (Fig. 11.3).

**Discussion**

Radiocarbon data from pink ling otoliths provide a excellent validation of the otolith section method of age estimation for the species and also provide insight into ocean circulation in the southwest Pacific Ocean. $\Delta^{14}C$ data from ling indicate that peak values reached about 130‰ in the late 1970s, similar to $\Delta^{14}C$ measured in otolith cores from redfish hatched between about 1975 and 1985 (Kalish 1995b). During this period, peak $\Delta^{14}C$ measured in snapper otoliths was 98.4±8.8‰ in 1980, significantly lower than that measured in both ling and redfish. Ling, redfish, and snapper otoliths used for radiocarbon measurements were obtained from fish collected around 39°S, 35°S, and 39°S, respectively. Snapper were collected from off the eastern coast of the North Island, New Zealand, whereas both the ling and redfish were collected off the coast of south eastern Australia. The results suggest that there are distinct differences in radiocarbon flux in these two regions after about 1975 with greater input of radiocarbon to Pacific Ocean off south eastern Australia. Furthermore, the similarity of radiocarbon records from the cores of ling and redfish otoliths suggests similar habitats for these two species during the first year of life.

**Conclusions**

This study validates the otolith section method of age estimation for ling between 8 and 26 years of age on the basis of $\Delta^{14}C$ values in otolith material deposited early in life. Birth dates assigned to individual fish on the basis of otolith sections agree with birth dates predicted from $\Delta^{14}C$ values measured in the otoliths. Although the age estimation method for ling less than 8 years of age was not validated directly, it is concluded that the counts of annual increments formed at younger ages must be correct in order to arrive at the correct age estimates for older fish. Furthermore, the results indicate that age estimates from ling otoliths are likely to be very accurate (±2 years). This study supports the application of the age and growth model for ling presented in Smith and Tilzey (1995). The number of radiocarbon analyses, however, was inadequate to confirm the existence of a difference in growth rate between males and females.

**Acknowledgements**
We thank members of the Central Ageing Facility for their cooperation with this research and the Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences (New Zealand) for carrying out the radiocarbon analyses.

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Withell, A.F. and Wankowski, J.W.J. 1989. Age and growth estimates for pink ling, *Genypterus blacodes* (Schneider), and gemfish, Rexea solandri (Cuvier), from eastern Bass Strait, Australia. Australian Journal of Marine and Freshwater Research 40: 215-216.

**Table 11.1.** Fish and otolith data from *Genypterus blacodes* collected from Western Bass Strait between February 1987 and April 1989. Fish ages were estimated by four readers; two from the CAF and two from ANU. All age and birth date estimates presented in the table are determined from counts of presumed annual increments in otolith thin sections. Values of $\Delta^{14}C$, mean age, and mean birthdate are reported with ±1 standard deviation. Sample weights indicate the weight of otolith material separated for individual analyses of stable carbon and radiocarbon and are representative of less than the first year of otolith growth for an individual fish.

| Sample No. | Laboratory and laboratory accession number | Sex | Fish length (cm) | Otolith wt. (g) | Sample wt. (mg) | $\delta^{13}C$ (‰, PDB) | $\Delta^{14}C$ (‰) | Reader (Age,Birth date) | | | | Mean age (years) | Mean Birth date (years, A.D.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | CAF 1 | CAF 2 | ANU 1 | ANU | | |
| PL1 | NZA5527 | M | 82 | 0.375 | 15.1 | -2.5 | 124.9±9.4 | 9(1980) | 9(1980) | 9(1980) | 11(197 | 9.5±1.0 | 1979.5±1.0 |
| PL2 | NZA5528 | F | 83 | 0.422 | 18.9 | -2.8 | 120.3±9.6 | 11(1978) | 10(1979) | 11(1978) | 12(197 | 11±0.8 | 1978±0.8 |
| PL5 | NZA5529 | M | 88 | 0.427 | 18.4 | -2.4 | 129.6±10.6 | 8(1979) | 8(1979) | 8(1979) | 9(1978 | 8.3±0.5 | 1978.8±0.5 |
| PL10 | NZA 5553 | F | 88 | 0.5 | 16.5 | -2.6 | 118.6±12.9 | 12(1976) | 11(1977) | 11(1977) | 13(197 | 11.8±0.9 | 1976.6±0.9 |
| PL14 | NZA 5554 | F | 91 | 0.663 | 15.1 | -2.6 | 79.2±10.6 | 19(1970) | 18(1971) | 18(1971) | 18(197 | 18.3±0.5 | 1970.8±0.5 |
| PL15 | NZA 5555 | F | 99 | 0.718 | 16.9 | -2.5 | -3±11 | 25(1964) | 24(1965) | 25(1964) | 24(196 | 24.5±0.6 | 1964.5±0.6 |
| PL19 | NZA 5556 | M | 90 | 0.574 | 15.6 | -2.6 | 94.8±13.1 | 13(1974) | 13(1974) | 13(1974) | 14(197 | 13.3±0.5 | 1973.8±0.5 |
| PL24 | NZA 5557 | M | 103 | 0.669 | 14.2 | -1.8 | 101.5±11.2 | 18(1971) | 18(1971) | 18(1971) | 18(197 | 18±0 | 1971±0 |
| PL25 | NZA 5558 | F | 100 | 0.782 | 19.2 | -4.2 | 36.9±11.9 | 21(1966) | 22(1965) | 22(1965) | 19(196 | 20.5±1.3 | 1966.5±1.3 |
| PL27 | NZA 5559 | F | 101 | 0.776 | 16.6 | -2.5 | -46.4±9.9 | 28(1959) | 28(1959) | 26(1961) | 23(196 | 26.3±2.4 | 1960.8±2.4 |
| PL28 | NZA 5560 | M | 108 | 0.997 | 13.9 | -2.4 | -41±15.4 | 25(1962) | 25(1962) | 25(1962) | 24(196 | 24.8±0.5 | 1962.3±0.5 |

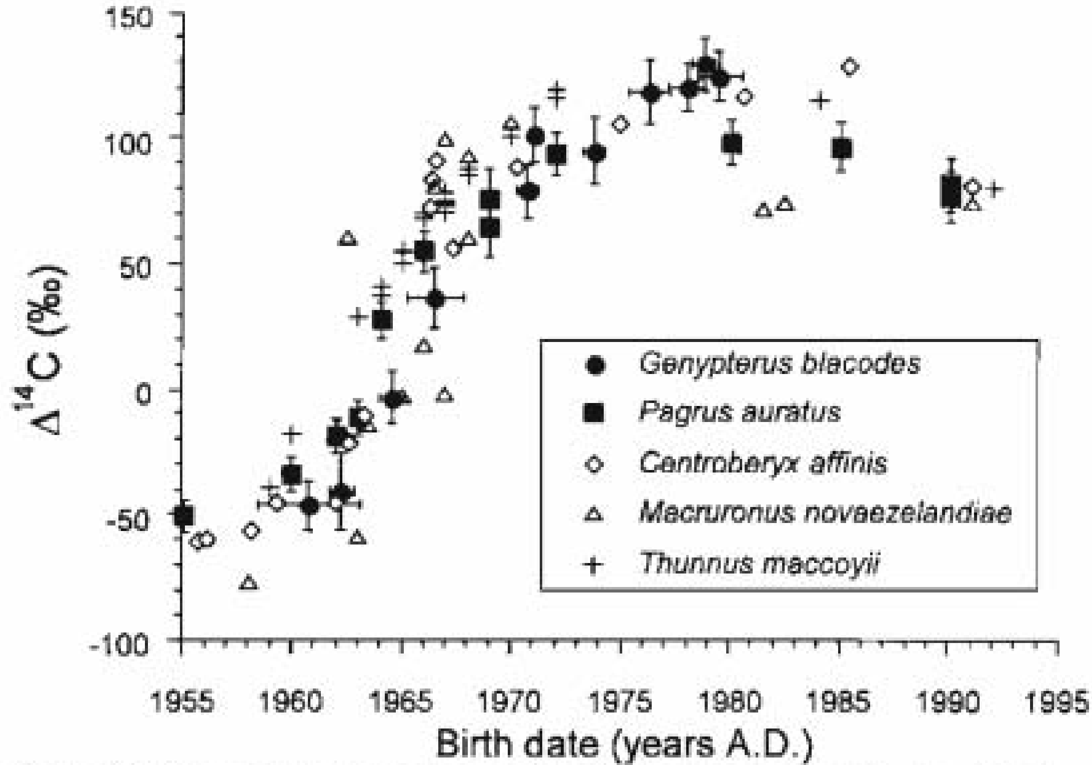


Figure 11.1. $\Delta^{14}$C data from five fish species. Data for *Genypterus blacodes* are plotted against the mean birth date estimated by four independent readers from Australian National University (ANU) and the Central Ageing Facility (CAF). Data from *Pagrus auratus* are plotted against the true birth date for those fish (Kalish 1993). Both *Centroberyx affinis* and Macruronous novaezelandiae $\Delta^{14}$C values are plotted versus mean ages estimated by multiple readers from the CAF and ANU (Kalish 1995, Kalish and Johnston 1995). $\Delta^{14}$C values from *Thunnus maccoyii* otoliths are plotted versus the birth date determined from a model describing variation in $\Delta^{14}$C levels over time in the eastern Indian Ocean (Kalish et al. submitted). Error bars plotted with the *G. blacodes* and P. auratus data are one standard deviation.

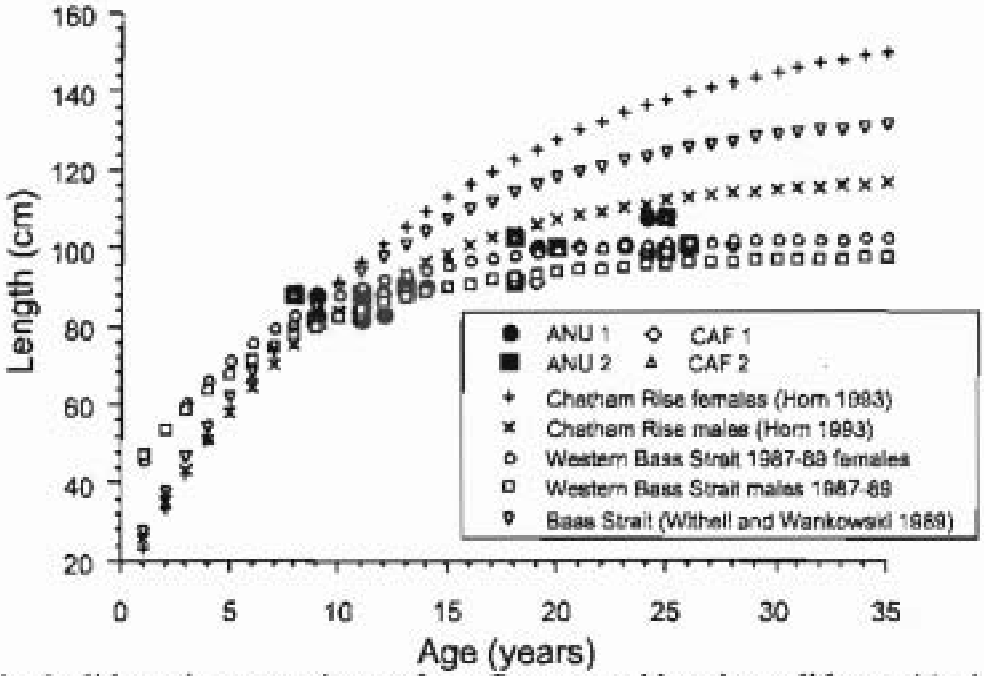


Figure 11.2. Otolith section age estimates from *Genypterus blacodes* otoliths used in this study plotted versus total length. The age versus length data are plotted with several von Bertalanffy growth models for ling. The model for Western Bass Strait ling was presented in Smith and Tilzey (1995).

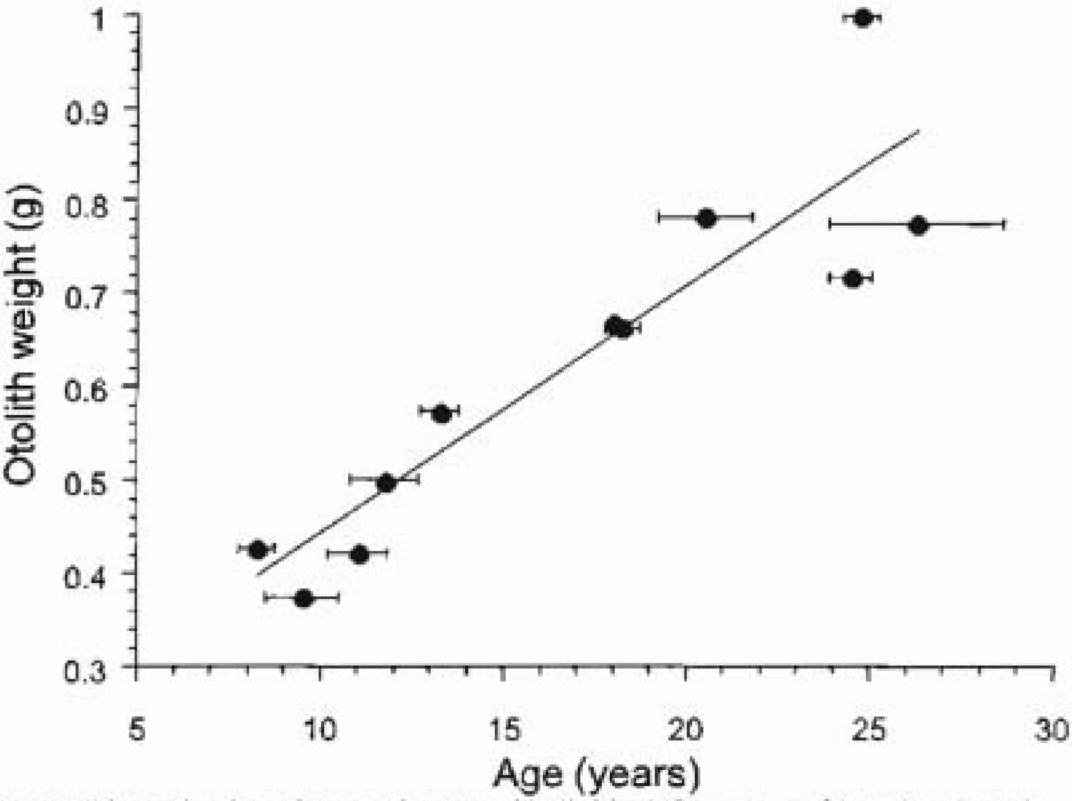


Figure 11.3. Otolith section based age estimates of individual *Genypterus blacodes* plotted against otolith weight. These data are from the otoliths used for the radiocarbon analyses. The line described by liner regression is: otolith weight = 0.18035 + 0.026447.age; (r2 = 0.83).

# Chapter 12 Application of the bomb radiocarbon chronometer to the validation of king dory (*Cyttus traversi*) age

John Kalish and Justine Johnston

**Summary**

Measurements of natural and bomb-produced radiocarbon in the otoliths of king dory (*Cyttus traversi*) indicate that estimates of age based on otolith sections are accurate. In addition, radiocarbon data from king dory otolith cores suggest that this species is likely to occur between about 45°S and 50°S latitude during the first year of life. King dory otoliths are relatively small and this study involved refinement of methods for the preparation of otolith calcium carbonate for radiocarbon analysis. The range of prepared otolith sample weights was 3.8 to 5.7 mg (mean=4.5±0.52 mg). Graphite targets prepared for radiocarbon analysis from these otolith samples contained less than 0.5 mg of carbon. This represents a significant advance in the use of the bomb radiocarbon chronometer for the determination of fish age and demonstrates the suitability of the method for species with small otoliths. Furthermore, improvements in sample preparation and the analysis of small samples will reduce the likelihood of contamination as discussed in the study of southern bluefin tuna age.

**Introduction**

The bomb radiocarbon chronometer represents a significant advancement in the determination of fish age and the validation of age estimation methods when compared with radiometric methods (e.g. 210Pb/226Ra) due to its accuracy and the ability to complete an analysis with individual otoliths or otolith "cores". There are, however, numerous species with relatively small otoliths that present novel complications in relation to the analysis of radiocarbon. Examples of commercially important species with small otoliths include john dory (Zeus faber), mirror dory (Zenopsis nebulosus), smooth oreo (*Pseudocyttus maculatus*), black oreo (*Allocyttus niger*), warty oreo (*Allocyttus verrucosus*), spiky oreo (*Neocyttus rhomboidalis*), jackass morwong (*Nemadactylus macropterus*), and others. Otolith mass for many of these species can be less than 100 mg for adult fish and under 5 mg for fish about 1 year of age. The small size of these otoliths made it necessary for Stewart et al. (1995) to use as many as 143 otoliths (mean otolith mass 7.4±1.9 mg) to make a single radiometric age determination for warty oreo. Of course, this precludes the possibility of determining age on an individual fish and imposes limitations on the interpretation of radiometric data in relation to the age estimation method being validated.

Methods have been developed for the analysis of radiocarbon in very small samples (Vogel et al. 1989) and the levels of precision associated with these methods has improved in recent years to the point where they are suitable for the determination of fish age. However, there are only a few accelerator mass spectrometry (AMS) facilities in the world that are able to complete high precision radiocarbon analysis with samples that contain less than 0.5 mg of carbon. Furthermore, the physical isolation of the relevant portion of otolith calcium carbonate for analysis can be problematic.

This study uses the bomb radiocarbon chronometer (Kalish 1993, 1995) to determine the age of individual king dory (*Cyttus traversi*), a species with relatively small otoliths. To complete this task, we explored alternate methods of sample preparation in order to isolate physically the earliest formed portions of the otolith and collaborated with U.S. National Science Foundation/University of Arizona Accelerator Mass Spectrometry Facility for the high precision analysis of small otolith samples.

**Materials and Methods**

In earlier studies of fish otolith radiocarbon, sample preparation procedures for the isolation of otolith cores involved the progressive sculpting of adult otoliths until they resembled otoliths from fish of about one year of age (Kalish 1993, Kalish 1995, Kalish et al. 1996a). The otoliths (sagittae) of king dory are relatively small (Smith and Stewart 1994) and are not amenable to these sculpting methods. Furthermore, previous studies of otolith radiocarbon typically involved the analysis of samples with weights ranging from about 10 to 25 mg (about 1.2 to 3.0 mg of carbon). Preparation of samples with this weight from king dory otoliths would result in the isolation of otolith material deposited during the first 0.9 to 6.9 years of life. This estimate is based on Smith and Stewart (1994) where otolith weight = 2.47 x (age) + 7.871. This would not provide an adequate estimate of $\Delta^{14}C$ in the otolith during the first year of life and is likely to be poorly suited as a general indication of birth date.

We modified earlier sculpting techniques to accommodate the small size of king dory otoliths and to make it feasible to prepare samples with a weight of less 4 mg. The region of interest for the otolith to be sculpted was determined by viewing the sample with transmitted light microscopy and the use of the section from the sister otolith as a reference. Opaque and translucent zones on the whole otoliths were suitable as reference points and could be related to the thin sections. After the sculptor determined the otolith's structure and key features, it was glued, distal side up, with Crystalbond on to standard microscope slide. The otolith was not "embedded" in the Crystalbond. The slide was placed on a microscope-type stage with slow motion controls that allowed for passage of light through the whole otolith (to facilitate viewing of opaque and translucent zones) and viewed with a magnifying lens/lamp. A thin outer layer (less than 50 μm) of the otolith's distal surface was ground away with a fine carborundum bit attached to a dental-type drill. This step was necessary because otoliths of both dories and oreos often incorporate a layer of older otolith material on the distal surface. After this material was removed, the Crystalbond was partly dissolved in reagent grade acetone so that the otolith could be removed from the slide. The otolith was then affixed to the slide with the proximal (sulcus acusticus) surface upward. We were careful to ensure that the entire distal surface of the otolith was covered with Crystalbond so that it was fixed securely to the glass slide. A thin pencil line was drawn on the proximal surface of the otolith to outline the outer edge of the region of interest. Fine carborundum drill bits were used to reduce the effective diameter of the otolith to a point just inside of the pencil line. This effectively removed otolith material deposited along the sagittal plane. The final step involved thinning the otolith by grinding in the lateral plane. We determined the thickness of the otolith with a micrometer and compared this thickness with estimates of the desired thickness determined from the otolith thin section. In some cases we were able to sculpt otoliths to a the point where the region of interest weighed 1.8 mg. After sculpting was complete the otolith was placed in acetone to remove the Crystalbond.

For some species, the otoliths were too brittle to sculpt to a mass of <5 to 8 mg. This appears to be due to the structural nature of the earliest deposited otolith material and may be related to the relatively high levels of organic material in these regions. For those species where it was impractical to grind the otolith to the small sizes required, we found that a combination of sculpting and acid dissolution was most effective for preparing small samples. Brittle otoliths were sculpted to a weight of about 10 mg. This involved initial removal of the thin distal layer of older otolith material, and grinding in both the sagittal and lateral planes so that the sample was similar in shape to the final shape desired, although somewhat larger.

The otolith was placed in acetone to remove the Crystalbond, allowed to dry and then placed into 5% HCl for a period of controlled digestion. Gas evolution from otolith calcium carbonate was slow enough to allow observation of the state of the otolith during digestion. The digestion process was observed until it was judged that the otolith was small enough to have attained the prescribed weight (e.g. 3.0 mg). The otolith was removed from the acid bath; put through several washes of distilled water, cleaned in an ultrasonic bath, and finally placed in plastic tubes for drying and storage. It was found that otolith digestion did not proceed along the desired axes if the otolith was not ground initially to a relatively small size (<10 mg). This was probably related to the uneven distribution of organic matrix in the later formed portions of the otolith. Relatively uniform distribution of organic matrix in the earliest formed portions of the otolith may result in more uniform acid digestion.

Graphite targets were prepared from the sculpted/acid digested otolith fragments and radiocarbon analyses were carried out at the U.S. National Science Foundation/University of Arizona Accelerator Mass Spectrometry Facility (NSF-AMS).

The second sagitta from each otolith pair was prepared for otolith reading. Thin sections were prepared by the Central Ageing Facility (Queenscliff, Victoria) and read by two independent readers at the Central Ageing Facility.

**Results and Discussion**

Otoliths from fourteen king dory were analysed for radiocarbon. Two samples included otolith cores from two individuals resulting in a total of twelve radiocarbon values (Table 12.1). One sample was contaminated and resulted in a $\Delta^{14}C$ value that was more than three times the maximum $\Delta^{14}C$ measured in southeast Australian waters. Contamination occurred when the sample was transferred from the CO2 line to the graphitisation line in a glass vessel that previously contained a sample that had twenty times modern levels of atmospheric radiocarbon (personal communication, G.S. Burr, NSF-AMS; NSF-AMS has agreed to run two free samples to replace the sample lost due to their error). The range of prepared otolith sample weights was 3.8 to 5.7 mg (mean=4.5±0.52 mg). Graphite targets prepared for radiocarbon analysis from these otolith samples contained less than 0.5 mg of carbon. The precision of the analyses ranged from 5.0 to 8.2% (mean=6.4±1.1%) and demonstrates the feasibility of carrying out routine high precision analyses of radiocarbon on small samples.

The results of the radiocarbon analyses were plotted versus the birth date estimated by CAF otolith readers and with data from snapper (*Pagrus auratus*) (Kalish 1993) and redfish (*Centroberyx affinis*) (Kalish 1995) (Fig. 12.1). On the basis of the birth dates estimated by the otolith readers, the $\Delta^{14}C$ values from king dory otoliths fall within the curve that defines the bomb radiocarbon increase in surface waters of the southwest Pacific Ocean. This indicates that ages estimated by the otolith readers are good estimates of the true fish age.

The radiocarbon data derived from the otolith cores of king dory suggest that this species might occur south of 45°S latitude during the first year of life. Estimates of pre-bomb $\Delta^{14}C$ from king dory of -48.9±5.0‰ are consistent with other values of $\Delta^{14}C$ obtained from the temperate south Pacific Ocean between about 30°S and 50°S latitude (Kalish 1993, 1995). Pre-bomb radiocarbon levels are not particularly useful in delineating the distribution of a fish in surface waters, however, post-bomb radiocarbon levels can be more useful in determining latitude of occurrence. Peak $\Delta^{14}C$ for king dory appears to be about 50‰ or less and this is consistent with $\Delta^{14}C$ measured in seawater dissolved inorganic carbon around 50°S latitude (Fig. 12.2). There are no significant differences between $\Delta^{14}C$ curves for snapper, redfish, and king dory between about 1958 and 1967. After 1967, the snapper and redfish curves show far greater increases in $\Delta^{14}C$ than the king dory data. These results are consistent with reduced transport of radiocarbon to higher latitude waters. Furthermore, the low $\Delta^{14}C$ values from king dory otolith cores calcified in 1988 and 1992 (Fig. 12.1) provide additional evidence that king dory less than one year of age spend the majority of their early life in waters. Although peak $\Delta^{14}C$ appears lower in king dory otolith cores, the rate at which $\Delta^{14}C$ decreases during the late 1980s to early 1990s appears similar to the rate suggested from radiocarbon analyses on the otolith cores of other southern temperate fish species. This suggests radiocarbon was redistributed to other pools (e.g. dissolved organic carbon, particulate organic carbon) at similar rates across the temperate waters of the South Pacific Ocean.

**Conclusions**

This study validates the otolith section method of age estimation for king dory on the basis of $\Delta^{14}C$ values in otolith material deposited early in life. Birth dates assigned to individual fish on the basis of otolith sections agree with birth dates predicted from $\Delta^{14}C$ values measured in the otoliths and expected rates of change in $\Delta^{14}C$. Results suggest that king dory less than one year of age may occur south of the Subtropical Convergence. Finally, this study demonstrates the suitability of the bomb radiocarbon chronometer for determining fish age for species with small otoliths and the feasibility of applying the method to samples containing <4 mg $CaCO_3$ (<0.5 mg of carbon). This increases the applicability of the bomb radiocarbon chronometer to a broader range of species (eg oreos, dories, morwong) and reduces the likelihood of contamination for those species where otolith sculpting is relatively difficult (Kalish et al submitted).

**Acknowledgments**

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Table 12.1. Fish and otolith data from *Cyttus traversi* collected in the southeast trawl fishery in February 1989 and June 1993. Fish ages were estimated by readers from the CAF. All age and birth date estimates presented in the table are determined from counts of presumed annual increments in otolith thin sections. Values of $\Delta^{14}C$ are reported with ±1 standard deviation. Sample weights indicate the weight of otolith material separated for individual analyses of stable carbon and radiocarbon and are representative of the first year of otolith growth for an individual fish.

| Sample No. | Laboratory accession no. | Collection date | Sex | Fish length (cm) | Fish weight (g) | Otolith wt. (g) | Sample wt. (mg) | $\delta^{13}C$ (‰,PDB) | $\Delta^{14}C$(‰) | Otolith section age (birth date) |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 | AA16786 | 6/93 | Imm | 11.0 | 28 | 0.003 | 3.0 | -3.0 | 18.6±6.7 | 1 (1992) |
| 4 | AA16786 | 6/93 | Imm | 10.2 | 26 | 0.0027 | 2.7 | " | " | " |
| 8 | AA16787 | 2/89 | Imm | 10.9 | 30 | 0.0043 | 4.3 | -4.7 | 52.8±7.2 | 1 (1988) |
| 19 | AA16788 | 2/89 | M | 43.3 | 1920 | 0.0681 | 4.2 | -1.3 | 31.7±6.8 | 20 (1969) |
| 31 | AA16789 | 2/89 | M | 39.4 | 1690 | 0.0592 | 4.6 | -3.3 | 45.4±6.8 | 20 (1969) |
| 49 | AA16790 | 2/89 | M | 42.6 | 2070 | 0.056 | 4.5 | -4.2 | 26.6±6.7 | 22 (1967) |
| 50 | AA16791 | 2/89 | M | 33.1 | 850 | 0.0427 | 3.8 | -1.5 | 17.6±7.5 | 23 (1966) |
| 56 | AA16792 | 2/89 | M | 40.9 | 1430 | 0.067 | 4.4 | -3.3 | 39.5±5.3 | 22 (1967) |
| 79 | AA16793 | 2/89 | F | 50.0 | 3380 | 0.1054 | 2.9 | -4.1 | -48.9±5.0 | 32 (1957) |
| 164 | AA16794 | 2/89 | F | 45.5 | 2070 | 0.0773 | 2.2 | " | " | " |
| 106 | AA16795 | 2/89 | M | 43.5 | 2030 | 0.0512 | 4.5 | -3.8 | 38.9±8.2 | 16 (1973) |
| 120 | AA16796 | 2/89 | M | 46.2 | 2090 | 0.0561 | 4.7 | -5.3 | 12.8±5.3 | 24 (1965) |
| 226 | AA16797 | 2/89 | F | 50.1 | 3320 | 0.059 | 4.2 | -3.0 | -35.1±5.1 | 30 (1959) |

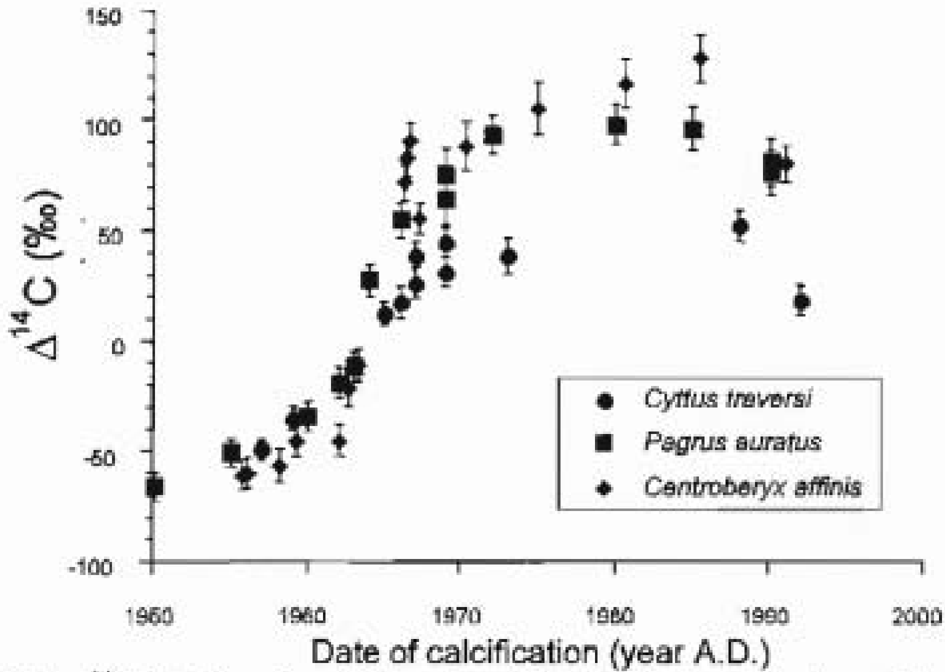


Figure 12.1. $\Delta^{14}C$ data from three fish species. Data for *Cyttus traversi* are plotted against the mean birth date estimated by a reader from the Central Ageing Facility (CAF). Data from *Pagrus auratus* are plotted against the true birth date for those fish (Kalish 1993). *Centroberyx affinis* $\Delta^{14}C$ values are plotted versus mean ages estimated by multiple readers from the CAF and ANU (Kalish 1995). Error bars are one standard deviation.

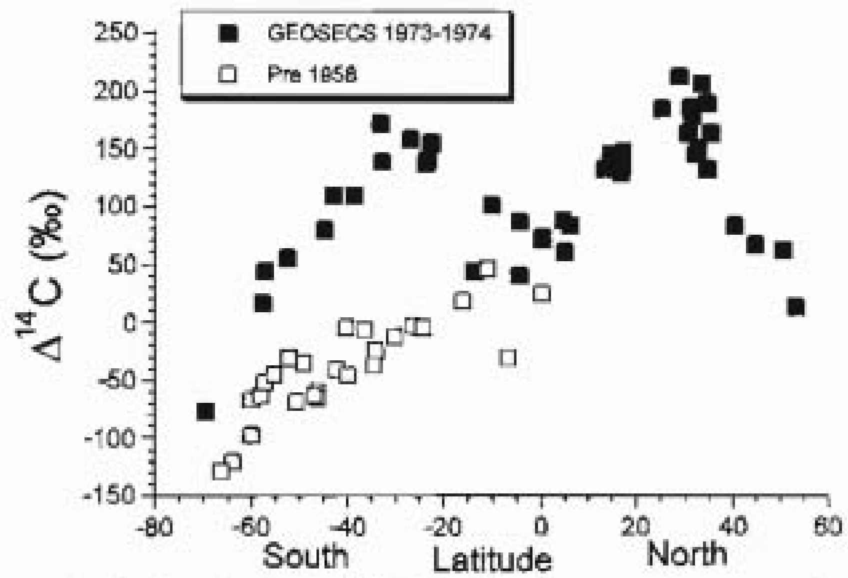


Figure 12.2. Latitudinal variation in $\Delta^{14}C$ in Pacific Ocean surface waters both before and after significant inputs due to the atmospheric testing of nuclear weapons. Figure from Broecker and Peng 1992.

# Chapter 13 Application of the bomb radiocarbon chronometer to the validation of blue-eye trevalla (*Hyperoglyphe antarctica*) age

John Kalish and Justine Johnston

**Summary**

Increased catches of blue-eye trevalla (*Hyperoglyphe antarctica*) associated with targeted and non-targeted trawling have highlighted the urgent need to determine the status of blue-eye stocks in southeast Australia. It is not possible to estimate yields from these stocks until further information is obtained. Age estimation and the requisite age validation are a key element of the stock assessment process; however, a validated method of age estimation does not exist for trevalla. Although some research has been carried out on age estimation of trevalla there has been concern regarding the accuracy of ages assigned to this species. Measurements of natural and bomb-produced radiocarbon in the otoliths of blue-eye trevalla indicate that there are significant errors when trevalla age is estimated by reading otolith sections. Furthermore, there are significant differences in age estimates made by different otolith readers from different laboratories. It is necessary to carry out further research on age estimation procedures for blue-eye trevalla and to establish otolith reading protocols for the species. Given the difficulty inherent in reading blue-eye otoliths it will be important to ensure that there is agreement among laboratories involved in age estimation. This can be achieved through inter-laboratory calibration exercises. Further measurements of radiocarbon in trevalla otoliths are planned.

**Introduction**

Catches of blue-eye trevalla (*Hyperoglyphe antarctica*) have increased steadily since the beginning of the dropline fishery that began targeting the species in the 1970s. Further increases in catch have resulted from by-catch associated with deepwater trawling and current trends suggest that commercial fishing pressure on trevalla will increase (Williams 1994). Increased catch rates and a lack of information on the productivity of this species highlight the need for developing a validated age estimation method for the species.

The depth distribution of blue-eye trevalla changes dramatically during their life-history. Trevalla belong to the Centrolophidae, a family of fishes where behaviour of juveniles can be characterised by their association with floating objects such as jellyfishes, salps, and other flotsam (Haedrich 1967). Because of the association that many species have with jellyfish, centrolophids are often called medusafishes. Juvenile blue-eye trevalla from 30-60 mm in length were found only recently drifting in association with rafts of kelp (Last et al. 1993). It is unknown how long these juveniles remain in the surface waters before descending to the deeper (200-900 m) habitats occupied by the adults. Horn (1988) estimated that blue-eye maintain a pelagic existence until a length of about 47 cm, after which blue-eye recruit to the commercial fishery in New Zealand waters. Trawl and drop-line catches of blue-eye in Australia are limited to fish greater than 45 cm FL so it appears likely a similar ontogenetic migration is occurring in Australian waters (Baelde 1995).

The depth distribution of juvenile blue-eye trevalla makes this species a suitable candidate for age validation based on the bomb radiocarbon chronometer (Kalish 1995a, 1995b). Estimates of age and growth for blue-eye trevalla in New Zealand suggest that a 47 cm trevalla is, on average, two years of age (Horn 1988). If this is correct, than a significant amount of otolith calcium carbonate would be deposited by this species while they remain in the surface mixed layer of the ocean. Radiocarbon derived from atmospheric testing of nuclear weapons is readily identified in the surface ocean after about 1960 and would be incorporated into the otoliths of juvenile blue-eye spawned after that year. Movement of the larger juvenile trevalla into deeper waters would reduce the amount of bomb-derived radiocarbon incorporated into the later-formed segments of the otolith. Despite the change in the dynamics of radiocarbon incorporation in the older life history stages, the radiocarbon deposited in early life in the surface waters would remain unaltered in the calcium carbonate lattice of the otolith. This study is designed to determine the suitability of the reading of otolith sections for estimating the age of blue-eye trevalla.

**Materials and methods**

Sample preparation procedures are similar to those described in Kalish (1993, 1996a et al, 1996b et al.). The otoliths of blue-eye trevalla are relatively large and this simplified the process of otolith sculpting to produce a sample of about 15 mg for analysis of radiocarbon by accelerator mass spectrometry (AMS). Analyses were carried out by the Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences (New Zealand). Details of sample pre-treatment and radiocarbon analysis are as reported in previous work (Kalish 1995, Kalish et al. 1995) and, results are reported as $\Delta^{14}C$ (Stuiver and Polach 1977).

The second sagitta from each otolith pair was prepared for otolith reading. Thin sections were prepared by the Central Ageing Facility (Queenscliff, Victoria) and read by three independent readers at Australian National University (designated Reader 1 and Reader 2) and the Central Ageing Facility (designated CAF).

**Results and Discussion**

Otoliths from fifteen blue-eye trevalla were analysed for radiocarbon (Table 13.1). The results of the radiocarbon analyses were plotted versus the birth date estimated by the three independent otolith readers and with data from snapper (*Pagrus auratus*) from an earlier study (Kalish 1993) (Fig. 13.1). There were significant differences among otolith-based age estimates from each of the readers . Furthermore, almost all estimates of birth date from otolith sections resulted in the data falling to the right of the radiocarbon calibration curve defined by P. auratus otoliths. The relationship between otolith section birth dates and radiocarbon indicates that there are significant errors in the age estimates from otolith sections.

Observations of otolith sections of blue-eye trevalla made by a range of readers indicate that interpretation of zones on these structures is relatively difficult. This appears to be due to the large number and irregular spacing of zones in the otoliths from larger trevalla. Estimates of age and birth dates for individual blue-eye differed dramatically among readers; however, the largest discrepancy was between readers from ANU and the reader from CAF. This suggests that the general interpretation of the zones within the otoliths is different between the two facilities. This is not due simply to differences in the interpretation of individual zones.

Interpretation of the blue-eye radiocarbon data is dependent on the use of an appropriate radiocarbon calibration for the region where fish less than one year of age occur. This study uses the snapper radiocarbon calibration obtained from the east coast of the North Island of New Zealand (1993). When radiocarbon data from a range of species are plotted with the blue-eye data (Fig. 13.2), the blue-eye data appear as outliers falling to the right of most of the data. This may result from: 1) occurrence of blue-eye trevalla juveniles at more southern latitudes where the quantities of bomb radiocarbon are significantly lower than at about 35° to 40° S latitude (i.e. south of the Subtropical Convergence in the southwest Pacific); 2) occurrence of blue-eye trevalla juveniles at greater depths than previously assumed (i.e. not in the surface mixed layer); 3) sample contamination, or; 4) errors in the estimation of age from otolith sections.

Because of the rarity of small blue-eye juveniles, there are few data on the occurrence of this life-history stage (Last et al. 1993); however, individuals of between 30-60 mm were recently collected in the Tasman Sea at 42°37' S, 148°41'E (Nicoll 1993) in rafts of floating seaweed. This collection puts the few juveniles that have been collected near the boundary of the Subtropical Convergence (around 42°S in the southwest Tasman Sea), a region across which post-bomb radiocarbon levels show marked spatial variation (Fig. 13.3). Although few data are available it can be estimated that pre-bomb variations in $\Delta^{14}C$ values between 35°S and 45°S latitude in the South Pacific were on the order of 10‰. Given the precision of the AMS analyses carried out in this study, this difference would be barely detected in trevalla otoliths. Radiocarbon measurements made by the GEOSECS (Geochemical Ocean Section Study) expeditions in the early 1970s along north-south transects indicate that, during that time, $\Delta^{14}C$ values between 35°S and 45°S latitude might differ by as much as 50‰. These estimates are based on radiocarbon data collected in the central Pacific Ocean (Ostlund and Stuiver 1980), and it is possible that this variation would be slightly less along the east coast of Australia due to radiocarbon transported to the south by the East Australian Current (Kalish 1995). Although the magnitude of the effect cannot be determined with the available data, it is possible that juvenile blue-eye trevalla occur far enough south that they are in a region where $\Delta^{14}C$ values are significantly lower than that of snapper, redfish (*Centroberyx affinis*), or blue grenadier (Macruronous novaezelandiae).

Another possible explanation for the discrepancy between $\Delta^{14}C$ values and section ages may be related to the depth of occurrence of blue-eye trevalla. Blue-eye belong to a family of fish that is characterised by a pelagic juvenile stage and the few collections of juveniles have been made in surface waters with floating seaweed. On this basis, it seems unlikely that the fish were occurring below the surfaced mixed layer. Furthermore, when plotted against CAF birth dates estimated from otolith sections the blue-eye data fall to the right of the blue grenadier data. Research on blue grenadier (Kalish et al. 1996b) indicated that juvenile blue grenadier are likely to be found at depths between 200 and 400 m and that bomb radiocarbon required about two years to penetrate to these depths. When the CAF birth dates are applied to the blue-eye radiocarbon data, the points fall to the right of the blue grenadier data. This suggests an additional lag of about 4 years before bomb radiocarbon reached the juvenile blue-eye habitat and, concomitantly, a depth of over 600 m, an unlikely habitat for small juvenile blue-eye.

Sample contamination could affect the radiocarbon results from blue-eye trevalla; however, this possibility seems unlikely. Blue-eye trevalla otoliths are relatively large and this simplifies the process of sculpting the otoliths so that the material deposited in the first year of life, or less, is retained. In fact, because the otoliths of this species are relatively large even at a presumably young age, it was possible to isolate material that was deposited during what was presumed to be less than the first 8 months of life. Nevertheless, it is possible that some material deposited in later life was retained on the sculpted otolith sample. If this situation had occurred that additional bomb radiocarbon would be included in the otolith samples and the estimate of age/birth date based on the level of bomb radiocarbon would have been lower. If this were the case than the bomb radiocarbon estimates of age/birth date would be lower than the CAF otolith section ages. This is a scenario that is opposite to the data presented in this study. The problem of sample contamination during the sculpting process was also discussed in relation to the preparation of southern bluefin tuna otoliths for radiocarbon analysis (Kalish et al. 1996a).

The data indicate that there are significant errors in the estimation of age from the reading of otolith sections. There are several indicators that make it necessary to question the accuracy of the section-based age estimates including the variability among otolith readers and the resulting relationship between birth date and radiocarbon. There is a significant bias among the otolith readers, most notably that fish estimated to be older by Reader 1 and Reader 2 were considered significantly younger by the CAF reader (Table 13.1 and Fig. 13.4). The similarity between age estimates from Readers 1 and 2 would be expected since these readers were trained to read trevalla otoliths at the same time, although they read otoliths sections independently for this study. The corroboration of age estimates by Readers 1 and 2 does not provide any indication that these readings are correct, both Readers 1 and 2 may be inaccurate.

Deviations from the expected relationship between $\Delta^{14}C$ values and age based on otolith sections provide further evidence of inaccuracies associated with the reading of blue-eye trevalla otoliths. Of the fifteen blue-eye trevalla otoliths analysed, six had radiocarbon levels that would be classified as pre-bomb due to $\Delta^{14}C$ values of less than -40‰ (Table 13.1, Figs. 13.1 and 13.2). These six samples had $\Delta^{14}C$ values that ranged from -81±9.2‰ to -44.7±8.3‰. The sample with the lowest $\Delta^{14}C$ value (-81±9.2‰ for ANU7) is low, but it is within the range of $\Delta^{14}C$ that might be expected between about 45°S and 50°S, particularly if it is possible for blue-eye larvae and juveniles to develop in throughout the northern regions of the Southern Ocean. Central Ageing Facility and ANU (Readers 1 and 2) estimates of birth dates for the six "pre-bomb" fish range from 1965 to 1969 and 1945 to 1963, respectively (Table 13.1). Birth dates as late as 1969 are extremely unlikely for blue-eye trevalla whose otoliths had $\Delta^{14}C$ values of less than -40‰. A summary of the latitudinal variation in radiocarbon in the Pacific (Linick 1980) indicated that mean values for $\Delta^{14}C$ at 50°S were about -25‰ and 50‰ in 1962 and 1969, respectively. Other results provide strong evidence that some of the age estimates are incorrect. For example, CAF age estimates suggested that several fish were 23 years of age (birth date 1969) (ANU1, ANU81, ANU171) and these fish had $\Delta^{14}C$ values of 39‰, -60.7‰, and -44.7‰ (Table 13.1, Figs. 1 and 2). The occurrence of pre-bomb and clearly post-bomb radiocarbon values for fish of the same age is extremely unlikely, if not impossible. If these values were correct than it would have been necessary for these fish to spend the first year of life in extremely different habitats (e.g. depth and/or latitude).

Age estimates from otolith sections were plotted against otolith weight in Fig. 13.5. A nearly linear relationship between otolith weight and age would be expected due to the continuous growth of otoliths throughout the life of the fish. The data from the blue-eye trevalla otoliths are inadequate to determine if there is significant deviation from a linear relationship, but there are some differences between the relationships between otolith weight and age estimated by the two laboratories (ANU and CAF). Otolith weight versus age estimated by Readers 1 and 2 was best described by a linear relationship (otolith weight = 0.1158 + 0.025938 age; r2=0.79) rather than an exponential relationship (otolith weight = 0.24829 e0.040277 age; r2=0.66). Otolith weight versus age estimated by CAF was best described effectively by both an exponential (otolith weight = 0.24829 e0.059033 age; r2=0.73) and linear relationship (otolith weight = 0.016251 + 0.037796 age; r2=0.72). This suggests that the ages of older fish may be underestimated.

**Conclusions**

This study indicates that there are significant errors in age estimates determined from otolith sections and that these age estimates are often inconsistent with measurements of radiocarbon in the otoliths. Furthermore, readers from different age determination laboratories obtained significantly different ages from individual otolith sections. Further research is necessary to determine the source of the discrepancies among otolith readers and to develop appropriate protocols for reading blue-eye trevalla otoliths. Additional measurements of bomb-radiocarbon will be essential to identify an accurate method of otolith reading and these measurements should be carried out in conjunction with inter-laboratory calibration of the preferred reading method.

Additional measurements of radiocarbon are planned for both young and old blue-eye trevalla and these measurements will be carried out in conjunction with otolith readers at CAF, ANU, and other laboratories involved in the management of blue-eye trevalla. Otolith sections used in this study will be re-read by readers from both the CAF and ANU to determine the repeatability and consistency of the interpretation of blue-eye otolith sections.

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**Table 13.1.** Fish and otolith data from *Hyperoglyphe antarctica* collected off southeast Australia between January 1992 and April 1994. Fish ages were estimated by three readers; one from the CAF and two from ANU. All age and birthdate estimates presented in the table are determined from counts of presumed annual increments in otolith thin sections. Values of $\Delta^{14}C$, mean age, and mean birthdate are reported with ±1 standard deviation. Sample weights indicate the weight of otolith material separated for individual analyses of stable carbon and radiocarbon and are representative of less than the first year of otolith growth for an individual fish.

| Sample No. | Laboratory and laboratory accession no. | Sex | Fish length (cm) | Fish wt. (g) | Otolith wt. (g) | Sample wt. (mg) | $\delta^{13}C$ (‰, PDB) | $\Delta^{14}C$ (‰) | Reader (Age, Birthdate) CAF | ANU1 | ANU2 | Mean age (years) | Mean Birthdate (years, A.D.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ANU 1 | RRL NZA5412 | F | 71 | 500 | 0.5871 | 17.8 | -2.5 | 39±9.3 | 23 (1969) | 24 (1968) | 22 (1970) | 23.8±2 | 1968.2±2 |
| ANU 7 | RRL NZA5413 | F | 90 | 571 | 0.9439 | 13 | -3.1 | -81±9.2 | 26 (1967) | 35 (1958) | 33 (1960) | 33±5 | 1960±5 |
| ANU 13 | RRL NZA5414 | F | 59 | 471 | 0.3722 | 16.2 | -2.7 | 89.6±9.2 | 10 (1983) | 12 (1981) | 11 (1982) | 11.8±1 | 1981.2±1 |
| ANU 17 | RRL NZA5415 | M | 62 | 651 | 0.4857 | 16.8 | -3.1 | 66.2±9.1 | 17 (1976) | 20 (1973) | 20 (1973) | 19.4±1 | 1973.6±1 |
| ANU 25 | RRL NZA5416 | F | 53 | 853 | 0.2577 | 17.7 | -3.3 | 72.8±9 | 5 (1988) | 7 (1986) | 4 (1989) | 6±2 | 1987±2 |
| ANU 27 | RRL NZA5417 | F | 74 | 432 | 0.7444 | 16.3 | -3.2 | 44±8.8 | 20 (1972) | | | 20 | 1972 |
| ANU 28 | RRL NZA5418 | M | 71 | 598 | 0.6954 | 15 | -2.5 | -20.6±9.3 | 27 (1966) | 35 (1958) | 33 (1960) | 33±4 | 1960±4 |
| ANU 35 | RRL NZA5419 | F | 87 | 645 | 1.2446 | 19.8 | -3 | -56.2±7.6 | 28 (1965) | 47 (1946) | 48 (1945) | 43.6±9 | 1949.4±9 |
| ANU 36 | RRL NZA5521 | F | 79 | 559 | 0.7244 | 16.1 | -3.3 | 59±8.9 | 19 (1973) | | | 19 | 1973 |
| ANU 54 | RRL NZA5522 | M | 66 | 497 | 0.5708 | 16.9 | -2.3 | 68.9±9.8 | 12 (1980) | | | 12 | 1980 |
| ANU 74 | RRL NZA5523 | F | 91 | 971 | 1.0947 | 12.6 | -2.5 | -65.3±11.1 | 25 (1967) | 34 (1958) | 32 (1960) | 31.8±5 | 1960.2±5 |
| ANU 78 | RRL NZA5524 | F | 90 | 586 | 0.9554 | 15.2 | -3.6 | -27.3±10.6 | 22 (1970) | 32 (1960) | 29 (1963) | 29.4±5 | 1962.6±5 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ANU 81 | RRL NZA5525 | F | 95 | 442 | 0.9667 | 15.3 | -3.4 | -60.7±9.3 | 23 (1969) | 32 (1960) | 32 (1960) | 30.4±4 | 1961.6±4 |
| ANU 171 | RRL NZA5526 | F | 92 | 457 | 0.9417 | 14 | -2.1 | -44.7±8.3 | 23 (1969) | 34 (1958) | 29 (1963) | 30.8±5 | 1961.2±5 |
| T47 | RRL NZA4390 | N/A | 79 | 454 | 1.3002 | 22 | -2.6 | -53.9±8.3 | 28 (1966) | 34 (1960) | 33 (1961) | 31.6±3 | 1962.3±3 |

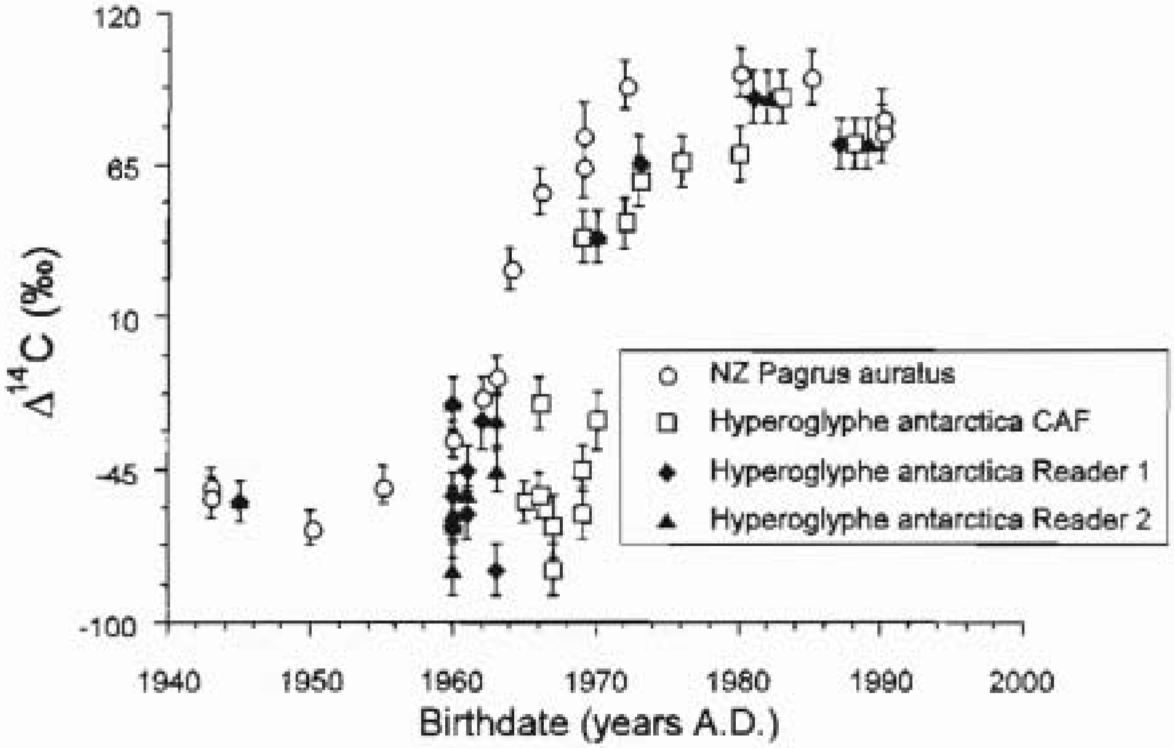


**Figure 13.1.** $\Delta^{14}C$ of *Hyperoglyphe antarctica* otolith cores plotted against the birth date determined by three independent readers from otolith sections. $\Delta^{14}C$ data from *Pagrus auratus* otolith cores are plotted against the true birth date (Kalish 1993). $\Delta^{14}C$ values are based on otolith material deposited over a time period equivalent to about the first eight months of life. Errors are ±1 sd.

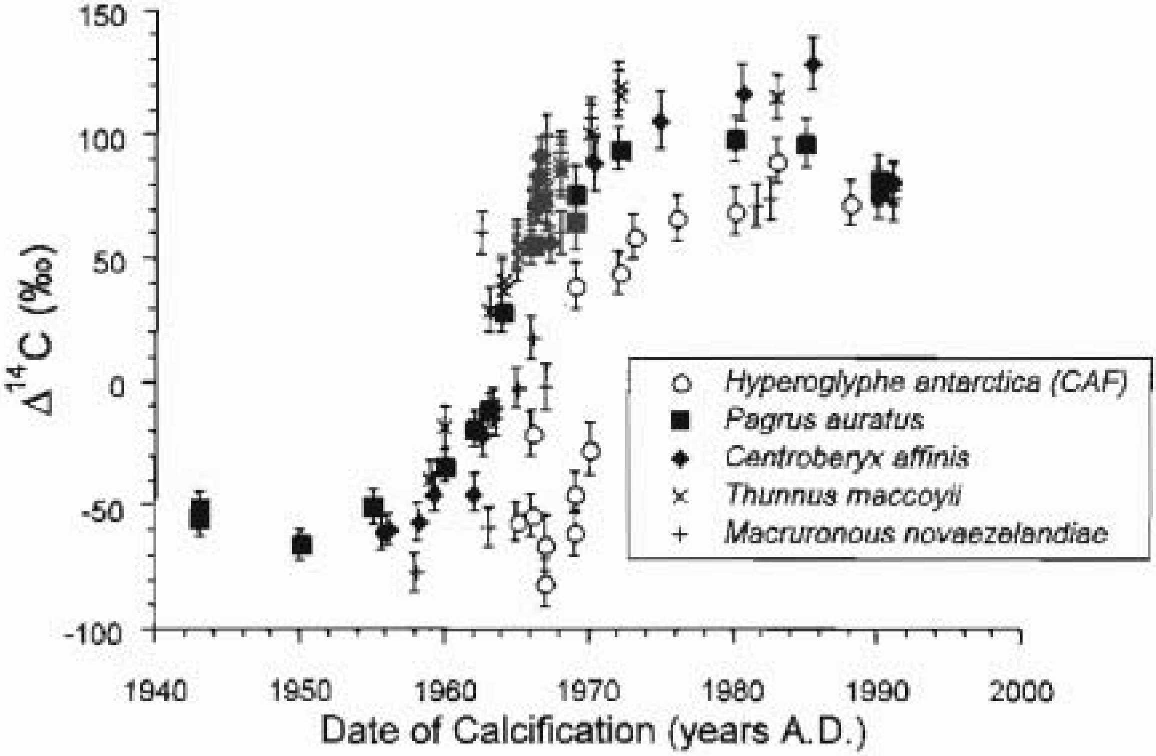


**Figure 13.2.** $\Delta^{14}C$ data from five fish species. Data for *Hyperoglyphe antarctica* are plotted against birth date estimated by the CAF. Data from *Pagrus auratus* are plotted against the true birth date for those fish (Kalish 1993). Both *Centroberyx affinis* and Macruronous novaezelandiae $\Delta^{14}C$ values are plotted versus mean ages estimated by multiple readers from the CAF and ANU (Kalish 1995, Kalish and Johnston 1995). $\Delta^{14}C$ values from *Thunnus maccoyii* otoliths are plotted versus the birth date determined from a model describing variation in $\Delta^{14}C$ levels over time in the eastern Indian Ocean (Kalish et al. 1996).

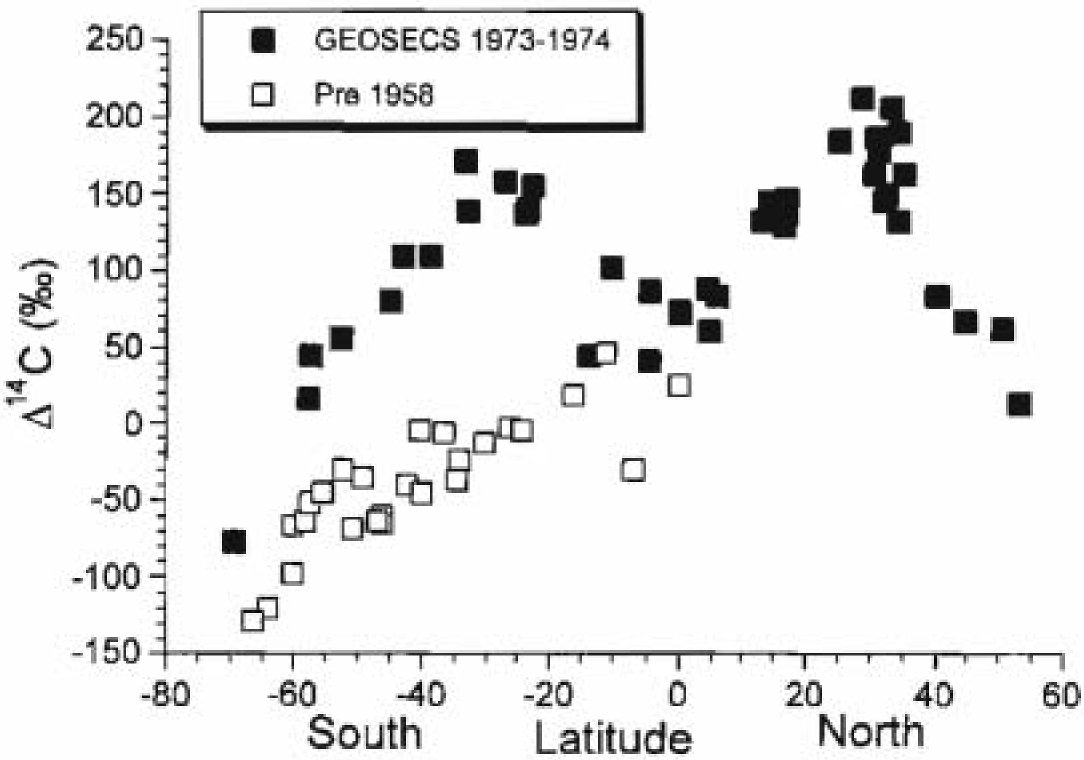


Figure 13.3. Latitudinal variation in $\Delta^{14}C$ in Pacific Ocean surface waters both before and after significant inputs due to the atmospheric testing of nuclear weapons. Figure from Broecker et al. (1985).

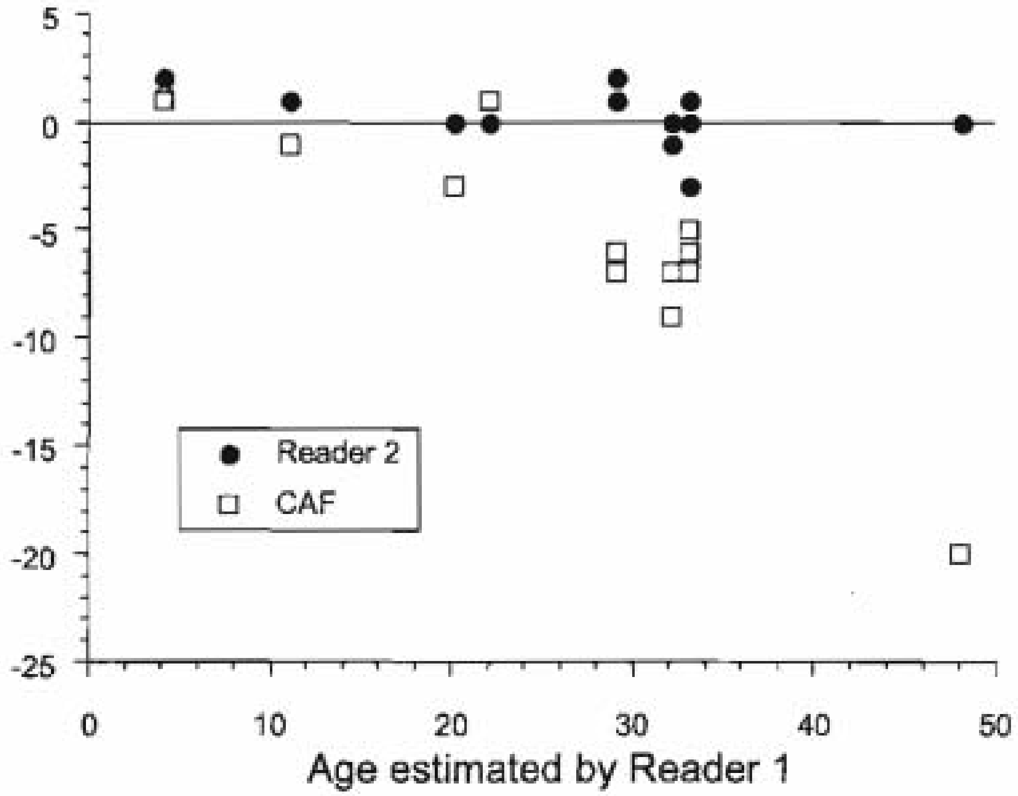


Figure 13.4. Differences (years) between age estimates determined from radiocarbon data and two independent otolith readers.

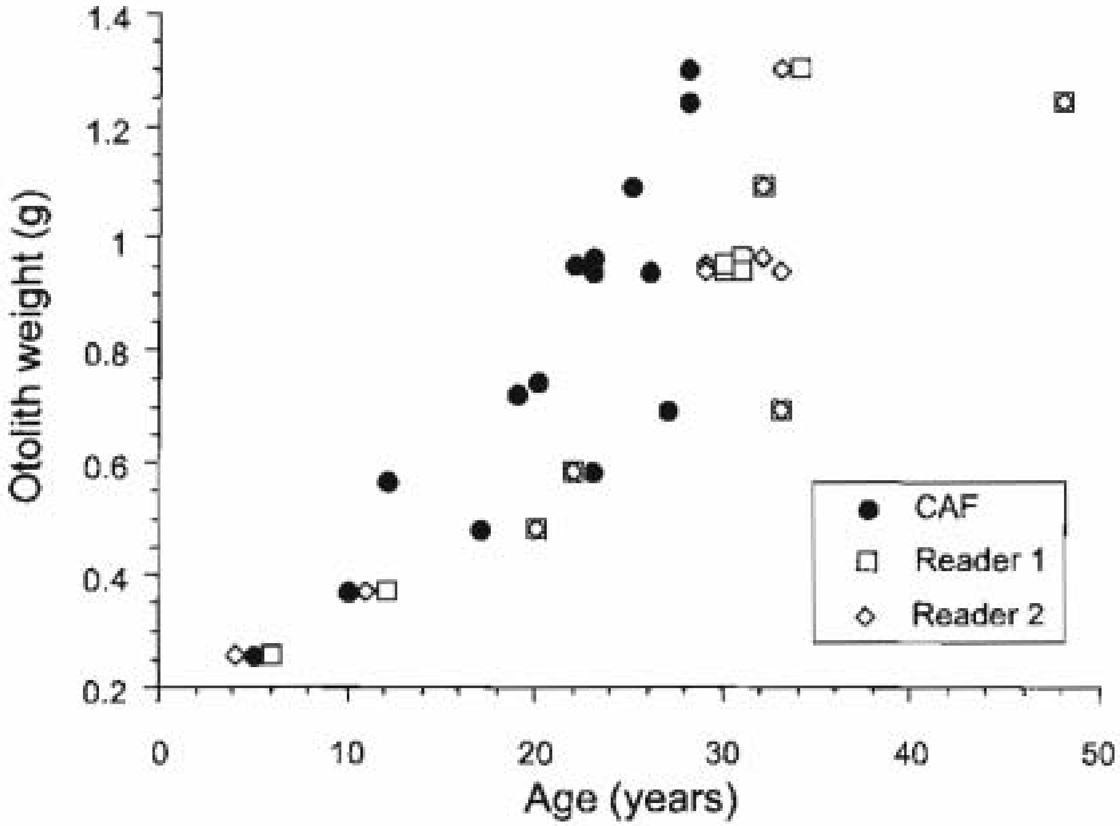


**Figure 13.5.** Otolith section age estimates, from three independent readers, of individual *Hyperoglyphe antarctica* plotted against otolith weight.

# Chapter 14 Validation and direct estimation of age and growth of Patagonian toothfish *Dissostichus eleginoides* based on otoliths

John Kalish, Trent Timmiss, Janet Pritchard, Justine Johnston and Guy Duhamel

**Summary**

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 $L\infty$ 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 14.1). 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. 14.1). 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. 14.2 and 14.3). 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. 14.3). 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 CO2 by reaction in vacuo with 100% phosphoric acid. An aliquot of the CO2 was used to determine $\delta^{13}C$ for each sample and the remaining CO2 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. 14.3). 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. 14.6). 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 □m aluminium oxide and 0.25 □m 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, t0 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 RSSP = residual sum of squares of the VBGF fitted by pooled growth data, RSSs=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 14.1) are plotted versus birth date estimated from otolith thin sections in Figure 14.7. 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. 14.7). 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 14.1). 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. 14.8) 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 14.9), 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

14.10 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 x 10-8 x total length2.856 (Fig 11). There was a strong linear relationship between fish weight and otolith weight (r2=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 14.2, Fig. 14.14). 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 14.2). After removal of these points from the 1996/97 data, VBGF parameter estimates for both fishing seasons were similar. The values estimated for t0 were adequately small (Table 14.2) 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 14.2). 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 14.2). 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. 14.14). 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. 14.15). 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. 14.15). Differences between repeat age estimates were slightly larger in older fish, though the percentage error is smaller (Fig. 14.15). 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 14.1). 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. 14.16 and 14.17). 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. 14.16 and 14.17). 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. 14.16.

The greatest bias occurred between age estimates from the ANU and ODU (Fig. 14.16 and 14.17). 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. 14.18 and 14.19). 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. 14.15) 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 $L\infty$ 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 $L\infty$ 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.

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Table 14.1. 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 14.2.** 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 | $L_\infty$ | | | $t_0$ | | | K | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Estimate | Lower CI | Upper CI | Estimate | Lower CI | Upper CI | Estimate | Lower CI | Upper CI |
| | combined | 500 | 1892 | 1458 | 3737 | -3.53 | -5.89 | -1.95 | 0.040 | 0.015 | 0.066 |
| 95/96 | 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 |
| | combined | 498 | 2999 | 2248 | 5154 | -2.42 | -3.44 | -1.59 | 0.025 | 0.012 | 0.038 |
| 96/97 | male | 171 | 1384 | 1126 | 2114 | -1.37 | -3.01 | -0.27 | 0.072 | 0.035 | 0.114 |
| (all) | female | 246 | 3298 | 2210 | 10405 | -2.74 | -4.52 | -1.42 | 0.022 | 0.005 | 0.041 |
| | combined | 496 | 2217 | 1818 | 3094 | -1.77 | -2.7 | -1.03 | 0.039 | 0.024 | 0.054 |
| 96/97 | male | 171 | 1384 | 1126 | 2114. | -1.37 | -3.01 | -0.27 | 0.072 | 0.035 | 0.114 |
| (-2) | female | 245 | 2053 | 1640 | 3300 | -1.54 | -3.09 | -0.45 | 0.045 | 0.022 | 0.070 |

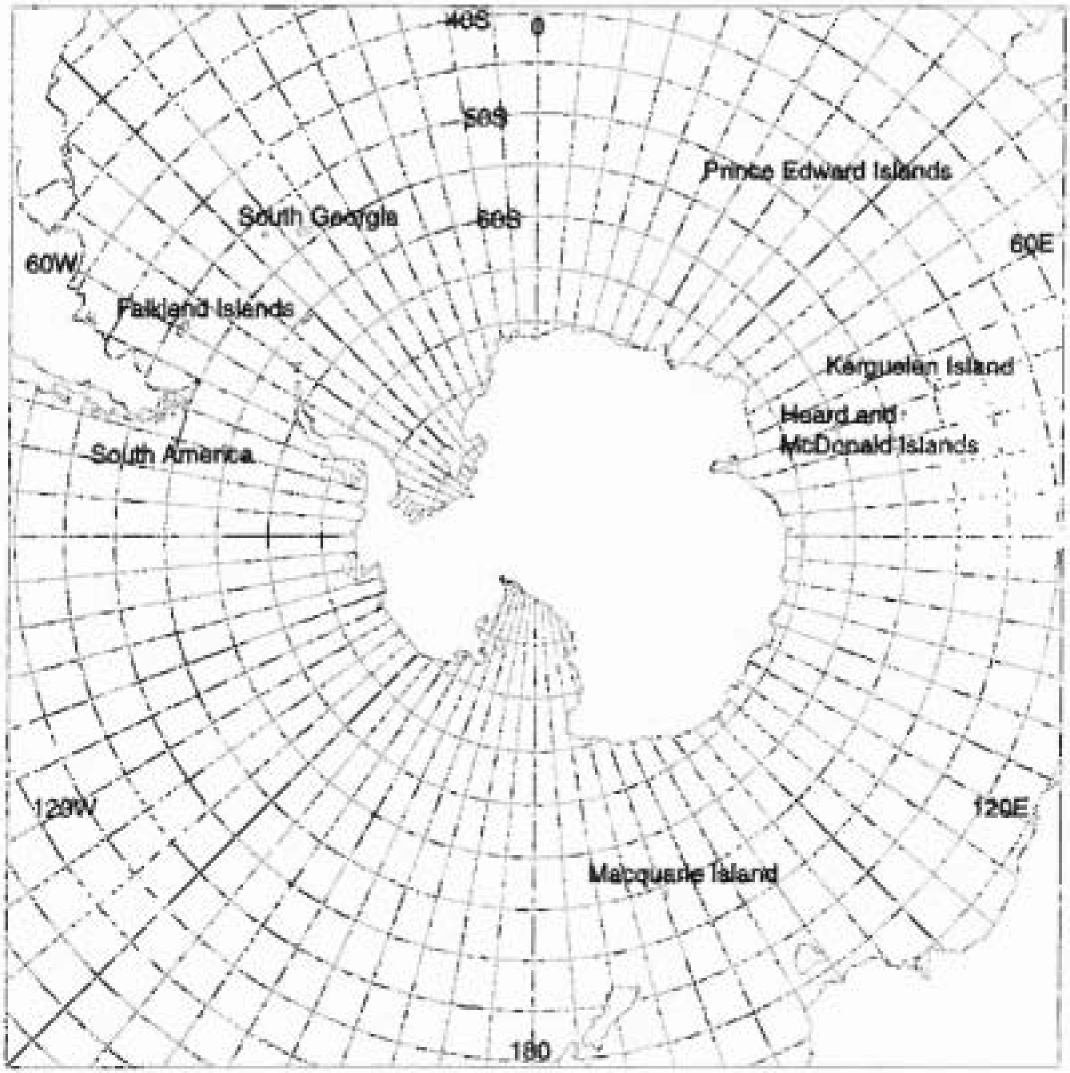


Figure 14.1. Antarctica and the Southern Ocean region with the locations of Patagonian toothfish *Dissostichus eleginoides* fisheries sampled for the validation and age and growth study.

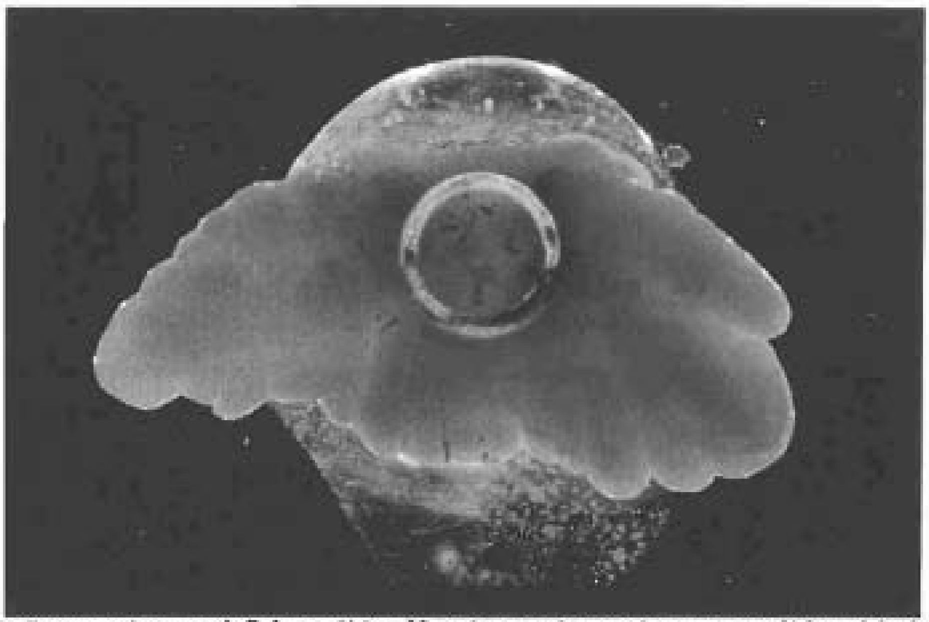

Figure 14.2. Patagonian toothfish otolith affixed to a glass microscope slide with Crystalbond and shown after isolation of core region with the ultrasonic disc cutter. The diameter of the core is 3.0 mm.

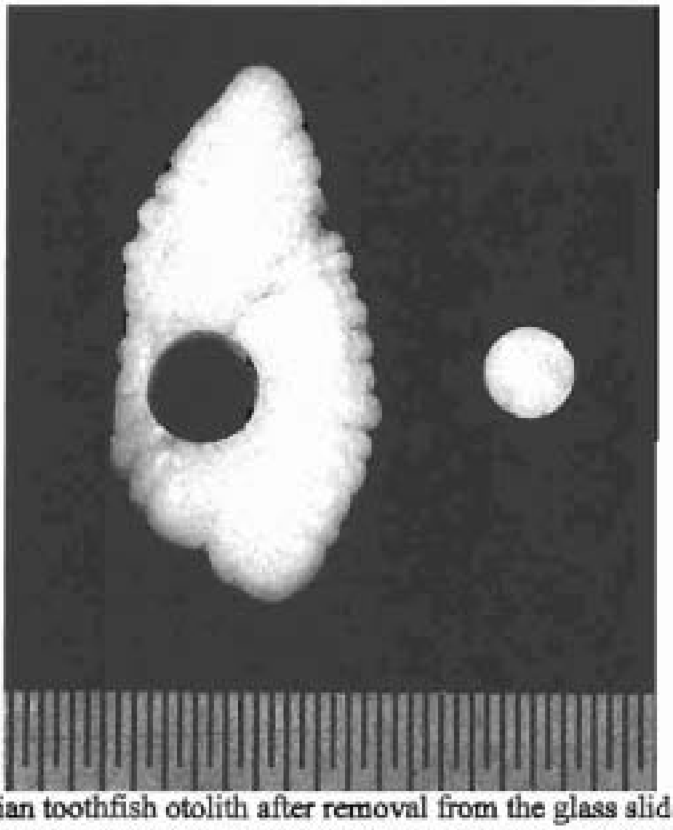

Figure 14.3. Patagonian toothfish otolith after removal from the glass slide. Crystalbond was removed by dissolving in acetone. The core, isolated with the ultrasonic disc cutter, comes free after removal of the Crystalbond and is now ready for further grinding with the mechanical jig described in the text. The diameter of the core is 3.0 mm.

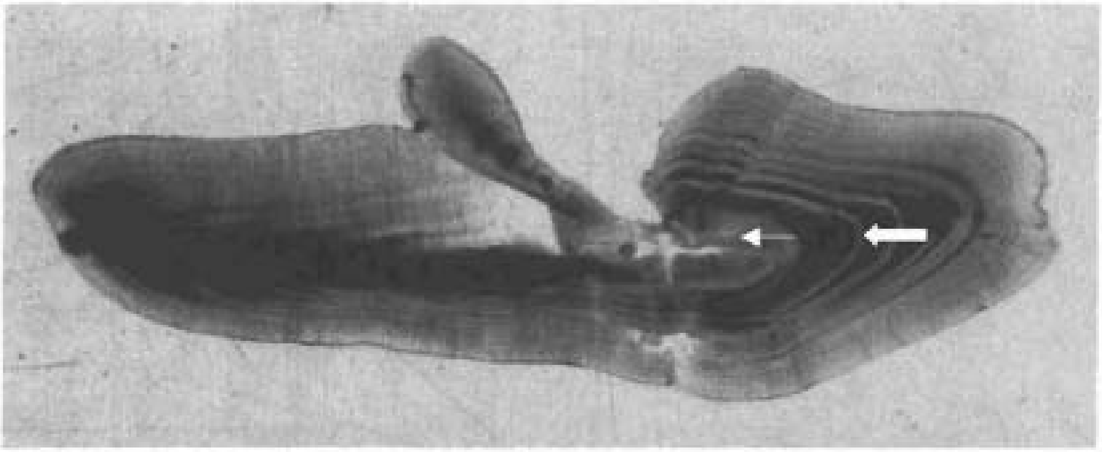

Figure 14.4. Transverse section of an otolith from a Patagonian toothfish estimated to be 14 years of age. The broad and narrow arrows indicate the first continuous translucent zone, estimated to be the completion of the first annual increment by the ANU and CAF, respectively.

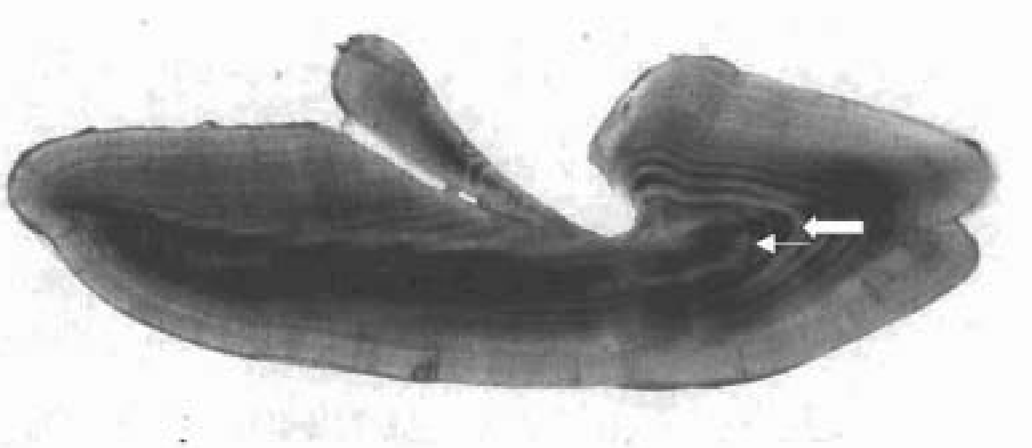

Figure 14.5. Transverse section of an otolith from a Patagonian toothfish estimated to be 15 years of age. The broad and narrow arrows indicate the first continuous translucent zone, estimated to be the completion of the first annual increment by the ANU and CAF, respectively.

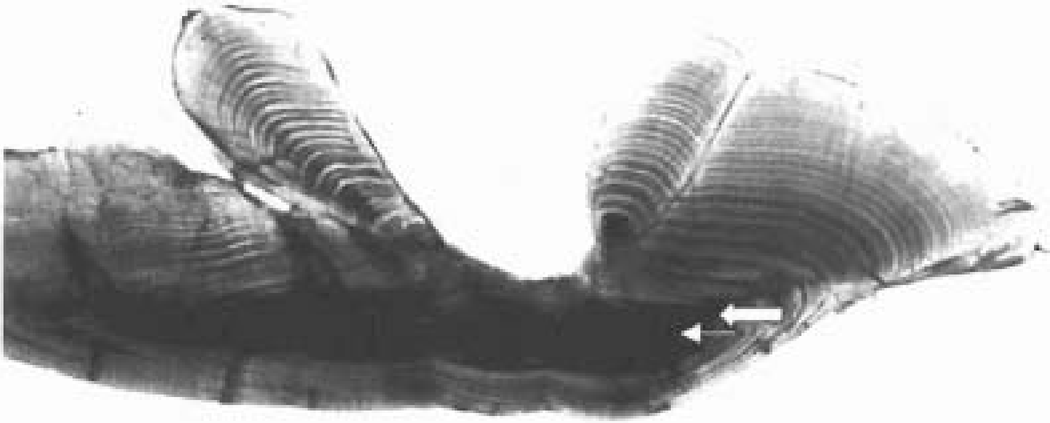

Figure 14.6. Transverse section of an otolith from a Patagonian toothfish estimated to be 25 years of age. The broad and narrow arrows indicate the first continuous translucent zone, estimated to be the completion of the first annual increment by the ANU and CAF, respectively.

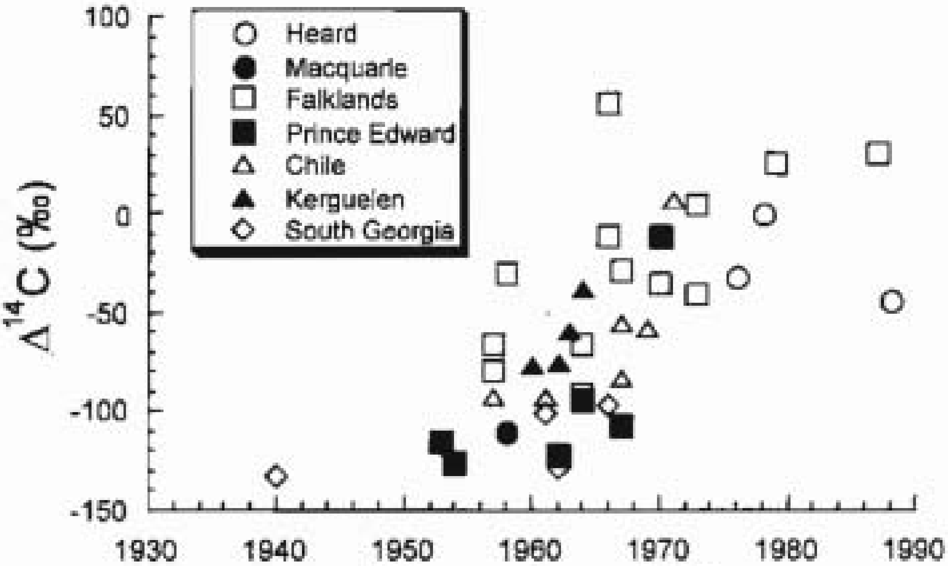


Figure 14.7. $\Delta^{14}C$ of Patagonian toothfish otolith cores plotted against the birth date determined by Reader 2 (see Table 14.1). Errors plotted are 1 standard deviation, but most are too small to be seen due to the size of the data points.

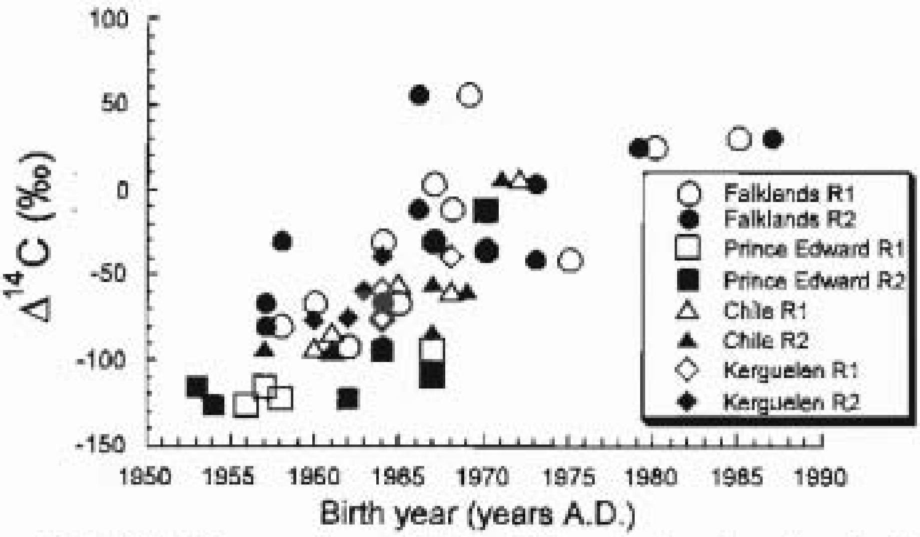


Figure 14.8. $\Delta^{14}C$ of Patagonian toothfish otolith cores plotted against the birth dates determined by Reader 1 and Reader 2 (see Table 14.1). Age has been clearly underestimated for at least two of the Falkland Islands toothfish by Reader 1. Errors plotted are 1 standard deviation, but most are too small to be seen due to the size of the data points.

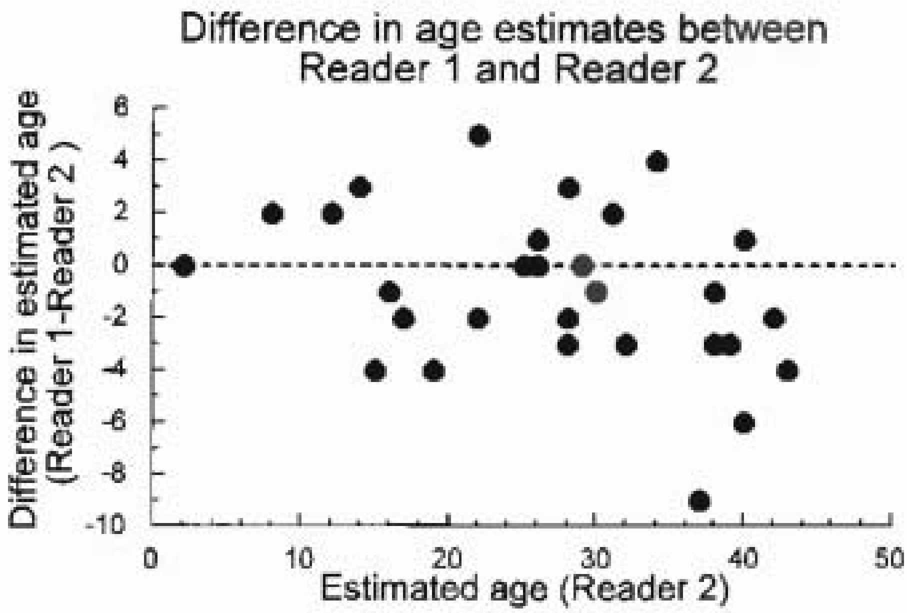


Figure 14.9. Differences (years) between age estimates determined for the validation set of otoliths (Table 14.1) by Reader 1 and Reader 2.

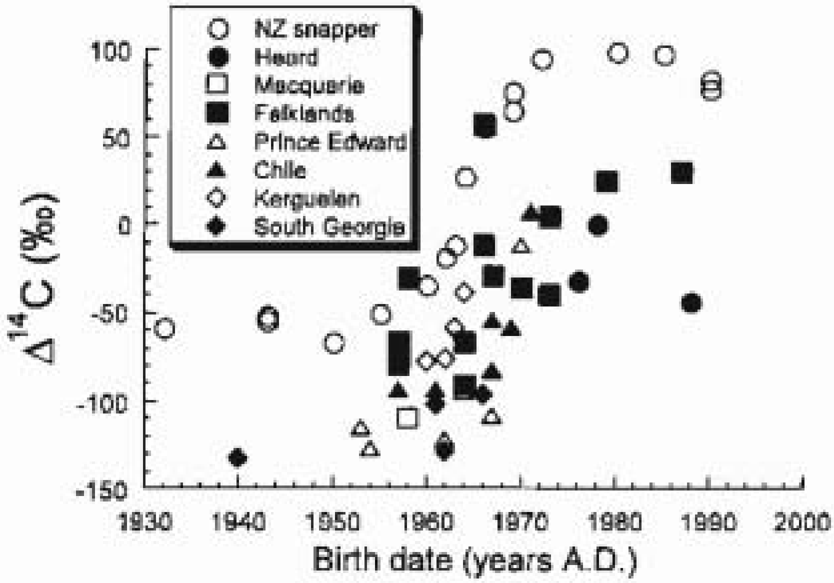


Figure 14.10. $\Delta^{14}C$ of Patagonian toothfish otolith cores plotted against the birth date determined by Reader 1 (see Table 14.1). $\Delta^{14}C$ data from New Zealand snapper *Pagrus auratus* otolith cores are plotted against the true birth dates (Kalish 1993) and demonstrate the difference 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.

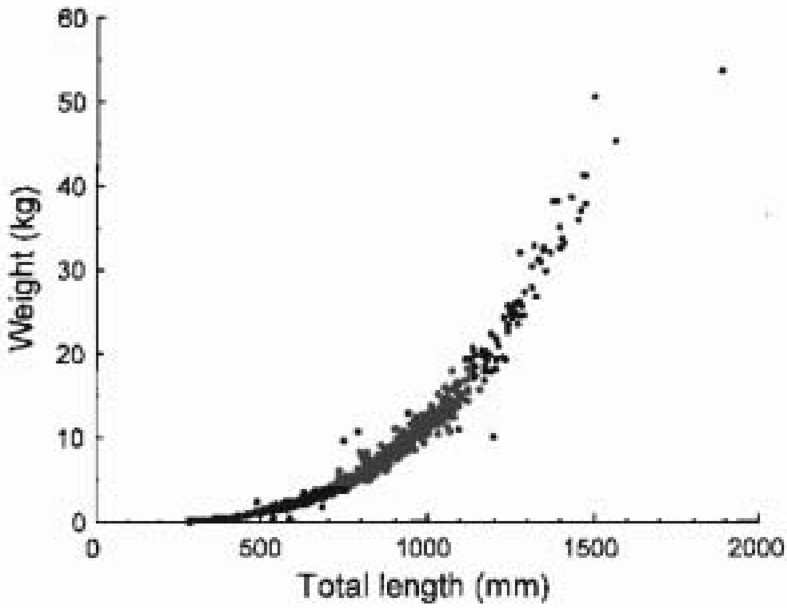


Figure 14.11. Length plotted against weight for Patagonian toothfish captured at Macquarie Island in the austral summers of 1995/96 and 1996/97. The exponential relationship between these data is weight = 3.06 x 10-8 x total length2.856 . Otolith samples from these fish were used in the study of age and growth.

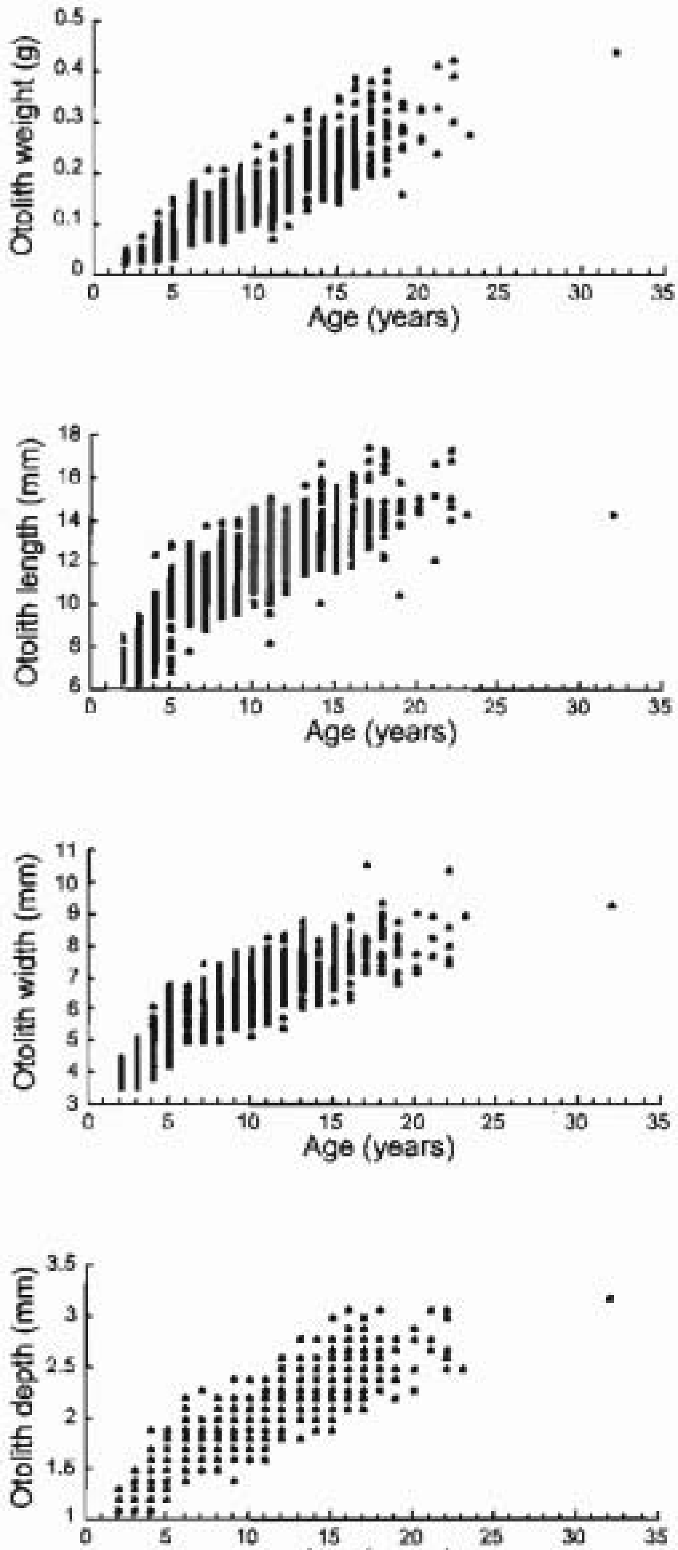


Figure 14.12. Relationships between Patagonian toothfish age estimated from otolith thin sections and otolith weight and otolith morphometrics.

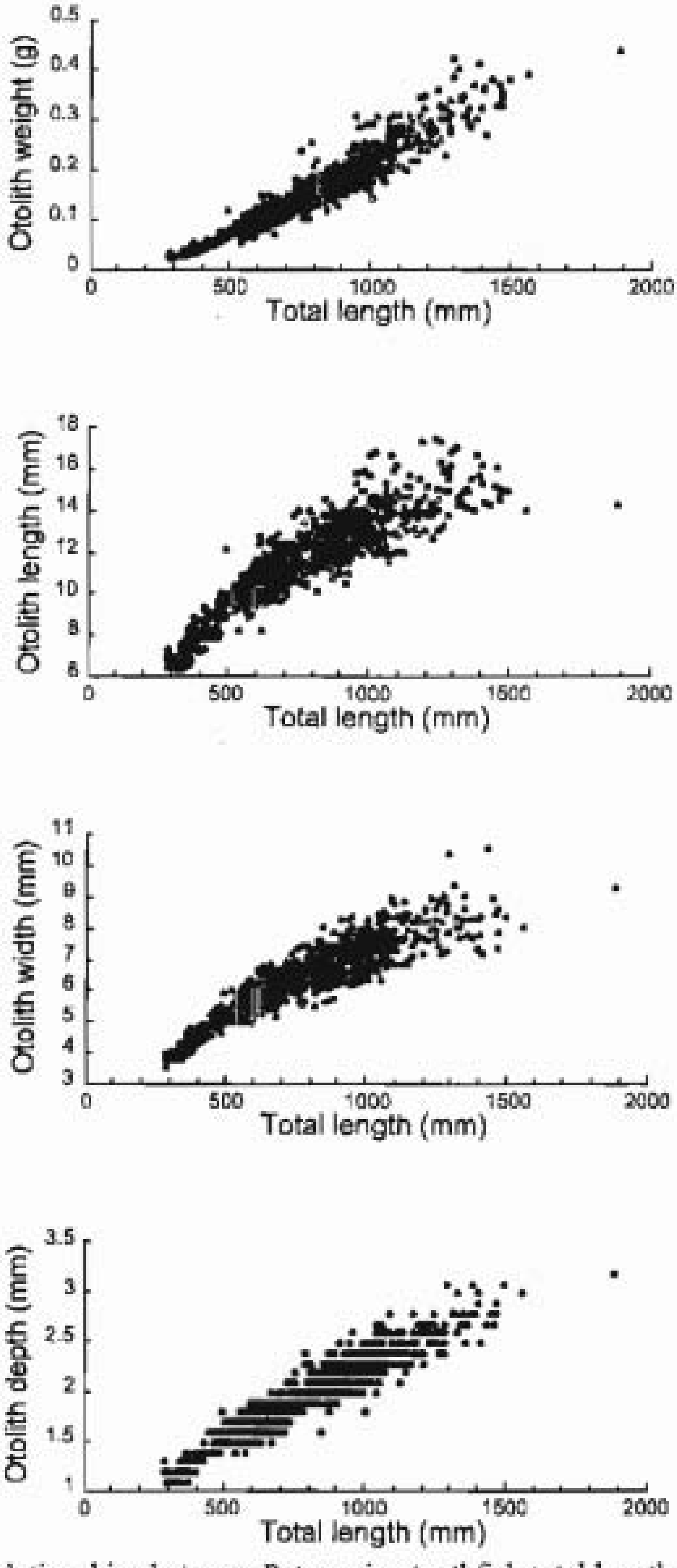


Figure 14.13. Relationships between Patagonian toothfish total length and otolith weight and otolith morphometrics.

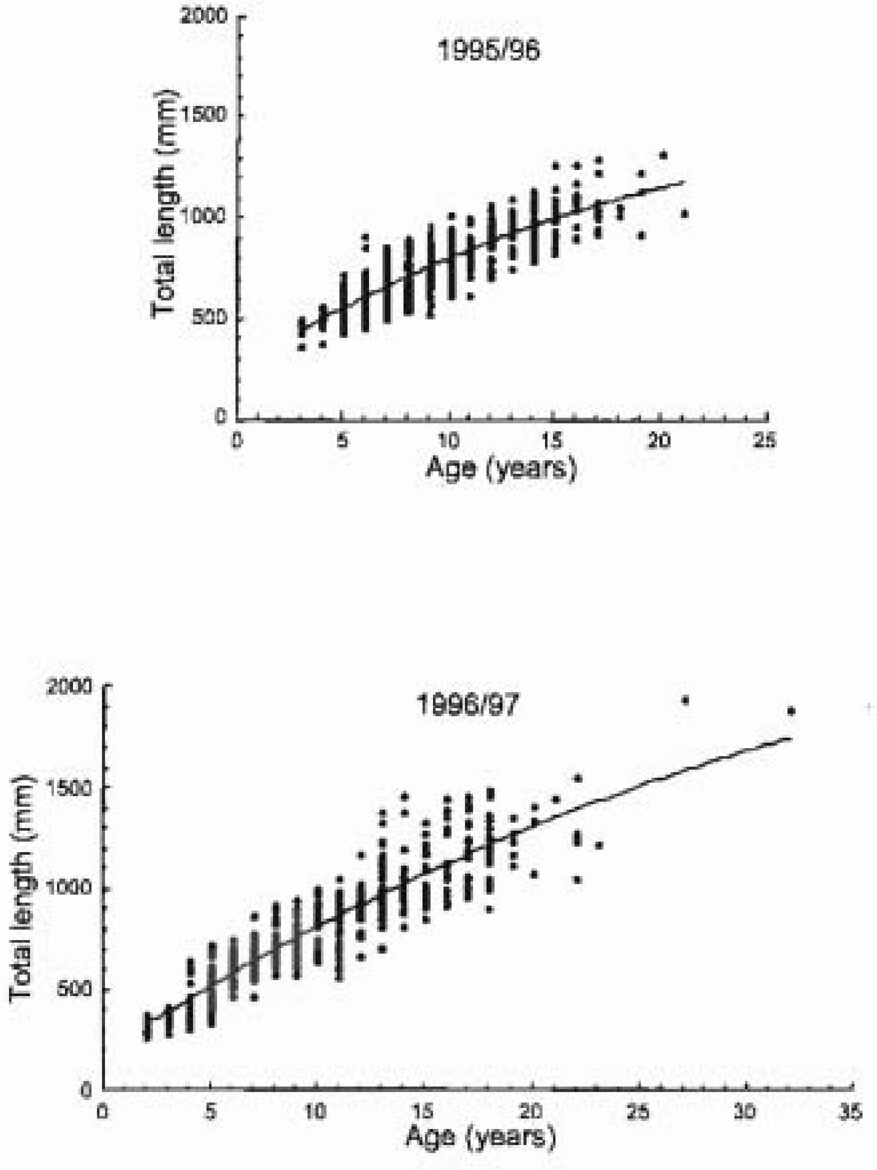


Figure 14.14. Age estimated from otolith thin sections plotted against total length and von Bertalanffy growth functions for Patagonian toothfish captured at Macquarie Island in the austral summers of 1995/96 (upper) and 1996/97 (lower). Parameter estimates for the von Bertalanffy growth functions are presented in Table 14.2.

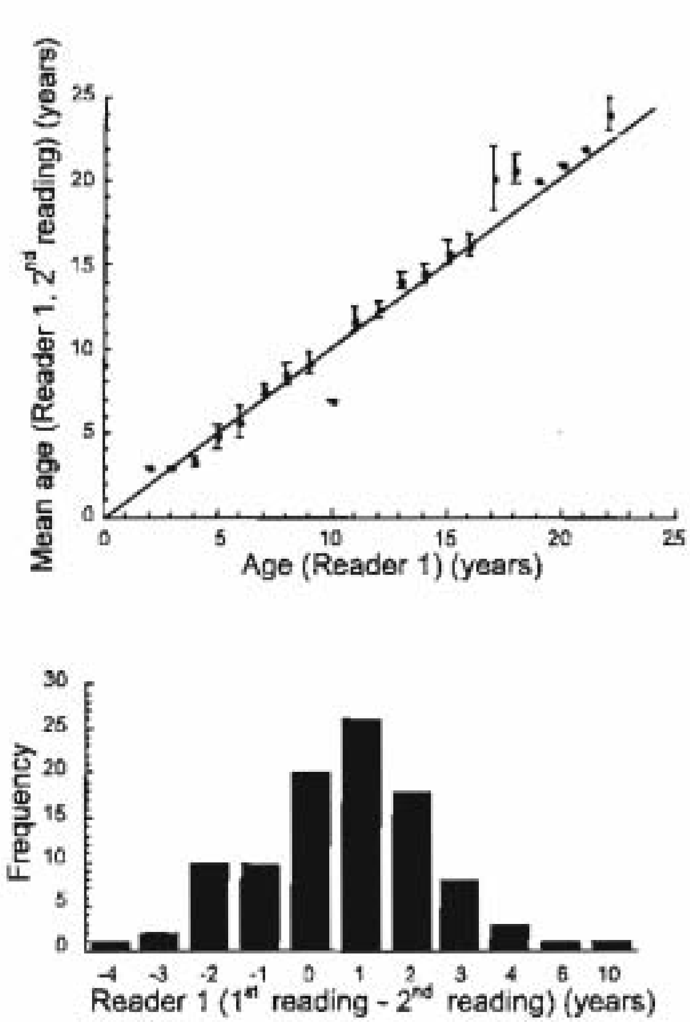


Figure 14.15. Age bias graph (upper) for a pair-wise comparison of repeat age estimates by Reader 1. Error bars represent the 95% confidence interval about the mean age assigned by Reader 1 (2nd reading) for all fish assigned a given age by Reader 1 (1st reading). Distribution of age differences between otolith readings (lower). Differences were calculated by subtracting the first age estimate from the second.

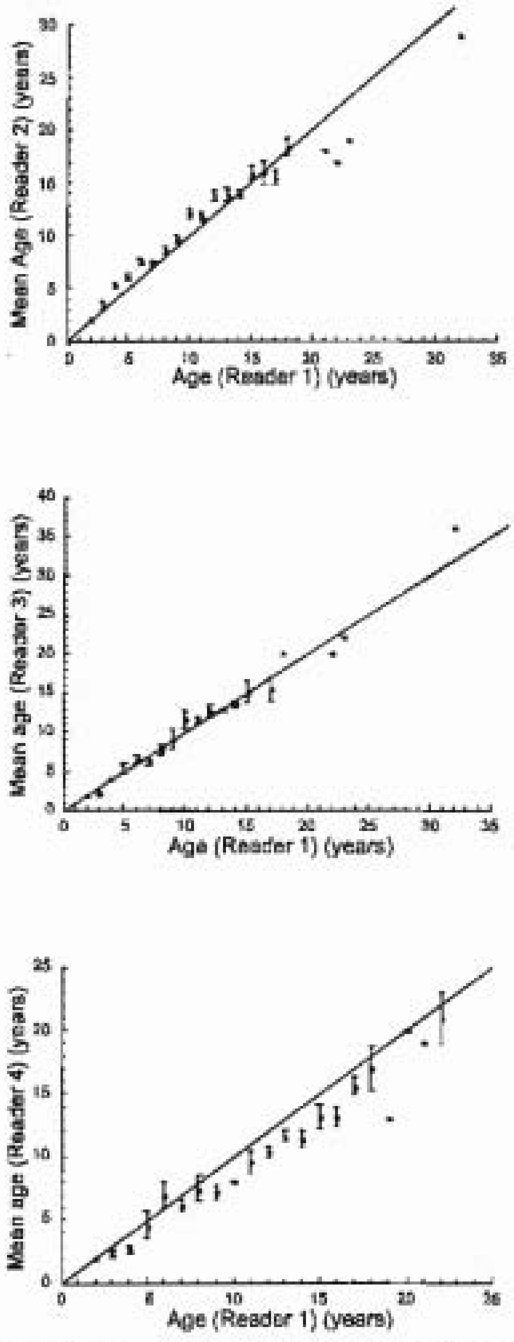


Figure 14.16. Age bias graphs for pair-wise comparisons of repeat age estimates by Reader 1 (Australian National University), Reader 2 (Central Ageing Facility) (upper), Reader 3 (National Institute for Water and Atmospheric Research) (centre) and Reader 4 (Old Dominion University) (lower). Error bars 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.

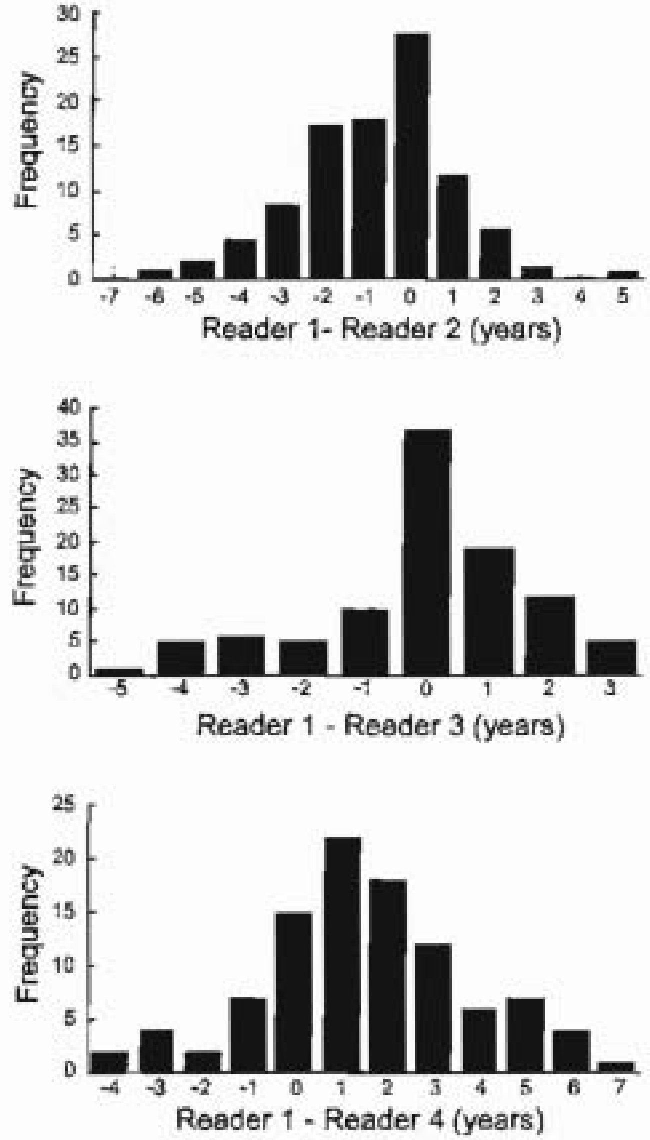


Figure 14.17. Distribution of age differences between otolith readings based on pair-wise comparisons of age estimates by Reader 1 (Australian National University), Reader 2 (Central Ageing Facility) (upper), Reader 3 (National Institute for Water and Atmospheric Research) (centre) and Reader 4 (Old Dominion University) (lower). Age estimate comparisons were based on randomly selected samples from Macquarie Island. Samples were prepared by Reader 1.

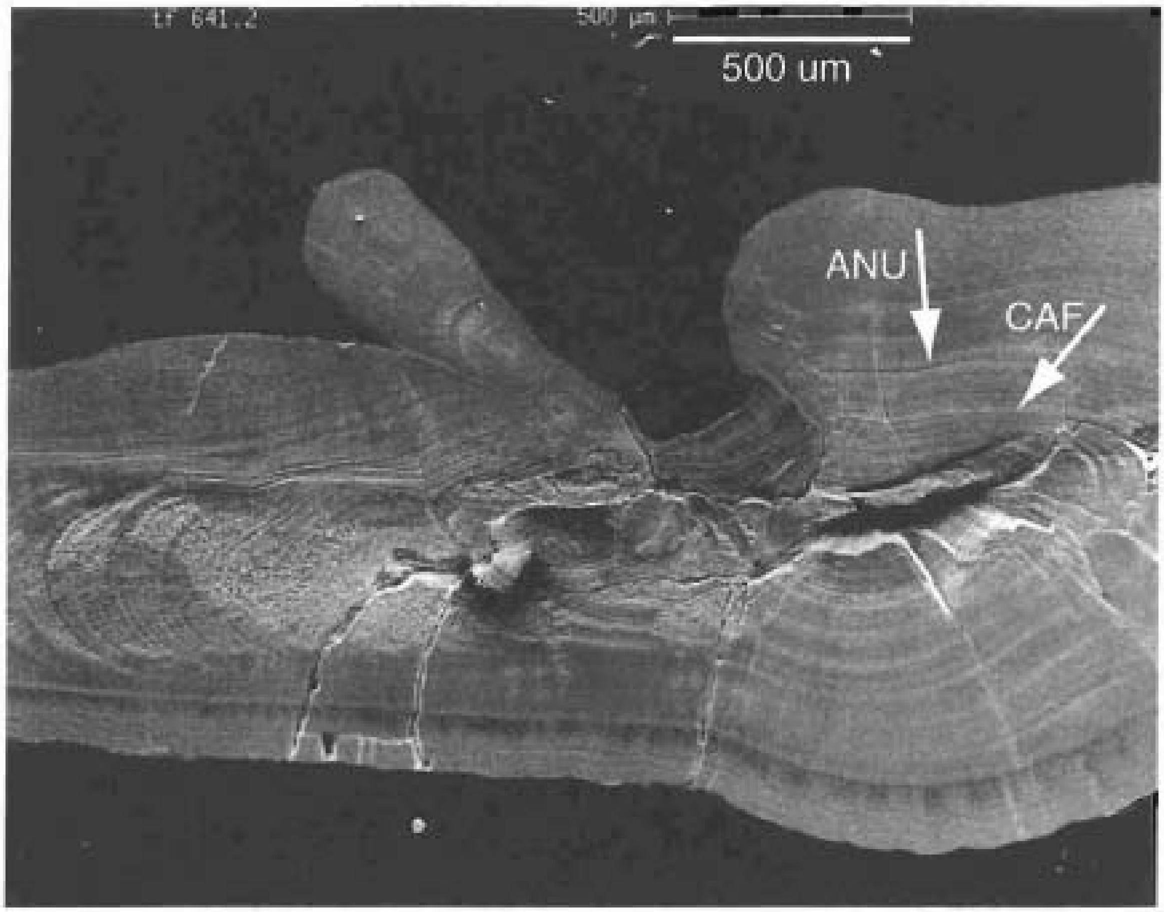


Figure 14.18. Scanning electron micrograph of an acid etched thin section of an otolith from a Patagonian toothfish estimated to be 16 years of age. The arrows labelled ANU and CAF identify the estimated position of the outer edge of the first annual increment as determined by those two groups. Counts of presumed daily increments were made from the otolith core to these positions.

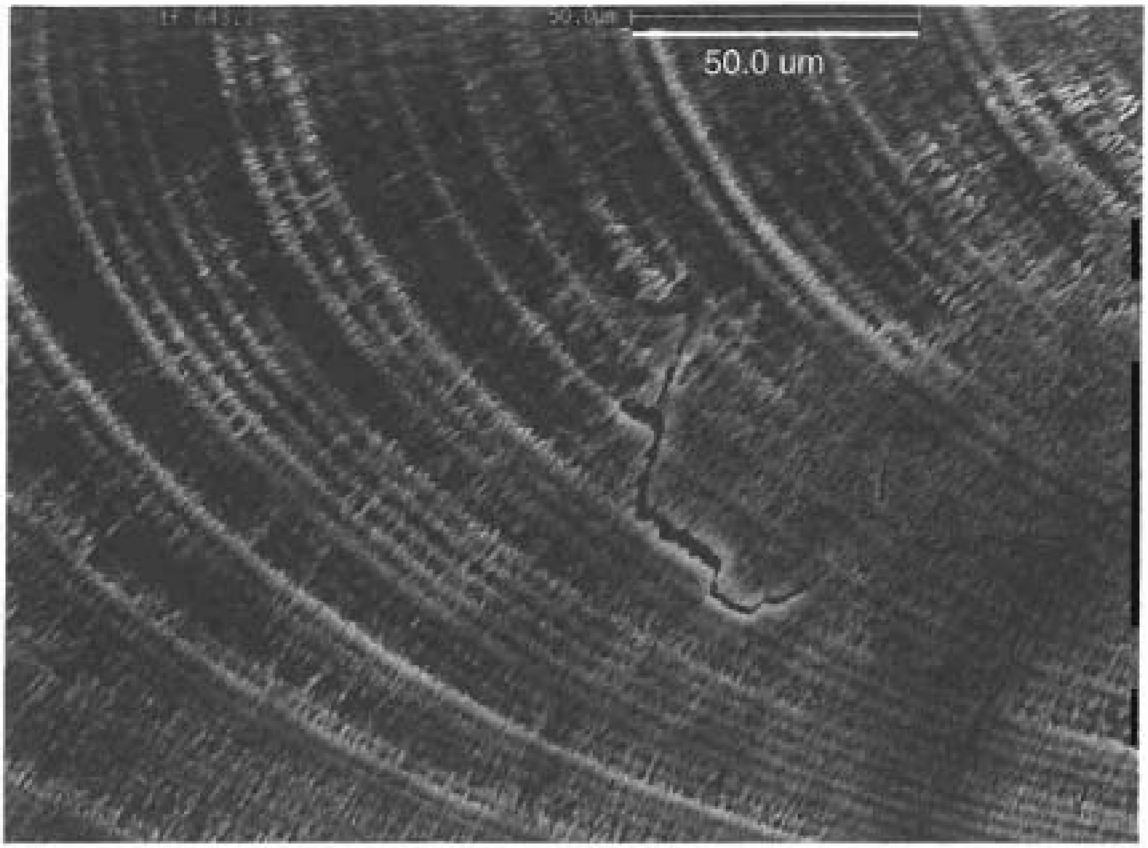


Figure 14.19. Scanning electron micrograph of an acid etched thin section of an otolith from a Patagonian toothfish estimated to be 16 years of age. The presumed daily increments shown here are dorsal to the otolith core.

# Chapter 15 Use of the bomb radiocarbon chronometer to validate estimates of age for black oreo (*Allocyttus niger*), smooth oreo (*Pseudocyttus maculatus*), spikey oreo (*Neocyttus rhomboidalis*) and warty oreo (*Allocyttus verrucosus*)

John Kalish, Justine Johnston, Sandy Morison and Corey Green

**Summary**

Measurements of radiocarbon in the earliest formed segments of black, smooth, spikey and warty oreo otoliths were compared with age estimates based on counts of presumed annual increments in thin sections of the 'sister' otoliths for each fish. Measurements of radiocarbon in the earliest formed segments of otoliths from these four oreosomatid fishes provide varying degrees of validation for age estimates derived from otolith thin sections. $\Delta^{14}C$ data from otolith cores of smooth oreo plotted against otolith section birth date describes temporal changes in radiocarbon that are indicative of the bomb radiocarbon increase in the 1960s and provide evidence that otolith based age estimates for this species are the most accurate of the four species investigated. The results provide conclusive evidence of minimum potential longevities of 35, 35, 29 and 28 years for black, smooth, spikey and warty oreos, respectively. However, there is no qualitative change to the appearance of increments that might suggest that the periodicity of increment formation is altered for fish older than 35 years and far older ages for these species are supported on the basis of radiocarbon analyses in conjunction with counts of presumed annual increments. Relatively low $\Delta^{14}C$ in relation to time for all four species and, in particular, for smooth oreo provides strong support that young juvenile habitats of these oreosomatid fishes are far removed from the adult habitats. Large negative values for $\Delta^{14}C$ from the smooth oreo otolith cores indicate that it is extremely unlikely that the adult smooth oreos spent their juvenile lives in surface waters at mid-latitudes. The radiocarbon levels measured in the otoliths are consistent with those expected for a fish living in the surface mixed layer at about 65°S latitude. This conclusion is supported by the limited ecological data on smooth oreo. Radiocarbon data from black oreo otolith cores also indicates that these fish inhabit high latitudes, around 60°S latitude, as juveniles. Both spikey and warty oreos may also inhabit surface waters of the Southern

Ocean based on $\Delta^{14}C$ measured in otolith cores. Further research is required to ensure effective management of these commercially important, but poorly understood fish species.

**Introduction**

Age estimation for deep-sea fishes is problematic due to difficulties involved in the interpretation of marks on hard parts used to age fishes, predominantly otoliths. Furthermore, logistical problems associated with the deep-sea habitats and methods used to harvest these species make tagging studies and, concomitantly, the validation of a "preferred" age estimation procedure impractical, if not impossible. Despite the complications associated with age estimation and the related validation for commercially important deep sea fishes, these areas of research have a high priority and require the application of novel methodologies.

A critical requirement for all age estimation methods is that of validation (Beamish and McFarlane 1983), where the validation process provides an estimate of the level of accuracy of the age estimation method (Francis et al. 1992, Kalish et al. 1995). Validation has often relied on the demonstration of annual cycles in the marginal growth of structures. Such cycles were often poorly defined and highly variable. Other means of validation involved matching age estimates to modes in length-frequency distributions, a method that was applied successfully to determine age of small juvenile orange roughy from one to three years of age (Mace et al. 1990). Both these techniques are often only applicable to younger, faster growing species or age classes. Validation may also be achieved by tracking the progression of sequences of strong and weak year-classes over several years of sampling. This method has been applied to young orange roughy (Doonan unpublished data, cited in Tracey and Horn 1999) and to other species (Morison et al. 1998a). Calciphilic fluorochrome dyes, such as oxytetracycline hydrochloride (OTC), create an unequivocal time mark on structures such as otoliths, and since the method was first suggested (Weber 1962) has become one of the most commonly employed methods of validation. However, this method is dependent usually on a successful capture-mark-recapture study.

The development of age estimation methods for deepwater fish species has proved to be a difficult problem. Unvalidated increment counts on hardparts, have indicated that many species are long-lived and slow-growing. However, validation of these age estimates is inherently difficult for deepwater fish species (Stewart et al. 1995). Methods that rely on recapture of tagged or marked individuals are usually not applicable because of the low probability of capturing, tagging and releasing fish alive to their deepwater habitats. Methods such as marginal increment analysis and interpretation of modes in length-frequency distributions are usually only applicable to juveniles or young adults, when growth is more rapid and increments are larger. The otoliths themselves are often small and the increments in them are frequently narrow and more difficult to interpret. The appearance of increments may also change with age as in the otoliths of species of oreo (Family Oreosomatidae), for which there is a major habitat shift from a juvenile pelagic phase to a demersal adult phase (George et al. 1998).

Using scanning electron microscopy, Davies et al. (1988) examined the otolith ultrastructure of black oreo (*Allocyttus niger*) and smooth oreo (*Pseudocyttus maculatus*) using whole otoliths, very thinly sectioned (20 μm) otoliths, and acetate peels and identified a close similarity in otolith structure between the two species. They were unable to develop a technique for age determination citing complex crystal morphology that obscured the sequences of increments. More recently, estimates of age and growth for black and smooth oreo from New Zealand, made using presumed annual increments in otolith sections, have suggested that both species are also long-lived and slow-growing (Annala 1992). Similar results were obtained from thin sections (approximately 300 μm) of sagittal otoliths of these two species collected from southern Australia (Smith and Stewart 1994) as well as for warty oreo (*Allocyttus verrucosus*) and spikey oreo (*Neocyttus rhomboidalis*) (Smith and Stewart 1994). Smooth oreo otoliths have been reported to be easier to read than black oreo otoliths (Doonan et al. 1995), although Smith and Stewart (1994) reported similar estimates of precision for these four oreosomatid species. Despite the lack of rigorous validation for all of these species, age estimation has relied on the use of counts of presumed annual increments in otolith thin sections.

The application of radiometric age estimation techniques, based on radioisotopic disequilibria (e.g. 210Pb/226Ra activity ratios) (Bennett et al. 1982; Campana 1990) provided a new method for validating ages for relatively old species. The method has been applied to orange roughy (Fenton et al. 1991) and warty oreo (*Allocyttus verrucosus*) (Stewart et al. 1995) and has shown consistency in age estimates with those from increment counts on otoliths. Errors associated with radiometric age estimation, however, are relatively large. Furthermore, sample sizes required for analysis of low-level radioisotopes by □-

spectrometry are large and often require samples from several fish for an individual analysis. For example, analyses of warty oreo otoliths required the pooling of a large number of samples (Stewart et al. 1995). This makes it difficult to apply radiometric age estimation data to validation of the 'routine' age estimation procedure for oreosomatid fishes that has been based on interpretation of presumed annual increments in otolith thin sections.

Some aspects of the radiometric method of age validation have been challenged (West and Gauldie 1994) on the basis of a failure to test or meet essential assumptions. According to West and Gauldie (1994) the radiometric method did not address adequately: (1) the potential loss of a 226Ra daughter product, gaseous 222Rn, from the otolith; (2) the potential for changes in the rate of uptake of 210Pb over the life of the fish; and (3) the critical dependence of the method on the otolith mass growth-in-time model. They concluded that these points disqualified the 210Pb:226Ra disequilibrium technique as a means of validating teleost ages. Radiometric methods that do not employ cores also have a serious potential for circularity when otolith growth is being estimated using the method of direct age estimation that is being validated (Kimura and Kastelle 1995). However, radiometric estimates of the ages of black, smooth and spiky oreo have been completed successfully (Fenton 1996). These analyses confirmed the longevity of these species and produced age estimates that were similar to those obtained from increment counts in sectioned otoliths. These radiometric analyses were independent of the increment counts, as the method of data analysis required no modelling of the otolith mass growth rate.

It has also been shown that substantial changes in the rate of uptake of 210Pb only slightly reduce the maximum ages estimated (Campana et al. 1990; Smith et al. 1995), and that a loss of 222Rn would increase the maximum age (Stewart et al. 1995). Similarly, Francis (1995) modelled the effects of the form of otolith growth model adopted, and the assumption of constant relative uptake of 210Pb and 226Ra, on the resulting estimated maximum ages for orange roughy. He estimated a lower bound on the maximum age of orange roughy (using the reported variability in activity ratios) as 84 years. Nevertheless, as a result of these concerns there are still some uncertainties about the maximum age of orange roughy, in particular and deep-sea species in general.

Validation of methods used for fish age estimation can also be achieved by two different techniques that are based on the measurement of radiocarbon in fish otoliths. These methods are the 'bomb radiocarbon chronometer' and 'radiocarbon dating' (Kalish 1993; Kalish 1995b). Application of these methods to studies of fish otoliths is dependent on application of a technique known as accelerator mass spectrometry (AMS), a technique that allows precise measurements of radiocarbon to be made on relatively small samples (<1 mg of carbon). The ability to measure low levels of radiocarbon with high precision and in small samples is essential for the success of both methods of validation.

The bomb radiocarbon chronometer can be used to estimate the age of individual fish and the technique is well suited to estimating the age of long-lived fish such as black, smooth, spikey and warty oreos. The bomb radiocarbon chronometer relies on measurements of radiocarbon in selected portions of fish otoliths and relates these data to radiocarbon levels found in the environment. Under "normal" circumstances these measurements would not appear to be useful due to the relative constancy of environmental radiocarbon levels; however, human activities have altered the levels of radiocarbon in the environment both rapidly and drastically. The most dramatic change in radiocarbon levels was due to the atmospheric testing of nuclear weapons between 1958 and 1963 (Kalish 1995b). These atmospheric tests released large quantities of radiocarbon into the atmosphere and ultimately increased atmospheric levels of radiocarbon by over 100%. This increase was so rapid and great that the entry of this material into the ocean effectively marked (in a manner analogous to marking with OTC) all fish living in the surface mixed layer of the ocean during the 1960s and 1970s. This radiocarbon mark is useful because we have a precise knowledge of the time when the mark would have become incorporated into a fish or, more specifically, the fish's otoliths. In addition, the age of an individual fish can be broadly classified as either pre-bomb or post-bomb, where the earliest formed regions of otoliths from pre-bomb fish have $\Delta^{14}C$ values that are typically below -50‰, a value that is representative of the surface ocean prior to significant atomic testing at temperate latitudes (Broecker et al. 1985, Kalish 1995b). This is a practical validation technique for deepwater species where the juvenile stage occurs in the surface mixed layer (Kalish 1995b) where it is exposed to the elevated levels of $^{14}C$. The method would not be applicable to deepwater species without early life history stages that inhabited the surface mixed layer of the ocean for at least six months (dependent on the size of the otolith). The bomb radiocarbon chronometer has been applied successfully to several teleost species that inhabit a range of depths in the south west Pacific Ocean (Kalish 1993, Kalish 1995a; Kalish et al. 1996; Kalish et al. 1997) and also to species in the northern hemisphere (Campana 1997; Campana and Jones 1998). These studies have demonstrated that the bomb radiocarbon method is a relatively inexpensive and rapid technique for age validation.

The relationship between time and $\Delta^{14}C$ is straightforward in relation to fish living all or, at least the early stages (e.g. first 6 months) of their lives in the surface mixed layer of the ocean. Black, smooth, spikey and warty oreo have life histories that are poorly understood. However, limited collections of the juvenile life history stages indicate that these species live in the surface mixed layer of the ocean for, in some cases, several years before migrating to deeper habitats as adults (James et al. 1988, George et al. 1998). Interpretation of presumed annual increments in otolith thin sections from smooth oreo suggested that these species live in the surface mixed layer for about three to six years (George et al. 1998) and similar changes in increment structure are evident in the other oreo species. Due to the fact that these species are likely to deposit adequate quantities of otolith material for radiocarbon analysis while living in the surface mixed layer of the ocean, the bomb radiocarbon chronometer provides a means of validating the otolith thin section method of age estimation for black, smooth, spikey and warty oreo.

**Materials and methods**

Otoliths were weighed dry and then prepared for radiocarbon and stable carbon isotope analysis. The earliest formed segments of individual otoliths were isolated with a fine, high speed drill. This was achieved by "sculpting" from the larger otolith, an otolith that was representative of a smooth oreo or black oreo otolith of about 4 years of age (Figure 15.1). During the sculpting process the position of "landmarks" such as the otolith core and zones associated with the presumed first four annual increments were monitored frequently. This ensured that the sculpted otolith contained material only deposited during the early pelagic phase of the fish's life which is spent in the surface mixed layer of the ocean. The final product was a single piece of otolith aragonite (Fig. 15.1). Sample weights ranged from about 4.0 mg to 4.8 mg from smooth oreos, 5.0 mg to 6.6 mg from black oreos; 2.4 mg to 4.4 mg for warty oreo and 1.8 mg to 4.2 mg for spikey oreos.

In the early 1990s the smallest sample of carbon that state-of-the-art accelerator mass spectrometry laboratories were able to routinely analyse with high precision (< 10% error) for radiocarbon was approximately 1.0 mg. Otoliths are mostly aragonite, a mineral of calcium carbonate (Ca CO3) that is 12% carbon by weight. Therefore, an otolith sample weighing 10 mg was close to the size limit for a high precision analysis; this is the approximate size of many of the samples analysed during this FRDC project. In recent years various accelerator mass spectrometry laboratories have worked to reduce the minimum size of the sample of carbon required for a high precision analysis. Several laboratories already involved with this FRDC funded research program on age validation based on the bomb radiocarbon chronometer were willing to analyse very small samples of otolith aragonite. These laboratories included the Radiocarbon Accelerator Unit, Research Laboratory for Archaelogy and the History of Art, Oxford University (OxA), the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, University of California (CAMS LLNL) and the U.S. National Science Foundation Accelerator Mass Spectrometry Facility, University of Arizona (AA). These laboratories analysed selected small samples (< 5 mg of aragonite) for this research on radiocarbon in otoliths of oreosomatid fishes. The majority of the radiocarbon analyses for this study were carried out by the Australian Nuclear Sciences and Technology Organisation, Lucas Heights, New South Wales.

Established procedures were followed for the preparation of graphite targets from calcium carbonate. After pre-preparation, otolith carbonate was converted to CO2 by reaction in vacuo with 100% phosphoric acid. An aliquot of the CO2 was used to determine $\delta^{13}C$ for some samples and the remaining CO2 was converted to graphite for analysis of radiocarbon. For those samples that were not analysed for $\delta^{13}C$, a value of –3.00 ‰ was estimated on the basis of earlier measurements of stable carbon isotopes in deepwater teleosts, including oreos. 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.

Details of methods used to estimate age for black, smooth, spikey and warty oreos on the basis of thin sections can be found in Smith and Stewart (1994), Doonan et al. (1995), Stewart et al. (1995) and Morison et al. (1999).

**Results**

*Smooth oreo*

$\Delta^{14}C$ data from smooth oreo otolith cores from fish collected in the Pacific Ocean off New Zealand and Tasmania (Table 15.1) are plotted against otolith section birth date in Figure 15.2. These data define a curve with the basic characteristics of a pre-bomb and post-bomb radiocarbon curve and show the increase in $\Delta^{14}C$ during the 1960s and 1970s.

Pre-bomb radiocarbon ranged from -111.9‰ to -127.2‰ for smooth oreo with estimated birth dates during the years between 1917 and 1956. Radiocarbon increased to -108.5‰ in the core of a smooth oreo otolith with an otolith section birth date of 1962 and reached -62.0‰ for an individual with an estimated birth date of 1964. After 1964 $\Delta^{14}C$ remained elevated significantly above pre-1962 levels and varied between -36.2‰ and -77.3‰. The highest $\Delta^{14}C$ measured was –36.2 ‰. from a fish with an estimated birth date of 1978. Two samples (sample nos. SMO8(/9) and SMO1(/10)) had high $\Delta^{14}C$ relative to the birth dates estimated from the otolith thin sections and fell above the general trend defining the increase in bomb derived radiocarbon during the 1960s. Unlike the measurements from *Pagrus auratus*, $\Delta^{14}C$ did not reach an asymptote in the smooth oreo samples analysed with the oldest smooth oreo having an estimated birth date of 1978. There was no evidence of a significant difference in the relationship between $\Delta^{14}C$ and age estimated from otolith thin sections for the samples from Tasmanian and New Zealand fish (Figure 15.2).

The difference in $\Delta^{14}C$ between *Pagrus auratus* and smooth oreo was relatively constant across all years with $\Delta^{14}C$ about 60‰ lower in the smooth oreo otolith cores.

*Black oreo*

$\Delta^{14}C$ data from black oreo otolith cores plotted against otolith section birth dates (Table 15.2) show a general increase in $\Delta^{14}C$ after 1960 consistent with the effects of atmospheric testing of atomic weapons (Figure 15.3). However, several samples (Sample nos. BOE16, BOE29 and BOE13) had relatively low otolith $\Delta^{14}C$, characteristic of the pre-bomb period prior to about 1960, despite the post-bomb birth dates estimated from otolith thin sections for these samples (Figure 15.3, Table 15.2). $\Delta^{14}C$ ranged between -93.3‰ and -14.4‰ in the cores of otoliths from black oreos with presumed birth dates between 1950 and 1978. Between 1950 and 1966 $\Delta^{14}C$ was less than -50‰ and increased to -25.9‰ in 1968. In 1970 there was a large decrease in $\Delta^{14}C$ to -89.3‰ and in 1972 $\Delta^{14}C$ returned to the previously elevated levels. In most years, black oreo $\Delta^{14}C$ was higher for a particular year than $\Delta^{14}C$ measured in the smooth oreo otoliths.

*Warty oreo*

$\Delta^{14}C$ data from warty oreo otolith cores from fish collected in the Pacific Ocean off Tasmania (Table 15.3) are plotted against otolith section birth date in Figure 15.4. The oldest fish had a birth date of 1873 (age of 115 years) and a $\Delta^{14}C$ of –89.1‰, the lowest for any of the warty oreo otolith cores. Eight fish had birth dates, estimated from otolith thin sections, between 1956 and 1960, but a relatively wide range of $\Delta^{14}C$ of from -75.8‰ to 38.3‰. Warty oreo with otolith based birth dates estimated to be more recent than 1960 had $\Delta^{14}C$ consistently well above pre-bomb levels.

*Spikey oreo*

$\Delta^{14}C$ data from spikey oreo otolith cores plotted against otolith section birth dates (Table 15.4) show a general increase in $\Delta^{14}C$ after 1960 consistent with the effects of atmospheric testing of atomic weapons (Figure 15.4). Two spikey oreo otolith cores, from fish with estimated birth dates prior to 1900 had $\Delta^{14}C$ of –72.5‰ and -75‰ and a third fish with a pre-bomb birth date of 1958 had a $\Delta^{14}C$ of –64‰. Samples with birth dates later than 1958 had clearly elevated $\Delta^{14}C$ measured in otolith cores relative to pre-bomb values. Post bomb $\Delta^{14}C$ ranged between -10‰ to 36‰ for samples from fish with estimated birth dates of 1969 and 1985, respectively. Despite the clear differentiation between fish with estimated pre-bomb and post-bomb birth dates, there was no clear pattern among the post-bomb results.

**Discussion**

The results provide validations of varying degrees for the smooth, black, spikey and warty oreo age estimates derived from otolith thin sections. This interpretation is based on the fact that most samples with birth dates prior to 1960 had significantly lower $\Delta^{14}C$ than those measured in samples with birth dates estimated to be after 1960. This pattern is particularly evident for the smooth and spikey oreos. For the most part, black and warty oreo samples demonstrate similar patterns with separate groupings of pre- and post-bomb fish, however, several samples deviate from this general pattern (Figures 15.3 and 15.4). The $\Delta^{14}C$ measurements from black oreo samples that did not fit the expected pattern are consistent with the conclusion that the ages were underestimated (up to about 8 years for BOE13) from the thin sections. There is no evidence to suggest that any of the black oreo otolith thin section ages were over estimated. The potential errors in age estimation based on the warty oreo sections appear to be less extreme; however, it is not possible to determine the precise extent of the errors. In the case of the warty oreo analyses, there is evidence that several ages may have been over estimated.

Five of the oreo samples used for these analyses had estimated birth dates prior to 1930 and a total of 17 samples had age estimates that were indicative of a birth date prior to 1960, around the time before it would be possible to detect bomb radiocarbon in the surface waters of the Southern Ocean or temperate South Pacific (Figure 15.5). Research on radiocarbon in the Southern Hemisphere demonstrated that it would be unlikely to detect bomb radiocarbon before 1961 south of 50°S latitude in most ocean regions (Bien et al. 1965; Broecker et al. 1985), but bomb radiocarbon would be detectable about 1958 at lower latitudes to the north of the Subtropical Convergence Zone. On this basis, it becomes impossible to distinguish among fish with a birth date prior to about 1960; however, this would be dependent on the species and the associated habitat of the early juvenile stages. This birth date equates to ages older than 37 years of age with the 1997 collection date of the majority of smooth and black oreo samples and 28 or 29 years of age for the warty and spikey oreos, respectively, with collection dates of 1988 and 1989. . In the most generally sense, the fact that the vast majority of oreo samples with otolith based birth dates prior to 1960 had low $\Delta^{14}C$, consistent with their estimated birth dates demonstrates the minimum longevities for these species and indicates that these longer lived individuals can be identified from counts of opaque and translucent zones in otolith thin sections. Apart from the narrowing of increments after the first 3-6 years, the pattern of opaque and translucent zones, which was counted to estimate ages for these samples, is the same on fish estimated to be 20 years old and 90 years old. There is no qualitative change to the appearance of increments that might suggest that the periodicity of increment formation alters for fish older than, say, 30 years.

Age validation of otolith thin section method was most effective for smooth oreo (Figure 15.2). The results provide clear evidence of a sharp increase in radiocarbon after 1960 and, for the most part, show an increase in $\Delta^{14}C$ at a stable rate between 1962 and 1978. Only two points deviate from this trend, suggesting that ages of these two fish may have been overestimated. In addition, the four smooth oreo with birth dates during the 1950s show a constant decline in $\Delta^{14}C$ during this period, consistent with a Suess effect of about -2.5‰ during this period. The decrease in atmospheric and ocean radiocarbon levels, attributable to the burning of radiocarbon free fossil fuels, between about 1850 and the present is called the Suess effect (Se) (Kalish 1995b). A value of –2.5‰ is consistent with estimates of Se from other ocean regions and estimated from analyses of radiocarbon in corals (Druffel and Suess 1983), but it is considerably higher than the only other estimate made at high latitudes.

Kalish et al (2001) found evidence for a minor Suess effect of only 0.2‰ per year between 1919 and 1950 for another fish species at high latitudes, the Arcto-Norwegian cod.

The inability to discern clearly detail of the rapid increase in radiocarbon during the 1960s for black, spikey and warty oreo suggests that the accuracy of the age estimates for these species from otolith thin sections is relatively low. For black oreo, samples were selected with estimated birth dates at intervals of two years in order to take advantage of the bomb increase period from 1962 to about 1970, the period with the finest level of temporal resolution. Despite this selection of samples, the black oreo data did not produce a curve characteristic of the rapid increase in $\Delta^{14}C$ during the 1960s. This conclusion, however, assumes that a series of 'cores' (i.e. first four years of otolith growth) from oreo otoliths with precisely known ages would provide an accurate record of radiocarbon variation in an 'homogeneous' ocean of the Southern Hemisphere. Due to the complex early life history of oreo species, this last assumption may not be easily resolved, as is discussed below.

The world's oceans are not homogenous in relation to the distribution of radiocarbon. Radiocarbon in the ocean varies spatially and temporally and this is the basis for the value of this radioisotope as a tracer of ocean circulation and carbon flux between the ocean and atmosphere. 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; Kalish et al. 2001), data that are important to research on ocean and atmosphere dynamics.

The distribution of the pelagic juvenile stages of smooth, black, spikey and warty oreo is poorly known, however, data that are available suggest that these early life history stages inhabit a broad region of the Southern Ocean and the high temperate latitudes of the Pacific, Atlantic and Indian Oceans. Although distributional information is limited, occurrence of these fish across a vast area would expose them to a wide range of water masses with $\Delta^{14}C$ that varies spatially and temporally. Subsequent recruitment to the adult populations, for example, within the EEZs of Australia and New Zealand, may derive from across the range of the pelagic juveniles and increase the difficulty of reconstructing a smooth 'bomb radiocarbon curve', even from fish of known age.

Due to the potentially broad distribution of oreo juveniles across all sectors of the Southern Ocean, it was not feasible to construct a general calibration curve of radiocarbon variation that would be applicable to each of these species. Therefore, it was not feasible to determine statistically, deviations of $\Delta^{14}C$ measured in oreo otolith cores from an estimated bomb radicarbon curve. In a previous study on *Centroberyx affinis* (Kalish 1995a), an age estimation error was considered likely if a point fell outside the 95% confidence intervals for the curve describing the relationship between year and $\Delta^{14}C$. For this study on oreos, it was only feasible to provide a crude validation of the age estimation method. For black, spikey and warty oreo the birth dates could only be classified as pre-bomb or post-bomb and, with limited reliability, 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 of 10 years or more. The data for smooth oreo are far more convincing in reconstructing a bomb radiocarbon curve, however, no time series of radiocarbon is available for the region tentatively identified as the juvenile habitat of smooth oreo and, again, it is difficult to determine the magnitude of age estimation errors.

Interpretation of radiocarbon data from the oreo otoliths, in conjunction with available data on the species' life histories and the temporal and spatial variation of radiocarbon in the southern hemisphere, can provide information that is critical to an understanding of the dynamics of oreo fisheries. Although there are few data on the habitats of the juvenile life history stages of smooth, black, spikey and warty oreo and age estimates based on otolith thin sections provide reasonable, but imprecise age estimates, these data are essential to development of a firm understanding of the observed variation in otolith radiocarbon.

Adult smooth oreo are most abundant at latitudes between 35°S and 52°S (James et al. 1988) and have only recently been found in high latitudes of the northern hemisphere (Post and Jonsson 1996). In New Zealand about 18,000 t of smooth oreo are trawled each year from depths of around 800 to 1200 m and a far smaller amount in Australian waters. The species is also a significant bycatch in trawl fisheries that target orange roughy (*Hoplostethus atlanticus*) on the high seas. Despite the abundance of smooth oreo adults (>135 mm SL), the juveniles are extremely rare and appear to have a distribution that is quite different from that of the adults. The majority of juvenile smooth oreos (32 specimens) (<135 mm SL) have been collected between 60°S and 68°S latitude in association with krill or in the stomachs of fin whales (Abe 1957; Svetlov 1978; Abe and Suzuki 1981). Three other juveniles have been collected: two at about 44°S latitude near New Zealand and one at about 48°S latitude near Patagonia. Adults are adapted to deepwater habitats whereas the juveniles display characteristics that would be expected of fish living in surface waters due to their silver colouration with an irregular pattern of bluish blotches and small eyes. From the results of the few juvenile collections and their physical appearance, it has been assumed that these fish occur in surface waters.

Relatively large negative values for $\Delta^{14}C$ from all smooth oreo samples (Table 15.1) indicate that the smooth oreos, collected as adults in deep water off New Zealand, spent their juvenile lives in surface waters at highlatitudes. Comparison with data on $\Delta^{14}C$ measured in seawater DIC (Figures 15.6, 15.7, 15.8 and 15.9) provides support for two hypotheses: 1) adult smooth oreos collected in New Zealand water's use waters south of 60°S latitude as a juvenile nursery ground and; 2) juvenile smooth oreos collected at mid-latitudes have strayed from the normal nursery ground or are at the northern edge of their range. If the smooth oreos with post-bomb birth dates had been in about the top 200 m of the water column at mid-latitudes (~35°S to 45°S) as juveniles, a $\Delta^{14}C$ value of about 100‰ (Broecker et al. 1985; Kalish 1993) would have been expected (Figures 15.6, 15.7 and 15.8). This is demonstrated through comparison of oreo otolith data with pre-bomb radiocarbon data collected from the surface oceans, largely between 1958 and 1960 and post-bomb surface ocean data from GEOSECS expeditions (1971-1974).

For the older smooth oreos, with a presumed pre-bomb hatching date before 1960 $\Delta^{14}C$ values are significantly lower than any pre-bomb values in low and mid latitude (<45°S) surface waters (Figures 15.6, 15.7, and 15.8). On this basis, it must be concluded that the juvenile smooth oreos either live well below the surface mixed layer or they occur at high latitudes in the Southern Ocean. Vertical profiles of $\Delta^{14}C$ from the temperate South Pacific and the Pacific sector of the Southern Ocean are available from the GEOSECS expedition in 1974 (Östlund and Stuiver 1980). This date corresponds with the estimated birth dates for some of the younger smooth and black oreos analysed in this study. The $\Delta^{14}C$ for the smooth oreo with a birth date of 1974 was -71.7±4.4.‰ and this value is plotted with the $\Delta^{14}C$

vertical profiles from the Southern Ocean (Figure 15.9). The smooth oreo datum corresponds with a depth of about 1000m at 45°S and 53°S, with a depth of about 500m at 57°S to 58°S, and with surface waters at 69°S latitude (Figure 15.9). Three factors make a deepwater distribution below about 250m for smooth oreos unlikely: 1) the disruptive color pattern and small eye indicative of a fish living in surface waters; 2) the occurence of the copepod *Neocalanus tonsus* in the stomachs of the two smooth oreo juveniles caught in New Zealand waters; and 3) the fact that the majority of juvenile smooth oreos were collected in shallow trawls for krill in the Southern Ocean. Furthermore, the radiocarbon levels measured in the otoliths are consistent with those expected for a fish living in the surface mixed layer at about 65°S latitude, in agreement with the capture location for 32 of 35 collections of juvenile smooth oreo.

Interpretation of the radiocarbon data from black oreo otolith cores, in a similar manner to the smooth oreo data indicates that these fish are also likely to reside at very high latitudes as juveniles. Reference to data presented in Figures 15.6, 15.7 and 15.8 supports the hypothesis that the pelagic juvenile stages of this species occur in the region of 50°S to 60°S latitude.

All spikey and warty oreo with post-bomb birth dates after 1960 had $\Delta^{14}C$ results that were greater than those measured in both smooth and black oreo (Figure 15.5). Furthermore, for those samples that were clearly from fish with pre-bomb birth dates, the spikey and warty oreo also had $\Delta^{14}C$ higher than in the smooth and black oreo otolith cores. Two, presumably young spikey oreo with estimated ages of four years (1985 birth date) had a mean $\Delta^{14}C$ of 34±2.8‰, far below $\Delta^{14}C$ of 94‰ measured in *Pagrus auratus* with a birth date of 1972 (Kalish 1993). This suggests that juvenile spikey oreo spend there first few years of life at latitudes south of about 45°S or at depths below the surface mixed layer. The morphology of juvenile spikey oreo was described from the single known specimen (James 1988). The specimen had a relatively large eye and darker colouration with some large blotches, potentially indicating a deepwater habitat. Unlike smooth and black oreos, it is plausible that this species inhabits depths below the surface mixed layer during its early life history although a more southerly habitat cannot be ruled out. Unfortunately, the extreme rarity of juveniles of this species makes it impossible to draw any definitive conclusion.

Juvenile warty oreo, like other oreosomatid juveniles, are extremely rare and James (1988) described the one specimen known. The relatively small eye and silver colouration with some blotches suggest a relatively shallow habitat, but, again, the lack of samples makes it difficult to draw any definitive conclusion. Of the four oreo species investigate, warty oreo provided the highest $\Delta^{14}C$ measured in an oreo otolith core, 61±8.2‰. Nevertheless, this value is well below that measured in *Pagrus auratus* from temperate latitudes (Kalish 1993).

**Conclusion**

Measurements of radiocarbon in the earliest formed segments of black, smooth, spikey and warty oreo otoliths were compared with age estimates based on counts of presumed annual increments in thin sections of the 'sister' otoliths for each fish. Measurements of radiocarbon in the earliest formed segments of otoliths from these four oreosomatid fishes provide varying degrees of validation for age estimates derived from otolith thin sections. $\Delta^{14}C$ data from otolith cores of smooth oreo plotted against otolith section birth date describes temporal changes in radiocarbon that are indicative of the bomb radiocarbon increase in the 1960s and provide evidence that otolith based age estimates for this species are the most accurate of the four species investigated. The results provide conclusive evidence of minimum potential longevities of 35, 35, 29 and 28 years for black, smooth, spikey and warty oreos, respectively. However, there is no qualitative change to the appearance of increments that might suggest that the periodicity of increment formation is altered for fish older than 35 years and far older ages for these species are supported on the basis of radiocarbon analyses in conjunction with counts of presumed annual increments. Relatively low $\Delta^{14}C$ in relation to time for all four species and, in particular, for smooth oreo provides strong support that young juvenile habitats of these oreosomatid fishes are far removed from the adult habitats. Large negative values for $\Delta^{14}C$ from the smooth oreo otolith cores indicate that it is extremely unlikely that the adult smooth oreos spent their juvenile lives in surface waters at mid-latitudes. The radiocarbon levels measured in the otoliths are consistent with those expected for a fish living in the surface mixed layer at about 65°S latitude. This conclusion is supported by the limited ecological data on smooth oreo. Radiocarbon data from black oreo otolith cores also indicates that these fish inhabit high latitudes, around 60°S latitude, as juveniles. Both spikey and warty oreos may also inhabit surface waters of the Southern

Ocean based on $\Delta^{14}C$ measured in otolith cores. Further research is required to ensure effective management of these commercially important, but poorly understood fish species.

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Table 15.1. Fish, otolith, and radiocarbon data for Smooth Oreo (*Pseudocyttus maculatus*) collected from off Tasmania and New Zealand. 'Internal' laboratory numbers indicate that a sample had unacceptably large errors during radiocarbon analysis and the result of those measurements should be treated with caution. These sample results have not been given 'official laboratory accession numbers.

| Sample No. | Laboratory and laboratory accession number | Collection date | Collection location | Fish length (cm)SL | Sex | Otolith weight (g) | Sample weight (mg) | $\Delta^{14}C$ (‰) | ANU age (years) | CAF age (years) | Birth-year |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SMO 17 | Oxford OxA7576 | 5/2/93 | Tasmania | 38.5 | M | 0.0231 | 3.6 | -89±6 | 24 | | 1969 |
| SMO 26 | Oxford OxA7577 | 5/2/93 | Tasmania | 37 | M | 0.0172 | 2.3 | -102±9 | 27 | | 1966 |
| SMO 62 | Oxford OxA8242 | 5/2/93 | Tasmania | 49 | F | 0.042 | 1.8 | -118±10 | 69 | | 1924 |
| SMO 18 | Oxford OxA8243 | 18/10/92 | NZ | 49.1 | F | 0.0293 | 2.2 | -111±9 | 85 | | 1917 |
| SMO 4 | ANSTO OZE099 | 18/9/97 | NZ | 41 | F | 0.0177 | 4.6 | -36.2±4.5 | | 20 | 1978 |
| SMO 8(/10) | ANSTO OZE100 | 1/9/97 | NZ | 36 | F | 0.0168 | 4.8 | -63.7±4.1 | | 21 | 1976 |
| SMO 20 | ANSTO OZE101 | 23/3/97 | NZ | 45 | F | 0.0198 | 4.6 | -71.7±4.4 | | 25 | 1974 |
| SMO 3 | ANSTO OZE102 | 23/3/97 | NZ | 43 | F | 0.0225 | 4.4 | -77.3±4 | | 28 | 1970 |
| SMO 5(/9) | ANSTO OZE103 | 23/3/97 | NZ | 42 | F | 0.0216 | 4 | -75.2±3.8 | | 30 | 1968 |
| SMO 8(/9) | ANSTO OZE104 | 23/3/97 | NZ | 44 | F | 0.0252 | 4.6 | -47.4±4.3 | | 31 | 1966 |
| SMO 1(/10) | ANSTO OZE105 | 1/9/97 | NZ | 42 | M | 0.0248 | 4.8 | -62±4.2 | | 34 | 1964 |
| SMO 18 | ANSTO OZE106 | 23/1/97 | NZ | 40 | F | 0.0221 | 4.1 | -108.5±3.7 | | 36 | 1962 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SMO 19 | ANSTO OZE107 | 23/3/97 | NZ | 49 | F | 0.0403 | 4.7 | -127.2±4.1 | 42 | 1956 |
| SMO 5(/7) | ANSTO OZE108 | 23/1/97 | NZ | 43 | M | 0.0335 | 4.6 | -122.3±3.7 | 44 | 1954 |
| SMO 10(/7) | ANSTO OZE109 | 23/1/97 | NZ | 43 | F | 0.0308 | 4.5 | -120.2±3.3 | 47 | 1952 |
| SMO 10(/9) | ANSTO OZE110 | 23/3/97 | NZ | 53 | F | 0.0408 | 4.7 | -111.9±3.3 | 48 | 1950 |

Table 15.2. Fish, otolith, and radiocarbon data for black oreo (*Allocyttus niger*) collected off New Zealand.

| Sample no. | Laboratory and laboratory accession no. | Collection location | Collection date | Fish Length (cm)SL | Sex | Otolith weight (g) | Sample weight (mg) | $\delta^{13}C$ (‰) | $\Delta^{14}C$ (‰) | $\Delta^{14}C$ error (±) | CAF Age (years) | Birth-year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BOE 25 | ANSTO OZE087 | NZ | 28/1/98 | 34 | m | 0.0357 | 6.5 | | -93.3 | 5 | 48 | 1950 |
| BOE 27 | ANSTO OZE088 | NZ | 28/1/98 | 37 | m | 0.046 | 5.8 | -3.55 | -75.0 | 4.6 | 44 | 1954 |
| BOE 40 | ANSTO OZE089 | NZ | 24/1/97 | 32 | m | 0.0316 | 6.3 | -2.78 | -72.9 | 4.1 | 40 | 1958 |
| BOE 36/7 | ANSTO OZE090 | NZ | 23/1/97 | 37 | f | 0.03 | 5 | | -80.8 | 4 | 38 | 1960 |
| BOE 33 | ANSTO OZE091 | NZ | 23/1/97 | 37 | f | 0.0325 | 6.5 | -4.43 | -53.4 | 6 | 36 | 1962 |
| BOE 16 | ANSTO OZE092 | NZ | 24/1/97 | 32 | m | 0.0254 | 6 | -3.54 | -75.3 | 3.9 | 34 | 1964 |
| BOE 29 | ANSTO OZE093 | NZ | 28/1/98 | 37 | f | 0.0264 | 6 | -3.56 | -79.0 | 4.5 | 32 | 1966 |
| BOE 10 | ANSTO OZE094 | NZ | 24/1/97 | 36 | f | 0.0302 | 5.8 | | -25.9 | 6.6 | 30 | 1968 |
| BOE 13 | ANSTO OZE095 | NZ | 29/1/97 | 32 | f | 0.0212 | 6.6 | -3.38 | -89.0 | 3.5 | 28 | 1970 |
| BOE 15 | ANSTO OZE096 | NZ | 29/1/97 | 26 | f | 0.0278 | 5.6 | | -35.6 | 4.7 | 26 | 1972 |
| BOE 3 | ANSTO OZE097 | NZ | 24/1/97 | 30 | f | 0.0209 | 5.7 | | -14.4 | 4.5 | 22 | 1976 |
| BOE 14 | ANSTO OZE098 | NZ | 25/1/97 | 31 | f | 0.0214 | 6.2 | -2.90 | -17.8 | 6.2 | 20 | 1978 |

Table 15.3. Fish, otolith, and radiocarbon data for warty oreo (*Allocyttus verrucosus*) collected from off Tasmania.

| Sample No. | Laboratory and laboratory accession number | Collection location | Collection date | Fish length (cm) SL | Sex | Otolith weight (g) | Sample weight (mg) | $\Delta^{14}C$ (‰) | Age (years) | Birth-year |
|---|---|---|---|---|---|---|---|---|---|---|
| wo47 | AA16798 | Tasmania | 5/12/88 | 28.8 | M | 0.0742 | 4.4 | -89.1±7.2 | 115 | 1873 |
| wo48 | CAMS46786 | Tasmania | 5/12/88 | 28 | M | 0.0194 | 4 | -64.3±7.1 | 32 | 1956 |
| wo76 | AA16801 | Tasmania | 5/12/88 | 27.8 | F | 0.0246 | 4.1 | 9.2±7.9 | 32 | 1956 |
| wo64 | AA16800 | Tasmania | 5/12/88 | 26.2 | M | 0.0244 | 4 | -6.0±7.8 | 30 | 1958 |
| wo154 | CAMS46790 | Tasmania | 5/12/88 | 29.3 | M | 0.0239 | 4 | -75.8±7.3 | 29 | 1959 |
| wo38 | CAMS46785 | Tasmania | 5/12/88 | 25.6 | M | 0.0254 | 3.9 | -13.7±7.8 | 28 | 1960 |
| wo52 | CAMS46787 | Tasmania | 5/12/88 | 27.5 | M | 0.0251 | 3.4 | -47.5±8.2 | 28 | 1960 |
| wo59 | AA16799 | Tasmania | 5/12/88 | 24.6 | M | 0.0168 | 3.1 | 38.3±8.6 | 28 | 1960 |
| wo73 | CAMS46789 | Tasmania | 5/12/88 | 27.2 | F | 0.0248 | 3.9 | -49.8±6.3 | 28 | 1960 |
| wo54 | CAMS46788 | Tasmania | 5/12/88 | 21.4 | M | 0.021 | 4.2 | -17.6±6.5 | 26 | 1962 |
| wo175 | CAMS46791 | Tasmania | 5/12/88 | 27.9 | M | 0.0229 | 2.4 | 11.6±20 | 26 | 1962 |
| wo173 | AA16802 | Tasmania | 5/12/88 | 25.4 | M | 0.0218 | 3.7 | 61±8.2 | 23 | 1965 |
| wo185 | AA16803 | Tasmania | 5/12/88 | 24 | M | 0.0189 | 4.1 | 12.8±7.9 | 18 | 1970 |
| wo235 | CAMS46792 | Tasmania | 5/12/88 | 16.9 | I | 0.0126 | 4.2 | 15.6±8.8 | 14 | 1974 |
| wo193 | AA16804 | Tasmania | 5/12/88 | 16.2 | F | 0.0081 | 3.2 | 39±8.7 | 10 | 1978 |

Table 15.4. Fish, otolith, and radiocarbon data for spikey oreo (*Neocyttus rhomboidalis*) collected from off Tasmania.

| Sample No. | Laboratory and laboratory accession number | Collection location | Collection date | Fish length (cm)SL | Sex | Otolith weight (g) | Sample weight (mg) | $\Delta^{14}C$ (‰) | Age (years) | Birth-year |
|---|---|---|---|---|---|---|---|---|---|---|
| spo38 | AA16805 | Tasmania | 2/2/89 | 34.8 | M | 0.067 | 4.1 | -72.5±7.4 | 100 | 1889 |
| spo48 | Oxford | Tasmania | 2/2/89 | 35.2 | M | 0.0636 | 2.8 | -75±9.0 | 90 | 1899 |
| spo98 | Oxford | Tasmania | 2/2/89 | 29.9 | M | 0.0262 | 4.2 | -64±10 | 32 | 1958 |
| spo50/66 | AA16806 | Tasmania | 2/2/89 | 25.4/25.8 | F/F | 0.0185 / 0.0152 | 2.2+1.5 | 31.0±8.0 | 20/20 | 1969 |
| spo56 | Oxford | Tasmania | 2/2/89 | 26.8 | F | 0.0157 | 2.5 | -10±10 | 20 | 1969 |
| spo31 | Oxford | Tasmania | 2/2/89 | 24.2 | M | 0.0131 | 3.4 | 12±10 | 19 | 1970 |
| spo18 | Oxford | Tasmania | 2/2/89 | 19.4 | M | 0.0123 | 3.4 | -9±10 | 17 | 1972 |
| spo141/154 | AA16807 | Tasmania | 2/2/89 | 10.3/9.9 | I/I | 0.0016 / 0.0021 | 1.6+2.1 | 36.0±8.1 | 4 | 1985 |
| spo144 | Oxford | Tasmania | 2/2/89 | 10.7 | I | 0.0018 | 1.8 | 32±15 | 4 | 1985 |

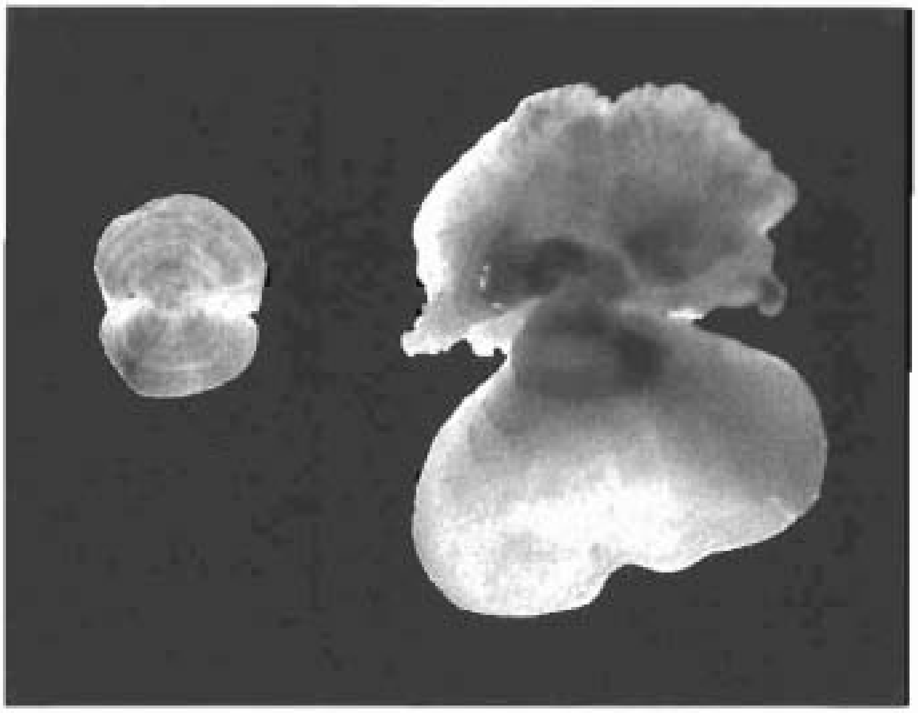

Figure 15.1a. Sculpted (left) and whole (right) smooth oreo (*Pseudocyttus maculatus*) otoliths viewed with transmitted light on a dark field. The sculpted otolith (sample no. 1(/10)) weighed 4.8 mg and contained the first four years of otolith growth. It was sculpted from a whole smooth oreo otolith with a weight of 24.8 mg and an estimated age of 34 years. The sculpted otolith is 2.4 mm in height and 1.8 mm wide.

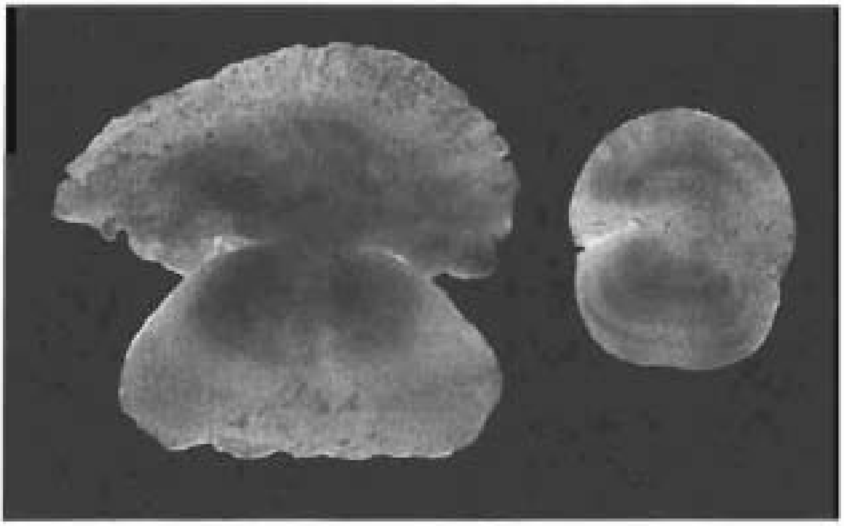

Figure 15.1b. Sculpted (right) and whole (left) black oreo (*Allocyttus niger*) otoliths viewed with transmitted light on a dark field. The sculpted otolith (sample no. 33) weighed 6.5 mg and contained the first four years of otolith growth. It was sculpted from a whole black oreo otolith with a weight of 32.5 mg and an estimated age of 36 years. The sculpted otolith is 3.2 mm in height and 2.7 mm wide.

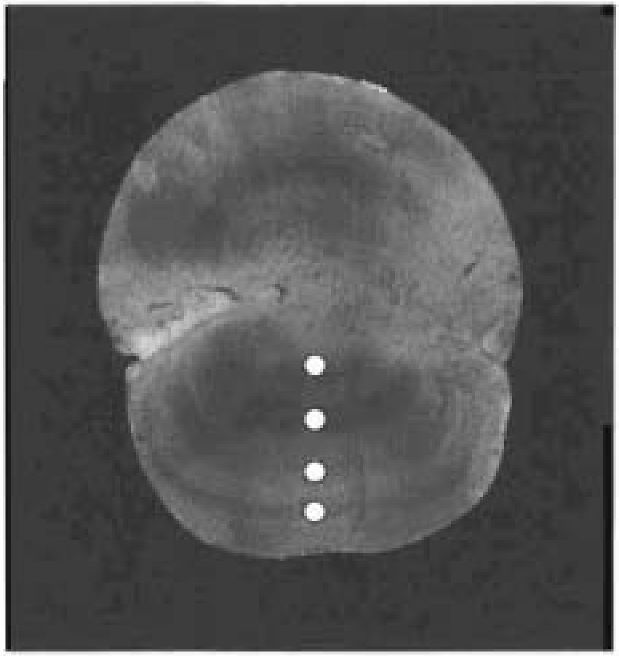

Figure 15.1c. Sculpted black oreo (*Allocyttus niger*) otolith viewed with transmitted light on a dark field. Sample no. 33, as in Fig. 15.1b, but at a higher magnification. . The sculpted otolith (sample no. 33) weighed 6.5 mg and contained the first four years of otolith growth. The four opaque zones are marked. It was sculpted from a whole black oreo otolith with a weight of 32.5 mg and an estimated age of 36 years. The sculpted otolith was 3.2 mm in height and 2.7 mm wide.

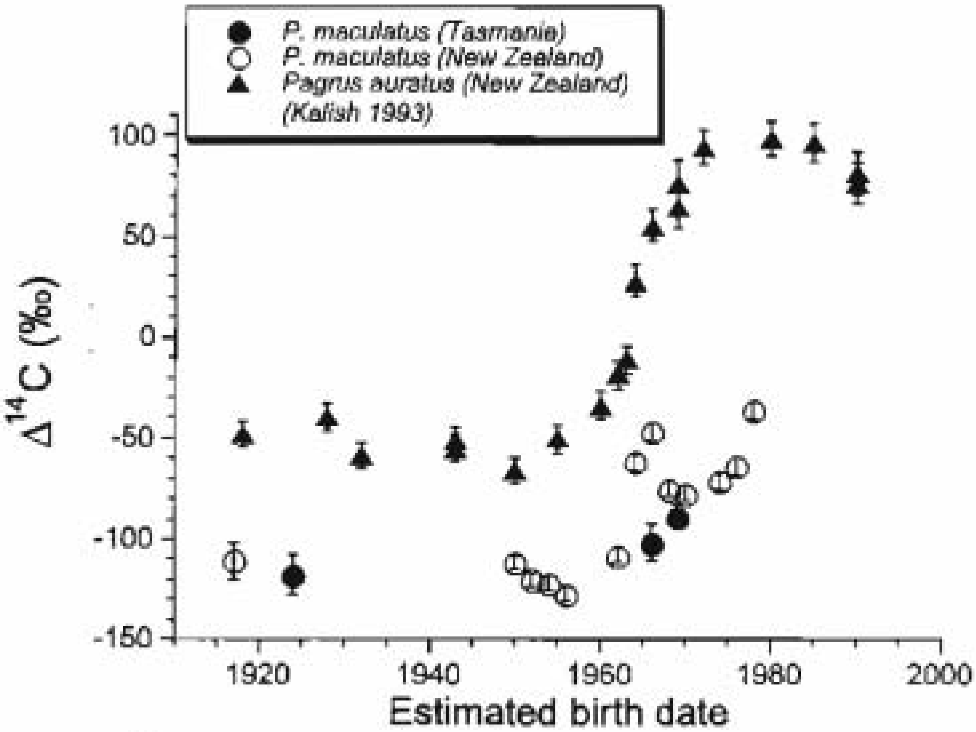


Figure 15.2. $\Delta^{14}C$ data from smooth oreo (*Pseudocyttus maculatus*) otolith cores plotted against otolith birth dates estimated from counts of presumed annual increments in otolith thin sections. P. maculatus samples from the ocean off New Zealand and off Tasmania are distinguished in the graph. Three samples with very large errors (see Table 15.1), but plotted in the graph should be treated with caution. These samples were not allocated official laboratory accession numbers and are indicative only. $\Delta^{14}C$ data from New Zealand *Pagrus auratus* (Kalish 1993) provide calibrations of $\Delta^{14}C$ versus year for the south western Pacific Ocean. $\Delta^{14}C$ values are based on otolith material deposited over a time period equivalent to about the first four years of life. Errors are ±1 sd.

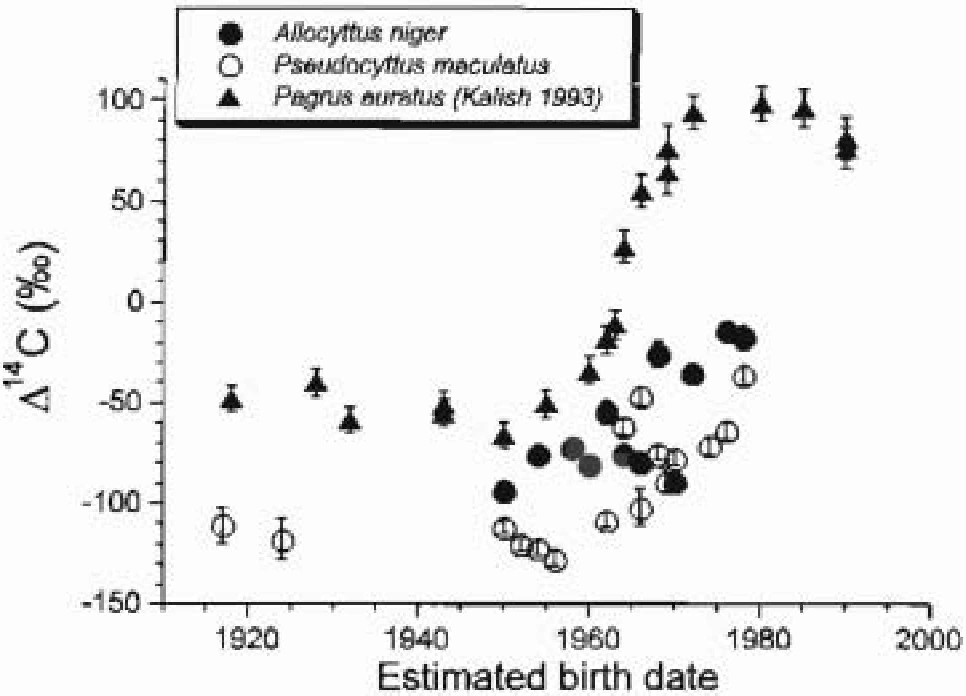


Figure 15.3. $\Delta^{14}C$ data from black oreo (*Allocyttus niger*) and smooth oreo (*Pseudocyttus maculatus*) otolith cores plotted against otolith birth dates estimated from counts of presumed annual increments in otolith thin sections. $\Delta^{14}C$ data from New Zealand *Pagrus auratus* (Kalish 1993) provide calibrations of $\Delta^{14}C$ versus year for the south western Pacific Ocean. $\Delta^{14}C$ values are based on otolith material deposited over a time period equivalent to about the first four years of life. Errors are ±1 sd.

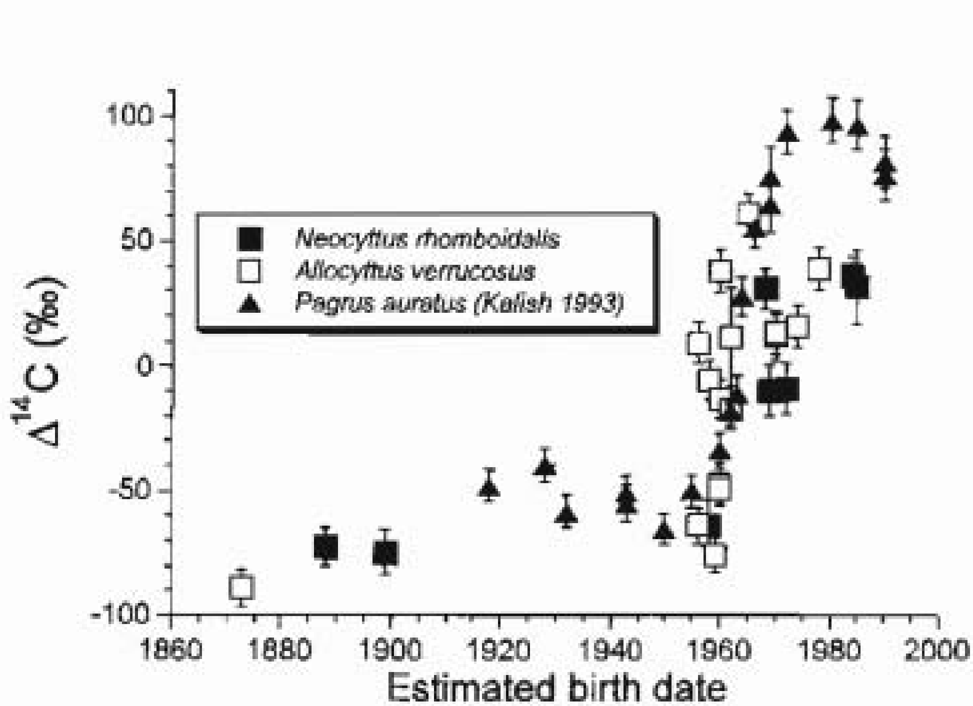


Figure 15.4. $\Delta^{14}C$ data from spikey oreo (*Neocyttus rhomboidalis*) and warty oreo (*Allocyttus verrucosus*) cores plotted against otolith birth dates estimated from counts of presumed annual increments in otolith thin sections. $\Delta^{14}C$ data from New Zealand *Pagrus auratus* (Kalish 1993) provide calibrations of $\Delta^{14}C$ versus year for the south western Pacific Ocean. $\Delta^{14}C$ values are based on otolith material deposited over a time period equivalent to about the first four years of life. Errors are ±1 sd.

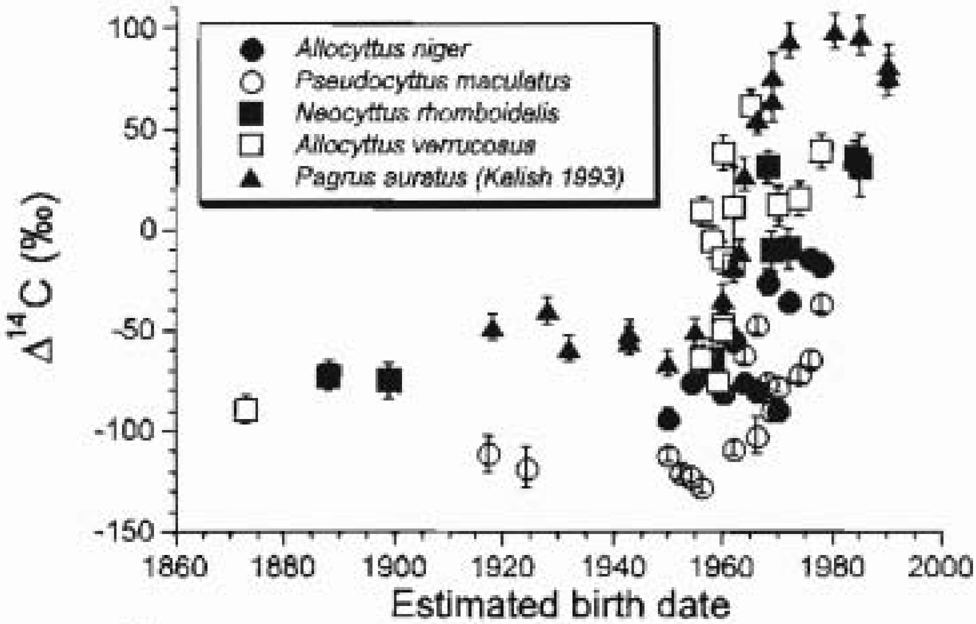


Figure 15.5. $\Delta^{14}C$ data from black oreo (*Allocyttus niger*), smooth oreo (*Pseudocyttus maculatus*), spikey oreo (*Neocyttus rhomboidalis*) and warty oreo (*Allocyttus verrucosus*) cores plotted against otolith birth dates estimated from counts of presumed annual increments in otolith thin sections. $\Delta^{14}C$ data from New Zealand *Pagrus auratus* (Kalish 1993) provide calibrations of $\Delta^{14}C$ versus year for the south western Pacific Ocean. $\Delta^{14}C$ values are based on otolith material deposited over a time period equivalent to about the first four years of life. Errors are ±1 sd.

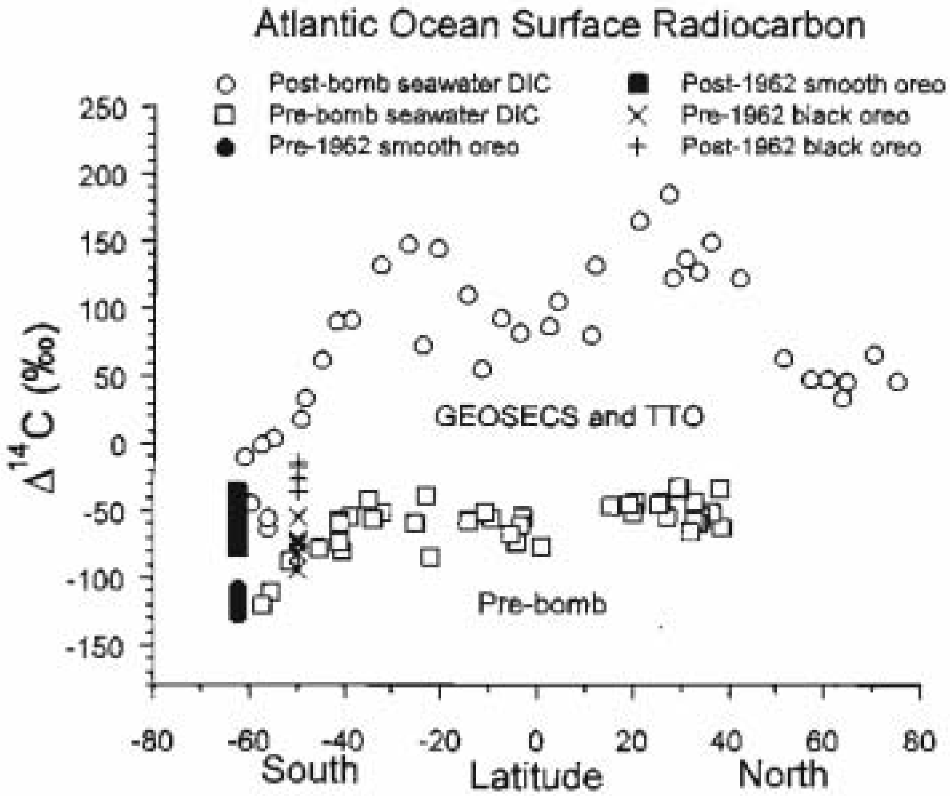


Figure 15.6. Latitudinal variation of $\Delta^{14}C$ measured in seawater dissolved inorganic carbon (DIC) from surface waters of the Atlantic Ocean prior to significant inputs of radiocarbon to surface waters (pre-bomb =prior to 1960) and after extensive bomb inputs (post-bomb=after 1960). $\Delta^{14}C$ data from otolith cores of *Pseudocyttus maculatus* and *Allocyttus niger* with birth dates estimated as pre-1962 or post-1962 on the basis of otolith thin sections are plotted at latitudes of 62.5°S and 50°S, respectively.

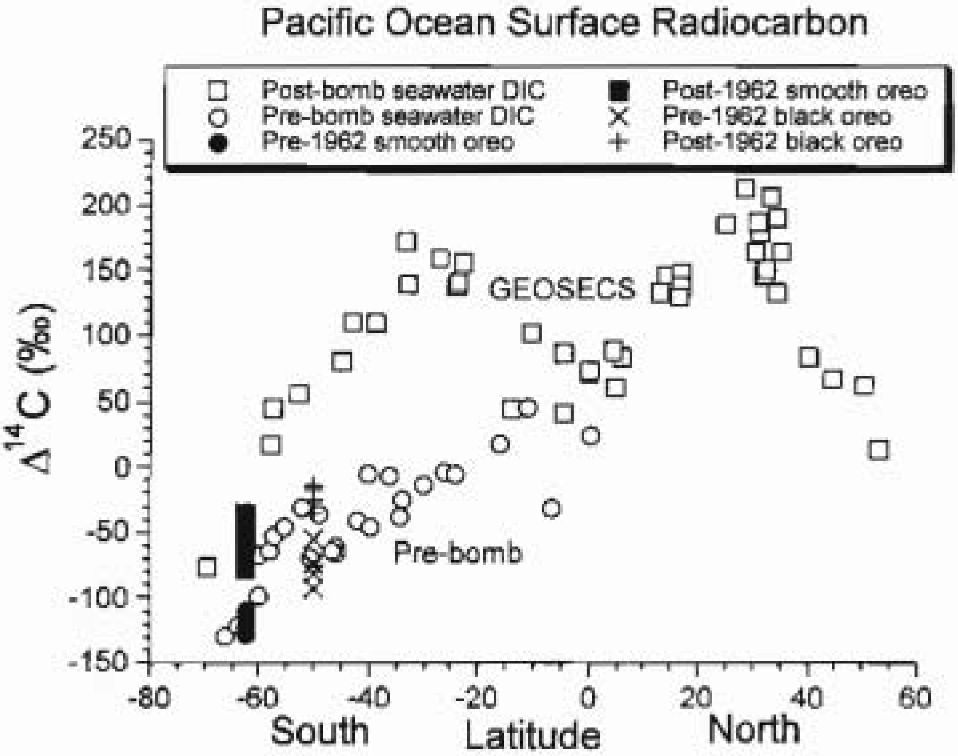


Figure 15.7. Latitudinal variation of $\Delta^{14}C$ measured in seawater dissolved inorganic carbon (DIC) from surface waters of the Pacific Ocean prior to significant inputs of radiocarbon to surface waters (pre-bomb =prior to 1960) and after extensive bomb inputs (post-bomb=after 1960). $\Delta^{14}C$ data from otolith cores of *Pseudocyttus maculatus* and *Allocyttus niger* with birth dates estimated as pre-1962 or post-1962 on the basis of otolith thin sections are plotted at latitudes of 62.5°S and 50°S, respectively.

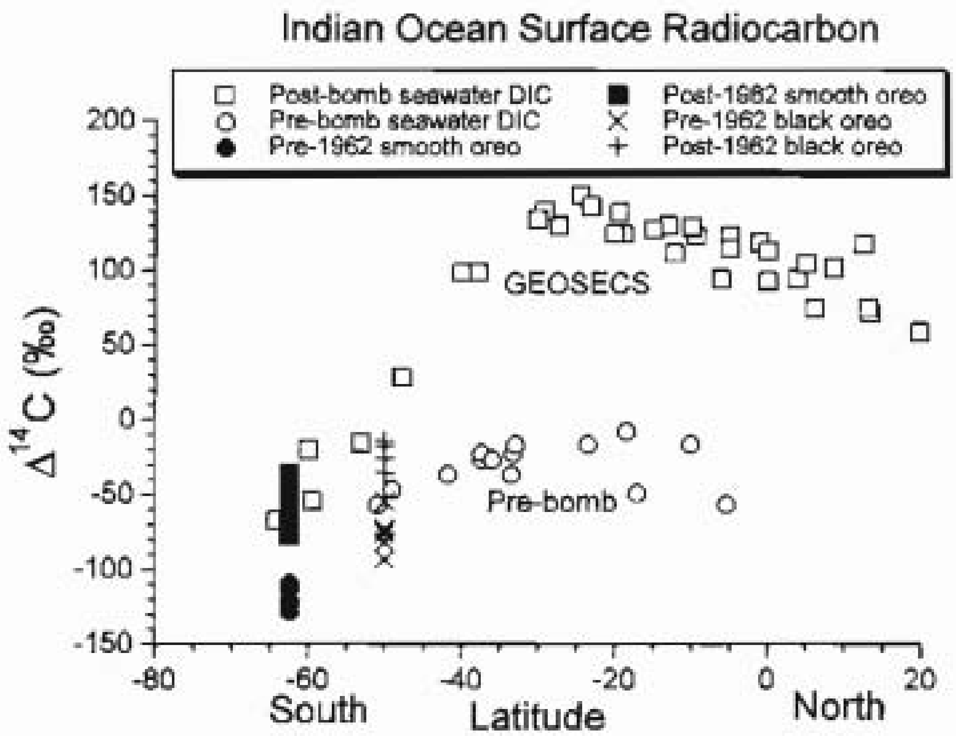


Figure 15.8. Latitudinal variation of $\Delta^{14}C$ measured in seawater dissolved inorganic carbon (DIC) from surface waters of the Indian Ocean prior to significant inputs of radiocarbon to surface waters (pre-bomb =prior to 1960) and after extensive bomb inputs (post-bomb=after 1960). $\Delta^{14}C$ data from otolith cores of *Pseudocyttus maculatus* and *Allocyttus niger* with birth dates estimated as pre-1962 or post-1962 on the basis of otolith thin sections are plotted at latitudes of 62.5°S and 50°S, respectively.

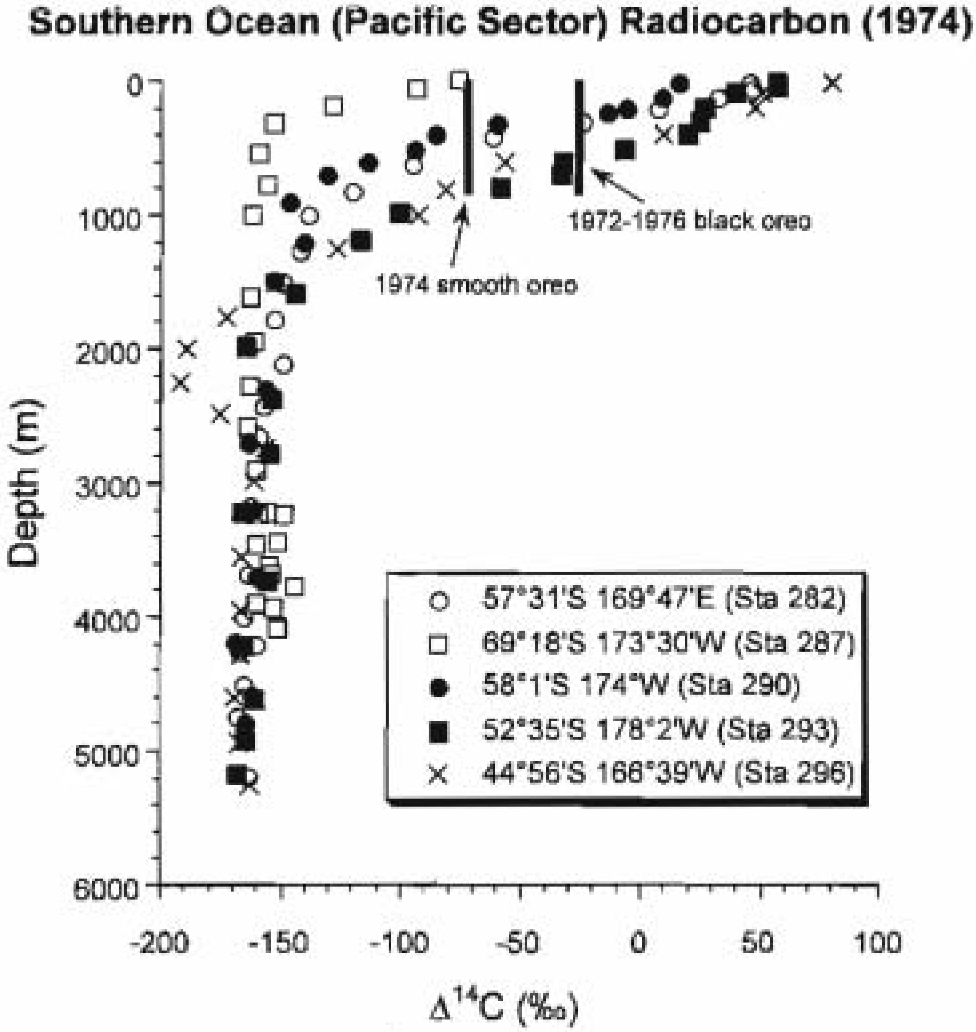


Figure 15.9. Vertical profiles of $\Delta^{14}C$ measured in seawater dissolved inorganic carbon (DIC) from the Pacific sector of the Southern Ocean. Data were collected during the GEOSECS expedition in 1974 (Ostlund and Stuiver, 1980). $\Delta^{14}C$ data from the otolith core of a smooth oreo (*Pseudocyttus maculatus*) with a birth date of 1974 (-71.7‰) and mean $\Delta^{14}C$ for two otolith cores from black oreos (*Allocyttus niger*) with birth dates of 1972 and 1976 (-25.0‰) are plotted.

# Chapter 16 Validation of Orange Roughy (*Hoplostethus atlanticus*) Age by High Precision Radiocarbon Dating

John Kalish

**Summary**

This research demonstrates that it is feasible to estimate the age of orange roughy on the basis of radiocarbon dating of otolith carbon. Radiocarbon age decreased from the otolith core to the otolith edge and radiocarbon decay was detected in individual orange roughy otoliths. Radiocarbon dating of individual orange roughy otoliths is feasible. Measurements of $\Delta^{14}C$ made at the otolith edge are not significantly different from $\Delta^{14}C$ measured in seawater dissolved inorganic carbon at orange roughy depths near the time of sample collection. High precision radiocarbon dating of orange roughy otoliths suggests that these fish are long-lived with maximum ages of 100 years or more Two otoliths that were free of any analytical or contamination problems yielded age estimates of 165 ± 118 years and 192 ± 96 years. Age estimates from increment counts for these fish were 138 years and 154 years, respectively. Reservoir corrected ages ranged from 22 to 160 years. However, reservoir corrected age estimates are difficult to interpret due to variability in the radiocarbon reservoir in different orange roughy habitats. The age estimates are consistent with estimates based on increment counts in thin sections and with estimates of age from radiometric techniques in that they support the hypothesis that orange roughy are very long-lived. Samples collected from orange roughy living at depths of about 1000 m and as long ago as 1985 can be contaminated by bomb radiocarbon and this makes it extremely difficult to determine the absolute age of individual fish. It is unlikely that it will be possible to overcome this problem as orange roughy were not collected with any regularity prior to the early 1980s.

**Introduction**

Validation of methods used for fish age estimation can be achieved by two different techniques that are based on the measurement of radiocarbon in fish otoliths. These methods are the "bomb radiocarbon chronometer" and "radiocarbon dating" (Kalish 1995). Application of these methods to studies of fish otoliths requires that measurements be carried out using a technique known as accelerator mass spectrometry (AMS), a technique that allows precise measurements of radiocarbon to be made on relatively small samples (<10 mg of calcium carbonate). The ability to measure low levels of radiocarbon with high precision and in small samples is essential for the success of both methods of validation.

Research on snapper *Pagrus auratus*) (Kalish 1993), redfish *Centroberyx affinis* (Kalish 1995), southern bluefin tuna *Thunnus maccoyii* (Kalish et al. 1996) and blue grenadier Macruronus novaezealandiae (Kalish et al. 1997) has demonstrated that the bomb radiocarbon method is a relatively inexpensive and rapid technique for age validation. The bomb radiocarbon chronometer relies on measurements of radiocarbon in selected portions of fish otoliths and relates these data to radiocarbon levels found in the environment. Under "normal" circumstances these measurements would not appear to be useful due to the relative constancy of environmental radiocarbon levels; however, human activities have altered the levels of radiocarbon in the environment both rapidly and drastically. The most dramatic change in radiocarbon levels was due to the atmospheric testing of nuclear weapons between 1958 and 1963. These atmospheric tests released large quantities of radiocarbon into the atmosphere and ultimately increased atmospheric levels of radiocarbon by over 100%. This increase was so rapid and great that the entry of this material into the ocean effectively marked (in a manner analogous to marking with oxytetracyline hydrochloride) all fish living in the upper 500 m of the ocean. This radiocarbon mark is useful because we have a precise knowledge of the time when the mark would have become incorporated into a fish and, more importantly, the fish's otoliths.

Properties of the movement and distribution of bomb-produced radiocarbon in the oceans make the bomb radiocarbon chronometer unsuitable for investigations of age in fish species, such as orange roughy, that live at depths greater than about 500 m (exceptions are those deep-sea species such as smooth oreo and black oreo with juvenile stages that live at shallower depths). This is due to the slow penetration of inorganic carbon into the deep ocean. Despite this problem, radiocarbon still has great potential for estimating the age of orange roughy. For orange roughy, the method of choice would be the technique known as radiocarbon dating. Radiocarbon dating is the standard technique used to determine the age of objects of interest to archaeologists and anthropologists and is most useful for material that is that is less than 30,000 years old. Radiocarbon dating is based on the known rate of decay of radiocarbon, which has a half-life of 5730 years. In most fish species this decay would not be detectable due to the relatively long half-life relative to the "typical" age of a fish, and the precision of radiocarbon methods for small samples (e.g. small portions of otoliths). However, there is some evidence that fish species such as orange roughy are extremely long-lived and, therefore, may be amenable to radiocarbon dating techniques, particularly with the high levels or precision that are attainable with AMS. The first consideration is to achieve the highest precision practical. For AMS, this is typically between 0.4% and 0.6% for small samples, or equivalent to about 35 to 50 years. In recent years, analytical precision has improved significantly and some facilities have developed the ability to routinely achieve precision of about 0.3% or about 24 years. This level of precision should be effective to determine the age of orange roughy if this species exhibits the longevity that has been reported previously (Mace et al. 1990, Fenton et al. 1991; Smith et al. 199??). Furthermore, age estimates of orange roughy based on radiocarbon dating would be independent of other age estimation procedures. This report describes a study to determine the age of orange roughy on the basis of high precision radiocarbon dating.

**Materials and methods**

Orange roughy otoliths were obtained from otolith archives maintained at NIWA (Di Tracey, personal communication). Orange roughy otoliths are weighed routinely and this provided an opportunity to obtain heavy otoliths that would have come from presumably old fish. The relationship between otolith weight and fish age is well established (Boehlert 1985) and data are available that indicate this relationship also applies to orange roughy otoliths (Francis and Smith 1995). Otoliths weighing in excess of 0.5 g were selected from fish collected in the "survey box" on the North Chatham Rise (between 175°W to 178°W longitude and 42°10'S to 44°S latitude) (Table 16.1). In a study of orange roughy age determination on the basis of 210Pb/226Ra in otoliths (Fenton et al. 1991) the heaviest individual otolith weighed 0.43 g and the mean otolith weight of the pooled sample containing the heaviest otoliths was 0.37 g (sample LH749 in Fenton et al. 1991). Examination of otolith weights indicates that 0.6 g is near the upper limit of otolith weights collected on the North Chatham Rise. In addition, two otoliths removed from orange roughy captured during the 1985 fishery off Sandy Cape, Tasmania were included due to their very large size.

A second criterion, date of collection, was also considered critical in the sample selection process. The otolith pairs were selected from fish collected between 1985 and 1992 (Table 16.1) with a view to identifying and avoiding bomb radiocarbon contamination in the most recently deposited aragonite in the samples. In the first instance, it was hoped that samples collected early in the fishery would be free of bomb radiocarbon, whereas samples collected at the later date (1992) might provide evidence for bomb contamination. In addition, the two Tasmanian orange roughy otoliths included the earliest samples available that were above the minimum weight and also included the heaviest otolith. Two of the samples, ORH1 and ORH10 were analysed as part of an pilot study of radiocarbon dating of orange roughy and was funded by the New Zealand Fishing Industry Board (Kalish 1994).

Otolith calcium carbonate for radiocarbon analyses was removed from each otolith by drilling with fine dental-type drill bits. Otolith calcium carbonate was obtained from 5 distinct regions of each otolith: 1) a region, including the otolith core, that encompassed no more than the presumed first 4 years of otolith growth designated here as "Position 1" or "core"; 2) a region mid-way between the otolith core and the "transition zone", a structure that is assumed to result from physiological changes associated with the onset of maturity and spawning and estimated to occur at about 30-34 years of age (Kalish unpublished data; Francis et al. 1993; (Smith et al. 1998); Francis and Horn 1997), and designated here as "Position 2" or "Juvenile 1"; 3) a region encompassing the transition zone designated here as "Position 3" or "Juvenile 2"; 4) a region just distal of the medial face of the otolith designated here as "Position 4" or "Adult 1", and; 5) the outer medial face of the otolith representing the most recently deposited calcium carbonate and designated here as "Position 5" or "Edge". An orange roughy otolith with Positions 1, 2 and 3 indicated, is shown in Fig. 16.1 and a section with Positions 1-5 indicated is shown in Fig. 16.2. .

Samples are designated as ORHx/1, ORHx/2, ORHx/3, ORHx/4 and ORHx/5 where the final number relates to the position sampled. A layer of otolith calcium carbonate about 100 μm thick was removed from the lateral (distal) surface of the otolith before material was sampled from Positions 1, 2 and 3 to eliminate the thin layer of older calcium carbonate that covers the lateral surface of otoliths from presumably older orange roughy. After grinding, otolith powder was placed in individual plastic centrifuge tubes. Each sample was sorted at 40X magnification and under clean room conditions to remove any impurities (e.g. dust, fibres, etc.) that might have become incorporated during the sampling process.

Further sample preparation and analysis was completed at the National Ocean Sciences Accelerator Mass Spectrometry facility (NOSAMS) at the Woods Hole Oceanographic Institute, Massachusetts and at the United States National Science Foundation-Arizona Accelerator Mass Spectrometry Facility at the University of Arizona in Tucson, Arizona. Preparation of graphite targets was accomplished using the method established by Slota et al. (1987) and details of the operation of AMS facilities can be found in Linick et al. (1989). For the analyses completed at Arizona (Samples ORH1 and ORH10), three graphite targets were produced from each sample and multiple carbon isotope measurements were made on each target. A weighted average was calculated to produce the final estimates of radiocarbon levels. Single targets were produced by the NOSAMS laboratory and multiple measurements were carried out on each sample. Reported errors for radiocarbon data and age estimates are one standard deviation and include both counting errors and laboratory random errors.

**Results**

The results of the 29 high precision analyses of radiocarbon in samples from orange roughy otoliths are presented in Table 16.1. A total of 32 samples was prepared and of these samples three encountered problems during the target preparation process and were not suitable for further radiocarbon analysis. Another sample, ORH4/1 was contaminated by a 'hot' sample digested in the same reaction vessel on a previous occasion. The otolith radiocarbon data are reported in three ways to simplify comparison with other radiocarbon data. Radiocarbon data may be expressed in several forms with the convention selected being dependent on the sample type. Radiocarbon measurements made on oceanographic samples (e.g. carbon extracted from seawater or animal tissues) are typically expressed as $\Delta^{14}C$ (‰), whereas measurements made for the purposes of radiocarbon dating are reported as conventional radiocarbon ages (years). In each case, the results are calculated on the basis of procedures outlined in Stuiver and Polach (1977).

The results, in relation to possible ages of the orange roughy samples, are most readily interpreted as radiocarbon age. The radiocarbon age data are presented in two forms a conventional radiocarbon age, and a reservoir –corrected age (Table 16.1). The conventional radiocarbon age presents the age of the sample in relation to known age standards (the standards are based on 1890 AD tree-ring carbon in equilibrium with 1890 AD atmospheric CO2) with the level of radiocarbon in 1890 AD atmospheric CO2 acting as the reservoir (or source) of radiocarbon. Using this convention, analysis of radiocarbon in samples from orange roughy otoliths yielded conventional radiocarbon ages that range from a maximum of 960±85 years to a minimum 240±40 years (Fig. 16.3). These conventional radiocarbon ages are a function of the age of the carbon reservoir, essentially the age of the carbon present in seawater dissolved inorganic carbon (DIC) when it is incorporated into the otolith and the age of the fish. The conventional radiocarbon ages can still be used to estimate the age of the orange roughy samples simply by looking at the differences in conventional radiocarbon age between samples and this was determined to be the most appropriate method to consider these data.

The old ages that result, before differencing, are an effect of the reservoir of carbon ultimately incorporated into orange roughy otoliths. The reservoir of carbon in the deep sea, and at the depths where orange roughy occur, is very old due to the dynamics of ocean circulation (Bien et al. 1965; Broecker et al. 1985; Kalish 1995b). Dissolved inorganic carbon is incorporated into water masses while at the ocean's surface and when in contact with the atmosphere. Processes that result in the movement of water masses to the deep sea are relatively slow and several hundred to several thousand years may pass before these water masses are transported to the depths beyond the surface mixed layer. During the time over which transport takes place, the radiocarbon incorporated at the ocean's surface decays. Data presented in this study support earlier research that indicates a conventional radiocarbon age of more than 500 years for seawater at depths of about 1000 m in the temperate Southern Hemisphere oceans.

An estimate of the age of the radiocarbon reservoir, essentially the specific radiocarbon content of the reservoir in the habitat from which the orange roughy were sampled, can be obtained from several sources and some of these data are presented in Table 16.3. These data suggest that the $\Delta^{14}C$ of the reservoir could range from about –50 to -120‰. A mean value for the reservoir was estimated based only on samples collected in 1974 and from orange roughy depths that are typically trawled. The mean $\Delta^{14}C$ at depths between 800 m and 1300 m in 1974 was –99.5±19‰, equivalent to an age of 800±165 years (n=5) (Table 16.3).The large error associated with this estimate is due to the rapid change in $\Delta^{14}$C with depth and for this reason, it is considered extremely difficult to estimate a single reservoir value for all orange roughy sampled. On this basis, the preferred method to estimate the reservoir value was based on the measurements of $\Delta^{14}$C made at Position 5 (or Position 4) from the individual otoliths as this would take into account potential differences in habitat selected by individual orange roughy.

Radiocarbon measured between Position 1 to Position 5 in each otolith shows the same general pattern with the oldest radiocarbon ages measured at Position 1 and the youngest ages measured at Position 5 (Table 16.1). Measurements of radiocarbon at Positions 1 and 3 are clustered relative to measurements in the adult regions of the otolith in Positions 4 and 5; the basis for these relative distributions is critical to interpretation of these radiocarbon data. Samples from otoliths taken at Position 5 yielded radiocarbon measurements that are representative of the time when the fish were collected between 1985 and 1992, more than 20 years after the influence of bomb radiocarbon was detected in the surface ocean. Based on an earlier pilot study it was expected that bomb radiocarbon would not be evident in samples collected at orange roughy depths prior to about 1990. It is clear, however, that many of the samples from both Positions 4 and 5 were influenced by bomb radiocarbon as evidenced by the very young age of the majority of these samples (Table 16.1).

Ocean circulation and the relatively slow mixing processes associated with the movement of surface water masses to the deeper ocean indicate that bomb radiocarbon would not be detectable in these deeper waters until many years after they were manifest in surface waters. This is discussed in detail in the literature that deals with the use of radiocarbon as a tracer for studies of ocean circulation (e.g Broecker and Peng 1982; Broecker et al. 1985; Kalish 1995b). Measurements of radiocarbon in seawater dissolved inorganic carbon (DIC) from the temperate South Pacific, in the region of the Chatham Rise, and at depths where orange roughy are likely to occur (Table 16.3) demonstrate the possible variation in radiocarbon in these habitats. The data from Östlund and Stuiver (1980) are based on radiocarbon measurements made in 1974 and these data are considered representative of pre-bomb conditions despite the fact the measurements were made more than a decade after bomb radiocarbon was detected in the surface ocean. This is due to slow ocean mixing and the conclusion that bomb radiocarbon had yet to penetrate into these regions of the deep sea by

1974. Also, the data from 1974 show a clear trend of decreasing $\Delta^{14}C$ with increasing depth (Table 16.3). $\Delta^{14}C$ is about -80‰ around 800 m depth and decreases to less than -110‰ below 1100 m. Radiocarbon data from the region of the Chatham Rise, collected by Sparks et al. (1992) show a similar relationship between $\Delta^{14}C$ and depth (Table 16.3), however, there is one measurement made at 800m in November 1987 of -41‰ that shows clear evidence for the presence of bomb radiocarbon in deeper waters. There is no further evidence in these seawater DIC measurements for the presence of bomb radiocarbon at orange roughy depths (>800 m) prior to 1987.

Over the last 500 years, prior to atomic weapons testing, $\Delta^{14}C$ in the atmosphere was relatively constant at around the 0‰. Over the same period, $\Delta^{14}C$ in the ocean was also constant at about -45‰. The difference is due to the balance between the input of $^{14}CO2$ to the ocean, the production of radiocarbon in the atmosphere, and the transport of radiocarbon to the deeper ocean. In addition to exchange with the atmosphere, the surface mixed layer of the ocean is constantly losing radiocarbon, in the form of dissolved inorganic carbon, to other carbon pools through several physical and biological processes. However, $\Delta^{14}C$ in these deeper waters is still significantly lower than at the surface at similar locations. The complexity of $\Delta^{14}C$ distribution in the ocean is clearly demonstrated by recent measurements made as part of the World Ocean Circulation Experiment (e.g. NOSAMS 1999)

Several measurements of $\Delta^{14}C$ at Positions 4 and 5 provide clear evidence for contamination by bomb radiocarbon. Although it is not possible to provide a definitive time for the earliest input of bomb radiocarbon to orange roughy depths, it is feasible to provide a threshold of $\Delta^{14}C$ above which bomb inputs are clearly present. On the basis of data from Table 16.3 and similar measurements throughout the world's oceans, it is concluded that a $\Delta^{14}C$ of -80‰ or a radiocarbon age of 600 years represents a reasonable threshold. This value for $\Delta^{14}C$ was selected on the basis of measurements of $\Delta^{14}C$ and tritium in seawater in the South Pacific Ocean at orange roughy depths and temperate latitudes (Table 16.3). A $\Delta^{14}C$ measurement of –81.4‰ was made on a sample of seawater collected at 803 m depth in the temperate South Pacific and no tritium, a definitive tracer of bomb products, was detected in this sample (Östlund and Stuiver 1980). Despite the selection of this estimate, it can only be considered a crude estimate for all the orange roughy analysed in this study. Values of $\Delta^{14}C$ above this level (and radiocarbon ages of less than 600 years) would be indicative of the presence of bomb derived radiocarbon in the sample. On the basis of this criterion, 3 samples from

Position 4, and 5 samples from Position 5 were contaminated. Several samples (ORH10/5, ORH31/5, ORH66/5) present conclusive evidence for contamination with $\Delta^{14}C$ of about 10‰ up to 45‰.

The sampling strategy for this project was designed to take into account possible effects of bomb contamination, but was not completely successful in this endeavour. This was to be achieved through the sampling of the most recently formed otolith material from the medial surface of the otolith (Position 5) and the adjacent, but older otolith material from Position 4. Since samples were not available that would have no chance of bomb contamination (i.e. collected before 1960), it was determined prudent to analyse the material at Position 5 with possible bomb contamination and, as a precautionary measure, analyse material from Position 4. However, it appears that some samples from Position 4 (ORH7/4, ORH31/4, ORH66/4) were also affected by bomb radiocarbon. Based on counts of presumed annual increments, it is estimated that a maximum of 10 years would have separated the most recently deposited calcium carbonate at Position 5 from the most recently deposited material at Position 4.

In addition to demonstrating the effects of bomb contamination, the contaminated samples provide evidence for significant regional variation in $\Delta^{14}C$. The samples with the earliest collection dates of 1985 show the highest values for $\Delta^{14}C$, indicating the greatest level of contamination. From these measurements, it can be concluded that detectable levels of bomb radiocarbon had reached orange roughy habitats off western Tasmania prior to reaching similar habitats in the Chatham Rise region. It was anticipated that these samples would be the least likely to be contaminated due to the early collection date, however, this was proven to be an incorrect assumption. These samples yielded the oldest fish age estimates of 690 years and 670 years for ORH31 and ORH66, respectively. The similarity of these estimates is likely to be associated with the common collection location off western Tasmania and the similar date of collection. This also reinforces the effectiveness of radiocarbon data as a tracer of water masses, a point that is critical to the interpretation of the otolith data from smooth and black oreos. The fact that these two samples from Tasmanian waters yielded similar $\Delta^{14}C$ at Positions 1 and 5 suggests that they were likely to have experienced similar oceanographic regimes over the course of their lives and that they were of similar age.

Due to the differences in oceanography and concomitantly, $\Delta^{14}C$ experienced during the life of the individual fish used in this study, it is most appropriate to view the results on a fish by fish basis. Although reservoir corrected ages are presented in Tables 16.1 and 16.2, these estimates are considered problematic due to the variable depth distribution of orange roughy and the lack of pre-bomb radiocarbon data at orange roughy depths from the region of the Chatham Rise. Comments on the results from each otolith are presented below and these relate directly to data presented in Table 16.1.

*ORH1*

The sample from Position 1 failed, therefore, it was only possible to estimate the difference between ORH1/3 and ORH1/5 of 192±96 years. This age would represent a minimum estimate for this sample due to the failure of the analysis of ORH1/1. Bomb radiocarbon contamination was not evident in sample ORH1/5 with a $\Delta^{14}C$ of −83.6‰. The reservoir corrected age for the otolith core was 93 years. The otoliths section age was 154 years and overlaps with the minimum age estimate for radiocarbon dating of this otolith.

*ORH4*

The sample from Position 1 failed, however, $\Delta^{14}C$ was measured successfully from Position 3. For the majority of samples these two measurements were not statistically significantly different. The difference between ORH4/3 and ORH4/5 was 345±176 years, however, the Position 5 sample was beyond the threshold for bomb contamination with a value of -55‰. The difference between ORH4/3 and ORH4/4 was 65±72 years and represents an estimate of the number of years between the formation of the transition zone (around first spawning) and the period estimated to be about 10 to 20 years before death (the estimated time covered by sample ORH4/5). With a $\Delta^{14}C$ of -87‰, sample ORH4/4 was unlikely to be contaminated by bomb carbon. The reservoir corrected age for the otolith core could not be estimated due to contamination of the core sample. ORH4 had an otolith section age of 128 years.

*ORH7*

The difference between ORH7/1 and ORH7/3 was 5±21 years and between ORH7/1 and ORH7/4 the difference was 325±166 years. The sample from Position 5 failed and bomb radiocarbon was present in ORH7/4 based on a $\Delta^{14}C$ of -70‰. Therefore, the estimated age of 325 years for this fish is invalid due to bomb contamination in both ORH7/4 and ORH7/5from the adult region of the otolith. The reservoir corrected age for the otolith core was 60 years. The otolith section age for this sample was 120 years.

*ORH8*

Samples from all five positions were measured from sample ORH8. There was no significant difference among ORH8/1, ORH8/2 and ORH8/3 with differences of -55±68 years and -45±62 years. The differences between ORH8/1, from the core, and ORH8/4 and ORH8/5 were 70±77 years and 165±118 years, respectively. The reservoir corrected age for the otolith core was 25 years. ORH8 yielded an otolith section age of 138 years.

*ORH10*

The sample from Position 5, with a $\Delta^{14}C$ of -43‰, shows clear evidence of bomb contamination and, without a sample from Position 4 it is not possible to make an estimate of age for this fish on the basis of radiocarbon decay. The difference between samples ORH10/1 and ORH10/3 was 43±48 years and represents an estimate for the time period between the first few years of life and first spawning. The reservoir corrected age for the otolith core was 22 years. The otolith section age for ORH10 was 156 years.

*ORH15*

The age difference between the samples from Position 1 and Position 3 was 50±55years, very similar to that estimated for ORH10. The difference between Position 1 and Position 4 was 105±94years. The sample from the otolith edge was contaminated by bomb carbon based on a $\Delta^{14}C$ of -64‰. The reservoir corrected age for the otolith core was 60 years. The otolith section age for ORH15 was 118 years.

*ORH31*

This was, by far, the heaviest otolith at almost 1 g, and would be expected to yield the oldest age. Bomb radiocarbon contamination of the samples from both Positions 4 and 5, with $\Delta^{14}C$ of -57‰ and -35‰, respectively, made an age estimate impossible. The reservoir corrected age for the otolith core was 130 years. ORH31 had an otolith section age of 180 years.

*ORH66*

Problems with bomb contamination, similar to those for ORH31, were evident in this sample and an age estimate was not possible. ORH66/4 and ORH66/5 yielded $\Delta^{14}C$ of -68‰ and -

41‰, respectively Nevertheless, the radiocarbon ages from both Position 1 and Position 5 in ORH31 and ORH66 clustered and suggests that these fish were exposed to similar ambient radiocarbon levels or, at least, levels distinct from those in the region of the Chatham Rise. The reservoir corrected age for the otolith core was 160 years. ORH66 was estimated to be the oldest fish based on an otolith section age of 185 years.

Two orange roughy otoliths, ORH1 and ORH8 were not contaminated with bomb radiocarbon at any of the positions sampled. Unfortunately, the sample from Position 1 from ORH1 was lost and there was no measurement of radiocarbon in the core. However, as samples from Positions 1, 2 and 3 indicate from all otoliths, there is little difference between measurements of $\Delta^{14}C$ at these positions and ORH1/3 can be used to provide an estimate of the minimum $\Delta^{14}C$ (maximum age) for this sample. On this basis ORH1 produced a minimum age estimate of 192±96 years and radiocarbon dating of ORH8 produced an age estimate of 165±118 years. These age estimates suggest longevities on the order of 100 years for orange roughy.

Two additional samples, ORH4 and ORH15 were contaminated with bomb radiocarbon at Position 5, but were not contaminated at Position 4. For sample ORH4, there was the additional loss of ORH4/1 due to contamination from an earlier sampled that was processed in the same reaction vessel, however, as for ORH1, ORH4/3 can be used to provide an estimate of the minimum $\Delta^{14}C$ (maximum age) for this sample. Therefore, a minimum age can be estimated for ORH4 and ORH15 from the difference between the radiocarbon age at Position 3 and Position 4 and Position 1 and 4, respectively. The resultant minimum age estimates are 65±72 years for ORH4 and 105±94 years for ORH15.

ORH 31 and ORH66 had the two oldest conventional radiocarbon ages (most negative $\Delta^{14}C$ ) measured at Position 1 of any of the otoliths at 930 years and 960 years, respectively, and this suggests the oldest ages for these fish based on radiocarbon decay. Also, these fish displayed the youngest ages (highest $\Delta^{14}C$ ) at Position 5, consistent with relatively rapid exchange of carbon between the surface ocean and the deepsea habitat where these fish were collected. If these fish remained in a similar location throughout their lives, then a low $\Delta^{14}C$ (an old conventional radiocarbon age) might be expected in early life.

*Otolith weight versus otolith $\Delta^{14}C$*

The validity of the radiocarbon-based age estimates for the orange roughy samples can be investigated further by consideration of the relationship between otolith weight and the radiocarbon age estimated from the core sample at Position 1. Otolith weight is considered a suitable, although imprecise, proxy for orange roughy age (Francis and Tracey 1994; Francis and Smith 1995). In addition, the measurement of radiocarbon age at Position 1 should provide an indication of the relative age of individual fish if it is assumed that radiocarbon levels were similar for each fish when this core otolith material was deposited. If this was the case, than any differences in radiocarbon age measured among the samples would be the result of radiocarbon decay over the life of the fish. There was a significant relationship between otolith weight and the conventional radiocarbon ages measured at Position 1 that suggests that the radiocarbon data may be related to the biological age of each fish. However, some consideration must be given to the possible influence of regional oceanography and the effects on $\Delta^{14}C$ in samples from different regions. Notably, the orange roughy from Sandy Cape, Tasmania were segregated in relation to the other samples based on radiocarbon measurements at Positions 1 and 5. This could be attributable to the fact that these were estimated to be the oldest of the samples, based on otolith section age estimates, the influence of regional oceanography on radiocarbon distribution, or a combination of these factors.

## Discussion

Radiocarbon dates from selected regions of orange roughy otoliths provide further support for the hypothesis that these fish are long-lived with maximum ages in excess of 100 years; however, these estimates must be considered in light of the significant uncertainties associated with the radiocarbon reservoir at orange roughy depths. Consistent trends in $\Delta^{14}C$ from the otolith core to the otolith edge provide evidence for the detection of significant levels of radiocarbon decay. The greatest amount of decay (longest time period) is evident between the transition zone samples (Position 3) and the adult samples (Position 4 and 5), consistent with the highly compressed appearance of presumed annual increments between these regions. However, many of the adult samples were contaminated with bomb radiocarbon and this makes it difficult to determine radiocarbon ages with a high degree of certainty, as discussed in more detail below. Rejection of samples presumed to be contaminated with bomb radiocarbon still suggests the greatest decay between the transition zone and adult samples. The extent of radiocarbon decay between samples from the first few years of life (Positions 1) and what is presumed to be the 'mid-juvenile' period (Position 2) and the late juvenile period at the transition zone (Position 3) was far less. This is consistent with interpretation of presumed annual increments in orange roughy otoliths and the differences in the width of these increments across the otolith.

The major problem in estimating radiocarbon dates from orange roughy otoliths appears to be linked to the difficulty in obtaining samples that do not contain bomb radiocarbon. The only way to ensure the samples do not contain bomb radiocarbon is to obtain samples collected prior to 1960. This requirement would be excessive given the deepsea habitat of orange roughy and the fact that a fishery for this species did not exist at that time. Our understanding of radiocarbon in the deep sea (e.g. Östlund and Stuiver 1980; Kalish 1995b) indicates that it is likely that fish collected prior to 1975 would satisfy the same requirement; however, the complex nature of ocean circulation would result in some uncertainty even with samples collected up to that date. The only definitive means of determining the presence of bomb products (e.g. radiocarbon) is to use a tracer that does not occur naturally, but results from atomic testing. Tritium satisfies this requirement and several studies (e.g. Broecker et al. 1985) have investigated tritium as an ocean tracer. Studies of tritium in the ocean show no evidence that this tracer penetrated to depths of 1000 m by 1980 and this would indicate that bomb radiocarbon had also failed to reach these depths. Data on tritium are not widely available and it would be difficult to extend these findings to other habits in later years (e.g. the Chatham Rise in 1988). Alternatively, a solution to the positive identification of bomb radiocarbon at the otolith edge would be the measurement of tritium in the same samples. Unfortunately, with currently available technology, it is not feasible to measure low levels of tritium in such small samples.

Due to the uncertainty in identification of bomb radiocarbon, it was necessary to estimate a threshold for $\Delta^{14}C$ (-80‰) below which samples were considered to be uncontaminated by bomb radiocarbon. This value for $\Delta^{14}C$ was selected on the basis of measurements of $\Delta^{14}C$ and tritium in seawater in the South Pacific Ocean at orange roughy depths and temperate latitudes (Table 16.2 and 16.3). A $\Delta^{14}C$ measurement of –81.4‰ was made on a sample of seawater collected at 803 m depth in the temperate South Pacific and no tritium was detected in this sample (Östlund and Stuiver 1980). Despite the selection of this estimate, it can only be considered a crude estimate for all the orange roughy analysed in this study. If the -80‰ threshold is accepted then some orange roughy collected as recently as 1988 may be free of bomb radiocarbon contamination at the otolith edge, but this cannot be established with certainty

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Table 16.1. Fish, otolith, and radiocarbon data for Orange roughy (Hoplostethus atlanticus ) collected from the Southern Ocean.

| Sample No. | Collection location | Collection date | Fish length (cm)SL | Sex | Otolith weight (g) | Sample weight (mg) | $\Delta^{14}C$ (‰) | $\Delta^{14}C$ Error | Age | Age Error |
|---|---|---|---|---|---|---|---|---|---|---|
| ORH 1/2 | 42°10'S 175°-178° W | 1987 | 45.1 | F | 0.71 | 21.6 | -105.2 | 3.0 | 893 | 25 |
| ORH 1/3 | North Chatham Rise | | | | | 15.1 | -83.6 | 3.0 | 701 | 23 |
| ORH 4/1 | 42°10'S 175°-178° W | | | | | 13.9 | 233.1378 | 3.64 | >mod | 0 |
| ORH 4/2 | North Chatham Rise | 1987 | 39.2 | F | 0.66 | 14.1 | -94.48769 | 6.24 | 750 | 55 |
| ORH 4/3 | | | | | | 12.4 | -87.09945 | 3.07 | 685 | 25 |
| ORH4/4 | | | | | | 32.4 | -55.00658 | 3.99 | 405 | 35 |
| ORH7/1 | | | | | | 8.0 | -106.7272 | 6.0 | 860 | 55 |
| ORH7/2 | 42□54 .S 175□33 .2W | 1988 | 35.1 | M | 0.6366 | 14.3 | -106.5349 | 3.43 | 855 | 30 |
| ORH7/3 | | | | | | 19.2 | -70.00999 | 3.72 | 535 | 30 |
| ORH7/4 | | | | | | 14.0 | | | | |
| ORH8/1 | | | | | | 12.3 | -102.83 | 5.10 | 825 | 45 |
| ORH8/2 | 42□52 .S 176□00 .W | 1988 | 38.8 | F | 0.5361 | 17.5 | -108.88 | 4.36 | 880 | 40 |
| ORH 8/3 | | | | | | 6.8 | -107.73 | 4.42 | 870 | 40 |
| ORH8/4 | | | | | | 13.6 | -95.17 | 4.57 | 755 | 40 |
| ORH8/5 | | | | | | 20.6 | -84.47 | 4.31 | 660 | 40 |
| ORH10/1 | 42°10'S 175°-178° W | | | | | 15.6 | -97.3 | 3.0 | 822 | 26 |
| ORH 10/2 | North Chatham Rise | 1992 | 41.4 | F | 0.61 | 18.4 | -92.4 | 3.0 | 779 | 27 |
| ORH 10/3 | | | | | | 12.2 | -43.2 | 3.0 | 355 | 27 |
| ORH15/1 | 42°10'S 175°-178° W | | | | | 10.1 | -106.7386 | 3.12 | 860 | 30 |
| ORH15/2 | North Chatham Rise | 1992 | 38.6 | F | 0.62 | 10.9 | -101.1571 | 3.53 | 810 | 30 |
| ORH15/3 | | | | | | 11.6 | -95.00 | 6.09 | 755 | 55 |
| ORH15/4 | | | | | | 12.0 | -64.54781 | 3.13 | 490 | 25 |
| ORR31/1 | | | | | | 8.7 | -114.64 | 6.35 | 930 | 55 |
| ORR31/2 | Cascade Plateau | 1985 | 50.8 | M | 0.9250 | 14.6 | -113.9367 | 8.82 | 925 | 80 |
| ORR31/3 | | | | | | 12.4 | -57.56 | 4.60 | 430 | 40 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ORR 31/4 | | | | | | 15.4 | -35.12 | 4.73 | 240 | 40 |
| ORR 66/1 | | | | | | 10.4 | -117.95 | 9.15 | 960 | 85 |
| ORR66/2 | Cascade Plateau | 1985 | 47.3 | 1 | 0.6749 | 10.7 | -100.5538 | 4.01 | 805 | 35 |
| ORR66/3 | | | | | | 10.6 | -68.11102 | 3.26 | 520 | 30 |
| ORR66/4 | | | | | | 11.2 | -41.37 | 6.40 | 290 | 55 |

Table 16.2. Data used to estimate mean reservoir ages for seawater at orange roughy depths.

| Sample position | Region sampled | Mean radiocarbon age±1 s.d. (years) | Mean difference calculation | Number of samples | Mean difference (years) |
|---|---|---|---|---|---|
| Position 1 | Core | 876 | - | 6 | - |
| Position 2 | Juvenile 1 | 880 | Pos.1-Pos.2 | 1 | -4 |
| Position 3 | Juvenile 2 | 836 | Pos.1-Pos.3 | 8 | 40 |
| Position 4 | Adult 1 | 613 | Pos.1-Pos.4 | 6 | 263 |
| Position 5 | Edge | 449 | Pos.1.-Pos.5 | 7 | 427 |

Table 16.3. Measurements of radiocarbon in seawater dissolved inorganic carbon from samples collected near the orange roughy fishing grounds of the Chatham Rise.

| Date | Latitude | Longitude | Depth (m) | $\Delta^{14}C$ (‰) | Source |
|---|---|---|---|---|---|
| Mar 1974 | 44° 56'S | 166° 39'E | 803 | -81.4 | Östlund and Stuiver 1980 |
| Mar 1974 | 44° 56'S | 166° 39'E | 1002 | -92.9 | Östlund and Stuiver 1980 |
| Mar 1974 | 44° 56'S | 166° 39'E | 1252 | -126.6 | Östlund and Stuiver 1980 |
| Mar 1974 | 44° 56'S | 166° 39'E | 1503 | -153.1 | Östlund and Stuiver 1980 |
| Mar 1974 | 38° 22'S | 170° 4'E | 898 | -85.6 | Östlund and Stuiver 1980 |
| Mar 1974 | 38° 22'S | 170° 4'E | 1097 | -111.0 | Östlund and Stuiver 1980 |
| Mar 1974 | 38° 22'S | 170° 4'E | 1348 | -143.4 | Östlund and Stuiver 1980 |
| Nov 1987 | 42.0°S | 179°E | 800 | -41.1±8.4 | Sparks et al. 1992 |
| Nov 1987 | 42.0°S | 179°E | 1000 | -98.4±7.3 | Sparks et al. 1992 |
| Nov 1987 | 45.0°S | 179°E | 800 | -112.0±53.2 | Sparks et al. 1992 |
| Nov 1987 | 45.0°S | 179°E | 1000 | -82.7±9.4 | Sparks et al. 1992 |
| July 1989 | 42.7°S | 177.5°E | 900 | -93.8±8.6 | Sparks et al. 1992 |
| July 1989 | 44.5°S | 177.5°E | 1268 | -94.5±12.7 | Sparks et al. 1992 |
| July 1989 | 45°S | 177.5°E | 750 | -74.2±10.1 | Sparks et al. 1992 |
| July 1989 | 45.5°S | 177.5°E | 800 | -92.7±9.0 | Sparks et al. 1992 |
| July 1989 | 45.5°S | 177.5°E | 1500 | -153.5±8.7 | Sparks et al. 1992 |

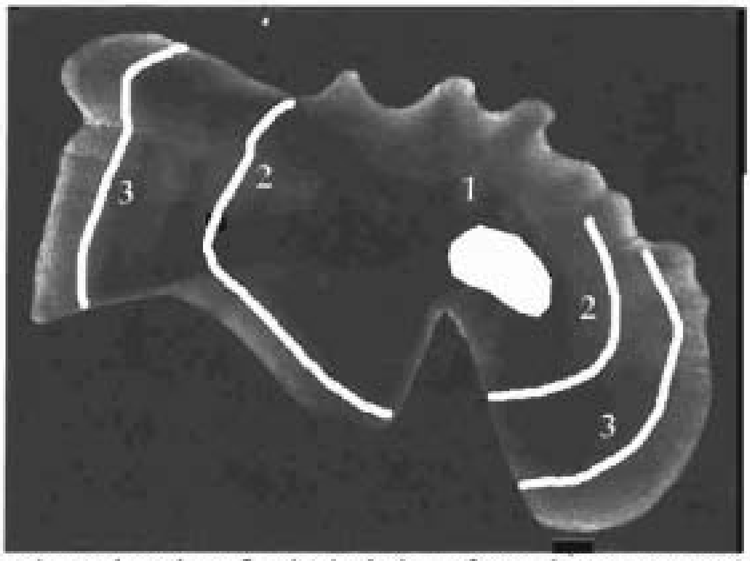


Figure 16.1. Approximate locations for the isolation of samples representative of Positions 1, 2 and 3 used for radiocarbon analysis of orange roughy otoliths. View is of the distal surface of an orange roughy otolith. Position 1 encompassed no more than the presumed first 4 years of otolith growth. Position 2 (Juvenile 1) is a region mid-way between the otolith core and the "transition zone". Position 3 (Juvenile 2) is a region encompassing the transition zone. Further details are provided in the Methods.

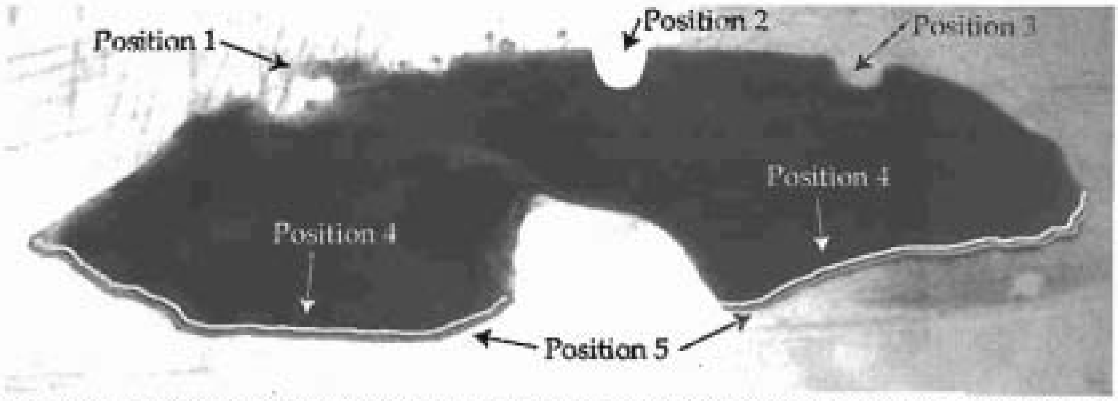


Figure 16.2. Positions of radiocarbon samples in a transverse thin section of an orange roughy otolith (ORH15) prepared after removal of the radiocarbon samples from the whole otolith. Position 1 is from the core region and the presumed first four years of growth. Position 2 (not removed from this sample) midway between the core region and the transition zone has been added to the figure. Position 3 is from the region of the transition zone. Position 4 is adjacent (distal) to the medial surface of the otolith and is removed after the sample from Position 5 is isolated. Position 5 is isolated from the medial surface of the otolith.

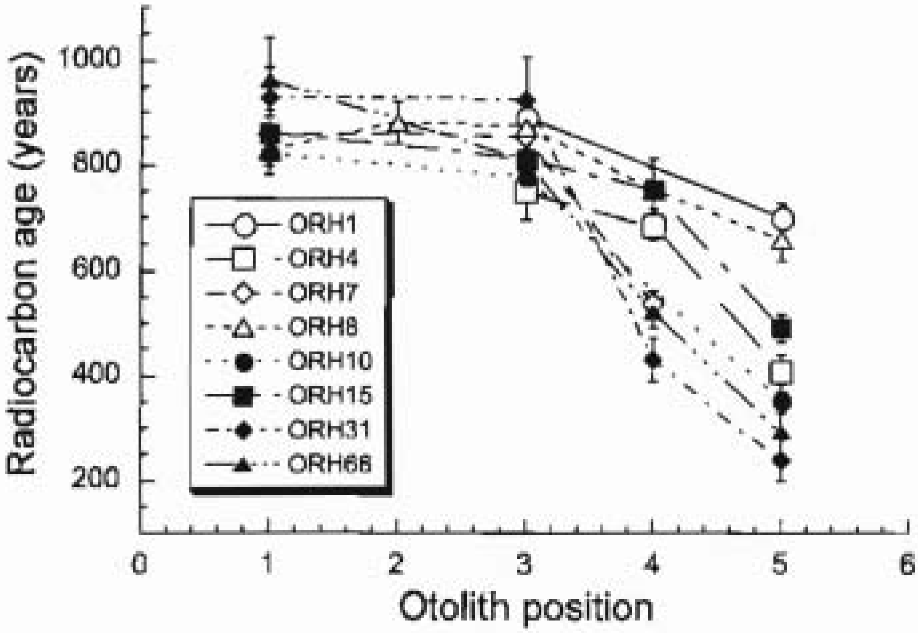


Figure 16.3. Conventional radiocarbon ages determined from samples of orange roughy otoliths. Each radiocarbon age was measured on a sample isolated from a specified location (Positions 1 to 5) on each otolith. The positions are defined in the Materials and Methods and details of the otoliths from each fish are presented in Table 5. Errors are one standard deviation.

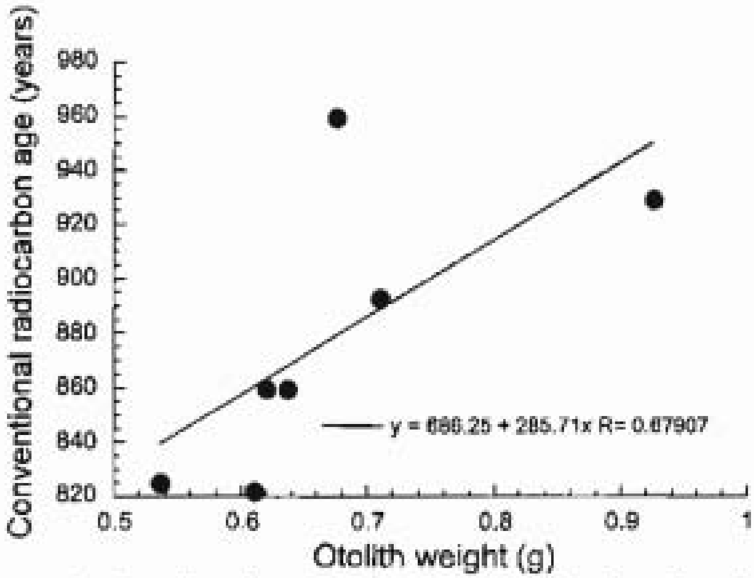


Figure 16.4. Conventional radiocarbon age for orange roughy plotted against otolith weight.

# Chapter 17 Use of the bomb radiocarbon chronometer to validate ages of four lutjanid species estimated from thin sections of otoliths

John Kalish, Mike Cappo and Stephen Newman

**Summary**

Lutjanids are important recreational and commercial fish species in tropical Australia. Contrasting results from biological studies have produced uncertainty in regard to the longevity and associated demographic parameters of these species and hence the potential for development in Australia's northern demersal fisheries. The use of the bomb radiocarbon chronometer in this study provided an independent and supplementary source of validation of the method of age estimation based on thin sections of otoliths of the four economically most important Lutjanus species from the central Great Barrier Reef. Measurement of radiocarbon in the cores of selected lutjanid otoliths supported longevities of at least 20 — 32 years estimated for these species in recent studies. Otolith $\Delta^{14}C$ varied from initially anticipated values based on a time series of $\Delta^{14}C$ derived from Great Barrier Reef corals with many results yielding $\Delta^{14}C$ considerably higher than that measured in corals. The variation in $\Delta^{14}C$ could be explained by existing knowledge of the early life history and ontogenetic habitat shifts of the species. Juveniles less than one year of age are found, to varying degrees, in coastal habitats that are influenced by freshwater runoff. Shallow freshwater habitats, notably lotic environments, display $\Delta^{14}C$ levels that are equivalent to atmospheric levels and, therefore, higher than that measured in marine environments. Varying degrees of mixing in nearshore habitats explains the $\Delta^{14}C$ series yielded from the otolith cores from the different lutjanid species. Variability in $\Delta^{14}C$ series both within and among species is attributable, in part, to marked variation in freshwater runoff prevailing during the first year of life of each specimen examined and differences in the habitats of individual fish. In addition to providing a validation of the thin section method of age estimation, the radiocarbon data collected from the otoliths provides insight into habitat selection by a range of lutjanid species.

**Introduction**

Large lutjanids form the basis of important recreational and commercial fisheries throughout tropical northern Australia and the Indo-Pacific (Williams and Russ, 1994). In northern Australia, exploitation of the "red snapper" complex, comprising *Lutjanus malabaricus*, *L. erythropterus*, and *L. sebae*, has increased in trawl, trap and line fisheries. Potential annual yield of red snappers from northern Australia has been estimated at 10,500 tonnes (Elliott 1996, Kailola et al. 1993, Ramm and McLoughlin 1995), based partly on growth and mortality parameters inferred from counts of increments visible on whole otoliths (McPherson and Squire, 1992; Milton et al. 1995). In addition, there is growing sportfishing interest in the spotted scale sea perch *L. johnii*, and a commercial line fishery has developed in parts of its range (Marriott and Cappo, 2000).

A radiometric analysis of otoliths to validate age estimates from whole otoliths was applied by Milton et al. (1995) to estimate longevities <10 years for the "red snappers" *Lutjanus erythropterus*, *L. malabaricus* and *L. sebae* in the Gulf of Carpentaria (latitude <14 oS ). The results of this study supported previous estimates of age from whole otolith readings by McPherson and Squire (1992). More recent studies using sectioned otoliths have produced estimates of longevity of at least 20 years for *L. malabaricus*, 22 years for *L. sebae* and 32 years for *L. erythropterus* (Newman et al. 2000), and at least 28 years for *L. johnii* (Marriott and Cappo 2000) from the central Great Barrier Reef region (lat. 18—20 °S). Direct validation of the temporal nature of the opaque and translucent zones in sectioned otoliths was demonstrated for these species by Cappo et al. (2000) and Marriott and Cappo (2000) based on oxytetracycline hydrochloride (OTC) marking. The differences in reported longevity based these two age estimation methods have produced uncertainty about the nature of potential development of northern Australian fisheries, and raised important questions regarding intraspecific, latitudinal variation in otolith interpretation and demographic parameters.

There is a clear need for further validation of age estimates from both regions, but there are several difficulties inherent in the most common, direct method using mark-release-recapture methods coupled with the injection of calciphilic fluorochromes to mark otoliths. Firstly, the majority of mark-release-recapture studies do not provide an indication of the absolute age of individual fish. These studies can only confirm the temporal nature of otolith zones used to estimate age, however, this does not necessarily provide a validation of all zones in the otolith (Kalish 1995). Secondly, large numbers of fish must be tagged and there is little control over the size, age and sex of fish released or recaptured. Thirdly, low recovery rates of fish at liberty for periods of more than 2-3 years are a feature of such tagging programs (Campana and Jones 1998). In addition, there may be tagging artefacts and sample sizes are usually small. A median of only 21 fish was reported by Francis et al. (1992) from 14 earlier OTC mark-recapture studies. Finally, the most common analyses of percentage agreement between the "observed" number and position of annual increments distal to fluorochrome marks with an "expected" number of annual increments exclude fish recovered at liberty for less than one year and do not allow exploration of the sources of error evident in the most extensive studies (see MacLellan and Fargo 1995, McFarlane and Beamish, 1995).

Use of the bomb radiocarbon chronometer in age validation studies offers several advantages including the ability to estimate, by independent means, the absolute age of individual fish. Furthermore, the method can be used to estimate the ages of larger and presumably older individuals, an outcome that is virtually impossible with traditional mark-recapture based methods. The benefits of the bomb radiocarbon method have been discussed extensively (Kalish 1993, Kalish 1995a, Kalish et al. 1996a, Campana 2001), and it is considered to be the most advanced and accurate method of fish age validation that is presently available (Campana 1999).

The purpose of this study is to provide an independent and supplementary source of validation of the otolith section based method of age estimation for the four most commercially important Lutjanus species from the central Great Barrier Reef. These results will be of value in further evaluation of age estimation of these species elsewhere in their Indo-Pacific range

**Materials and methods**

*Study area and species*

Otoliths were obtained from catches made by recreational anglers along the coast and amongst the reefs of the central Great Barrier Reef (GBR) (18.2o—19.4o South) and from research trawls in Cleveland Bay and the Arafura Sea (10o33.59' S, 134 o 31.02' E) (Figure 17.1, Table 1). Specimens for radiocarbon analyses were selected to represent mostly the presumed oldest fish in the populations fished in the central GBR, with two young individuals for contrast and to provide data that could be linked with current radiocarbon levels measured on the GBR.

Adult *L. erythropterus*, *L. malabaricus* and *L. sebae* occur offshore (McPherson and Squire, 1992) and were collected below the 22 m isobath amongst extinct reef edges and carbonate sediments. All life history stages of *L. johnii*, and the juveniles of the three "red snappers", occur inshore to varying extent in shallow, turbid bays that are lined with discontinuous mangrove forests (Newman and Williams, 1996). These bays contain terrigenous sediments and are subject to major, episodic inputs of freshwater from the Burdekin, Herbert and Haughton Rivers and from smaller coastal streams (Figure 17. 1). There is strong variability in this runoff at annual and decadal scales, linked to the passage of tropical cyclones and the strength and duration of the summer monsoon caused by El Niño–Southern Oscillation (ENSO) climate variability (Lough, 1998).

*Otolith Preparation*

For each whole fish or filleted carcass, the length to caudal fork (LCF) was measured where possible and both sagittae (hereafter referred to as the otoliths) were removed, weighed and measured. One otolith from each fish was selected at random and embedded in soft epoxy resin. After curing of the resin, three transverse sections were cut with a low speed saw and diamond wafering blade in the vicinity of the otolith primordium. The sections were 0.25 - 0.50 mm in thickness, depending on width of the otolith, and were lightly polished on wet carborundum paper (1000 grade) and lapping film (9 and 3 μm) and mounted on microscope slides. Ages were estimated by the first and second authors by counting opaque zones on the sections under magnifications from 7.5X to 25.2X with a stereo-dissecting microscope using transmitted white light, or under reflected light against a dark background (Newman et al., 1996). Birth years for both readings were estimated by subtracting the age from the year of collection for each fish.

The second otolith was prepared for radiocarbon analysis and the methods utilised were similar to those described elsewhere (e.g. Kalish 1995, Kalish et al. 1996a, Kalish et al. 1996b, Kalish et al. 2001). Otolith aragonite deposited during a period presumed to be less than the first 6 months of life was isolated from one sagitta from each fish. The earliest formed portion of individual otoliths was isolated with a fine, high-speed drill. This was achieved by "sculpting" from the larger otolith, an otolith that was representative of the particular Lutjanus spp. otolith at an age of about 6 months. The final product was a single piece of otolith aragonite. Sample weights ranged from about 5 to 17 mg (Table 1). Otolith carbonate was converted to CO2 by reaction in vacuo with 100% phosphoric acid. An aliquot of the CO2 was used to determine $\delta^{13}C$ for each sample and the remaining CO2 was graphitised for analysis of radiocarbon. Radiocarbon levels in each sample were determined by accelerator mass spectrometry (AMS) at the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) of the Woods Hole Oceanographic Institution (WHOI) and at Antares Mass Spectrometry Group of the Australian Nuclear Sciences and Technology Organisation (ANSTO). 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). Age corrections are based on the mean estimate of age determined from reading of otolith sections. Reported errors are 1 standard deviation for both radiocarbon data and age estimates based on the reading of otolith sections. Radiocarbon errors include both counting errors and laboratory random errors.

**Results**

The data from the otolith section based estimates of age for the four lutjanid species are plotted against the radiocarbon data obtained from the otolith cores and with a time series of radiocarbon derived from a hermatypic coral sampled on the GBR (Druffel and Griffin 1993) (Figure 17.2). The radiocarbon data from Abraham Reef on the central GBR are used as a calibration between surface ocean radiocarbon levels measured in tropical latitudes in the southern hemisphere and time and are believed to represent the true variation in radiocarbon in the tropical southwestern Pacific Ocean. The otolith samples analysed spanned a wide range of estimated birth dates based on the interpretation of the otolith thin sections with the oldest fish, a specimen of *L. erythropterus*, estimated to be 32 years and with a birth date of 1959.

For the most part, the data for otolith based age versus otolith radiocarbon deviates broadly from the coral-based curve defining radiocarbon variation on the central GBR, although the majority of data points from *L. malabaricus* fall relatively close to radiocarbon curve based on the hermatypic coral. Inspection of the age estimate and radiocarbon data for each species indicates some pattern among species. For example, data from *L. erythropterus* and *L. johnii* tend to be higher in radiocarbon than *L. sebae* and *L. malabaricus* during a particular year.

Freshwater in shallow and particularly flowing water bodies is in constant exchange with the atmosphere and there is little or no opportunity for stratification of radiocarbon due to relatively rapid mixing (Kalish 1995). Therefore, atmospheric radiocarbon levels can serve as a proxy for radiocarbon in freshwater. A time series of atmospheric measurements of radiocarbon from Wellington, New Zealand, the longest atmospheric radiocarbon record in the southern hemisphere (Manning and Melhuish 1994, Hua et al. 1999), can be used to estimate radiocarbon levels in mid-latitude freshwater environments as early as the 1950s. Figure 17.3 shows the Wellington atmospheric data plotted with the radiocarbon time series from Abraham Reef. In addition, these two time series have been used to model radiocarbon levels in waters of two intermediate salinities (17.5‰ and 24‰) that would result from mixing between freshwater equilibrated with the atmosphere in respect to radiocarbon and seawater. These estimated salinities would be representative of coastal waters with substantial freshwater inputs.

A plot that combines the radiocarbon time series from waters of different salinities with the otolith age and radiocarbon data shows that many of the time series of radiocarbon measured in otolith cores are consistent with mixed water masses of reduced salinity (Figure 17.4). The *L. johnii* and *L. erythropterus* otolith data are closest to radiocarbon levels indicative of mixed waters with the otolith data straddling the 24‰ radiocarbon time series. The otolith data from *L. sebae* and *L. malabaricus* fall close to the Abraham Reef time series or between those data and the 24‰ radiocarbon time series.

**Discussion**

The results support both the use of sectioned otoliths to accurately estimate lutjanid ages, and longevities of at least 22 years for *L. sebae*, 20 years for *L. malabaricus*, 32 years for *L. erythropterus* and 28 years for *L. johnii* as proposed by Newman et al. (2000). In contrast to these results, Milton et al. (1995) concluded from disequilibria in 210Pb: 226Ra ratios of pooled otoliths that longevities < 10 years from whole otolith readings (sensu McPherson and Squire, 1992) were more accurate than section counts. The disequilibria method had previously helped confirm far greater longevities estimated for long-lived, deep-water *Hoplostethus atlanticus* (Fenton et al. 1991) and Sebastes (Campana et al. 1990). However, Campana and Jones (1998) noted that radiometric dating was too imprecise for detailed or individual age determinations and West and Gauldie (1995) also concluded that the method was inadequate to validate fish ages. Of particular concern to West and Gauldie (1995) were a lack of knowledge of how much Pb 210 is allogenic (coming from outside the otolith) and how much is authigenic (produced from decay of Ra 226 within the otolith), inappropriate use of a single model for otolith growth in time, and especially emanation of Rn 222 from otoliths during the decay series of Ra 226 to Pb 210 and contamination. We consider that the use of the technique for three species of red snapper from the Gulf of Carpentaria may be prone to greater errors than when used to estimate ages of deepwater fish, due to the failure of key assumptions. In particular, tropical coastal habitats such as the Gulf of Carpentaria are characterised by steep environmental gradients of temperature and salinity. For the most part, this is linked to massive episodic inputs of freshwater and dissolved inorganic material characteristic of the shallow Gulf environment. Furthermore, as discussed below, these lutjanid species inhabit coastal habitats characterised by mixing with freshwater. Alternatively, there may be real latitudinal differences in the demography of these species that may be resolved by further validation using the bomb radiocarbon method, OTC marking or other independent techniques for age estimation.

*Life histories and exposure to freshwater*

The radiocarbon measurements obtained from the otoliths of the four lutjanid species are best explained by a separation amongst species in their degree of exposure to freshwater in the first six months of life. This proposal is strongly supported by our understanding, albeit limited, of lutjanid ecology in the region (Newman and Williams, 1996). In the GBR, *Lutjanus erythropterus*, *L. malabaricus* and *L. sebae* have cross-shelf differences in patterns of distribution and size (McPherson and Squire, 1992, McPherson et al., 1992) that indicate ontogenetic shifts in habitat.

Juvenile *Lutjanus johnii* are found only in brackish mangrove-lined estuaries and the largest individuals are caught in deeper waters at nearby headlands and rocky outcrops in 10-26 metres. The species does not extend offshore into the coral reef matrix of the central Great

Barrier Reef and has not been sighted there in depths greater than 31 metres (Williams and Russ, 1994; Newman and Williams, 1996; Malcolm et al., 1999).

Juvenile *L. malabaricus* and *L. erythropterus* >=2.5 cm TL have been commonly reported in Upstart, Cleveland and Halifax Bays, especially around sparse seagrass beds (Williams and Russ 1994, Newman and Williams 1996). Bycatch of juveniles in penaeid trawls were restricted to depths <15 m at stations closest to shore in a survey of the central GBR by Jones and Derbyshire (1988). Only these sites had high silt and clay fractions of terrigenous origins (Williams and Russ 1994). However, the overlap of *L. malabaricus* and *L. erythropterus* inshore is not precisely known because the smaller juveniles are so similar in form and habit that they are not readily separated on the basis of morphological features. For example, allozyme and mitochondrial DNA analysis by Elliott (1996) found that only 32 % of putative *L. malabaricus* juveniles proved to be that species from samples identified by four different laboratories. The remainder were juvenile *L. erythropterus*. Therefore, it is possible that *L. malabaricus* occurs slightly further offshore than *L. erythropterus* in the first six months of life. The radiocarbon data from the otoliths of these two species support this hypothesis as *L. malabaricus* otoliths tended to have lower levels of radiocarbon during a particular presumed time period, indicative of residence in more saline waters, than the otoliths of *L. erythropterus*.

Juvenile *L. sebae* have a much wider depth range than the other three species (Figure 17.5) and were found over both terrigenous and carbonate sediments in the range 15-62 m by Jones and Derbyshire (1988). They can be caught on the same inter-reef grounds as mature adults, and there is some evidence that they may be less common than *L. malabaricus* and *L. erythropterus* in turbid waters of 5-15 m (Williams and Russ 1994). It would be expected that the radiocarbon levels measured in the otoliths of *L. sebae* would reflect more the composition of full-strength seawater. The radiocarbon data from the cores of *L. sebae* otoliths suggest a range of habitats, but with a clear tendency for full-strength seawater environments.

Most of the freshwater discharge to the central GBR occurs in a few short floods in the austral summer between December and March. Flood plumes enter the GBR lagoon (mostly between 17o – 23o S) and typically flow northwards. The Burdekin River is dominant with mean annual flow of 9.272 x 106 megalitres, in the range 0.54 x 106 – 50.927 x 106 megalitres and with a Coefficient of Variation of 116.7% (Wolanski, 1994).

Three-dimensional modelling of the Burdekin River plume has shown wind-driven trajectories and patchiness in the far salinity field enhanced by discharges from the Haughton and Herbert Rivers and neighbouring streams and by tidal interactions with headlands (King et al. 2001). The residence times of dilute patches inside headlands are in the order of a few weeks. In the 1981 Burdekin River flood peak, the entire Upstart Bay was filled with freshwater and a plume of brackish water (<18 ppt) stretched 100 km northward along the coast. At this time the surface salinities over the 15-20 m isobaths in Bowling Green, Cleveland and Halifax Bays were 15—30 ppt (King et al. 2001), and significant seawater dilution at the seabed in these depths has been measured by Wolanski (1994).

The settlement inshore of juvenile lutjanids would coincide with floods in December – March. McPherson et al. (1992) reported spawning in spring and summer months, for seven months for *L. sebae*, five months for *L. malabaricus* and eight months for *L. erythropterus* and without any apparent lunar cycle in reproduction. Spawning peaks were evident in November-January for L.sebae and *L. malabaricus* and October-November for *L. erythropterus*. Evidence of spawning was found in depths of 20-70 m for *L. erythropterus* and *L. malabaricus* and 20-160 m for *L. sebae*. Unpublished studies of gonadosomatic indices of *L. johnii* in the central GBR show a sharp peak in January for fish collected at 3-15 m around rocky headlands such as Cape Cleveland.

There are several difficulties associated with the interpretation of radiocarbon data from the otoliths of these coastal lutjanid species. Firstly, the otolith cores isolated from each sample were representative of, at most, the first six months of the fish's life and, as a result, would have the potential to integrate information from a wide range of habitats (e.g. salinities). Individual fish may be exposed to varying salinities due to active movement between habitats (e.g. onshore to offshore) or through passive exposure to different water masses associated with the encroachment of, for example, freshwater plumes on the fish habitat.

The major rivers of north Queensland are characterised by high intra- and interannual variability in discharge and this can have a dramatic effect on the salinity of coastal waters in the region and the habitat of the individual specimens considered in this study. For example, three of the five *L. malabaricus* analysed for radiocarbon had birth years estimated (by both readers) from otolith thin sections that coincided with very dry years when the combined discharge of the major rivers (Table 2) was extremely low. Under these conditions, the otoliths would be expected to incorporate less radiocarbon than in those years when freshwater discharge was higher. Ultimately, the factors that impact on the radiocarbon content of lutjanid otoliths are extremely complex and a fully satisfactory explanation for the patterns observed is unlikely without the availability of additional data. Further insight into the nature of lutjanid habitat would be a valuable adjunct to this study and of particular significance to research on recruitment of these species. Useful data on these issues is likely to be obtained from further detailed studies of the chemical composition of lutjanid otoliths using ultra-trace techniques such as ICP-MS.

**Conclusions**

Measurement of the radiocarbon in the cores of selected lutjanid otoliths provides a validation for the thin section method of age estimation for these four species. The data also demonstrate that at least three of the four lutjanid species investigated inhabit coastal waters during early life and, notably, habitats characterised by mixing with freshwater. This finding supports the current limited understanding of the early life history of these lutjanid species. In addition, the significant freshwater input to the juvenile lutjanid habitat would have a dramatic impact on the trace element chemistry of the otoliths; this effect is demonstrated by the high $\Delta^{14}C$ measured in otolith cores. This same factor will result in violation of a critical assumption of the radiometric method of age validation and result in extreme variations in the incorporation of isotopes such as 226Ra and 210Pb which have very different distributions in fresh and marine waters.

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Table 17.1. Fish, otolith, and radiocarbon data for four lutjanid species collected on the central Great Barrier Reef, Australia.

| Sample No. | Laboratory accession no. | Species | Collection location | Collection date | Caudal-fork length (cm) | Otolith weight (g) | Sample weight (mg) | $\Delta^{14}C$ (‰) | Otolith section age (years) Reader 1 | Birth date (year A.D.) | Otolith section age (years) Reader 1 | Birth date (year A.D.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L.e 183 | ANSTO OZC627 | L.erythropterus | Robbery Shoals | Nov 1990 | 622 | 1.0436 | 13.9 | 127.3±11.54 | 25 | 1965 | 25 | 1965 |
| L.e 187 | ANSTO OZC628 | L.erythropterus | Robbery Shoals | Nov 1990 | 590 | 1.0684 | 14 | 119.1±7.4 | 26 | 1964 | 28 | 1962 |
| L.e 1719 | ANSTO OZC629 | L.erythropterus | Old Reef | 1994 | 582 | 1.0189 | 14 | 237.9±6.4 | 23 | 1971 | 24 | 1970 |
| L.e 1720 | ANSTO OZC630 | L.erythropterus | Old Reef | 1994 | 541 | 0.4905 | 13.9 | 149.6±5.9 | 4 | 1990 | 5 | 1989 |
| L.e 1730 | ANSTO OZC631 | L.erythropterus | Old Reef | Sept 1993 | 602 | 0.9556 | 14. | 178.4±6.2 | 24 | 1969 | 24 | 1969 |
| L.e 1773 | ANSTO OZC632 | L.erythropterus | Spoon Reef | Jan 1995 | 573 | 1.0164 | 16.7 | 256.5±8.1 | 27 | 1968 | 31 | 1964 |
| L.e 1780 | ANSTO OZC633 | L.erythropterus | Kelso Shoals | June 1993 | 574 | 0.9088 | 14.7 | 220.6±14. | 19 | 1974 | 23 | 1970 |
| L.e 1793 | ANSTO OZC634 | L.erythropterus | Kelso Shoals | June 1993 | 570 | 0.833 | 13. | 203.3±11.5 | 13 | 1980 | 13 | 1980 |
| L.e 1818 | ANSTO OZC635 | L.erythropterus | Kelso Shoals | June 1993 | 575 | 1.0334 | 9.9 | 116.3±13.5 | 25 | 1968 | 27 | 1966 |
| L.e 1829 | ANSTO OZC636 | L.erythropterus | Old Reef | Sept 1991 | 600 | 1.3971 | 5.3 | -19.5±14.5 | 32 | 1959 | 34 | 1957 |
| L.m 1852 | RRL NZA7001 | L.malabaricus | Old Reef | Oct 1991 | 705 | 2.2662 | 14.5 | 124.7±10.1 | 20 | 1971 | 19 | 1972 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L.m 147 | ANSTO OZC637 | L.malabaricus | Townsville offshore | Feb 1990 | 635 | 1.969 | 10.8 | 168.5±14.8 | 18 | 1972 | 21 | 1969 |
| L.m 167 | RRL NZA7002 | L.malabaricus | Townsville | Feb 1990 | 635 | 1.3322 | 12.8 | 153.3±12.3 | 10 | 1980 | 8 | 1982 |
| L.m 160 | ANSTO OZC638 | L.malabaricus | Robbery Shoals | Nov 1990 | 630 | 1.0199 | 11.4 | 155.±9.8 | 5 | 1985 | 7 | 1983 |
| L.m 650 | ANSTO OZC639 | L.malabaricus | Cleveland Bay | Mar 1991 | 76 | 0.0142 | 5.1 | 128.2±11.8 | 0+ | 1990 | 0+ | 1990 |
| L.s 128 | ANSTO OZC640 | L.sebae | Pith Reef | Nov 1990 | 680 | 2.63 | 9.8 | 111.5±13.9 | 22 | 1968 | 26 | 1964 |
| L.s 133 | ANSTO OZC641 | L.sebae | Pith Reef | Nov 1990 | 635 | 2.29 | 10.1 | 133.3±17.9 | 15 | 1975 | 15 | 1975 |
| L.s 1899 | ANSTO OZC642 | L.sebae | Old Reef | 1994 | 668 | 1.67 | 11.6 | 197.8±17.2 | 15 | 1979 | 18 | 1976 |
| L.s 1910 | ANSTO OZC643 | L.sebae | Old Reef | Aug 1991 | 470 | 0.88 | 11.2 | 169.2±13.4 | 6 | 1985 | 7 | 1984 |
| L.j 2009 | ANSTO OZC644 | L.johnii | Cape Ferguson | 28 Mar 1995 | 815 | 2.59 | 12.6 | 179.8±10.1 | 15 | 1980 | 16 | 1979 |
| L.j 2066 | ANSTO OZC645 | L.johnii | Arafura Sea | 2 Oct 1992 | 536 | 1.5187 | 6.5 | 235.6±19.0 | 16 | 1976 | 13 | 1979 |
| L.j 2109 | ANSTO OZC646 | L.johnii | Magnetic Is. | 29 Jan 1995 | 800 | 3.303 | 5.4 | 244.6±7.4 | 24 | 1971 | 25 | 1970 |
| L.j 18 | RRL NZA8014 | L.johnii | Cape Cleveland | 28 Nov 1989 | 990 | 5.8433 | 13.2 | 152.3±9.6 | 25 | 1964 | - | - |
| L.j 2705 | RRL NZA8015 | L.johnii | Cape Cleveland | 25 May 1995 | 810 | 3.7316 | 14.8 | 238.7±11.1 | 28 | 1967 | - | - |

*Otolith had part of the rostrum missing giving incomplete otolith weight.

Table 17.2. Deviation in flow during back-calculated birth years from average discharge of the Burdekin and Herbert Rivers combined (1958-1995).

| Sample no. | Species | Reader 1 Birth year | Flow deviation (megalitres) | Reader 2 Birth year | Flow deviation (megalitres) |
|---|---|---|---|---|---|
| L.e 1719 | L.erythropterus | 1971 | -414324 | 1970 | -4961907 |
| L.e 1720 | L.erythropterus | 1990 | 195748 | 1989 | 2195927 |
| L.e 1730 | L.erythropterus | 1969 | -9306958 | 1969 | -9306958 |
| L.e 1773 | L.erythropterus | 1968 | 6983127 | 1964 | -6111149 |
| L.e 1780 | L.erythropterus | 1974 | 49786851 | 1970 | -4961907 |
| L.e 1793 | L.erythropterus | 1980 | -4807768 | 1980 | -4807768 |
| L.e 1818 | L.erythropterus | 1968 | 6983127 | 1966 | -8803209 |
| L.e 1829 | L.erythropterus | 1959 | -1569053 | 1957 | |
| L.e 183 | L.erythropterus | 1965 | -6039229 | 1965 | -6039229 |
| L.e 187 | L.erythropterus | 1964 | -6111149 | 1962 | -7544364 |
| L.m 147 | L.malabaricus | 1972 | 10622815 | 1969 | -9306958 |
| L.m 160 | L.malabaricus | 1985 | -8132337 | 1983 | -1035209 |
| L.m 167 | L.malabaricus | 1980 | -4807768 | 1982 | -7795064 |
| L.m 1852 | L.malabaricus | 1971 | -414324 | 1972 | 10622815 |
| L.m 650 | L.malabaricus | 1990 | 195748 | 1990 | 195748 |
| L.s 128 | L.sebae | 1968 | 6983127 | 1964 | -6111149 |
| L.s 133 | L.sebae | 1975 | 3422999 | 1975 | 3422999 |
| L.s 1899 | L.sebae | 1979 | 10780873 | 1976 | 2253699 |
| L.s 1910 | L.sebae | 1985 | -8132337 | 1984 | -4051878 |
| L.j 18 | L.johnii | 1964 | -6111149 | | |
| L.j 2009 | L.johnii | 1980 | -4807768 | 1979 | 10780873 |
| L.j 2109 | L.johnii | 1971 | -414324 | 1970 | -4961907 |
| L.j 2705 | L.johnii | 1967 | -4673664 | | |

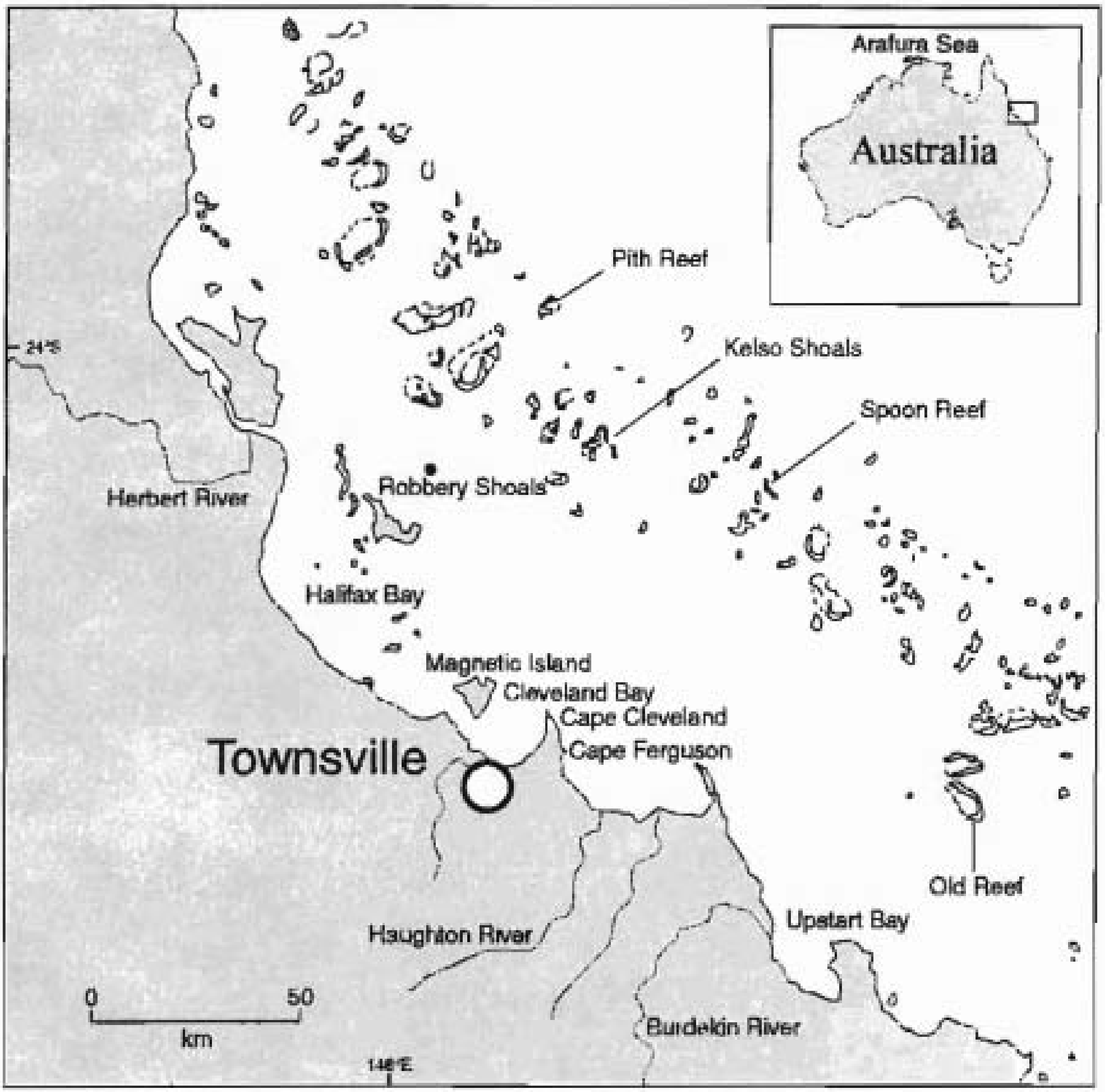



Figure 17.1. Sites in the central Great Barrier Reef region from which lutjanid fish samples were collected.

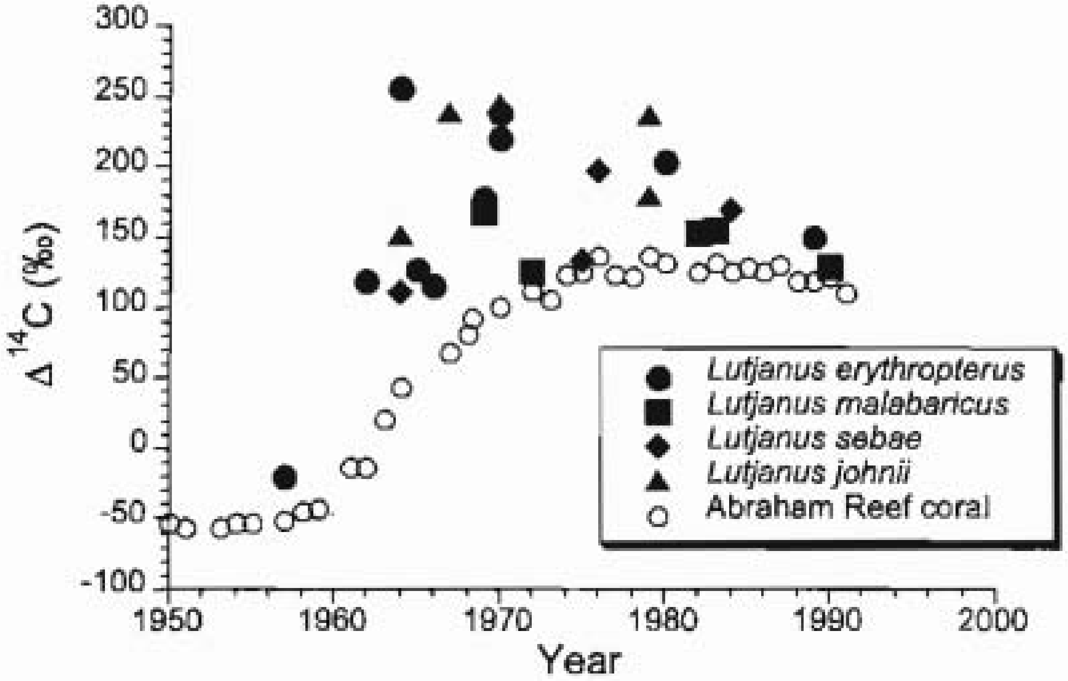



Figure 17.2. Plot of otolith thin section birth date versus otolith core radiocarbon for 4 lutjanid species. Abraham Reef coral data (Druffel and Griffin 1993) are provided as calibration for variations in radiocarbon over time on the central Great Barrier Reef.

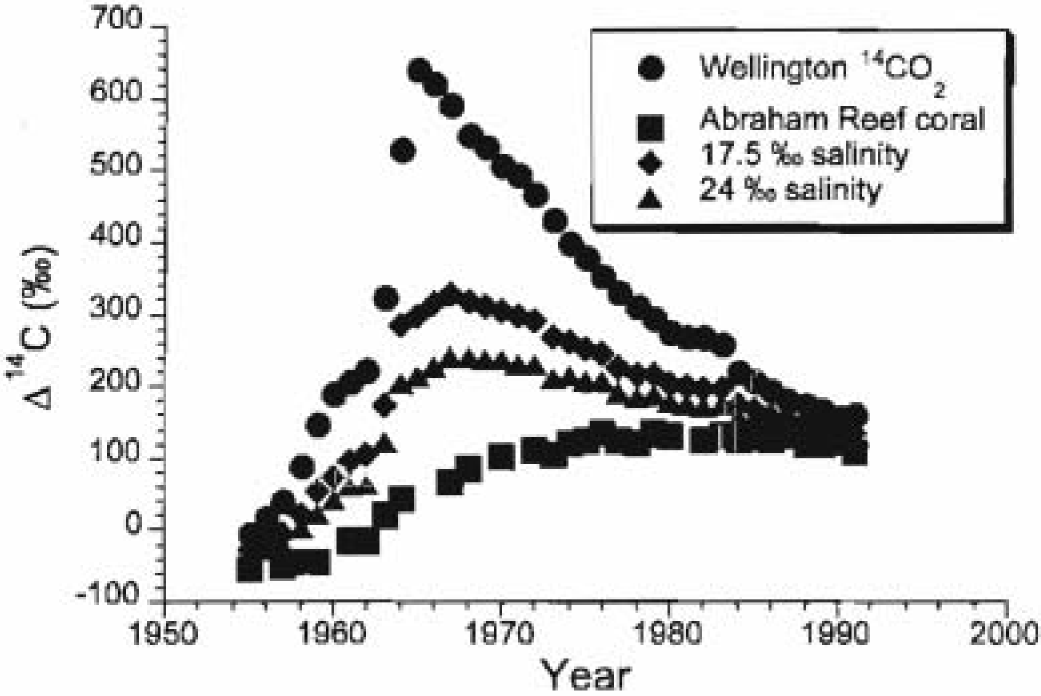



Figure 17.3. Mixing model of radiocarbon variation over time. Measurements of radiocarbon in atmospheric CO2 at Wellington, New Zealand (Manning and Melhuish 1994) and radiocarbon in seawater dissolved inorganic carbon, based on a coral proxy, are used to model predicted radiocarbon levels in coastal waters of 17.5 ‰ and 24.0 ‰ in the region of the central Great Barrier Reef.

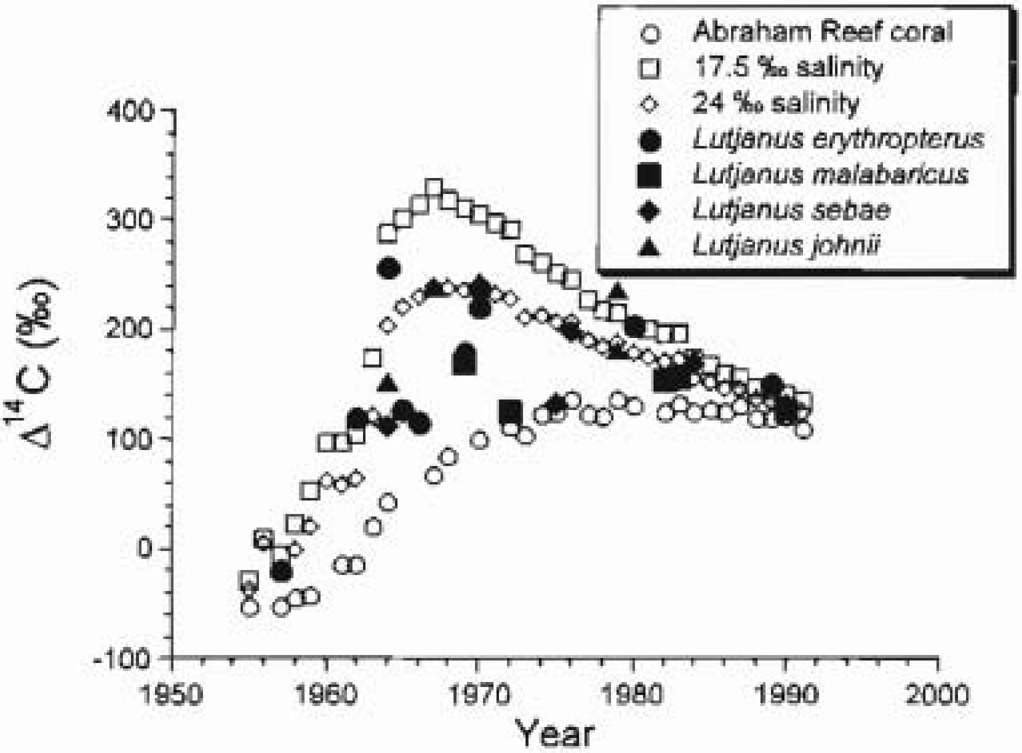
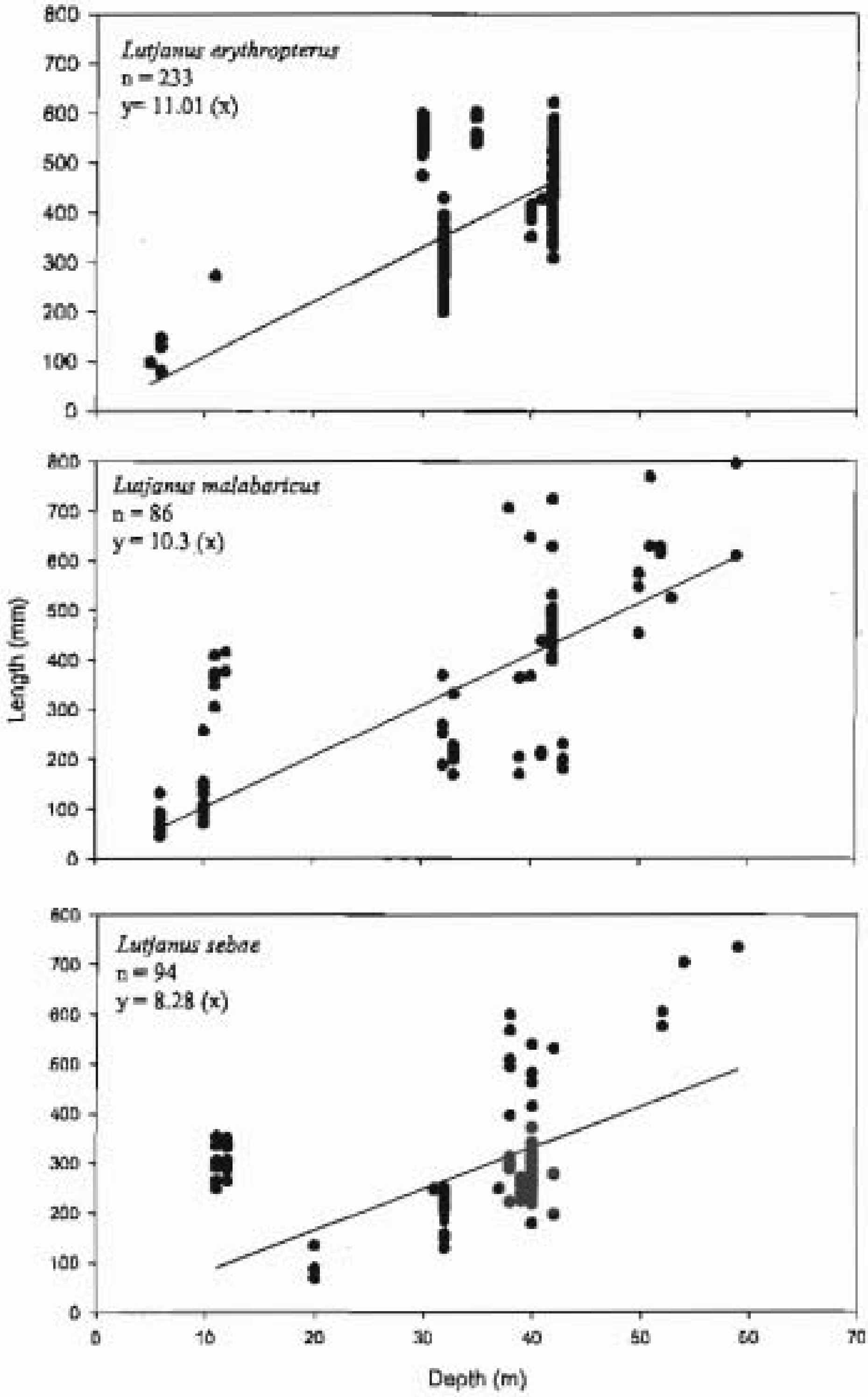



Figure 17.4. Plot of otolith thin section birth date versus otolith core radiocarbon and measured and modelled environmental radiocarbon for seawater and coastal waters of 17.5‰ and 24.0‰ salinity.

Figure 17.5. Relationships between depth and size of exploited "reds" in the central GBR region between latitudes –18.560 and –19.147 from trawl, trap and handline collections by Newman et al. (2000) and Cappo et al. (2000). Regression lines forced through the origin are shown with sample sizes.



# Chapter 18 Use of bomb radiocarbon to validate the age estimation method for *Epinephelus octofasciatus*, *Etelis carbunculus* and *Lethrinus nebulosus* from Western Australia

John Kalish, Stephen Newman and Justine Johnston

**Summary**

Measurement of radiocarbon in the cores of otoliths from *Epinephelus octofasciatus*, *Etelis carbunculus* and *Lethrinus nebulosus*, all commercially harvested in the Indian Ocean off northern Western Australia provided a validation of the age estimation method for these species. Validation of the age estimation method for the two deepwater species, *E. octofasciatus*, *E. carbunculus*, would be impractical by other methods frequently used for age validation. Although the otoliths were from three different genera, there was an overall similarity in the relationship between age estimated from the otolith thin sections and $\Delta^{14}C$. Some of the inter-annual variability in $\Delta^{14}C$ may be the result of intra- and inter-annual changes in the transport of North Pacific water via the Indonesian throughflow.

**Introduction**

The ruby snapper, *Etelis carbunculus* Cuvier, known also as red dog snapper and ehu is widely distributed throughout the tropical Indo-Pacific Ocean region from the Hawaiian Islands to East Africa from southern Japan south to Australia (Allen 1985). Along Western Australia, *E. carbunculus* is found as far south as the Abrolhos Islands (29°S) and has been landed in limited commercial quantities from the Ningaloo Reef area (23°30'S) northwards (Newman unpublished data). They inhabit hard bottom areas and areas of vertical relief such as pinnacles and areas of large epibenthos from depths of 90 to at least 440 m and are concentrated in depths from 200 to 300 m (Allen 1985, Brouard and Grandperri 1985, Newman unpublished data). The exploitation of deep slope species such as *Etelis carbunculus* is still in the developmental stage along the north-west coast of Western Australia. Most of the northern fisheries view the deep slope region as the next frontier for fishery development with only small catches landed to date from State based fishers of up to about 50 t.

The eightbar grouper, *Epinephelus octofasciatus* Griffin, known also as grey-banded cod or barred cod is distributed widely throughout the Indo-West Pacific region from South Africa to Japan, Australia and New Zealand (Heemstra and Randall 1993). In much of the literature on Indo-Pacific fishes this species has been misidentified as *Epinephelus septemfasciatus* (e.g. Dalzell et al. 1996) as discussed in Heemstra and Randall (1993). *Epinephelus septemfasciatus* is believed to occur only in the ocean around China, Japan and Korea. *Epinephelus octofasciatus* is a deep-water species important in numerous fisheries throughout the tropical Indo-Pacific region. It is also found along the Western Australian coastline from 35°S-12°S where it is caught in commercial fisheries. They inhabit rocky reef areas from depths of 150 to at least 400 m (Heemstra and Randall 1993, Newman unpublished data). This species is landed as part of the deep slope demersal fish catch and has recently been targeted by recreational anglers fishing deep offshore reefs.

Validation of age estimation methods for deepwater species such as *E. carbunculus* and *E. octofasciatus* is extremely difficult due to the distribution and biology of these species. Capture-mark-recapture studies that include marking with substances that leave a detectable mark on calcified tissues (e.g. oxytetracycline hydrochloride or strontium chloride) are impractical since in most cases these species are brought to the surface with ruptured swim bladders and popped or bulging eyes. Furthermore, it is difficult to obtain adequate (monthly) samples for marginal increment analysis due to the seasonality of the fishery. Due to the difficult of employing standard methods of age validation and the presumed longevity of these species, they are excellent candidates for age validation based on the bomb radiocarbon chronometer.

In contrast to the above two species, the spangled emperor, *Lethrinus nebulosus* (Forsskal) known also as nor-west snapper is a relatively shallow water reef species that is widespread throughout the Indo-West Pacific region (Carpenter and Allen 1989). It inhabits nearshore and offshore reefs and lagoons usually in depths less than 50 metres but can occur in depths of up to 100 m (Carpenter and Allen 1989, Newman and Williams 1996). This species is a very important commercial and recreational fish species throughout its range. *Lethrinus nebulosus* is a key commercial species in Western Australia with annual landings in 1999-2000 of more than 110 t (Penn 2001). In addition, *Lethrinus nebulosus* was the third most abundant fish species landed by recreational fishers in the Gascoyne region of mid Western

Australia (Sumner et al. in press). Similarly, in a recent recreational fish study of the Pilbara region of North-Western Australia, *Lethrinus nebulosus* was again the third most common demersal fish species landed by recreational fishers (Williamson, pers. comm.).

The importance of these species to commercial and/or recreational fisheries indicates a need for the development of stock assessments. Validated and accurate methods of age estimation are essential to reduce uncertainty in the stock assessment and ensure effective management. As demonstrated in earlier research, the bomb radiocarbon chronometer represents a cost-effective and efficient means of age validation. In addition, for the two deep-water species considered in this study it is likely to be the only reliable method.

**Materials and methods**

For each whole fish or filleted carcass, the length to caudal fork (LCF) was measured where possible and both sagittae (hereafter referred to as the otoliths) were removed, weighed and measured. One otolith from each fish was selected at random and embe2dded in soft epoxy resin. After curing of the resin, three transverse sections were cut with a low speed saw and diamond wafering blade in the vicinity of the otolith primordium. The sections were 0.25 - 0.50 mm in thickness, depending on width of the otolith, and were lightly polished on wet carborundum paper (1000 grade) and lapping film (9 and 3 µm) and mounted on microscope slides. Ages were estimated by the first and second authors by counting opaque zones on the sections under magnifications from 7.5X to 25.2X with a stereo-dissecting microscope using transmitted white light, or under reflected light against a dark background (Newman et al., 1996). Birth years for both readings were estimated by subtracting the age from the year of collection for each fish.

The second otolith was prepared for radiocarbon analysis and the methods utilised were similar to those described elsewhere (e.g. Kalish 1995; Kalish et al. 1996a; Kalish et al. 1996b; Kalish et al. 2001). Otolith aragonite deposited during a period presumed to be less than the first 6 months of life was isolated from one sagitta from each fish. The earliest formed portion of individual otoliths was isolated with a fine, high-speed drill. This was achieved by "sculpting" from the larger otolith, an otolith that was representative of the otolith from that species at an age of about 6 months. The final product was a single piece of otolith aragonite. Sample weights ranged from about 8.6 to 15.5 mg (Table 1, 2 and 3). Otolith carbonate was converted to CO2 by reaction in vacuo with 100% phosphoric acid. An aliquot of the CO2 was used to determine $\delta^{13}C$ for each sample and the remaining CO2 was graphitised for analysis of radiocarbon. Radiocarbon levels in each sample were determined by accelerator mass spectrometry (AMS) at the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) of the Woods Hole Oceanographic Institution (WHOI) (Woods Hole, USA), at Antares Mass Spectrometry Group of the Australian Nuclear Sciences and Technology Organisation (ANSTO) (Lucas Heights, NSW) or at the Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences (Wellington, New Zealand). 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). Age corrections are based on the mean estimate of age determined from reading of otolith sections. Reported errors are 1 standard deviation for both radiocarbon data and age estimates based on the reading of otolith sections. Radiocarbon errors include both counting errors and laboratory random errors.

**Results**

Radiocarbon data from otolith cores were graphed against otolith section based birth date estimates for *Epinephelus octofasciatus*, *Etelis carbunculus* and *Lethrinus nebulosus* (Figure 18.1) and the data are presented in Tables 18.1, 18.2 and 18.3. The combined data clearly defined a curve that shows the increase in bomb radiocarbon during the 1960s and 1970s and the subsequent gradual decrease in radiocarbon in the surface ocean as bomb-derived radiocarbon is sequestered to other pools. A model of radiocarbon variation derived for the eastern Indian Ocean (Kalish et al. 1996) and a calibration determined for the South Pacific (Kalish 1993) are plotted with these data (Figure 18.2) to provide an indication of the accuracy of the age estimates for *Epinephelus sp.*, *Etelis sp.* and Lethrinus sp. determined from otolith sections. The otolith data are in relatively good agreement with the calibrations and suggest that the majority of the age estimates from otolith thin sections are accurate. However, in a few instances there is evidence that there may be significant errors in some of the age estimates.

Radiocarbon data from otolith cores were graphed against otolith section based birth date estimates from multiple otolith readers for *Epinephelus sp.* and *Etelis sp.* in Figures 18.3 and 18.4, respectively. The *Epinephelus sp.* age estimates from both laboratories show an increase in radiocarbon during the 1960s-1970s, but there is considerable variation in the repeat age readings from the WA reader and with the age estimates from ANU. Three *Epinephelus sp.* otolith cores had $\Delta^{14}C$ levels of less than –60‰, levels that are clearly indicative of a pre-bomb (pre-1960) birth date. However, there were large differences in the otolith section based age estimates from the two laboratories for these three fish. The ANU reader estimated these fish to be 40, 42 and 46 years of age and with birth dates prior to 1960, while the WA reader's estimates suggested younger ages with birth dates of 1960 or latter, except for one age estimate of 41 (birth date 1956). Based on the information on the appearance of bomb-derived radiocarbon in the Indian and Pacific Oceans, these three fish were certainly spawned prior to 1960 and the WA age estimates are likely to be underestimates by at least five years.

A comparison of the *Epinephelus sp.* otolith cores with of $\Delta^{14}C$ in excess of 100‰ with the calibration data suggests that some of the ages for these fish were overestimated. For example, the ANU reader estimated an age of 36 years (birth date 1961) for an *Epinephelus sp.* with an otolith core $\Delta^{14}C$ of 116.2‰. This otolith-based age estimate is likely to overestimate this fish's true age by as much as 10 years.

Otolith-based age estimates for *Etelis sp.* from both laboratories were in better agreement than the age estimates for *Epinephelus sp.* Furthermore, the ages corresponded more closely to the calibration and model data for radiocarbon variation in the eastern Indian Ocean and South Pacific Ocean.

$\Delta^{14}C$ measured in otolith cores of Lethrinus sp. was compared with otolith readings from one reader only and, therefore, it is not feasible to consider potential differences in age estimates among readers at this time (Figure 18.2). Two Lethrinus sp. samples had very high $\Delta^{14}C$, almost 180‰, compared with the results from the other two species and there is some indication that the young juveniles of this species may inhabit different environments from juvenile *Epinephelus sp.* and *Etelis sp.*

**Discussion**

Several factors linked to the physical environment may impact upon the $\Delta^{14}C$ measured in the otolith cores of these three species from northern Western Australia and could make it relatively difficult to detect some age estimation errors. As for the Lutjanus spp. from the central Great Barrier Reef (Chapter 17), there is large seasonal and interannual variation in freshwater river inputs to areas that may include the habitat of juvenile *Epinephelus sp.*, *Etelis sp.* and Lethrinus sp. However, based on the radiocarbon data from otolith cores analysed, only Lethrinus sp. displays $\Delta^{14}C$ that may be suggestive of some freshwater influence.

Rapidly growing massive hermatypic corals can provide material that is suitable for the determination of intra-annual variation in $\Delta^{14}C$ and these data can be used to determine variability in ocean circulation. Analysis of small samples of known age from large colonies of *Porites* spp. corals have been used to determine intra- and inter-annual variations in ocean circulation in the southwestern Pacific Ocean (Druffel and Griffin 1993) and in the northeastern Indian Ocean (Moore et al. 1997).

Radiocarbon data from an Indonesian coral sampled off Langkai Island, Indonesia showed large intra- and inter-annual variation in $\Delta^{14}C$ with a seasonal range of from 15 to 60‰ (Moore et al. 1997). The variability is linked to changes in ocean transport via the Indonesian throughflow, which transports water from, predominantly, the North Pacific to the Indian Ocean (Lukas et al. 1996, Godfrey 1996). Transport through the throughflow can be highly variable between the El Niño-Southern Oscillation (ENSO) cool phase and the ENSO warm phase. For example, transport via the throughflow was estimated on the basis of hydrographic data between Bali and Port Hedland and showed westward transport of 18±7 Sv during the August 1989 cool phase and eastward transport 2.6±9 Sv during the February 1992 warm phase (Fieux et al. 1996). Bray et al. (1996) and Meyers (1996) estimated that, during warm phases, transport via the throughflow decreases by about 5 Sv. Data from the study by Moore et al. (1997) are plotted with data from otolith cores analysed in this study (Figure 18.5) and demonstrate that a large percentage of the variation in otolith $\Delta^{14}C$ could be due to seasonal changes in transport of North Pacific water via the Indonesian throughflow.

The large size of otoliths from some genera, such as Epinephelus, Etelis, Lethrinus and Lutjanus, can make validation of an age estimation method based on the bomb radiocarbon chronometer more difficult. For genera with relatively small otoliths (e.g. Centroberyx, Pseudocyttus, Thunnus) isolation of the otolith core often results in preparation of a sample that is presumed to contain otolith material deposited during the first year of life or more. As a result, radiocarbon analysis of those samples would average potentially large seasonal variations in $\Delta^{14}C$ and these measurements can be readily compared with measurements of annual variation derived from corals, otoliths or other proxies for radiocarbon in seawater dissolved inorganic carbon.

Intra-annual variability in seawater $\Delta^{14}C$ becomes even more problematic for species that combine large otoliths with an extended spawning season. For example, the spawning season for *Lutjanus erythropterus* has been estimated to extend over eight months of the year (McPherson et al. 1992). Therefore, it would be possible for otolith material deposited in the first six months to be representative of non-overlapping periods of the year. Given the large scope for seasonal variation in ocean $\Delta^{14}C$ over a relatively small area (e.g. 15-60‰ based on the coral from Langkai Island), this factor must be taken into account.

The most effective way to overcome the potential problem associated with high intra-annual variability may be to optimise the size of the otolith sample analysed for radiocarbon. In most cases, this would require that the radiocarbon analysis is carried out on a sample that contains otolith material deposited during the first year of life, rather than say the first six months. This step would only be necessary for those fish species that spend their early juvenile life in regions likely to be characterised be high intra-annual variability. For some fish species with very large otoliths this may create additional complications. Many accelerator mass spectrometry (AMS) facilities are set-up to operate on an optimum or maximum sample size. Therefore, if an otolith core containing material deposited during the first year of life weighed 40 mg it may be too large for AMS analysis. This is not necessarily a problem as in most situations, the entire sampled is dissolved in acid and the CO2 evolved from the sample will be well-mixed and sub-sampling should provide carbon representative of the entire sample. Alternatively, the large otolith core could be cut in half and the unused portion of the sample retained for other analyses or as 'insurance'. On occasion samples are destroyed or contaminated during preparation; the second half of the otolith could be used as a back-up in such a situation.

**Conclusion**

Measurement of radiocarbon in the cores of otoliths from three species commercially Measurement of radiocarbon in the cores of otoliths from *Epinephelus octofasciatus*, *Etelis carbunculus* and *Lethrinus nebulosus*, all commercially harvested in the Indian Ocean off northern Western Australia provided a validation of the age estimation method for these species. Validation of the age estimation method for the two deepwater species, *E. octofasciatus*, *E. carbunculus*, would be impractical by other methods frequently used for age validation. Although the otoliths were from three different genera, there was an overall similarity in the relationship between age estimated from the otolith thin sections and $\Delta^{14}C$. Some of the inter-annual variability in $\Delta^{14}C$ may be the result of intra- and inter-annual changes in the transport of North Pacific water via the Indonesian throughflow.

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Table18.1. Fish, otolith, and radiocarbon data for *Epinephilus octofasciatus* caught by line fishing at a depth of approximately 255 metres.

| Sample no. | Laboratory analysis number | Capture location | Reef name | Capture date | Fish length (mm) SL | Otolith weight (g) | Sample weight (mg) | $\Delta^{14}C$ (‰) | Otolith age (years) WA 1 | Birth date (year) WA 1 | Otolith age (years) WA 2 | Birth date (year) WA 2 | Otolith age (years) ANU1 | Birth date (year) ANU1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S283 | NOSAMS OS-15612 | 22°42.8'S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 949 | 1.2256 | 11.8 | 144.3±4.9 | 26 | 1971 | 26 | 1971 | 26 | 1971 |
| S296 | NOSAMS OS-15613 | 22°42.8'S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 688 | 0.8743 | 12 | 116.2±4.6 | 28 | 1969 | 18 | 1979 | 36 | 1961 |
| S2386 | NOSAMS OS-15614 | 14°02.5'S 121°45.5'E | Scott Reef WA | Nov 1997 | 1220 | 2.3716 | 10.4 | -60.7±47. | 36 | 1961 | 37 | 1960 | 42 | 1955 |
| S2389 | NOSAMS OS-15615 | 14°02.5'S 121°45.5'E | Scott Reef WA | Nov 1997 | 1240 | 3.0405 | 11.5 | -67.4±4.6 | 37 | 1960 | 41 | 1956 | 46 | 1951 |
| S2346 | NOSAMS OS-15616 | 22°42.8'S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 695 | 1.1128 | 12.3 | 137.6±8.7 | 24 | 1973 | 25 | 1972 | 31 | 1966 |
| S269 | NOSAMS OS-15617 | 22°42.8'S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 877 | 0.9181 | 10.5 | 145.7±4.7 | 19 | 1978 | 17 | 1980 | 17 | 1980 |
| S2365 | NOSAMS OS-15618 | 22°42.8'S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 516 | 0.3312 | 8.8 | 87.9±5.2 | 9 | 1988 | 8 | 1989 | 15 | 1982 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S270 | NOSAMS OS-15619 | 22°42.8'S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 1028 | 1.8202 | 11 | -68.9±5.4 | 32 | 1965 | 34 | 1963 | 40 | 1957 |
| S282 | NOSAMS OS-15620 | 22°42.8'S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 983 | 1.3791 | 10.2 | -9.4±4.8 | 34 | 1963 | 28 | 1969 | 37 | 1960 |
| S291 | NOSAMS OS-15621 | 22°42.8'S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 661 | 0.7125 | 12.2 | 111.5±4.4 | 30 | 1967 | 18 | 1979 | 32 | 1965 |

Table 18.2. Fish, otolith, and radiocarbon data for *Etelis carbunculus* caught by line fishing at a depth of approximately 255 metres.

| Sample no. | Laboratory analysis number | Capture location | Reef name | Capture date | Fish length (mm) SL | Otolith weight (g) | Sample weight (mg) | $\Delta^{14}C$ (‰) | Otolith age (years) WA 1 | Birth date (year) WA 1 | Otolith age (years) WA 2 | Birth date (year) WA 2 | Otolith age (years) ANU1 | Birth Date (year) ANU1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S400 | NOSAMS OS-15602 | 22°42.8' S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 268 | 0.034 | 12.8 | 83.2±4.7 | 5 | 1992 | 3 | 1994 | 8 | 1989 |
| S567 | NOSAMS OS-15603 | 22°42.8' S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 310 | 0.045 | 13.3 | 79±4.1 | 9 | 1988 | 6 | 1991 | 9 | 1988 |
| S485 | NOSAMS OS-15604 | 22°42.8' S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 512 | 0.1586 | 11 | 107.3±4.4 | 14 | 1983 | 17 | 1980 | 19 | 1978 |
| S444 | NOSAMS OS-15605 | 22°42.8' S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 712 | 0.2402 | 13.3 | 124.2±4.5 | 21 | 1976 | 20 | 1977 | 33 | 1964 |
| S477 | NOSAMS OS-15606 | 22°42.8' S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 663 | 0.165 | 8.6 | 120.5±4.6 | 18 | 1979 | 17 | 1980 | 18 | 1979 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S388 | NOSAMS OS-15607 | 22°42.8' S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 884 | 0.3291 | 12.8 | 84.7±4.3 | 24 | 1973 | 30 | 1967 | 27 | 1970 |
| S431 | NOSAMS OS-15608 | 22°42.8' S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 886 | 0.4502 | 11.5 | -21.2±4.1 | 33 | 1964 | 34 | 1963 | 33 | 1964 |
| S435 | NOSAMS OS-15609 | 22°42.8' S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 932 | 0.3601 | 13.1 | 75.1±5.0 | 38 | 1959 | 30 | 1967 | 28 | 1969 |
| S447 | NOSAMS OS-15610 | 22°42.8' S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 886 | 0.3154 | 13.9 | 106.7±4.7 | 28 | 1969 | 27 | 1970 | 24 | 1973 |
| S449 | NOSAMS OS-15611 | 22°42.8' S 113°26.8'E | Ningaloo Reef WA | Nov 1997 | 807 | 0.2711 | 12.2 | 120.7±4.5 | 29 | 1968 | 26 | 1971 | 25 | 1972 |

Table 18.3. Fish, otolith and radiocarbon data for *Lethrinus nebulosus* .

| Sample No. | Laboratory analysis no. | Capture location | Collection date | Length FL (mm) | Fish wt (g) | Sex | Otolith wt (g) | Sample wt.1 (mg) | Sample wt.2 (mg) | $\Delta^{14}C$ (‰) | $\Delta^{14}C$ (‰) error | Otolith section age (years) | Birth date (year) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N1 | RRL NZA8009 | Abrolhos WA | 18.9.91 | 520 | 2498 | M | 0.6558 | 14.4 | | 9.4 | 8.4 | 26 | 1965 |
| N2 | RRL NZA8010 | Abrolhos WA | 7.7.91 | 553 | 3138 | F | 0.6015 | 15.6 | | -19.8 | 8.1 | 27 | 1964 |
| N3 | RRL NZA8011 | Abrolhos WA | 15.5.91 | 522 | 3221 | F | 0.5857 | 14 | | -10.3 | 8.2 | 26 | 1965 |
| N4 | ANSTO OZC647 | Abrolhos WA | 26.1.91 | 503 | 2360 | F | 0.624 | 9.5 | 9.9 | 147.3 | 7.14 | 26 | 1965 |
| N5 | ANSTO OZC648 | Abrolhos WA | 22.6.90 | 585 | nd | F | 0.7004 | 9 | 15.3 | 4.8 | 8.19 | 27 | 1963 |
| N9 | ANSTO OZC649 | Abrolhos WA | 7.10.90 | 505 | 2090 | F | 0.4065 | 9.3 | 15.5 | 175.6 | 15.37 | 15 | 1975 |
| N10 | ANSTO OZC650 | Abrolhos WA | 21.6.90 | 517 | nd | M | 0.4373 | 6 | 13.8 | 178.9 | 16.27 | 15 | 1975 |
| N13 | RRL NZA8012 | Broome WA | 5.12.90 | 518 | nd | M | 0.4303 | 13.9 | | 117 | 9.3 | 10 | 1980 |
| N14 | ANSTO OZC651 | Abrolhos WA | 7.10.90 | 500 | 2464 | F | 0.2517 | 7.1 | 14.7 | 156.1 | 9.97 | 5 | 1985 |
| N16 | RRL NZA8013 | Exmouth WA | 2.10.90 | 297 | 481 | Imm | 0.1197 | 15.2 | | 103.5 | 9.5 | 2 | 1988 |

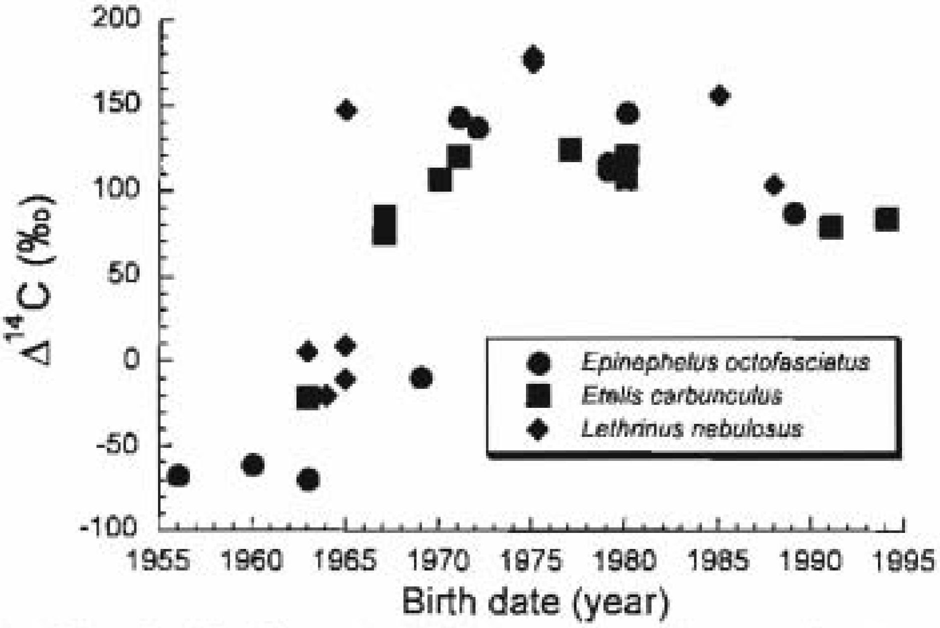


Figure 18.1. Plot of otolith thin section birth date versus otolith core radiocarbon for three species from the Indian Ocean off northern Western Australia.

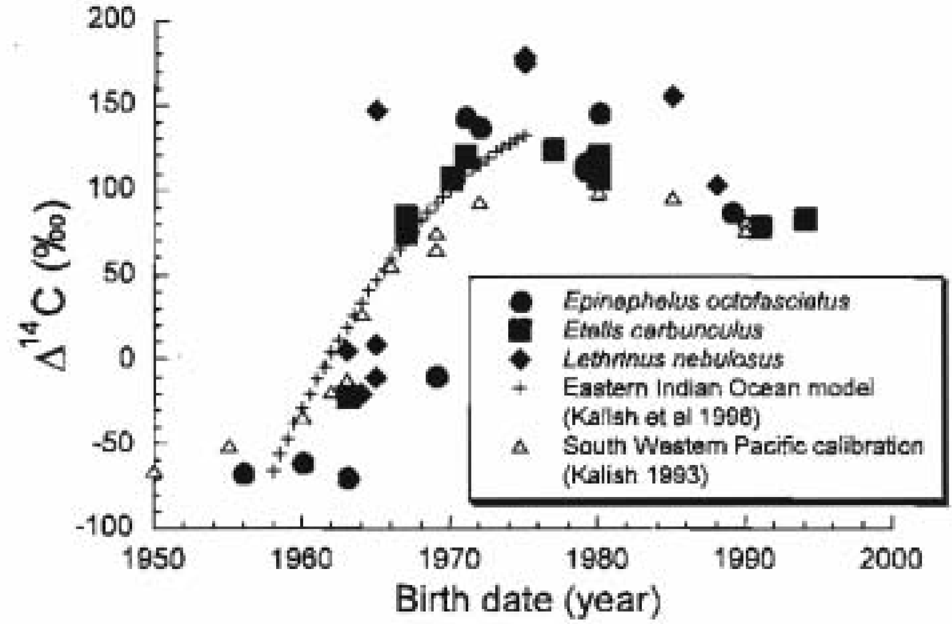


Figure 18.2. Plot of otolith thin section birth dates, estimated by a single reader, versus otolith core radiocarbon for three fish species from the Indian Ocean off northern Western Australia. A model of variation in radiocarbon over time for the eastern Indian Ocean (Kalish et al. 1996) and a radiocarbon calibration for the south western Pacific Ocean (Kalish 1993) are provided to estimate radiocarbon variation in the region of the eastern Indian Ocean.

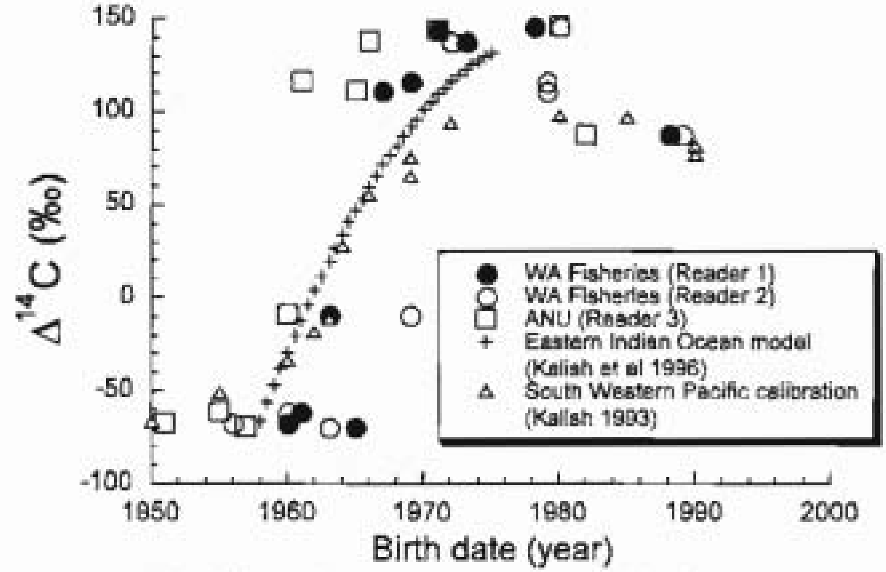


Figure 18.3. Plot of otolith thin section birth dates, estimated by three independent readers, versus otolith core radiocarbon for *Epinephelus octofasciatus* from the Indian Ocean off northern Western Australia. A model of variation in radiocarbon over time for the eastern Indian Ocean (Kalish et al. 1996) and a radiocarbon calibration for the south western Pacific Ocean (Kalish 1993) are provided to estimate radiocarbon variation in the region of the eastern Indian Ocean.

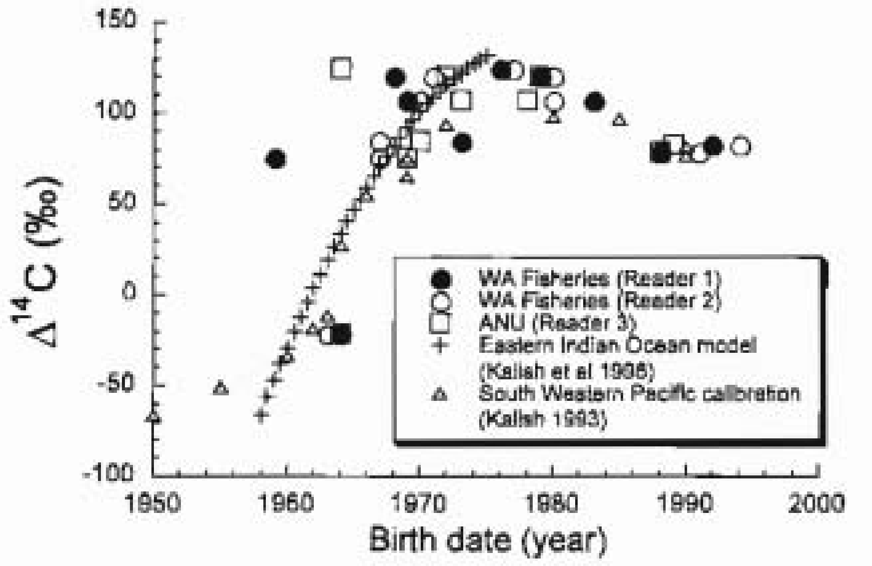


Figure 18.4. Plot of otolith thin section birth dates, estimated by three independent readers, versus otolith core radiocarbon for *Etelis carbunculus* from the Indian Ocean off northern Western Australia. A model of variation in radiocarbon over time for the eastern Indian Ocean (Kalish et al. 1996) and a radiocarbon calibration for the south western Pacific Ocean (Kalish 1993) are provided to estimate radiocarbon variation in the region of the eastern Indian Ocean.

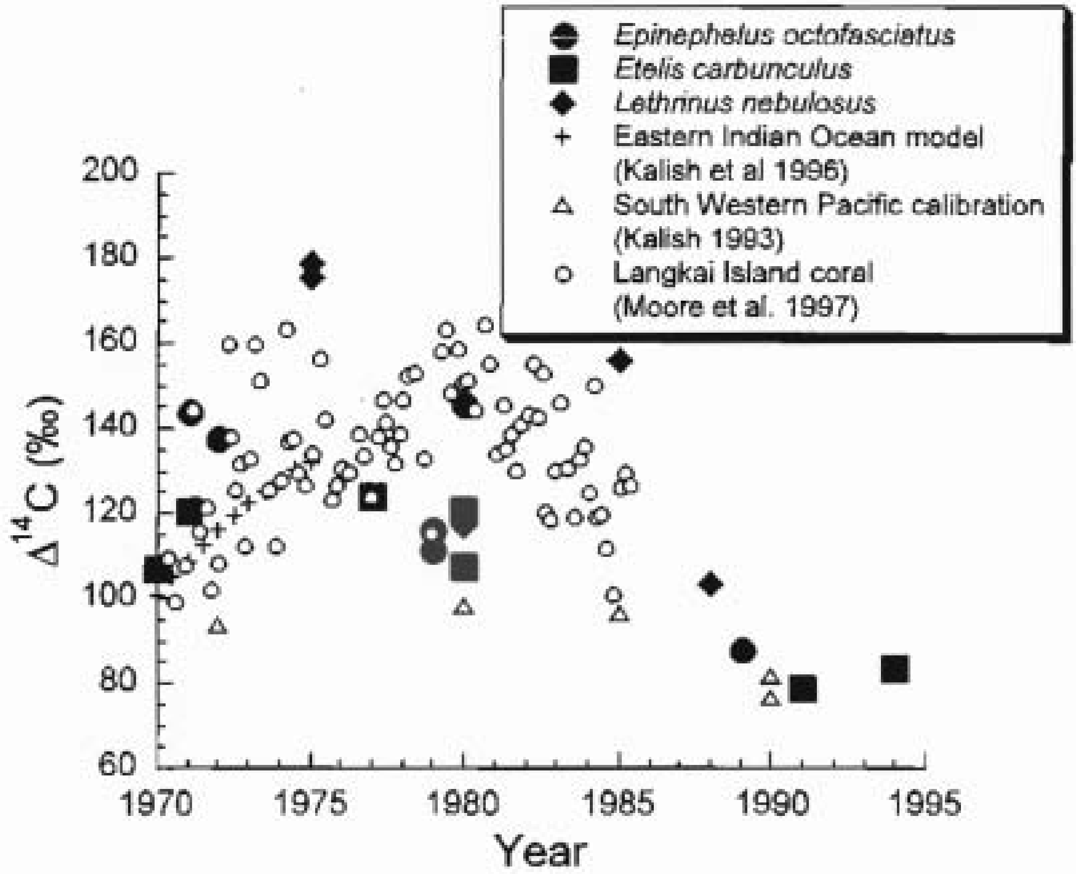


Figure 18.5. Plot of otolith thin section birth dates, estimated by a single reader, versus otolith core radiocarbon for three fish species from the Indian Ocean off northern Western Australia. Radiocarbon data from a hermatypic coral sampled at Langkai Island in the Makassar Strait (Moore et al. 1997) are plotted to indicate the intra- and inter-annual variation in $\Delta^{14}C$ in the regions influenced by the Indonesian throughflow. A model of variation in radiocarbon over time for the eastern Indian Ocean (Kalish et al. 1996) and a radiocarbon calibration for the south western Pacific Ocean (Kalish 1993) are provided to estimate radiocarbon variation in the region of the eastern Indian Ocean.

# Chapter 19 Pilot study on the application of the bomb radiocarbon chronometer to validation of age estimation methods for sharptooth jobfish (*Pristipomoides typus*) and goldbanded jobfish (*Pristipomoides multidens*) from northern Australia

John Kalish

**Summary**

A pilot study was undertaken to determine the feasibility of validating age estimates based on otolith sections for sharptooth jobfish (*Pristipomoides typus*) and goldbanded jobfish (*Pristipomoides multidens*) based on the bomb radiocarbon chronomteter. Analysis of radiocarbon in otolith cores from a small number of samples of these two species was unable to provide a validation of the age estimation method. Relatively low $\Delta^{14}C$ suggested influence of the South Equatorial Current on the habitats of juvenile jobfish or significant over estimates of fish age based on otolith sections. Complex oceanography in the region of the Arafura Sea and the potential influence of both the Indonesian Throughflow and the South Equatorial Current may make it difficult to validate age for these, and perhaps other, species from the Arafura Sea off the Northern Territory. $\Delta^{14}C$ is likely to vary seasonally and inter-annually in the region, based on the relative influence of these two water sources. Further research on the oceanography of the region and the early life history of these jobfish species (e.g. spawning season, juvenile habitats) would be required to resolve these issues.

## Introduction

The sharptooth jobfish (*Pristipomoides typus*) and goldbanded jobfish (*Pristipomoides multidens*) are deepwater snapper species that are caught in line, trawl and trap fisheries throughout tropical Indo-Pacific waters (Kailola et al. 1993). In Australia they are commonly called sharptoothed snapper and gold band snapper. Goldbanded jobfish are recorded from depths of about 40-200 m and sharptooth jobfish from about 40-100 m depth (Allen 1985).

Previous studies on the age and growth of sharptooth and goldbanded jobfish indicated that these species are relatively short lived with maximum ages of 11 and 14 years, respectively (Edwards 1985). More recent interpretation of otolith thin sections from large individuals of both species suggests that maximum longevity may be about twice these estimates (Julie Lloyd, Northern Territory Department of Primary Industry and Fisheries).

The potentially slow growth of these species, combined with their schooling habits and the discrete nature of stocks suggests that sustainable yields from these resources may be a relatively low proportion of stock biomass. Recent research on the goldbanded jobfish suggests that this species forms multiple discrete stocks within Australian waters (Newman et al. 2000). These characteristics highlight the need for accurate and validated estimates of age for these species.

This pilot study analysed cores of otoliths from selected, presumably old, sharptooth and goldbanded jobfish in an attempt to determine the suitability of the bomb radiocarbon chronometer for validation of the age estimation method used for these species.

**Materials and methods**

Goldbanded jobfish and sharptooth jobfish otoliths were supplied by the Northern Territory Department of Primary Industry and Fisheries. The fish were collected in the Arafura Sea off the Northern Territory and were selected to include a range of ages as well as one individual that was among the oldest sampled from the fishery. Age estimates were determined from otolith thin sections viewed with a stereo microscope and using transmitted light illumination.

Sample preparation procedures were similar to those described for earlier bomb radiocarbon validation research (e.g. 1995). Otolith aragonite deposited during a period presumed to be less than the first year of life was isolated from one sagitta from each fish. This was accomplished by cutting and grinding the otolith with a hand-held high speed drill to remove material deposited after what was presumed to be the first annual increment. Further grinding of the remaining portion of the otolith was used to "sculpt" the material into a structure with the shape and dimensions of the earliest formed portions of a jobfish sagitta. Sagittae from very small jobfish and zones visible on the otolith being sculpted were used as a guide. Otoliths of these two jobfish species, as well as other species in the Lutjanidae, are relatively large (Table 1) and, therefore, sculpting is a relatively simple process. A total of 8 otoliths were sculpted successfully including three goldbanded jobfish and five sharptooth jobfish. Sample weights for otoliths after sculpting ranged from 12.8 mg to 16.4 mg.

Otolith carbonate was converted to CO2 by reaction in vacuo with 100% phosphoric acid and the resultant CO2 was converted to graphite in the presence of catalyst. The graphatised samples were analysed for radiocarbon. Radiocarbon was determined in each sample by accelerator mass spectrometry (AMS) at the Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences (New Zealand). Radiocarbon values are reported as $\Delta^{14}C$, which is the age- and fractionation-corrected deviation (parts per thousand) from the activity of nineteenth century wood. Age corrections are based on the mean estimate of age determined from reading of otolith sections. Reported errors are 1 standard deviation for both radiocarbon data and age estimates based on the reading of otolith sections. Radiocarbon errors include both counting errors and laboratory random errors. $\delta^{13}C$ was not analysed in these samples but was estimated as –3.00‰ on the basis of results obtained from stable isotope analyses of other tropical lutjanids from Australian waters.

**Results and discussion**

Radiocarbon measurements (Table 19.1) are plotted versus otolith based age estimates for both jobfish species in Fig. 19.1. $\Delta^{14}C$ results from the two species fall within a relatively narrow range of 75.2 ‰ ± 10.6 to 100.0 ‰ ± 9.6. No trend is evident; furthermore, the results provide little, if any, indication that the relationship between age estimated from the otolith thin sections and the cores analysed for radiocarbon defines any segment of the increase in bomb-derived radiocarbon during the 1960s and 1970s.

The results are plotted with similar data from two tropical reef species from Western Australia (*Epinephelus octofasciatus* and *Etelis carbunculus*) (this report Chapter 18) and the calibration for the south western Pacific Ocean (Kalish 1993). It would be expected that radiocarbon data from these otoliths, collected in the Arafura Sea, would be similar to the data from the otoliths collected from the tropical Western Australia species, however, this is not the case. In fact, the data are more similar to the south western Pacific calibration. The relatively low $\Delta^{14}C$ measured in the otolith cores suggests that otolith section based ages for these fish are over estimates or that limited understanding of oceanography in the region and the early life history these species makes accurate interpretation of the data problematic.

The relatively low values for $\Delta^{14}C$ from the jobfish samples are difficult to explain with the limited data available. The Arafura Sea is a region characterised by complex patterns of ocean circulation and the water mass where these fish spent the first six months of life may be relatively difficult to define precisely. In particular, circulation of tropical North Pacific waters through the Indonesian Throughflow is highly variable, although these waters are likely to influence $\Delta^{14}C$ in the Arafura Sea region off the Northern Territory to a significant, albeit variable, extent (Fig. 19.2). Moore et al. (1997) determined seasonal and inter-annual variability of $\Delta^{14}C$ in the southern Makassar Strait based on analysis of corals from Langkai off the south western tip of Sulawesi and found that the season variability of $\Delta^{14}C$ ranged from 15 to 60‰. Seasonal variability in $\Delta^{14}C$ was highly correlated with □18O (a proxy for temperature) and in most years $\Delta^{14}C$ peaked during the summer months and declined during the autumn and early winter to a minimum in the late winter. Therefore, the time of spawning and, concomitantly, the seasons during which the earliest formed otolith material is deposited in these jobfish species would have a dramatic effect on $\Delta^{14}C$ measured in the otolith cores. For example, if spawning took place in spring, the earliest otolith growth would take place during the spring and summer months, resulting in relatively high $\Delta^{14}C$ for that particular year. Conversely, spawning in the autumn would result in early otolith growth during the autumn and winter and relatively low $\Delta^{14}C$ for that year. Ripe goldbanded jobfish are present on the North West Shelf of Western Australia from October to February (Kailola et al. 1993) and this would suggest that $\Delta^{14}C$ measured in the otoliths would be relatively high for a given year. However, the $\Delta^{14}C$ data from the jobfish species is relatively low compared with similar measurements, based on corals, made at these latitudes in the Indian Ocean (e.g. Moore et al. 1997).

The low $\Delta^{14}C$ measured in the jobfish otolith cores could result from a greater influence of the South Equatorial Current on the juvenile habitats of these species off northern Australia. Annual mean $\Delta^{14}C$ from the Indonesian corals at Langkai (Moore et al. 1997) are 50 to 60‰ higher that in corals from habitats influenced by the South Equatorial Current (Druffel 1987). Therefore, more definitive identification of the habitats of juvenile goldbanded and sharptooth jobfish off the Northern Territory may help to resolve some of this uncertainty.

Due to the inability to determine the nature of the water masses influencing the juvenile jobfish habitats, it is not possible to provide a solid basis for the observed variation in jobfish otolith $\Delta^{14}C$ relative to the ages estimated from otolith thin sections. Further research is required to resolve the problems associated with age validation for this species.

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I thank Julie Lloyd (Northern Territory Department of Primary Industry and Fisheries) for providing the otolith samples and Justin Johnston (Australian National University) for assistance with sample preparation.

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Table 19.1. Fish, otolith, and radiocarbon data for sharp toothed jobfish (*Pristipomoides typus*) and gold banded jobfish (*Pristipomoides multidens*) from northern Australia.

| Sample no. | Species | Analysis number | Collection location | Collection date | Length (mm) LCF | Otolith weight (g) | Sample weight (mg) | $\Delta^{14}C$ (‰) | Age (years) | Birth date (year) |
|---|---|---|---|---|---|---|---|---|---|---|
| NT.PM1 | *Pristipomoides multidens* | RRL NZA7978 | Arafura Sea | 26/3/93 | 643 | 0.7925 | 14.7 | 96.5±9.6 | 23 | 1970 |
| NT.PM3 | *Pristipomoides multidens* | RRL NZA7983 | Arafura Sea | 5/2/95 | 580 | 0.6341 | 15 | 85.6±9.1 | 15 | 1980 |
| NT.PM4 | *Pristipomoides multidens* | RRL NZA7984 | Arafura Sea | 20/3/95 | 610 | 0.702 | 16.4 | 91±8.9 | 12 | 1983 |
| NT.PT1 | *Pristipomoides typus* | RRL NZA7974 | Arafura Sea | 1/11/89 | 578 | 0.946 | 13.5 | 100±9.6 | 22 | 1967 |
| NT.PT2 | *Pristipomoides typus* | RRL NZA7975 | Arafura Sea | 1/11/89 | 526 | 0.9115 | 14.4 | 80.4±9.4 | 20 | 1969 |
| NT.PT3 | *Pristipomoides typus* | RRL NZA7973 | Arafura Sea | 10/11/90 | 516 | 0.5521 | 12.8 | 98.9±11.3 | 16 | 1974 |
| NT.PT4 | *Pristipomoides typus* | RRL NZA7976 | Arafura Sea | 7/2/95 | 482 | 0.5891 | 14.3 | 79.8±10.1 | 15 | 1980 |
| NT.PT5 | *Pristipomoides typus* | RRL NZA7977 | Arafura Sea | 15/3/95 | 472 | 0.4555 | 14.9 | 75.2±10.6 | 11 | 1984 |

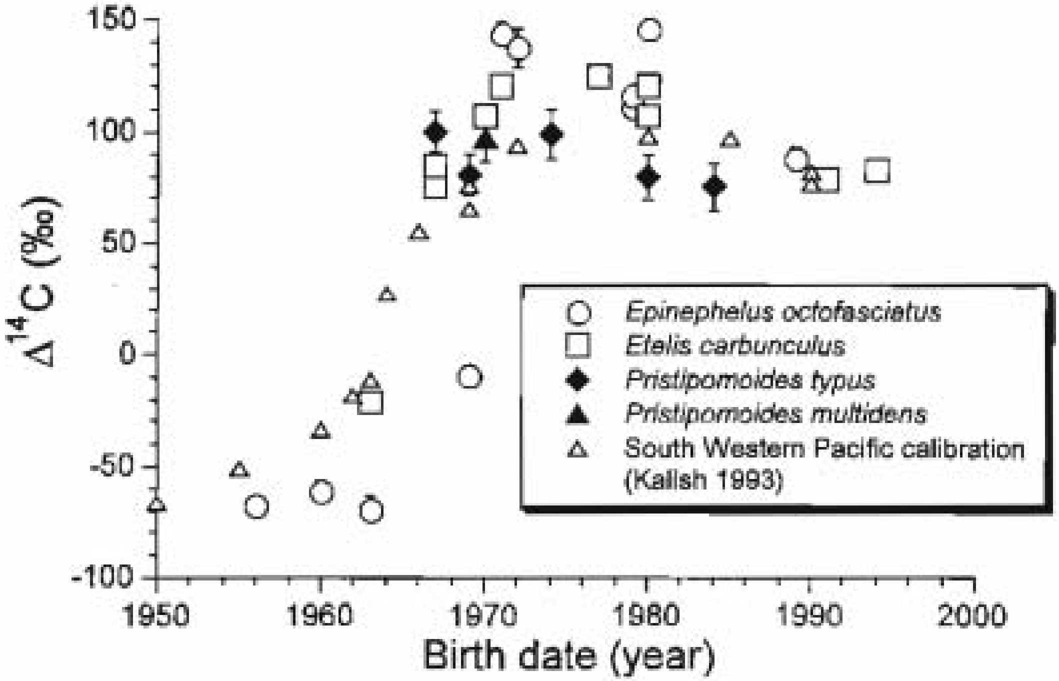


Figure 19.1. Plot of otolith thin section birth date estimates versus otolith core radiocarbon for two jobfish species (Pristipomoides spp.) from the Arafura Sea off the Northern Territory and two deepwater tropical species (*Epinephelus sp.* and *Etelis sp.*) collected off the Kimberly coast of Western Australia. $\Delta^{14}C$ data from New Zealand *Pagrus auratus* (Kalish 1993) provides a calibration of $\Delta^{14}C$ versus year for the south western Pacific Ocean. $\Delta^{14}C$ values are based on otolith material deposited over a time period equivalent to about the first six months of life. Errors are ±1 sd.

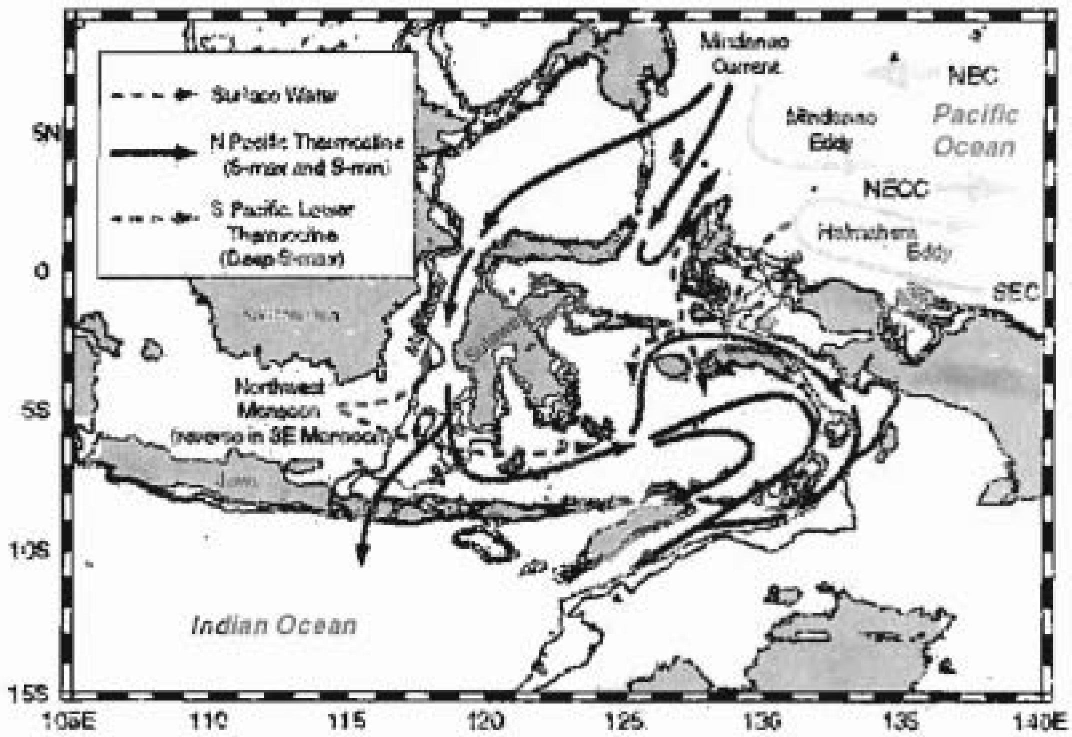


Figure 19.2. Diagram showing the movement of water from the Pacific Ocean to the Indian Ocean via the Indonesian Throughflow. The diagram highlights results that indicate that water masses transported via the Indonesian Throughflow are derived predominantly from the tropical North Pacific Ocean. The relative influence of the Indonesian Throughflow and the South Equatorial Current (SEC) on the Arafura Sea off the Northern Territory has not been investigated. Figure from: Lamont-Doherty Earth Observatory, http://www.ldeo.columbia.edu/physocean/proj_AM.html).

# Chapter 20 Use of the bomb radiocarbon chronometer to validate two age estimation methods for jack mackerel (*Trachurus declivis*)

John Kalish, Justine Johnston, Jeremy Lyle, Kyne Krusic-Golub and Sandy Morison

**Summary**

This study demonstrates that the otolith 'break and burn' and thin section methods of age estimation provide reasonably accurate estimates of age for Australian and New Zealand jack mackerel. Jack mackerel can live to ages in excess of 20 years, however, New Zealand jack mackerel may be longer-lived than fish caught off south eastern Australia. Although this study demonstrates the effectiveness of the methods, further research may be warranted to provide a more detailed understanding of the accuracy of age estimation methods used for jack mackerel.

**Introduction**

Jack mackerel (*Trachurus declivis*) is a small pelagic species broadly distributed across the waters off southern Australia and New Zealand. The species forms large schools and is the target species for a purse seine fishery that operates primarily off the eastern coast of Tasmania. The Tasmanian fishery developed rapidly from an annual catch of 6,000 t in 1984-85 to a peak of almost 42,000 t in 1986-87. Subsequent catches have been lower, ranging between 8,000 and 32,000 t. The majority of the catch is used to produce fishmeal for use by aquaculture enterprises in Tasmania (Tilzey et al. 2000).

Scientific assessment of jack mackerel is necessary to ensure effective management of this commercially important species and accurate estimates of age and growth are essential for effective assessments. The age and growth of jack mackerel has been studied previously (e.g. Horn 1993), however, there has been no detailed study of this species in Australian waters. Furthermore, age validation of the age estimation method used by Australian researchers for this species has not been achieved, although a limited age validation, based on marginal increment analysis, has been completed for New Zealand jack mackerel (Horn 1993).

Validation of age estimation methods for several species of marine fish based on the bomb radiocarbon chronometer has demonstrated the effectiveness of this method for age validation. Although the method is typically most appropriate for moderately long-lived or long lived species, the method was considered to be suitable for jack mackerel due to the availability of archived otoliths in both Australia and New Zealand.

The purpose of this study was to validate two methods, 'break and burn' and 'thin section', of age estimation for jack mackerel based on the bomb radiocarbon chronometer. The research was coordinated with another study of jack mackerel age and growth underway at laboratories in Tasmania and Victoria (Lyle et al. 2000).

**Materials and methods**

Otoliths were obtained from two sources to increase the likelihood that samples would encompass the time period from about 1960 to 1990 and include the period of rapid increase in radiocarbon. Initial estimates of age for jack mackerel caught off Australian suggested that these fish were relatively young with maximum ages of about 12 years. Samples were available from Australian jack mackerel caught as early as 1985, however, even these earlier collections would not provide samples with presumed birth dates during the period of the most rapid increase in bomb derived radiocarbon. Otoliths from presumably older jack mackerel were sourced from New Zealand were maximum ages in excess of 20 years had been estimated (Horn 1993). Furthermore, New Zealand samples were available from fish caught in the early 1980s. Peter Horn (NZ NIWA) provided a series of jack mackerel otoliths from large and presumably old fish collected in New Zealand waters. The remaining samples used in this study were from jack mackerel collected off the east coast of Tasmania and supplied by TAFI. Although the otolith samples were sourced from different regions, previous research on radiocarbon in otoliths from New Zealand snapper (Pagurs auratus) and redfish (*Centroberyx affinis*) from off the south eastern coast of Australia has demonstrated that the time series of $\Delta^{14}C$ are very similar for the two regions (Kalish 1995).

Sample preparation procedures were similar to those described in Kalish (1995) for redfish (*Centroberyx affinis*). Otolith aragonite deposited during a period presumed to be less than the first year of life was isolated from one sagitta from each fish. This was accomplished by cutting and grinding the otolith with a hand-held high speed drill to remove material deposited after what was presumed to be the first annual increment. Further grinding of the remaining portion of the otolith was used to "sculpt" the material into a structure with the shape and dimensions of the earliest formed portions of a jack mackerel sagitta. Sagittae from very small jack mackerel and zones visible on the otolith being sculpted were used as a guide. Sample weights ranged from about 3.1 to 8.5 mg (Table 20.1). Otolith carbonate was converted to CO2 by reaction in vacuo with 100% phosphoric acid. An aliquot of the CO2 was used to determine $\delta^{13}C$ for each sample and the remaining CO2 was converted to graphite. The graphatised samples were analysed for radiocarbon. Radiocarbon levels in each sample were determined by accelerator mass spectrometry (AMS) at the Center for Accelerator Mass Spectrometry (CAMS) Lawrence Livermore National Laboratory (LLNL) in Livermore, California. Radiocarbon values are reported as $\Delta^{14}C$, which is the age- and fractionation-corrected deviation (parts per thousand) from the activity of nineteenth century wood. Age corrections are based on the mean estimate of age determined from reading of otolith sections. Reported errors are 1 standard deviation for both radiocarbon data and age estimates based on the reading of otolith sections. Radiocarbon errors include both counting errors and laboratory random errors.

Different age estimation methods were used by the Australian and New Zealand laboratories involved in reading the fish otolith samples. The New Zealand laboratory (NIWA) used the 'break and burn' method (Horn 1993) and the Australian laboratories (ANU, MAFRI, TAFI) estimated jack mackerel age based on the 'thin section' method. Comparisons of these two otolith-based methods of age estimation for jack mackerel indicated that there are unlikely to be significant differences between these two methods when employed by an individual laboratory.

**Results and discussion**

Radiocarbon measurements (Table 20.1) are plotted versus otolith based age estimates for the longer-lived jack mackerel collected from New Zealand and shorter-lived individuals from the ocean off Tasmania in Fig. 20.1. The curve defined by the jack mackerel samples defines the increase in bomb-derived radiocarbon during the 1960s and 1970s and is consistent with similar data from *Pagrus auratus* and *Centroberyx affinis*. The results show that, with the exception of two New Zealand fish that were probably incorrectly aged, the estimated ages were consistent with the birth dates estimated by measurement of radiocarbon from the cores of the same otoliths. The results provide support for the conclusion that jack mackerel can live to ages in excess of 20 years and that ages estimated from counts of opaque and translucent zones in thin sections or 'break and burn' preparations are reliable methods to estimate age.

The combined data sets indicate good agreement between estimates of age derived from measurements of radiocarbon in the otolith cores and ages estimated from otolith increments. However, the data that cover the period of the most rapid rise in radiocarbon, when the method is most accurate for age estimation, were only from jack mackerel caught off New Zealand. The age of these fish was estimated by the 'break and burn' method and not thin sections. All the Australian samples were from younger fish and had estimated birth dates during the 1970s and 1980s. Unfortunately, the bomb radiocarbon chronometer has relatively little ability to provide accurate age estimates during this period. Despite this problem, the fact that the lowest $\Delta^{14}C$ for a jack mackerel caught off Australia was 87.4‰ provided clear evidence that these Australian samples were from relatively young fish and were very unlikely to have birth dates earlier than 1970. Therefore, it is important to resolve the issue of potential differences between the different methods of sample preparation used by the Australian and New Zealand laboratories.

The issue of potential differences between the 'break and burn' and thin section methods of age estimation was investigated by the researchers from New Zealand and the CAF (Lyle et al. 2000, CAF unpublished data). The study was based on preparing the left and right otoliths from the same fish, with the New Zealand laboratory preparing one otolith with the 'break and burn' method and the CAF preparing the other otolith with the thin section method. Both methods resulted in similar estimates of maximum age, although there were some relatively large discrepancies among estimates of age. Lyle et al (2000) suggested that this was due largely to the relative inexperience of one of the readers and concluded that New Zealand and Australian otolith readers were using similar structures to estimate the age of jack mackerel. Therefore, Lyle et al (2000) concluded that the age differences of the samples used for validation based on the bomb radiocarbon chronometer are real and not an artefact of the sample preparation process. This conclusion is also supported by the relationship between otolith radiocarbon and birth date estimates produced from this validation study.

**Acknowlegements**

We thank Peter Horn (New Zealand National Insitute for Water and Atmospheric Research) for providing the jack mackerel otoliths and age estimates from NZ jack mackerel.

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Tilzey, R., Williams, G., Caton, A., McLoughlin, K., Garvey, J., Larcombe, J. and Sahlqvist, P. 2000. Other fisheries, pp. 193-213. In: Fishery Status Reports 1999: Resource Assessments of Australian Commonwealth Fisheries, Caton, A. and McLoughlin, K. (eds). Bureau of Rural Sciences, Canberra.

Table 20.1. Fish, otolith, and radiocarbon data for Australian and New Zealand jack mackerel (*Trachurus declivis* ).

| Sample No. | Laboratory and laboratory accession number | Collection location | Collection date | Fish length (mm) | Otolith weight (g) | Sample weight (mg) | $\delta^{13}C$ | $\Delta^{14}C$ (‰) | Otolith section age (years) | Birth date (year A.D.) |
|---|---|---|---|---|---|---|---|---|---|---|
| JMKNZ 16 | CAMS46772 | New Zealand | 10/02/81 | 454 | 0.0663 | 3.1 | -4.2 | -18.5±7.7 | 22 | 1959 |
| JMKNZ 4 | CAMS46773 | New Zealand | 08/02/81 | 453 | 0.0821 | 5.3 | -4.0 | -44.4±6.4 | 23 | 1958 |
| JMKNZ 18 | CAMS46774 | New Zealand | 10/02/81 | 447 | 0.0955 | 4.3 | -4.0 | -46.2±18.7 | 16 | 1965 |
| JMKNZ 19 | CAMS46775 | New Zealand | 10/02/81 | 452 | 0.0985 | 4.7 | -3.7 | -38.5±6.6 | 18 | 1963 |
| JMKNZ 2 | CAMS46776 | New Zealand | 08/02/81 | 425 | 0.0673 | 3.9 | -5.4 | 69.9±7.9 | 10 | 1971 |
| JMKNZ 5 | CAMS46777 | New Zealand | 08/02/81 | 429 | 0.0824 | 5.2 | -4.2 | 44.2±6.9 | 14 | 1967 |
| JMK 30 | CAMS46778 | Tasmania | 14/07/86 | 157 | 0.0085 | 8.5 | -4.5 | 87.4±5.8 | 1 | 1985 |
| JMK 861 | CAMS46779 | Tasmania | 10/12/95 | 359 | 0.0580 | 4.6 | -3.9 | 116.3±6.5 | 10 | 1985 |
| JMK 558 | CAMS46780 | Tasmania | 12/12/95 | 351 | 0.0535 | 4.6 | -4.3 | 106.9±7.5 | 12 | 1983 |
| JMK 517 | CAMS46781 | Tasmania | 11/12/95 | 338 | 0.0545 | 5.1 | -7.1 | 110.1±8.8 | 8 | 1987 |
| JMK 594 | CAMS46782 | Tasmania | 18/06/85 | 341 | 0.0604 | 3.7 | -5.5 | 107.1±16.7 | 11 | 1974 |
| JMK 531 | CAMS46783 | Tasmania | 24/05/85 | 344 | 0.0526 | 6.1 | -4.9 | 121.8±6.7 | 7 | 1978 |
| JMK 525 | CAMS46784 | Tasmania | 24/05/85 | 351 | 0.0550 | 7 | -5.1 | 138.5±6.6 | 10 | 1975 |

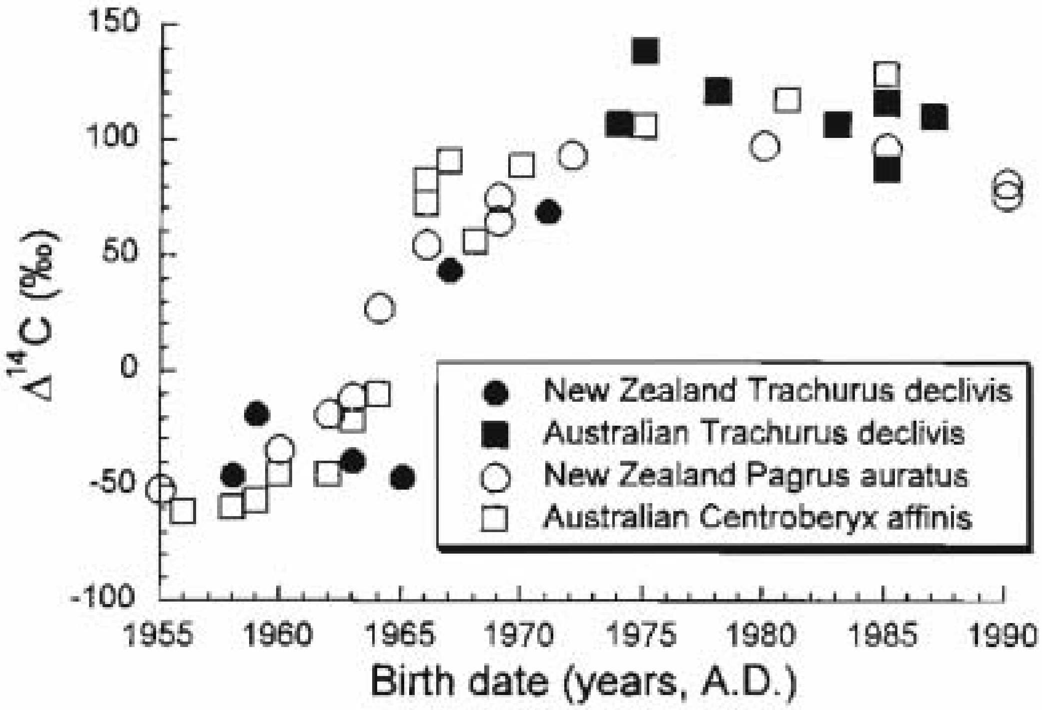


Figure 20.1. $\Delta^{14}C$ of Trachuru declivis otolith cores plotted against birth dates estimated from 'broken and burnt' otoliths (New Zealand *Trachurus declivis*) and otolith thin sections (Australian *Trachurus declivis*). $\Delta^{14}C$ data from New Zealand *Pagrus auratus* (Kalish 1993) and Australian *Centroberyx affinis* (Kalish 1995) provide calibrations of $\Delta^{14}C$ versus year for the south western Pacific Ocean. $\Delta^{14}C$ values are based on otolith material deposited over a time period equivalent to about the first year of life. Errors are ±1 sd.

# Chapter 21 Validation of age and growth in silver trevally (*Pseudocaranx dentex*) from Australian and New Zealand waters

John Kalish and Michael Johnston

**Summary**

Estimates of silver trevally *Pseudocaranx dentex* age off southeast Australia were determined from transverse sections of 207 otoliths. Age estimates were repeatable and there was a high level of agreement between otolith readers. Silver trevally are relatively slow growing with no detectable difference in growth rate between males and females. Von Bertalanffy growth parameters for both sexes combined are K=0.145, L∞=50.7 and t0=-1.823. Growth rates are slower for silver trevally in Australia when compared with New Zealand trevally. Bomb radiocarbon validation of the age estimation procedure based on thin sections of silver trevally otoliths determined that this method of age estimation is accurate. The high level of agreement between radiocarbon data from silver trevally and redfish (*Centroberyx affinis*) otoliths provides a more precise characterisation of radiocarbon levels off eastern Australia during the period 1975 to 1990 and increases the value of the bomb radiocarbon chronometer for age validation of younger fish with birth dates after peak radiocarbon levels. The standard length at 50% maturity was 20.9 cm for male and 20.3 cm for female trevally, considerably smaller than New Zealand trevally.

**Introduction**

This report summarises results from a study to validate age and growth in silver trevally *Pseudocaranx dentex*. Other components of the silver trevally study considered length at first maturity and population genetics, but these data are not considered in this report. Several sections of this report make reference to the work of James (1984) on trevally Caranx georgianus, a synonym of *P. dentex*.

**Materials and methods**

Silver trevally from NSW waters were obtained from two sources. Random samples were obtained from commercial catches taken from the Ulladulla region (n=43) in mid-July, late October and early November, 1994. The majority of samples (n=164) were collected in October and November, 1994 by the NSW Fisheries Research Institute vessel RV Kapala as part of their trawl research program. Samples obtained from the RV Kapala were collected from the waters off Newcastle, Wreck Bay, Ulladulla, Tathra and Green Cape. Samples were frozen at sea.

Otoliths were extracted from fish and stored dry in envelopes before further processing. Otoliths were weighed and the length, width and thickness measured. Estimates of age were made from transverse sections of sagittae and typically involved enumerating opaque zones along one or both sides of the sulcus acusticus. Additional information on individual zones was obtained from both the dorsal and ventral regions of the transverse section. An opaque zone was considered completed and counted only if translucent material was present on both sides. All sections were read by three independent readers. Two of the readers were 'experienced' readers (Readers A and B) and one was relatively 'unexperienced' (Reader C).

The relationships between standard length, total length or weight, and age were modelled by applying the von Bertalanffy growth function (VBGF) using maximum likelihood procedures in JMP, version 2 (SAS Institute 1989). Because of the nonlinear formulation of the VBGF, a general linear model could not be used for an analysis of covariance. Instead, parameter estimates of the VBGF were compared using an analysis of the residual sum of squares (ARSS) (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 RSSP=residual sum of squares of the VBGF fitted by pooled growth data, RSSs=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. To test if there was a difference between samples, the calculated F value was then compared with the critical F, with the degrees of freedom of the numerator and denominator equal to 3(K-1) and N-3K, respectively.

Age estimates were validated based on the bomb radiocarbon chronometer. Details of the method can be found elsewhere (Kalish 1993, 1995a, 1995b; Kalish et al. 1996) and are not considered here. Otoliths from 4 silver trevally were selected for measurement of bomb radiocarbon. These fish had ages of 5, 10, 15 and 20 years based on the quantification of opaque and translucent zones in thin sections and were selected because they covered most of the age range of fish in this study and could encompass both the ascending and descending limbs of the bomb radiocarbon curve. This would increase the likelihood of an interpretable result with a minimum of radiocarbon measurements.

**Age and growth**

Opaque and translucent zones in transverse sections of otoliths were similar in appearance to those observed in New Zealand silver trevally by James (1984). James (1984) provided evidence that opaque zones were deposited annually in *P. dentex* by several methods including marginal increment analysis, quantification of the number of opaque zones in consecutive cohorts separated by length-frequency analysis, and quantification of the number of opaque zones from a dominant cohort over successive years. These methods are not completely satisfactory for validation of an age estimation procedure and we validated our age estimates on the basis of the bomb radiocarbon chronometer.

Four otoliths were selected for the radiocarbon validation and details of these samples appear in Table 21.1. Silver trevally otoliths are relatively small, but due to our past success with radiocarbon analyses with sample weights less than 3 mg (eg FRDC funded research on radiocarbon in king dory and several oreo species) we felt that satisfactory results could be acheived. Previous data obtained from radiocarbon measurements in both snapper and redfish suggested that the data from the silver trevally should span the ascending and descending limb and incorporate peak levels of radiocarbon characteristic of the late 1970s or early 1980s off southeastern Australia.

Table 21.1. Details of fish and otoliths used for radiocarbon analyses.

| Sample no. | Date caught | Standard length (cm) | Otolith weight (mg) | Sample weight (mg) | $\Delta^{14}C$ (‰) | Otolith section age (yr) | Otolith section birth date |
|---|---|---|---|---|---|---|---|
| 31 | 26/7/94 | 32.5 | 12.6 | 2.3 | 83.0±9.0 | 5 | 1989 |
| 88 | 20/10/94 | 50.9 | 32.7 | 2.5 | 110.1±9.1 | 10 | 1984 |
| 93 | 20/10/94 | 49.5 | 36.9 | 3.0 | 101.5±8.9 | 20 | 1974 |
| 104 | 20/10/94 | 45.0 | 28.8 | 3.3 | 115.8±9.4 | 15 | 1979 |

The radiocarbon data from silver trevally are plotted with similar data from New Zealand snapper (Kalish 1993) and southeast Australian redfish (Kalish 1995) and provide good evidence that the otolith section age estimates are accurate for silver trevally (Fig. 21.1). The silver trevally data describe the peak region of the bomb radiocarbon curve and are in extremely good agreement with the data from snapper and redfish.

The successful validation of silver trevally based on bomb radiocarbon serves to characterise, more precisely, the bomb radiocarbon curve off southeast Australia. The data from trevally, redfish and snapper otoliths show that peak radiocarbon was not reached off southeastern Australia until the early 1980s. These data will help to increase the reliability of subsequent validations for relatively young fish of southeastern Australia. For many shorter-lived species, such as silver trevally, it is difficult to obtain otoliths from individuals with presumed birth dates during the 1960s. Samples from this time period are preferred for radiocarbon validations due to the high rate of change during this period and the possibility for higher temporal resolution. Nevertheless, precise characterisation of the bomb radiocarbon curve during later time periods makes it feasible to employ the method with greater confidence for younger marine animals.

Figures 21.2 and 21.3 show the relationship between otolith length, width, thickness and weight versus fish standard length or estimated age. Otolith growth in all dimensions is highly correlated to fish length and estimated age. Otolith weight appears to be the best proxy for estimated age.

The difference between age estimates was calculated for each pair of readers (Table 21.2). Readers A and B assigned individual fish to the same age class in over 70% of all cases and were within one year in about 95% of cases. The strong agreement between readers A and B indicates that the assigned ages can be replicated (Fig. 21.4a).

Table 21.2. The precision and replicability of the assigned age class of 207 otoliths from *Pseudocaranx dentex* determined by three independent readers.

| | Percentage agreement | | | |
|---|---|---|---|---|
| Readers | Exact | ±1 year | ±2 years | ≥±3 years |
| A:B | 71.5 | 23.2 | 4.8 | 0.5 |
| A:C | 50.0 | 31.5 | 9.2 | 9.3 |
| B:C | 43.1 | 34.1 | 12.7 | 10.1 |

The index of average percent error per age designation (Beamish and Fournier 1981) was calculated to be 0.46% (n=207) between readers A and B. The very low error was attributed to the clarity of the opaque and translucent zones in the otolith sections and the similar interpretation of these zones by the readers. No systematic bias was detected in the age estimates between readers A and B (Fig. 21.4c); however, reader C over-estimated the age of small fish and underestimated the age of larger fish relative to readers A and B (Fig. 21.4b).

Age estimates of 207 trevally (106 female, 80 male, 18 immature, and 10 unknown) made by reader A were used in all subsequent analyses. Parameters of the VBGF for both length and weight against age are given in Table 21.3 and the VBGF are plotted in Fig. 21.5a and 21.5b. Comparisons of the VBGF parameters for female and male trevally showed no significant differences for growth in length ($F(3,180)=0.82$, $P>0.05$), or growth in weight ($F(3,180)=2.14$, $P>0.05$). Therefore, all individuals were included in further analyses.

Table 21.3. Estimated parameters of the von Bertalanffy growth function for growth in length and growth in weight of *Pseudocaranx dentex*. Standard errors are given in parentheses.

| | Estimated parameters of the von Bertalanffy growth function | | | | |
|---|---|---|---|---|---|
| | K | t0 | $L_\infty$ (cm) SL | $L_\infty$ (cm) FL | $W_\infty$ (kg) |
| Growth in length (Australia | 0.145 (0.016) | -1.823 (0.31) | 50.7 (1.73) | 54.2 (2.1) | -- |
| Growth in weight (Australia) | 0.177 (0.017) | -1.636 (0.37) | – | -- | 2.24 (0.13) |
| Growth in length (New Zealand) | 0.341 (0.014) | -1.121 (0.12) | -- | 40.5 (0.18) | -- |

Age and length data for *P. dentex* collected off the west coast of New Zealand in 1973 were supplied by MAF Wellington. The VBGF does not appear to fit these data well (see James 1984), as fish length does not appear to reach an asymptote. The VBGF parameters for the NZ fish collected in 1973 are presented in Table 21.3.

Comparisons between Australian and New Zealand trevally reveal differences in growth between these populations for the years sampled. Age and growth of *P. dentex* from NZ was characterised by James (1984). The NZ analysis was calculated in terms of fork length, not standard length as in this study of southeast Australian trevally. Thus, Australian data were reanalysed using fork length to facilitate comparison with the NZ data. Quantitative comparisons were made between Australian samples and NZ samples from 1973 and there was a significant difference in growth characteristics ($F(3,806)=144.3$, $P<0.05$).

Trevally have been aged to 47 years in NZ (James 1984), more than twice the age of the oldest fish in this study. This may reflect insufficient sampling of larger fish in the present study, rather than differences in longevity between the two populations of trevally.

The VBGF for trevally from the West Coast (1973) and East Coast (1972, 1973, 1974) of the North Island of NZ are compared with Australian data (this study) in Fig. 21.6. New Zealand trevally show relatively rapid growth for about the first 5 years, to fork lengths of 35-40 cm and growth nears an asymptote before 10 years of age and at a fork length of about 40-45 cm. Southeast Australian trevally displayed slower growth and attained a significantly larger asymptotic length than any of the four NZ samples.

Australian and New Zealand trevally exhibited markedly different size and age at sexual maturity. NZ trevally did not mature until 35 cm FL and 4 years (James 1984), whereas, for southeast Australian trevally, the standard length at 50% maturity was 20.9 cm for males and 20.3 cm for females (Table 21.4) or an age of about 2 years.

Table 21.4. Parameter estimates for the logistic model of proportion mature at standard length (cm) and length at 50% mature for *Pseudocaranx dentex*.

| | Parameter estimates | | |
|---|---|---|---|
| Sex | a | b | SL50% (cm) |
| Male | 12.69 | -0.61 | 20.9 |
| Female | 16.81 | -0.83 | 20.3 |

Silver trevally are extremely fecund with the relative fecundity exceeding 3 million ova per kg of body weight (Fig. 21.7a) and absolute fecundities in excess of 10 million for fish of about 45 cm SL (Fig. 21.7b).

**Acknowledgments**

We thank Ken Graham (NSW Fisheries) and the crew of the FRV Kapala for providing silver trevally from New South Wales.

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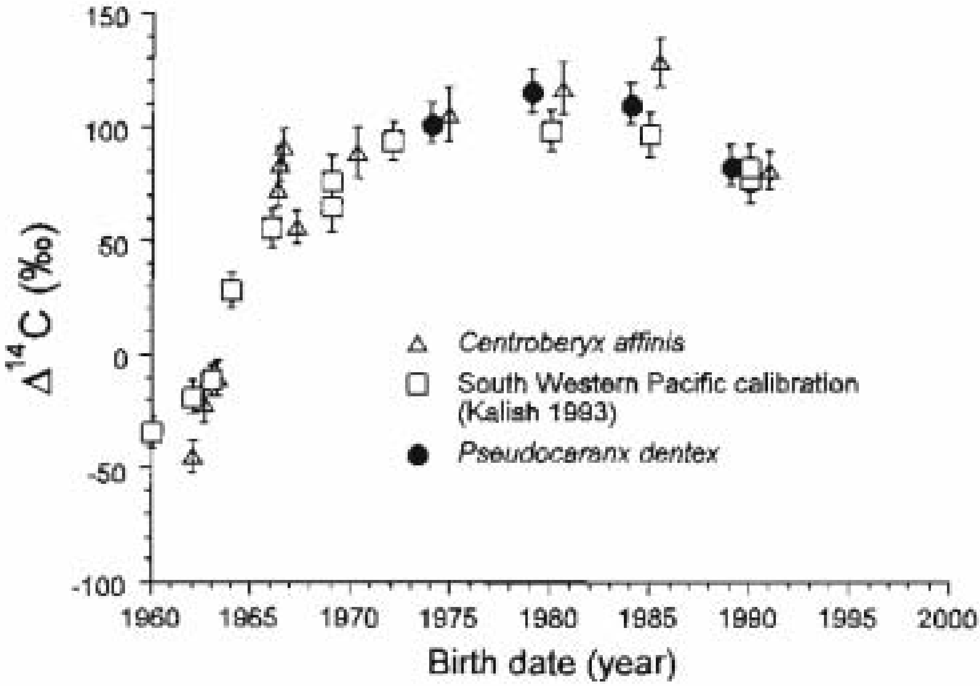



Figure 21.1. $\Delta^{14}C$ of silver trevally otolith cores plotted against the birth date estimated from otolith thin sections. $\Delta^{14}C$ from *Pagrus auratus* otolith cores are plotted against the true birth date (Kalish 1993) and $\Delta^{14}C$ from *Centroberyx affinis* (Kalish 1995) are plotted against birth dates determined from reading otolith sections.

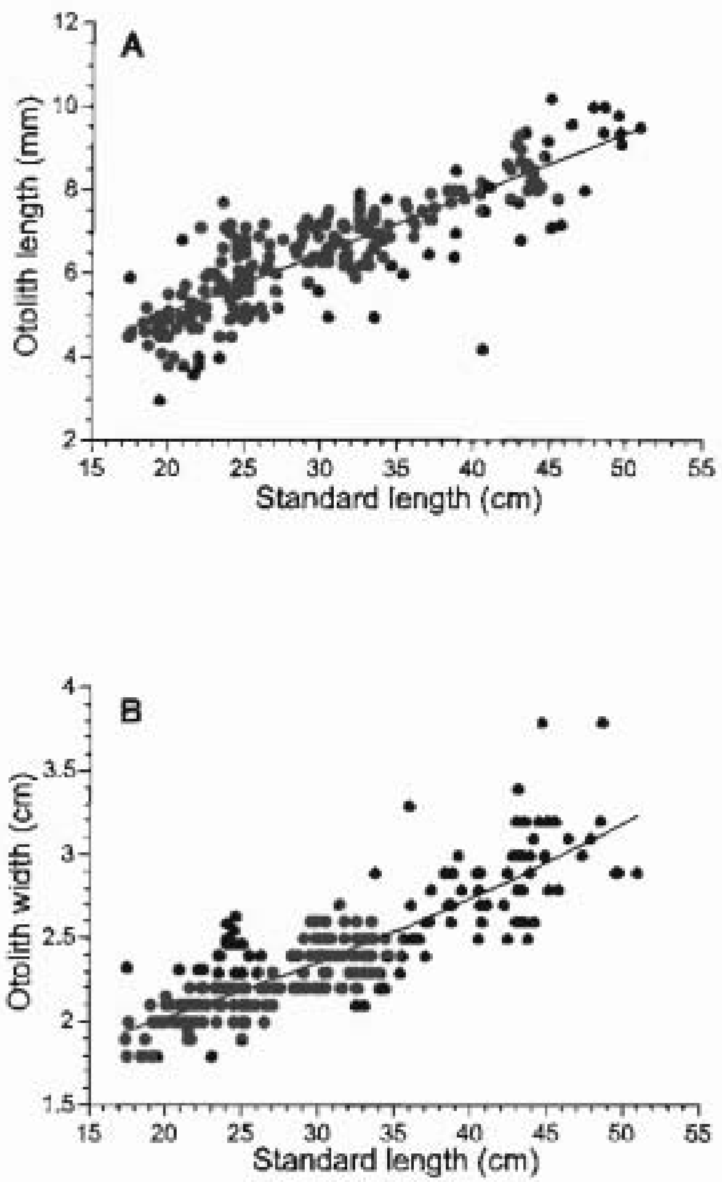
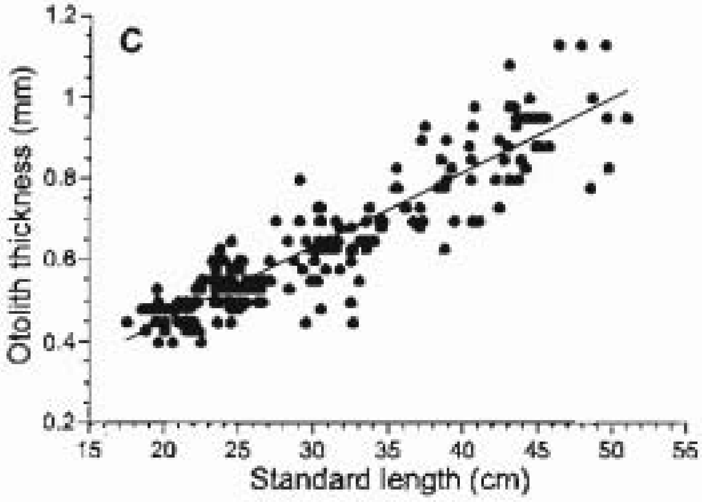



Figure 21.2. Plots of otolith length (OL) (A) (OL = 0.14254SL + 2.2042; r2 = 0.72) and otolith width (OW) (B) (OW = 1.4924 x e0.015178SL; r2 = 0.73) against fish standard length (SL) (n=207) for *Pseudocaranx dentex* sampled in southeast Australian waters between Newcastle and Green Cape.



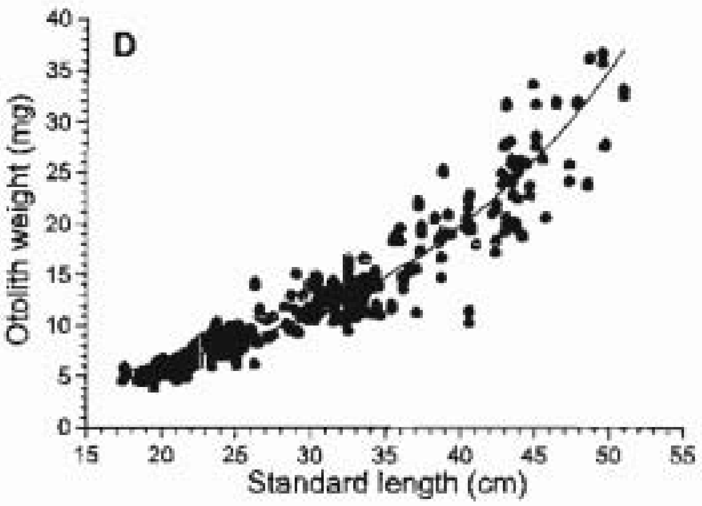




Figure 21.2. (continued). Plots of otolith thickness (OT) (C) (OT = 0.018264SL + 0.08555; r2 = 0.83), and otolith weight (OW) (D) (OW = 1.9834 x e0.057522SL; r2 = 0.91) against fish standard length (n=207) for *Pseudocaranx dentex* sampled in southeast Australian waters between Newcastle and Green Cape.

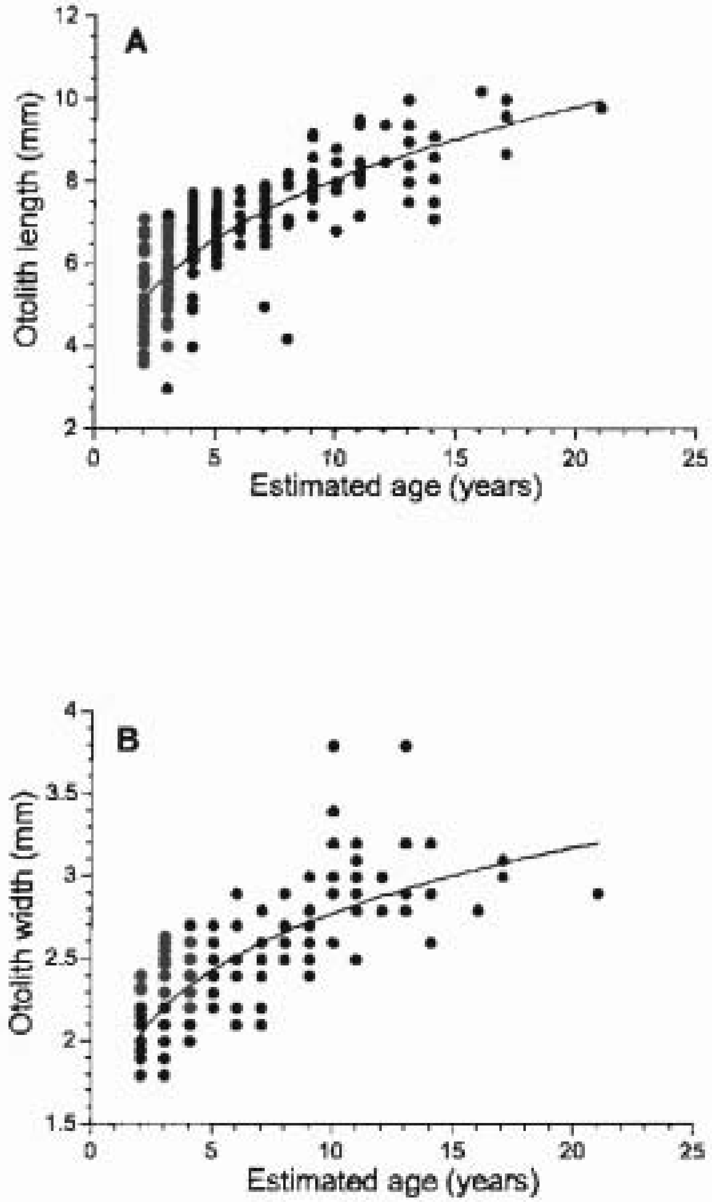
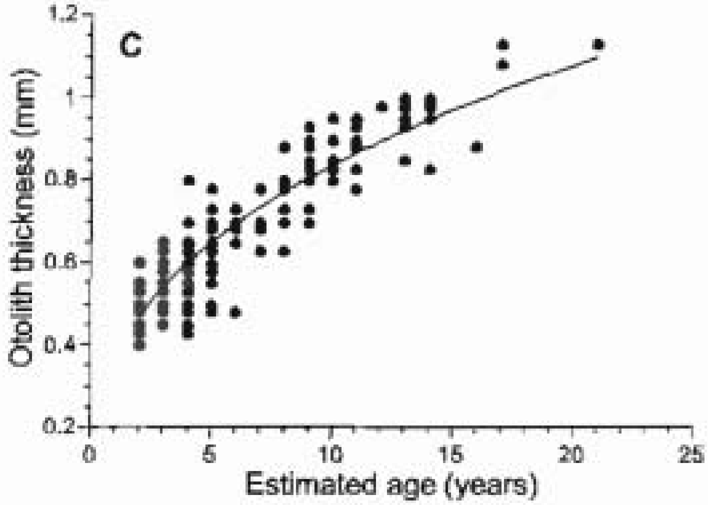



Figure 21.3. Plots of otolith length (OL) (A) (OL = 4.1681 x AGE0.28562; r2 = 0.68), otolith width (OW) (B) (OW = 1.7794 x AGE0.1933; r2 = 0.66) against estimated age (AGE) (n=207) for *Pseudocaranx dentex* sampled in southeast Australian waters between Newcastle and Green Cape.



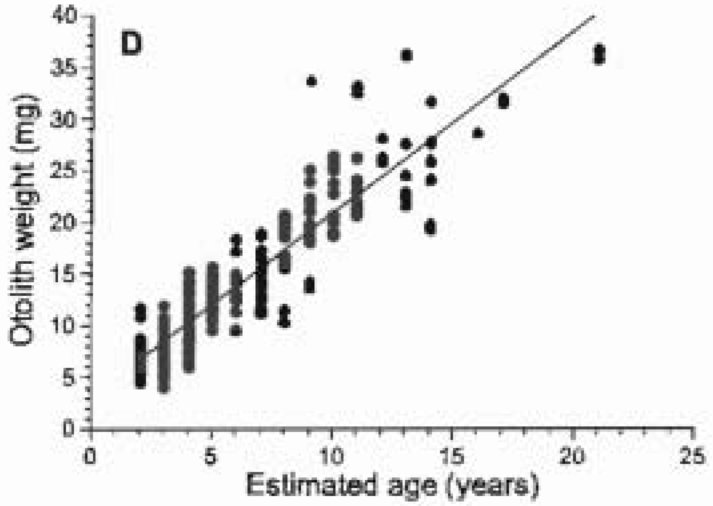




Figure 21.3. (continued). Plots of otolith thickness (OT) (C) (OT = 0.35969 x AGE0.36628; r2 = 0.86), otolith weight (OW) (D) (OW = 1.766AGE + 3.2043; r2 = 0.89) against estimated age (AGE) (n=207) for *Pseudocaranx dentex* sampled in southeast Australian waters between Newcastle and Green Cape.

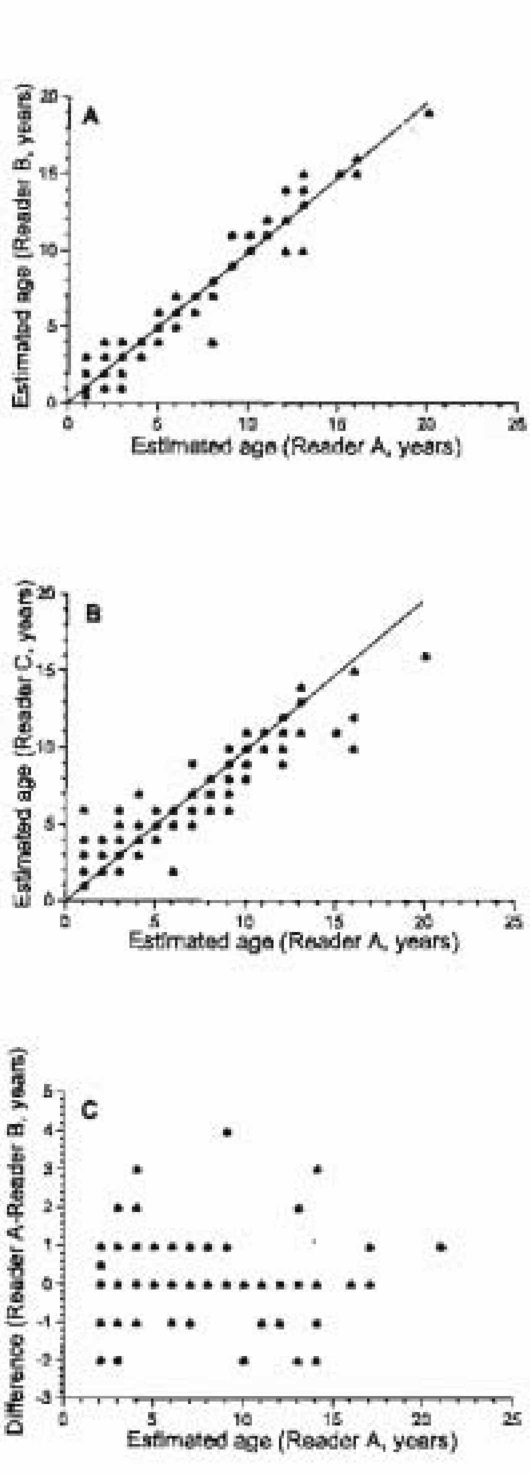
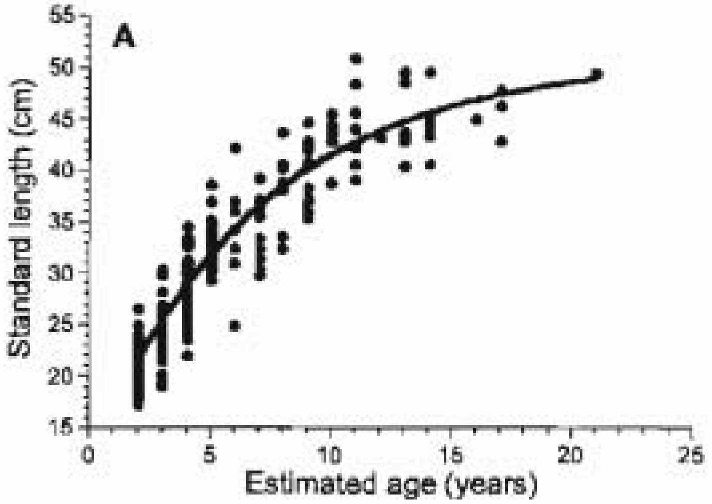



Figure 21.4. Comparison of the estimated age assigned to otolith transverse sections from *Pseudocaranx dentex* (n=207) by three independent readers. Plot (a) compares between readers A and B with the 1:1 line, (b) plots readers A and C with the 1:1 line. Plot (c) shows no systematic bias in the estimated age for readers A and B.



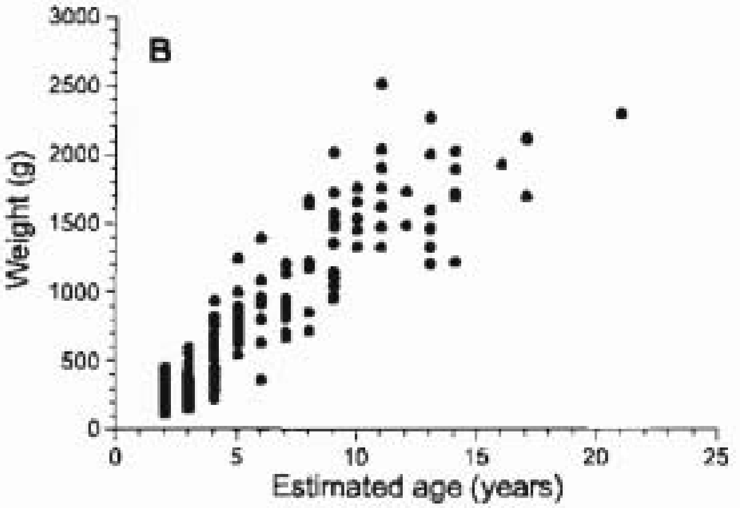




Figure 21.5. Relationships between estimated age and length, plotted with Von Bertalanffy growth curve (A) and age and weight (B) (n=207) for *Pseudocaranx dentex* sampled in southeast Australian waters between Newcastle and Green Cape.

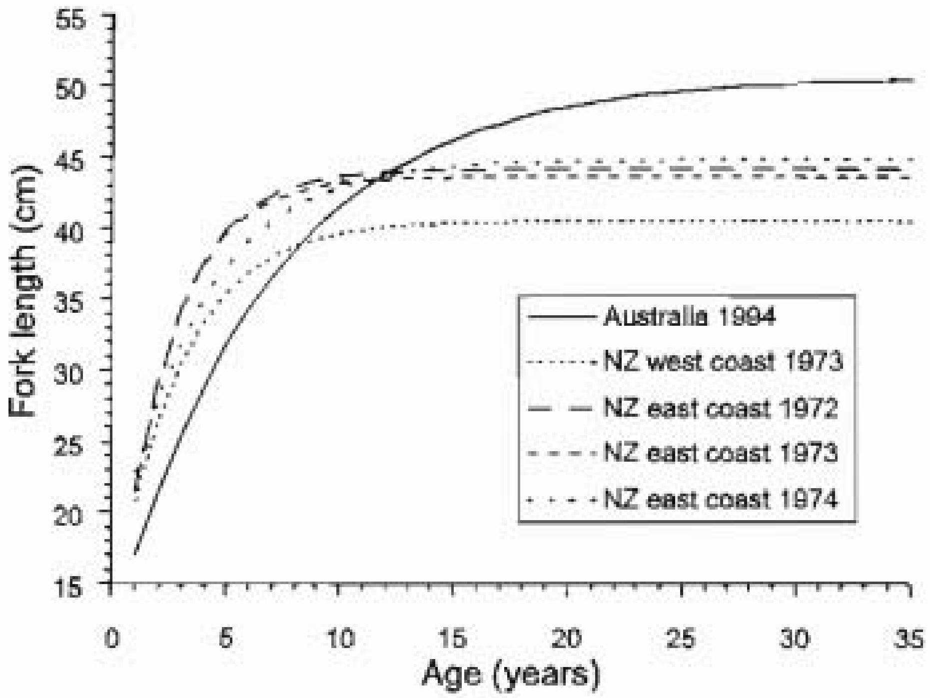



Figure 21.6. Comparison of the von Bertalanffy growth functions used to model growth in Australian (1994) and New Zealand (1972, 1973, 1974) *Pseudocaranx dentex*. Note that ages in excess of 21 years have not been observed for this species in the Australian region. The functions for the east coast data were obtained from James (1984) and are representative of females and males combined. The data from the west coast of New Zealand (1973) are the same as in Figure 6.

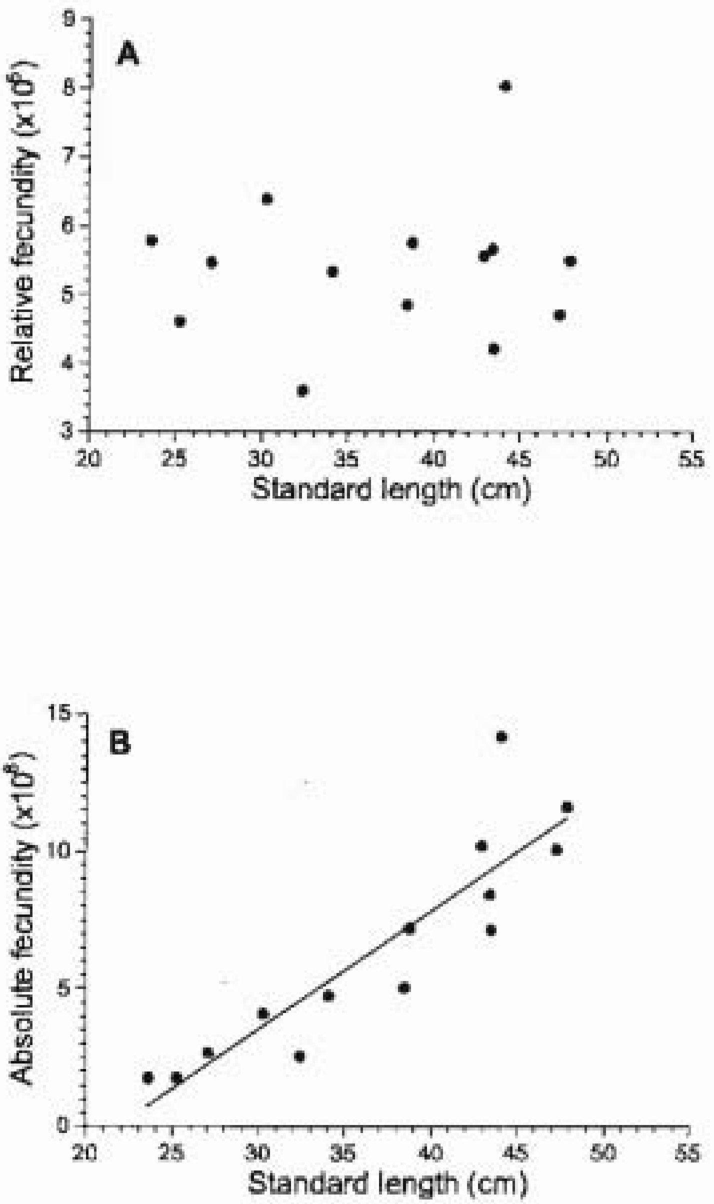



Figure 21.7. Relationship between relative (number of ova per kg body weight) and standard length (A) and absolute (total number of ova) fecundity (AF) estimates and standard length (SL) (B) (AF = 0.42964SL – 9.3372; r2 = 0.81) for *Pseudocaranx dentex* (n=14) sampled in southeast Australian waters between Newcastle and Green Cape.

# Chapter 22 Use of the bomb radiocarbon chronometer to validate age estimation methods for banded morwong (*Cheilodactylus spectabilis*) and jackass morwong (*Nemadactylus macropterus*) from south eastern Australia

John Kalish, Justine Johnston and Jeremy Lyle

**Summary**

High precision analyses of radiocarbon in small samples of otoliths from two morwong species provide good evidence to support the conclusion that age estimates based on quantification of presumed annual increments in otolith thin sections are accurate. This conclusion is well supported for banded morwong despite the small number of samples analysed and less so for jackass morwong. Further analyses are warranted to determine the accuracy of the routine age estimation method for these species, but emphasis should be placed on research that considers jackass morwong.

**Introduction**

Banded morwong (*Cheilodactylus spectabilis*) and jackass morwong (tarakihi in New Zealand) (*Nemadactylus macropterus*) are commercially important species with presumably long life spans. Both species are subject to commercial and recreational fisheries employing a range of fishing gears. The South East Fishery for jackass morwong is managed on the basis of Individual Transferable Quotas, however, the species is also taken in state fisheries and recreational fisheries. In recent years, total catches of jackass morwong from the area of the South East Fishery have been estimated at around 1000 t (Smith and Wayte 2001). Banded morwong is caught as part of state fisheries with the majority of catch from Tasmanian waters. The fishery is regulated through limited entry, minimum and maximum size limits and a closed season with catches from Tasmanian waters at about 80 t.

Several studies have investigated the age and growth of tarakihi in New Zealand (Tong and Vooren 1972, Vooren 1977) and these studies reported ages of up to 50 years. Smith (1982) reported maximum ages for male and female jackass morwong of 11 and 16 years, respectively on the basis of opaque and translucent zones in whole otoliths. These whole otolith ages were validated on the basis of marginal increment analysis (Smith 1982). Ages estimated from the whole otolith method were used for stock assessments for this species until 1994 when the Central Ageing Facility (MAFRI) determined that in Australia, this species lived longer than previously reported after examining thin sections of otoliths (Smith and Robertson 1994). Recent studies of sectioned jackass morwong otoliths have extended the maximum known age for this species to about 30 years (Morison 1996), more in line with estimates from New Zealand for the species. However, these new Australian age estimates have not been validated despite the need for this research.

Recent research on banded morwong estimated maximum ages on the basis of otolith thin sections of 86 and 81 years for females and males, respectively (Murphy and Lyle 1999). These age estimates were validated by a capture-mark-recapture experiment where fish were tagged and injected with the calciphilic fluorochrome, oxtetracycline hydrochloride. This technique is extremely valuable for validated the nature of annual increments in typically younger fish, however, it is usually impractical for validating ages of presumably very long-lived species such as banded morwong. The presumed longevity of banded morwong and the concomitant potential for over-exploitation warrants further investigation of age validation for this species.

The very small size of sagittal otoliths from fish of the Family Cheilodactylidae (morwongs) makes preparation and analysis of samples extremely difficult. This research on banded morwong and jackass morwong completed high precision analyses on samples that contained as little as 0.23 mg of carbon. In the early 1990s the smallest sample of carbon that state-of-the-art accelerator mass spectrometry laboratories were able to routinely analyse with high precision (< 10% error) for radiocarbon was approximately 1.0 mg. Otoliths are mostly aragonite, a mineral of calcium carbonate (Ca CO3) that is 12% carbon by weight. Therefore, an otolith sample weighing 10 mg was close to the size limit for a high precision analysis; this is the approximate size of many of the samples analysed during this FRDC project. In recent years various accelerator mass spectrometry laboratories have worked to reduce the minimum size of the sample of carbon required for a high precision analysis. Several laboratories already involved with this FRDC funded research program on age validation based on the bomb radiocarbon chronometer were willing to analyse very small samples of otolith aragonite. These laboratories included the Radiocarbon Accelerator Unit, Research

Laboratory for Archaelogy and the History of Art, Oxford University, the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, University of California and the U.S. National Science Foundation Accelerator Mass Spectrometry Facility, University of Arizona.

The objective of this study was to validate age estimates for two presumably long-lived morwong species. The research offers challenges due to the difficulties associated with the preparation and analysis of the samples. Success in these analyses on small samples has the potential to increase significantly the range of species that can be studied with radiocarbon.

**Materials and methods**

Banded morwong otoliths were supplied by the Department of Primary Industry, Water and Environment (DPIWE, Tasmania). Fish were collected off the east coast of Tasmania in the region of the Tasman Peninisula. Jackass morwong otoliths were supplied by the Marine and Fisheries Resources Institute (MAFRI, Victoria) with the fish collected through the Integrated Scientific Monitoring Program (ISMP) in the ocean off Eden, NSW (Table 22.1). Age estimates were determined from otolith thin sections viewed with a stereo microscope and using transmitted light illumination.

Sample preparation procedures were similar to those described in Kalish (1995) for redfish (*Centroberyx affinis*). Otolith aragonite deposited during a period presumed to be less than the first year of life was isolated from one sagitta from each fish. This was accomplished by cutting and grinding the otolith with a hand-held high speed drill to remove material deposited after what was presumed to be the first annual increment. Further grinding of the remaining portion of the otolith was used to "sculpt" the material into a structure with the shape and dimensions of the earliest formed portions of a banded or jackass morwong sagitta. Sagittae from very small morwong and zones visible on the otolith being sculpted were used as a guide. A total of 15 otoliths were sculpted successfully including 7 banded morwong and 8 jackass morwong otoliths. Sample weights for otoliths that were sculpted successfully ranged from 1.4 mg to 4.2 mg for banded morwong and from 2.5 mg to 5.7 mg for jackass morwong. However, many of the analyses of the small samples were not successful due to unsuccessful preparation of the graphite target, contamination or poor precision. Only the seven analyses that were successful are recorded in Table 22.1.

Otolith carbonate was converted to CO2 by reaction in vacuo with 100% phosphoric acid. An aliquot of the CO2 was used to determine $\delta^{13}C$ for each sample and the remaining CO2 was converted to graphite. The graphatised samples were analysed for radiocarbon. Radiocarbon was determined in each sample by accelerator mass spectrometry (AMS) at the Center for Accelerator Mass Spectrometry (CAMS) Lawrence Livermore National Laboratory (LLNL), University of California or at the Radiocarbon Accelerator Unit (OxA), Research Laboratory for Archaeology and the History of Art, Oxford University. Radiocarbon values are reported as $\Delta^{14}C$, which is the age- and fractionation-corrected deviation (parts per thousand) from the activity of nineteenth century wood. Age corrections are based on the mean estimate of age determined from reading of otolith sections. Reported errors are 1 standard deviation for both radiocarbon data and age estimates based on the reading of otolith sections. Radiocarbon errors include both counting errors and laboratory random errors.

**Results and discussion**

Radiocarbon measurements (Table 22.1) are plotted versus otolith based age estimates for both morwong species in Figure 22.1. Although few samples were analysed, the curve defined by the data from the morwong otoliths defines the increase in bomb-derived radiocarbon during the 1960s and 1970s and is consistent with similar data from *Pagrus auratus* (south western Pacific Ocean calibration) (Kalish 1993) and *Centroberyx affinis* (Kalish 1995). The sample with the oldest estimated age, a banded morwong of 54 years had a $\Delta^{14}C$ of –66.7‰, clearly indicative of pre-bomb (<1955) birth date. Two banded morwong of 37 and 32 years of age (sample nos. BM40 and BM48) with $\Delta^{14}C$ of –45.8‰ and –30.0‰, respectively, provide good evidence that the banded morwong otolith ages are accurate and record the earliest increase in bomb produced radiocarbon in the late 1950s and early 1960s. Four samples had age estimates based on presumed annual increments that indicated birth dates near the peak in bomb radiocarbon and measurements of $\Delta^{14}C$ are consistent with these age estimates.

Results from the jackass morwong are more difficult to interpret in isolation. The results are clearly consistent with the bomb radiocarbon time series defined for the south western

Pacific Ocean, however, the samples that were analysed successfully fall within the segment of the curve with the lowest temporal resolution. Therefore, for these samples, it is only possible to conclude that the age estimates based on otolith thin sections are not dramatically under estimated (e.g. by more than 5 years). Unfortunately, several of the older jackass morwong samples were not analysed successfully, due to inadequate carbon for graphite target preparation, sample contamination or unacceptably low precision. Further analyses are warranted as the ability to analyse these small samples has been demonstrated and is likely to be successful with recently developed improvements in techniques of target preparation.

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Table 22.1. Fish, otolith, and radiocarbon data for banded morwong (*Cheilodactylus spectabilis*) and jackass morwong (*Nemadactylus macropterus*) from south eastern Australia.

| Sample no. | Species | Laboratory and laboratory acquisition no. | Collection location | Collection date | Fish length (mm) | Otolith weight (g) | Sample weight (mg) | $\Delta^{14}C$ (‰) | Otolith section age (years) | Birth date (year A.D.) |
|---|---|---|---|---|---|---|---|---|---|---|
| BM21 | *Cheilodactylus spectabilis* | LLNL CAMS37836 | Tasmania | 1994 | nd | 0.0284 | 2.3 | -66.6±7.7 | 54 | 1940 |
| BM48 | *Cheilodactylus spectabilis* | LLNL CAMS37837 | Tasmania | 1994 | nd | 0.0249 | 2.1 | -45.8±8.0 | 37 | 1957 |
| BM40 | *Cheilodactylus spectabilis* | OxA8239 | Tasmania | 1994 | nd | 0.0278 | 4.2 | -30±9.0 | 32 | 1962 |
| BM50 | *Cheilodactylus spectabilis* | OxA8240 | Tasmania | 1994 | nd | 0.0151 | 1.9 | 84±8.0 | 16 | 1978 |
| JM16 | *Nemadactylus macropterus* | LLNL CAMS37834 | New South Wales | 20-Mar-92 | 300 | 0.0233 | 3.8 | 92.7±8.6 | 6 | 1986 |
| JM68 | *Nemadactylus macropterus* | LLNL CAMS37833 | New South Wales | 20-Mar-92 | 337 | 0.0483 | 2.7 | 98.1±8.7 | 20 | 1972 |
| JM72 | *Nemadactylus macropterus* | LLNL CAMS37835 | New South Wales | 20-Mar-92 | 342 | 0.035 | 2.5 | 92.2±8.7 | 14 | 1978 |

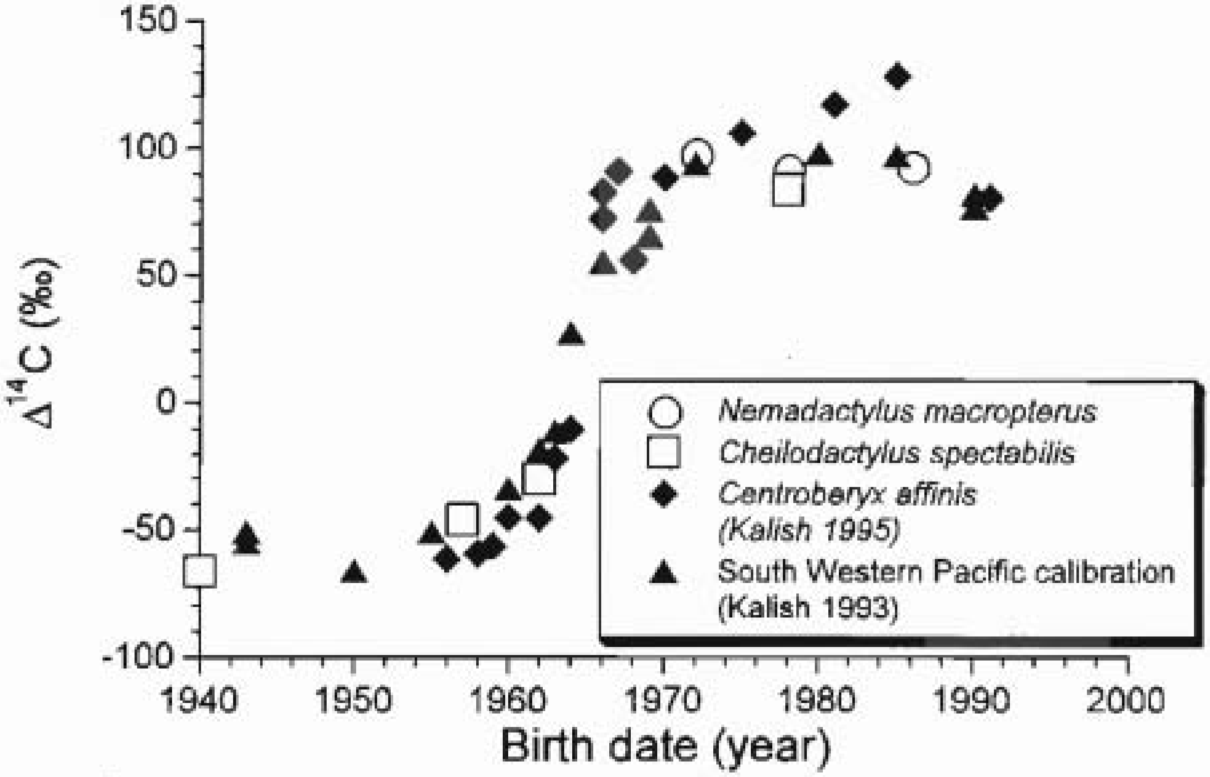


Figure 22.1. $\Delta^{14}C$ of *Cheilodactylus spectabilis*) and jackass morwong (*Nemadactylus macropterus*) otolith cores plotted against birth dates estimated from otolith thin sections. $\Delta^{14}C$ data from New Zealand *Pagrus auratus* (Kalish 1993) and Australian *Centroberyx affinis* (Kalish 1995) provide calibrations of $\Delta^{14}C$ versus year for the south western Pacific Ocean. $\Delta^{14}C$ values are based on otolith material deposited over a time period equivalent to about the first year of life. Errors are ±1 sd.

# Chapter 23 Determination of swordfish (*Xiphias gladius*) age based on analysis of radiocarbon in vertebral carbonate and collagen

John Kalish and Edward DeMartini

**Summary**

The ability to use measurements of radiocarbon in swordfish vertebrae to determine fish age appears to be limited due to the likelihood that swordfish vertebrae undergo significant reworking during development and growth. This result is very different from measurements of radiocarbon in the vertebrae of school shark which provided evidence that vertebral collagen is not reworked to any great extent, if at all (Chapter 10). The different results for swordfish and school shark suggest dramatic differences in the metabolism of bone in these distantly related fish species. There is a significant increase in the density of swordfish vertebrae during growth and this provides clear evidence that the vertebrae are going through a process of reworking during growth. As a result it seems likely that the vertebrae of swordfish violate a key assumption required for the successful application of the bomb radiocarbon chronometer, specifically that the material (carbonate or collagen) deposited in early life is not subject to resorption or reworking. The difference between $\delta^{13}C$ from carbonate and collagen in swordfish vertebrae clearly identifies a dominant metabolic source for carbon destined for collagen and an inorganic source for carbon with collagen more depleted in the heavier stable carbon isotope, $^{13}C$, than the carbonate fraction.

**Introduction**

The success of mineralised tissues for the estimation of fish age is dependent on the assumption that resorption of the tissue does not occur during the life of the fish. Among the mineralised tissues in fishes, otoliths are most likely to be fulfil this assumption due to the fact that these calcium carbonate structures are acellular. Development of otoliths is through accretion of successive mineralised layers invested with varying amounts of organic matrix; the organic matrix playing an essential role in the ultimate micro- and macrostructure of the otolith. No mechanisms are in place that facilitate the resorption of calcium from otoliths and they are unlikely to serve as sinks of calcium during the life of a fish.

Other mineralised tissues, including fin rays, cleithra, and vertebrae have been employed for the estimation of fish age and in many cases these methods have been validated, thereby confirming a satisfactory level of accuracy is achievable. Nevertheless, the application of bone to studies of age and growth may be problematic due to the potential for resorption, where resorption refers to either osteoclasia involving the breakdown of the bone surface by osteoclasts or osteolysis where osteocytes are responsible for resorption.

There are few data on the ability of teleosts to resorb and/or remodel bone, however, many teleosts have been shown to possess acellular bone due to the lack of osteocytes or osteoclasts in the mineralised tissue. There is limited research in this area; however, this topic has relevance to the development of aquaculture industries and the maintenance of adequate nutrition to captive fish.

Bone resorption is a critical issue in assessing the suitability of age estimates for swordfish, the majority of which are determined on the basis of zones discernible in anal fin rays (refs). Other studies have attempted to estimate ages of adult swordfish based on structure in vertebrae (refs) and otoliths (refs); however these methods have proven largely unsuccessful. Zones in both otoliths and vertebrae from swordfish are poorly defined and difficult to quantify. In addition, otoliths of all billfish are extremely small relative to otoliths of other fish species and swordfish otoliths are no different. Estimates of daily age have been successfully achieved in young swordfish based on daily increments in otoliths (e.g. Hawaiian study). Age estimates from these young fish indicate very rapid growth during early life, but the oldest age estimates based on this method were ??? days.

Initial assessment, through microscopic examination, of the second anal fin rays of swordfish supports the notion that resorption occurs in this mineralised tissue. The alternating opaque and translucent pattern of the anal ray appears to result from the density of vascular canals. Opaque zones contain a larger proportion of terminal vascular canals, suggesting that the rate of anal ray growth had been reduced at this time thereby decreasing the need for the vascular canals to distribute bone constituents to the ray periphery. It may be less costly, in an energetic sense, to terminate some centers of bone growth during slow growth periods and re-establish the sites of osteogenesis when growth rates increase.

The region of the swordfish anal fin ray characterised by opaque and translucent zones has secondary structure that differs dramatically from the ray core. The ray core appears to contain a far greater density of Haversian canals than later-formed regions of the ray and, more importantly, the orientation of these canals provides good evidence for bone resorption due to the nature of overlap of some of these canals. Furthermore, there is clear evidence of ray resorption based on simple observation of the ray macrostructure. The two lateral segments of the ray are clearly separated by a large vascularised lumen. In the second anal ray of large swordfish (>200 cm EFL) this lumen is large enough to contain the ray of a swordfish about 100 cm EFL. Clearly, resorption must occur in this region. The proximity of this region of resorption to the denser Haversian bone increases the likelihood that bone remodelling has occurred. Here, bone remodelling refers to the reshaping of bone associated with the physiological and mechanical demands of growth and later life.

Bone resorption is only one stage of the remodelling process; bone re-deposition must also occur. Bone remodelling is often associated with discrete zones of resorption and the opaque zones of the rays may, in fact, be manifestations of this process. Nevertheless, the quantity of resorption and remodelling may be insignificant as far as estimation of age is concerned and the microstructure of the anal fin ray suggests that this may be the case. Resolution of this issue would require histological analysis of ray tissue during different growth periods (e.g. winter vs. summer growth).

Although the potential resorption and remodelling of the ray may not introduce significant errors into the standard age estimation process, it could be problematic for the interpretation of natural or human-induced chemical marks. Radiocarbon in mineralised tissue is included in both organic (collagenous and non-collagenous components) and inorganic (carbonate fluorapatite, also known by the mineralogical name francolite) fractions with the far greatest proportion bound in an organic form. Although a particular region of the ray may appear intact, this does not provide evidence in relation to potential remodelling of the tissue. Collagen and carbonate fractions in a particular region may have been deposited recently and the likelihood of this occurrence is increased for ray tissue that shows evidence of remodelling.

Initial observations of thin sections of swordfish vertebrae do not display evidence of resorbtion or remodelling, but vertebrae may be a site of secondary mineralisation. This is based on the possibility of increased density of the innermost region of vertebrae in large swordfish (>200 cm EFL), when compared with vertebral density from a small individuals (<100 cm EFL). The high density components of the vertebrae are the inorganic constituents which are predominated by hydroxyapatite ( $Ca10(PO4)6(OH)2$). The lower density components of the vertebrae are comprised largely of the organic compound collagen.

**Materials and methods**

Vertebrae were selected from archived samples collected on research cruises in 1992 (Cruise TC-92-03) and 1993 (Cruise TC-93-03) of the Honolulu Laboratory, U.S. National Marine Fisheries Service.

Selected regions of vertebrae from larger swordfish were isolated by drilling and grinding with a dental-type drill. One region incorporated vertebral material representative of the first year of life and these samples are referred to as "inner", whereas a second sample from each large vertebra was cut from the outer periphery or growing edge of the vertebra. These samples are referred to as "outer". The vertebra from the small swordfish was cleaned of extraneous tissue and blood by grinding.

After isolation of the selected portion of the vertebra, samples were subject to pre-treatment to isolate the relevant chemical fraction of the bone (Table 23.1) at the Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences (New Zealand). Samples were analysed for radiocarbon by accelerator mass spectrometry at the Rafter Radiocarbon Laboratory and are reported as $\Delta^{14}C$ (‰).

Density of swordfish vertebrae was estimated from samples collected on cruises of the RV Townsend Cromwell (U.S. National Marine Fisheries Service, Honolulu). The sample included 24 female fish ranging in length from 81-219 cm EFL. Samples were cleaned of all tissue and had been air dried over several years. Analysis of the density of vertebrae from different size swordfish was tested as was the assumption that there is no significant difference in the density among vertebrae within individual swordfish compared with variation among swordfish. The volume of the vertebrae was determined by measuring the water displaced by a vertebra placed within a graduated cylinder. Weight of vertebrae was determined for dry vertebrae weighed in air. Measurement errors were <1% for dry weights (measured to 0.01 g) and 9% each for volume displacements (measured to the 0.1 ml) and density estimates.

A model fitting vertebral density versus eye-to-fork length (EFL, cm) was determined by non-linear regression. The regression was weighted by the number of replicate measurements taken for the specimens. Twenty four samples were used in the regression including 1, 21 and 2 samples of the 23rd, 24th and 25th vertebrae, respectively. A balanced, fixed-factor ANOVA was used to determine if there was significant variation in density among vertebrae within fish compared to among fish.

**Results**

Radiocarbon and stable carbon isotope data from selected regions of swordfish vertebrae and from different chemical fractions are presented in Table 23.2. The average $\delta^{13}C$ values were -4.21 (±1.45) and −15.7 (±0.92) for the carbonate and collagen fractions of the vertebrae, respectively. Vertebral $\delta^{13}C$ values were significantly different between collagen and carbonate fractions (t-test, $p<0.0001$) with the collagen fractions displaying far greater depletion in the heavier stable carbon isotope.

$\Delta^{14}C$ ranged from 20.8‰ to 123.8‰ with highest $\Delta^{14}C$ measured in collagen from the smallest and presumably youngest swordfish (sample no. 94). There was no evidence for systematic variation in $\Delta^{14}C$ between the collagen and carbonate samples nor between inner and outer samples (Fig. 23.1). Mean $\Delta^{14}C$ was 67.3±24.0‰ and 94.1±15.1‰ for vertebral carbonate and collagen, respectively.

The $\Delta^{14}C$ data from the swordfish vertebrae are plotted with $\Delta^{14}C$ data from North Pacific corals from Oahu and French Frigate Shoals (Druffel 1987) and from seawater dissolved inorganic carbon (DIC) collected during WOCE (World Ocean Circulation Experiment) cruises in the Pacific during the early 1990s (Key et al. 1996) (Fig. 23.2). The minimum and maximum values for surface water $\Delta^{14}C$ from between 10°N and 35°N latitude along the 135°W section (P17) are plotted in Fig. 23.2 and these data coincide with the majority of measurements made in the inner and outer segments of the swordfish vertebrae.

Birth date estimates for individual swordfish were determined directly from the vertebral radiocarbon data. A similar procedure was used in age validation for school shark (Chapter 10) with only minor differences. Rather than use a model derived from the Oahu and French Frigate Shoals coral data to calculate swordfish birth dates, estimates were made graphically (Figs. 23.3 and 23.4). As in the previous otolith and vertebrae studies, only $\Delta^{14}C$ measured in the earliest formed portions of the calcified tissues was used to estimate birth dates. Figure 23.3 shows estimated birth dates of around 1965 based on collagen from the three larger swordfish (Sample nos. 46, 58 and 108) and associated ages of around 30 years. Similar birth dates and ages are derived from an analogous interpretation of the carbonate data from the inner segment of the vertebrae from the large swordfish (Fig. 23.4).

There was a significant relationship between vertebra density and fish length (EFL) where: Vertebral density = $0.0218EFL^{0.7324}$; $r2 = 0.632$; $n = 24$) (Figure 23.5). Analysis of variance demonstrated that vertebra density varied insignificantly among the 23rd, 24th and 25th vertebrae within fish, but varied strongly among fish spanning the range of body sizes examined (Table 23.3).

**Discussion**

The interaction of factors that result in the radiocarbon levels measured in different fractions of individual swordfish vertebrae is likely to be complex. Significant differences in $\Delta^{14}C$ measured in swordfish vertebrae may result from the following factors: 1) habitat latitude and longitude; 2) depth of occurrence; 3) fish age; 4) diet; and, 5) chemical fraction analysed.

Knowledge of swordfish biology is relatively limited and it is particularly difficult to develop a clear understanding of individual presumed stocks of swordfish due to the cosmopolitan distribution of the species. It is known that during their first year of life swordfish in the North Pacific Ocean are likely to be found at middle to higher latitudes; therefore, it was considered suitable to compare the radiocarbon data from the vertebrae with radiocarbon time series derived from North Pacific corals.

Data are available that make it possible to infer the depth of occurrence of swordfish during their first year of life. Very small swordfish (<30 cm TL) have been identified in the stomach contents of dolphinfish (Coryphaena hippurus). Dolphinfish are surface daytime feeders; therefore, this provides evidence that very young swordfish are present in surface waters during the day. Small swordfish (<100 cm EFL) are commonly caught by longliners targeting larger swordfish with surface sets made during the night and recovered in the early morning. This provides further evidence for small swordfish inhabiting surface waters. However, there is no information on the daytime habitat of small swordfish (between 30 cm TL and 100 cm EFL) and these fish may move to greater depths at this time. Acoustic telemetry studies of large swordfish clearly show swordfish moving to depths of greater than 600 m during the daytime (Carey and Robison 1981, Carey 1990). If this was the case with smaller swordfish, it would have a large effect on the radiocarbon content of their vertebrae since the radiocarbon content of water masses below the surface mixed layer (~200 m in the North Pacific) are considerably lower than those at the surface. Despite the limited information available on swordfish, the high levels of radiocarbon measured in the vertebrae make it extremely unlikely that young swordfish spend large amounts of time in waters below the surface mixed layer.

$\Delta^{14}C$ of 123.8±9.4‰ measured in the vertebra from the 76.5 cm EFL swordfish collected in 1992 is indicative of radiocarbon levels in the upper 200 m in the North Pacific and is indicative of radiocarbon in seawater dissolved inorganic carbon (DIC). $\Delta^{14}C$ data collected during the WOCE section along 135°W (p17) (Keys et al. 1996) demonstrate that this value is clearly at, if not above, the maximum $\Delta^{14}C$ measured in the North Pacific in the early 1990s.

$\Delta^{14}C$ measured in both collagen and carbonate fractions from the outer regions of adult vertebrae collected in 1992 and 1993 do not provide a clear indication of the water mass inhabited by these large fish. It is not at all surprising that a distinct habitat is not evident and this is associated with the fact that there is almost no difference in $\Delta^{14}C$ measured in surface waters of the North Pacific between the equator and about 40°N latitude. However, the data from the single juvenile swordfish provide some indication that the habitats frequented by these life history stages are different. As stated above, the outer collagen datum from the juvenile swordfish is indicative of very high $\Delta^{14}C$ relative to ambient surface water levels. The lower $\Delta^{14}C$ measured in the outer collagen from the large swordfish may be the result of mixing due to vertebral deposition in both shallow and deeper waters in conjunction with the extensive vertical migration of this species. However, identifying the factors that could influence radiocarbon in the vertebrae of adult swordfish is complex and unlikely to resolvable without detailed data regarding the vertical and horizontal movements and diet of swordfish, data that are becoming available through archival and acoustical tagging studies.

Interpretation of these data is made even more complex by the lack of a relationship between the collagen and carbonate fractions of the vertebrae. This point is highlighted by the significant difference between both $\delta^{13}C$ and $\Delta^{14}C$ for collagen and carbonate from the same segments of each vertebra. The difference between $\delta^{13}C$ from carbonate and collagen is not surprising and clearly identifies a dominant metabolic source for carbon destined for collagen and an inorganic source for carbon that will ultimately go into the deposition of vertebral carbonate. Collagen fractions of vertebrae were more depleted in the heavier stable carbon isotope, $^{13}C$, than the carbonate (presumably francolite) fractions of vertebrae. Mean $\delta^{13}C$ for carbonate and collagen fractions of vertebrae was -4.2±1.2‰ and -15.5±1.0‰, respectively. The $\delta^{13}C$ for carbonate is within the range that might be expected for carbonate from otoliths, whereas $\delta^{13}C$ for collagen is similar to what would be expected in muscle tissue. This indicates that different proportions of carbon from multiple sources are involved in the production of the inorganic and organic fractions of the vertebrae with organic fractions deriving the bulk of carbon from metabolic sources. Furthermore, studies of otoliths have demonstrated that, at a daily level, calcium carbonate rich zones are deposited at different times from organic-rich zones. Similar process may be involved in the deposition of material in swordfish vertebrae and could play a role in the different $\Delta^{14}C$ measured in collagen and carbonate.

This study was initiated on the basis that interpretation of $\Delta^{14}C$ data from the inner portion of the vertebrae of large swordfish would provide an independent estimate of swordfish age. The data from the larger fish can be interpreted in relation to birth date; however, the results must be treated with caution for several reasons. Birth dates of around 1965 (ages of approximately 30 years) were estimated for two very large swordfish. A third moderately large fish was selected for analysis in order to determine if higher $\Delta^{14}C$ could be measured in vertebrae from a presumably younger fish with a presumed birth date closer to the peak in bomb radiocarbon. Unfortunately, $\Delta^{14}C$ measured in the vertebra this fish was very similar to that measured in the two larger swordfish and also yielded an estimated birth date of around

1965. This age does not seem plausible on the basis of the limited information that is currently available on swordfish age and growth.

During the preparation of vertebrae for radiocarbon analysis it became apparent that the composition of vertebrae differed among fish with the smaller fish seeming to have less dense vertebrae. This was tested on a sample of 24 female fish and clearly demonstrated that as female swordfish grow larger their vertebrae become denser. From a functional point of view there are two plausible interpretations of this phenomena. Firstly, as small juveniles swordfish appear to be surface dwellers and do not display the characteristics indicative of a deep diving. In larger juveniles and adults, however, there are dramatic changes in morphology and physiology (Palko et al. 1981, Carey 1982, Pepperell 2000) that indicate a change to the deep diving habits that have been well described through acoustic telemetry research (Carey and Robison 1981, Carey 1990). During this transition the swim bladder of swordfish is reduced to the point that it no longer functions as a gas-filled organ; such a structure would be a hindrance to the rapid vertical movements characteristic of this species. In fact, diving ability would be accentuated in a negatively buoyant fish, a characteristic of tunas as well as swordfish. Therefore, the increased density of the vertebrae may contribute to the overall increase in density as swordfish age and alter their feeding strategy.

A second explanation for the change in density of vertebrae may be related more directly to swimming. Significant increases in bone density are likely to occur through the deposition of additional bone mineral, hydroxyapatite, in place of the less dense collagen. Enhanced mineralisation leads to increased stiffness of the vertebrae (Currey 1984), an important property for large pelagic fish capable of high-speed swimming.

Changes in the density of the vertebrae are critical to the interpretation of the $\Delta^{14}C$ data obtained from the vertebrae. The change in density provides clear evidence that the vertebra is going through a process of reworking or remodelling during growth. As a result, the collagen and carbonate isolated from the inner segments of the vertebrae may have been deposited more recently due to reworking processes. The degree to which this reworking is associated with collagen or carbonate is difficult to determine, but it is possible that both materials are involved. As a result it seems likely that the vertebrae of swordfish violate a key assumption required for the successful application of the bomb radiocarbon chronometer, specifically that the material (carbonate or collagen) deposited in early life is not subject to resorption or reworking. This is different to the well-established finding that otoliths are not resorbed or reworked after deposition, a key attribute that makes them invaluable to research on the biology of fishes.

The ability to use measurements of radiocarbon in swordfish vertebrae to determine fish age appears to be limited due to the likelihood that swordfish vertebrae undergo significant reworking during development and growth. This result is very different from measurements of radiocarbon in the vertebrae of school shark which provided evidence that vertebral collagen is not reworked to any great extent, if at all (Chapter 10). Several school shark vertebrae yielded pre-bomb or early post-bomb values of $\Delta^{14}C$, indicating that these vertebrae retained collagen deposited several decades prior to death. The different results for swordfish and school shark suggest dramatic differences in the metabolism of bone in these distantly related fish species.

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Table 23.1. Sample pre-treatment methods to isolate inorganic and organic fractions of swordfish vertebrae prior to target preparation for accelerator mass spectrometry of radiocarbon.

| Sample No. | Sample type | Sample pre-treatment |
|---|---|---|
| 46i/col; 46o/col; 58i/col; 58o/col; 108o/col | inner vert; collagen | Chiselled and ground. Organic washes hexane-isopropanol-acetone 1 h each at room temperature. Demineralised with 0.5M HCl for 1 h. Vacuum dried. H3PO4 evolution of CO2. |
| 46i/car; 46o/car; 108o/car; 58i/car; 58o/car | inner vert; carbonate | Chiselled and ground. Organic washes hexane-isopropanol-acetone 1 h each at room temperature. Vacuum dried. H3PO4 evolution of CO2. |
| 108i/col | inner vert; collagen | Chiselled and ground. Ground to fine powder. Demineralised in 0.5M HCl for 1 h at room temperature. Vacuum dried. H3PO4 evolution of CO2. |
| 108i/car | inner vert; carbonate | Chiselled and ground. Ground to fine powder. H3PO4 evolution of CO2. |
| 94/col | whole vert; collagen | Ground to fine powder. Demineralised in 0.5M HCl for 1 h at room temperature. Vacuum dried. H3PO4 evolution of CO2. |

Table 23.2. *Xiphias gladius* fish, vertebra and radiocarbon data for specimens collected from the Hawaiian Islands longline fishery.

| Sample no. | Date of capture | Length (EFL, cm) | Weight (kg) | Sex | Sample location | Vertebra weight (g) | Pre-treatment sample weight (g) | Sample fraction | $\Delta^{14}C$ (‰) | $\delta^{13}C$ (‰,PDB) |
|---|---|---|---|---|---|---|---|---|---|---|
| 46i/col | 1 Apr 93 | 210 | 220 | F | inner vert | 58.2 | 4.4 | collagen | 91.2±9.1 | -15.7 |
| 46i/car | | | | | inner vert | | | carbonate | 67.0±9.5 | -3.5 |
| 46o/col | | | | | outer vert | | 1.3 | collagen | 80.8±9.0 | -16.4 |
| 46o/car | | | | | outer vert | | | carbonate | 84.3±9.2 | -5.5 |
| 108i/col | 30 Apr 92 | 219 | 180 | F | inner vert | 54.0 | 2.5 | collagen | 104.3±9.2 | -16.1 |
| 108i/car | | | | | inner vert | | 1.6 | carbonate | 87.0±9.0 | -3.0 |
| 108o/col | | | | | outer vert | | | collagen | 85.7±9.0 | -15.3 |
| 108o/car | | | | | outer vert | | | carbonate | 68.7±8.9 | -4.9 |
| 58i/col | 20 Apr 92 | 171 | 92 | F | inner vert | 19.8 | 1.4 | collagen | 84.1±9.5 | -16.7 |
| 58i/car | | | | | inner vert | | | carbonate | 54.4±9.8 | -2.0 |
| 58o/col | | | | | outer vert | | 0.9 | collagen | 88.9±9.2 | -15.8 |
| 58o/car | | | | | outer vert | | | carbonate | 20.8±8.9 | -6.1 |
| 94/col | 28 Apr 92 | 76.5 | 5.7 | F | whole vert | 1.6 (2 vert) | 1.5 (2 vert) | collagen | 123.8±9.4 | -13.9 |
| 94/car | | | | | whole vert | | | carbonate | 88.8±9.5 | -4.5 |

Table 23.3. Results of a balanced, fixed-factor ANOVA, with vertebrae nested within fish to determine if there were significant differences in vertebra density within swordfish compared to among swordfish.

| Source | df | SSQ | MSQ | F-value | Pr>F |
|---|---|---|---|---|---|
| Model | 8 | 1.3251 | 0.1656 | 5.77 | 0.001 |
| Error | 18 | 0.5171 | 0.2887 | | |
| Total | 26 | 1.8422 | | | |

| Source | df | ANOVA SS | MSQ | F-value | Pr>F |
|---|---|---|---|---|---|
| Vertebra within fish | 6 | 0.2352 | 0.0392 | 1.36 | 0.28 |
| Fish | 2 | 1.0899 | 0.5449 | 18.97 | 0.0001 |

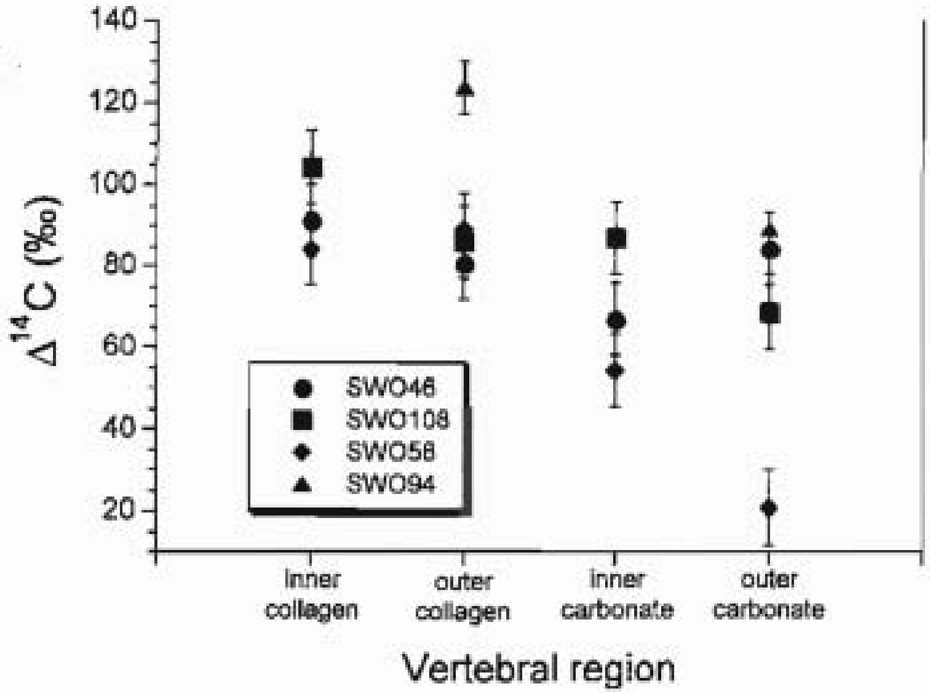


Figure 23.1. $\Delta^{14}C$ measured in different regions and from different constituents of vertebrae from four swordfish. Details of the four swordfish are in Table 23.2.

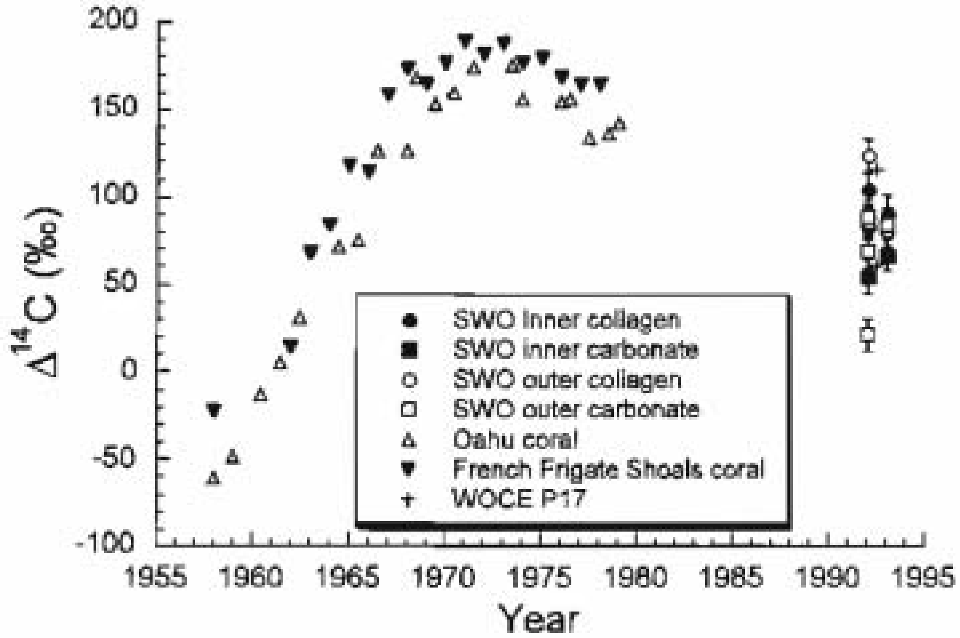


Figure 23.2. $\Delta^{14}C$ measured in different regions and from different constituents of vertebrae from four swordfish. The data are plotted with historical time series of radiocarbon variation in the tropical North Pacific Ocean determined from hermatypic corals collected at Oahu and French Frigate Shoals (Druffel 1987). The minimum and maximum values for surface water $\Delta^{14}C$ from between 10°N and 35°N latitude along the 135°W section (P17) of WOCE (World Ocean Circulation Experiment) are shown as a measure of more recent $\Delta^{14}C$. These data were collected during the early 1990s and are plotted here with at 1992.5.

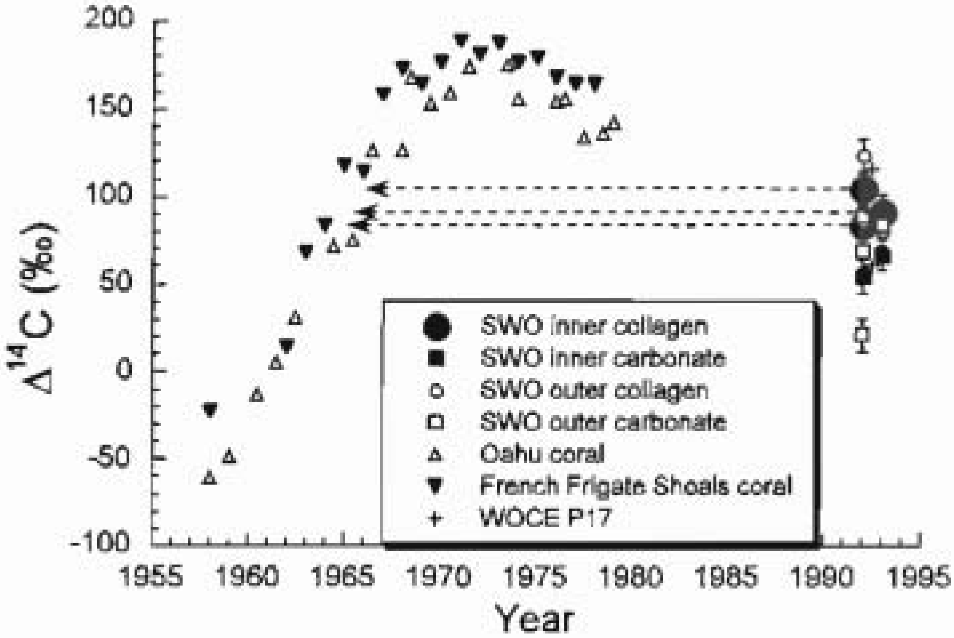


Figure 23.3. Same data as plotted in Figure 23.2, but with arrows to estimate graphically possible interpretation of the swordfish collagen $\Delta^{14}C$ data in relation to birth date. The arrowheads estimate the possible time of deposition (birth date) for collagen from the inner segment of the swordfish vertebra.

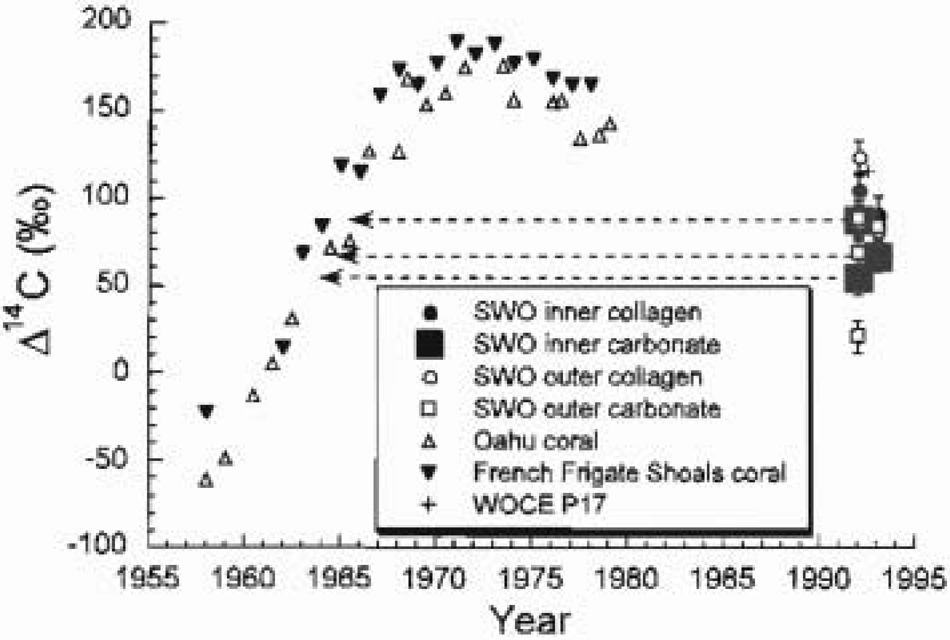


Figure 23.4. Same data as plotted in Figure 23.2, but with arrows to estimate graphically possible interpretation of the swordfish carbonate $\Delta^{14}C$ data in relation to birth date. The arrowheads estimate the possible time of deposition (birth date) for carbonate from the inner segment of the swordfish vertebra.

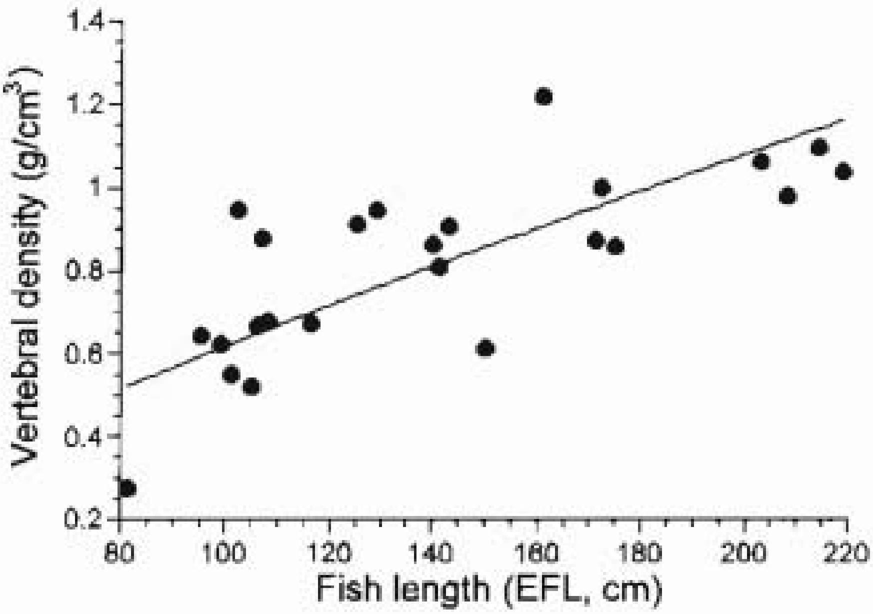


Figure 23.5. Relationship between swordfish length (eye-to-fork length) versus vertebra density (Vertebra density = $0.0218EFL^{0.7324}$; r2 = 0.632; n = 24). All fish in the sample were females.

# Chapter 24 Use of the bomb radiocarbon chronometer to validate age estimation methods for Bight redfish (*Centroberyx gerrardi*) from the Great Australian Bight

John Kalish

**Summary**

Measurements of radiocarbon in the cores of otoliths from presumably old Bight redfish confirm that this species lives to a maximum age of at least 35 years. Samples analysed were not suitable for a more detailed analysis of the accuracy of the otolith section method of age estimation. However, the appearance of opaque and translucent zones in thin sections of Bight redfish otoliths are very similar to those seen in redfish (*Centroberyx affinis*) otoliths. Therefore, it is extremely likely that accuracy similar to that for reading C. affinis will also be achieved for Bight redfish.

## Introduction

The Great Australian Bight Trawl Fishery (GABTF) extends from Kangaroo Island off South Australia (138o08′ E), to Cape Leeuwin in Western Australia (115o08′ E), encompassing an area of about 812 000 km2.. The fishery can be divided into a continental shelf fishery in depths of 200 m or less, and a slope fishery in depths between 200 m and 1000 m. Most shelf-waters trawling occurs in a narrow depth range—120–160 m—with deepwater flathead (Neoplatycephalus conatus) and Bight redfish (*Centroberyx gerrardi*) being the main target species (Tilzey In press).

The 1999 Bight redfish catch of 412 t was the highest since logbooks began in 1988 and landings remained comparatively high (317 t) in 2000. Mean annual catch rates rose to 47 kg/hr in 1999 and 46 kg/hr in 2000, continuing an improvement since 1995. Most redfish are usually taken as a bycatch of targeting deepwater flathead, but the increased landings from 1998 onwards suggest a shift towards targeting known redfish grounds. As noted above, most fishing effort occurred in the central zone where redfish are most abundant (Tilzey In press).

Bight redfish are believed to be a long lived species with longevities in excess of 50 years. They exhibit high variability in age among fish of similar size, such that commercial catches consist of fish 9 years to more than 60 years old. Age determination is by sectioned otoliths (Tilzey In press) and this method of age estimation requires validation for this species in order to reduce uncertainty in the stock assessments.

**Materials and methods**

Bight redfish otoliths were supplied by the Marine and Freshwater Resources Institute (MAFRI, Victoria). Age estimates were determined at the Central Ageing Facility (MAFRI) from otolith thin sections viewed with a stereo microscope and using transmitted light illumination.

Sample preparation procedures were similar to those described in Kalish (1995) for redfish (*Centroberyx affinis*) and other species analysed as part of this research. A total of five samples were analysed successfully for radiocarbon. Graphite target preparation and sample analysis was carried out at the Rafter Radiocarbon Laboratory, Instituted of Geological and Nuclear Sciences (Wellington, New Zealand). Radiocarbon values are reported as $\Delta^{14}C$, which is the age- and fractionation-corrected deviation (parts per thousand) from the activity of nineteenth century wood. Age corrections are based on the mean estimate of age determined from reading of otolith sections. Reported errors are 1 standard deviation for both radiocarbon data and age estimates based on the reading of otolith sections. Radiocarbon errors include both counting errors and laboratory random errors.

**Results and Discussion**

Five samples were analysed for radiocarbon and the results are presented in Table 24.1. Presumed sample ages were between 32 and 36 years with associated birth dates between 1962 and 1958. All radiocarbon results were indicative of pre-bomb radiocarbon levels with the highest $\Delta^{14}C$ -52.8‰ (Table 24.1)

Radiocarbon data from the region of the Great Australian Bight (GAB) are extremely limited, however, extensive data reported from this research for the oceans off the east and west coasts of Australia suggests that the time series of radiocarbon in the northern GAB is unlikely to be very different. On this basis, bomb radiocarbon is unlikely to be detected in the surface ocean of the region prior to about 1960. Therefore, these data provide conclusive evidence that Bight redfish have longevities of at least 35 years.

The narrow range of presumed ages selected for analysis does not make it possible to provide a more definitive indication of the accuracy of the age estimation procedure used for Bight redfish. However, the appearance of opaque and translucent zones in thin sections of Bight redfish otoliths are very similar to those seen in redfish (*Centroberyx affinis*) otoliths and it is extremely likely that accuracy similar to that for reading C. affinis (Kalish 1995) will also be achieved for Bight redfish.

**Acknowledgements**

We thank Sandy Morison and Simon Robertson (MAFRI) for providing the otolith samples used in this study.

**References**

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Table 24.1. Fish, otolith, and radiocarbon data for Bight redfish *Centroberyx gerrardi*.

| Sample No. | Laboratory and laboratory accession number | Collection location | Collection date | Otolith weight (g) | Sample weight (mg) | $\Delta^{14}C$ (‰) | Otolith section age (years) | Birth date (year) |
|---|---|---|---|---|---|---|---|---|
| 2 | RRL NZA7985 | Great Australian Bight | 31/1/94 | 1.23 | 14.7 | -58.6±7.9 | 32 | 1962 |
| 10 | RRL NZA7986 | Great Australian Bight | 8/3/94 | 1.233 | 14.2 | -65.0±7.6 | 33 | 1961 |
| 30 | RRL NZA | Great Australian Bight | 8/3/94 | 1.108 | 17.2 | -52.8±7.9 | 34 | 1960 |
| 42 | RRL NZA | Great Australian Bight | 8/3/94 | 1.296 | 15.4 | -65.6±8.1 | 36 | 1958 |
| 46 | RRL NZA | Great Australian Bight | 8/3/94 | 1.194 | 16.8 | -67.5±7.8 | 35 | 1959 |

# Chapter 25 Conclusions

This study provides conclusive support for comments made in a recent review of age determination and age validation methods which included the statement that: 'Bomb derived radiocarbon from nuclear testing provides one of the best validation approaches available for long-lived fishes' (Campana 2001). The method was first described in 1993 (Kalish 1993) and research by Kalish (1995) and Kalish et al. (1996a, 1996b) demonstrated that the method was applicable to a range of species. However, other researchers have been relatively slow to embrace the bomb radiocarbon chronometer with further applications of the method by Campana (1997), Campana and Jones (1998) and Baker and Wilson (2001). In each of these instances, it was concluded that the method was extremely well suited to age validation of long-lived and moderately long-lived fish species. Published applications of the method will increase dramatically in the near future due to several projects underway in North America and Europe that apply measurements of radiocarbon to studies of age validation.

The results of this intensive study of radiocarbon in calcified tissues demonstrate the broad application of the method and highlight the value of measurements of radiocarbon to investigations of fish biology and ecology. Details of the potential applications are indentified in the individual species summaries that follow. Furthermore, research presented here demonstrates the feasibility of applying radiocarbon dating to studies of extremely long-lived fish species (e.g. *Hoplostethus atlanticus*).

The project measured radiocarbon in 28 commercially important fish species with the primary objective to validate the preferred age estimation method for each species. The majority of measurements were made on otolith carbonate although vertebral collagen was analysed in two species (school shark and swordfish) and vertebral carbonate in one species (swordfish). The results of the analyses are summarised in Table 24.1 and in the text below.

Southern bluefin tuna (*Thunnus maccoyii*)
The growing otoliths of fish incorporate radiocarbon in concentrations that are equivalent to that found in ambient seawater dissolved inorganic carbon. Therefore, pulses of anthropogenic radiocarbon produced by the atmospheric detonation of nuclear weapons can ultimately be detected in otoliths. This study estimates the age of large southern bluefin tuna

*Thunnus maccoyii* using an age estimation procedure based on the determination of levels of bomb-derived radiocarbon in otoliths. Radiocarbon data from selected regions of southern bluefin tuna otoliths indicate that this species may reach ages in excess of 30 years. Furthermore, individuals that approach the asymptotic length are likely to be 20 years of age or older. The data agree generally with accepted models of southern bluefin growth, but show that these fish live longer than was believed previously. Comparisons between otolith section and bomb radiocarbon age estimates indicate that reading otolith sections is an effective method to estimate the age of larger southern bluefin. The presence of a significant number of individuals greater than 20 years of age in the southern bluefin population may alter estimates of natural mortality rates currently used in Virtual Population Analysis models for stock assessment of this species.

Redfish (*Centroberyx affinis*)

Validation of methods used to estimate fish age is a critical element of the fish stock assessment process. Despite the importance of validation, few procedures are available that provide unbiased estimates of true fish age and those methods that are available are seldom used. The majority of these methods are unlikely to provide an indication of the true age of individual fish, data that are best suited to the validation process. Accelerator mass spectrometry analyses of radiocarbon in selected regions of *Centroberyx affinis* otoliths, were used to validate the age estimation method for this species. Radiocarbon data from the otoliths of *Centroberyx affinis* with presumed birthdates between 1955 and 1985 described the increase in ocean radiocarbon attributable to the atmospheric detonation of nuclear weapons in the 1950s and 1960s. The results confirm the longevity of *Centroberyx affinis* and demonstrate the effectiveness of the bomb radiocarbon chronometer for the validation of age estimation methods.

Blue grenadier (*Macruronus novaezelandiae*)

Accelerator mass spectrometry was used to measure radiocarbon in the earliest formed portions of selected blue grenadier *Macruronus novaezelandiae* otoliths to provide a validation of fish age estimates based on the quantification of opaque and translucent zones in otolith thin sections. $\Delta^{14}C$ data from blue grenadier otoliths were compared with previous estimates of $\Delta^{14}C$ in seawater dissolved inorganic carbon at similar latitutes, longitudes, and depths to link variation in otolith $\Delta^{14}C$ to time. Minimum otolith $\Delta^{14}C$ was -76.9±7.7‰, indicative of pre-bomb radiocarbon levels below the surfaced mixed layer at latitudes where juvenile blue grenadier are found. When plotted versus fish age estimated from otolith sections, the majority of the $\Delta^{14}C$ data combined to define a curve indicative of the increase in bomb radiocarbon in temperate oceans of the Southern Hemisphere and indicates that age estimation procedures based on otolith thin sections are satisfactory for blue grenadier age. If otolith section age estimates were correct, peak otolith $\Delta^{14}C$ of 106.8±7.9‰ occurred during the late 1960s, earlier than expected. This may be a manifestation of an increase in mixed-layer depth associated with increased frequency of zonal westerly winds at this time.

School shark (*Galeorhinus galeus*)

Radiocarbon measured in school shark vertebrae provides strong evidence that age estimates determined from counts of presumed annual increments in stained vertebrae are gross underestimates of the true fish age. This result is reinforced by a comparison between the two independent estimates of fish age and vertebra weight. Vertebral growth is likely to be dramatically reduced or cease altogether when fish reach asymptotic length; as a result, shark vertebrae may not be well suited to estimation of age for larger and older individuals. Measurements of pre-bomb levels of radiocarbon in the earliest formed segments of school shark vertebrae provides evidence that elasmobranch vertebral tissue may be subject to limited reworking and, therefore, would be suitable for studies of temporal changes in vertebral composition as proxies for physiological and environmental changes experienced during the life of the shark.

Pink ling (*Genypterus blacodes*)

Measurements of natural and bomb-produced radiocarbon in the otoliths of pink ling (*Genypterus blacodes*) indicate that estimates of age based on otolith sections are accurate. The relationships between otolith section based age estimates and radiocarbon in otolith cores, and the radiocarbon calibration curve based on *Pagrus auratus* (Kalish 1993) was excellent and indicates that otolith based estimates are likely to very accurate. Furthermore, there was good agreement between otolith readers both within and among laboratories for those samples analysed for radiocarbon. This result supports the application of the age and growth model for ling presented in Smith and Tilzey (1995). The number of radiocarbon analyses, however, was inadequate to confirm the existence of a difference in growth rate between males and females.

King dory (*Cyttus traversi*)

Measurements of natural and bomb-produced radiocarbon in the otoliths of king dory (*Cyttus traversi*) indicate that estimates of age based on otolith sections are accurate. In addition, radiocarbon data from king dory otolith cores suggest that this species is likely to occur between about 45°S and 50°S latitude during the first year of life. King dory otoliths are relatively small and this study involved refinement of methods for the preparation of otolith calcium carbonate for radiocarbon analysis. The range of prepared otolith sample weights was 3.8 to 5.7 mg (mean=4.5±0.52 mg). Graphite targets prepared for radiocarbon analysis from these otolith samples contained less than 0.5 mg of carbon. This represents a significant advance in the use of the bomb radiocarbon chronometer for the determination of fish age and demonstrates the suitability of the method for species with small otoliths. Furthermore, improvements in sample preparation and the analysis of small samples will reduce the likelihood of contamination as discussed in the study of southern bluefin tuna age.

Blue-eye trevalla (*Hyperoglyphe antarctica*)

Increased catches of blue-eye trevalla (*Hyperoglyphe antarctica*) associated with targeted and non-targeted trawling have highlighted the urgent need to determine the status of blue-eye stocks in southeast Australia. It is not possible to estimate yields from these stocks until further information is obtained. Age estimation and the requisite age validation are a key element of the stock assessment process; however, a validated method of age estimation does not exist for trevalla. Although some research has been carried out on age estimation of trevalla there has been concern regarding the accuracy of ages assigned to this species. Measurements of natural and bomb-produced radiocarbon in the otoliths of blue-eye trevalla indicate that there are significant errors when trevalla age is estimated by reading otolith sections. Furthermore, there are significant differences in age estimates made by different otolith readers from different laboratories. It is necessary to carry out further research on age estimation procedures for blue-eye trevalla and to establish otolith reading protocols for the species. Given the difficulty inherent in reading blue-eye otoliths it will be important to ensure that there is agreement among laboratories involved in age estimation. This can be achieved through inter-laboratory calibration exercises. Further measurements of radiocarbon in trevalla otoliths are planned.

Patagonian toothfish (*Dissostichus eleginoides*)

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 $L\infty$ 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.

Black, smooth, spikey and warty oreos (Oreosomatidae)

Measurements of radiocarbon in the earliest formed segments of black, smooth, spikey and warty oreo otoliths were compared with age estimates based on counts of presumed annual increments in thin sections of the 'sister' otoliths for each fish. Measurements of radiocarbon in the earliest formed segments of otoliths from these four oreosomatid fishes provide varying degrees of validation for age estimates derived from otolith thin sections. $\Delta^{14}C$ data from otolith cores of smooth oreo plotted against otolith section birth date describes temporal changes in radiocarbon that are indicative of the bomb radiocarbon increase in the 1960s and provide evidence that otolith based age estimates for this species are the most accurate of the four species investigated. The results provide conclusive evidence of minimum potential longevities of 35, 35, 29 and 28 years for black, smooth, spikey and warty oreos, respectively. However, there is no qualitative change to the appearance of increments that might suggest that the periodicity of increment formation is altered for fish older than 35 years and far older ages for these species are supported on the basis of radiocarbon analyses in conjunction with counts of presumed annual increments. Relatively low $\Delta^{14}C$ in relation to time for all four species and, in particular, for smooth oreo provides strong support that young juvenile habitats of these oreosomatid fishes are far removed from the adult habitats. Large negative values for $\Delta^{14}C$ from the smooth oreo otolith cores indicate that it is extremely unlikely that the adult smooth oreos spent their juvenile lives in surface waters at mid-latitudes. The radiocarbon levels measured in the otoliths are consistent with those expected for a fish living in the surface mixed layer at about 65°S latitude. This conclusion is supported by the limited ecological data on smooth oreo. Radiocarbon data from black oreo otolith cores also indicates that these fish inhabit high latitudes, around 60°S latitude, as juveniles. Both spikey and warty oreos may also inhabit surface waters of the Southern Ocean based on $\Delta^{14}C$ measured in otolith cores. Further research is required to ensure effective management of these commercially important, but poorly understood fish species.

Orange roughy (*Hoplostethus atlanticus*)

This research demonstrates that it is feasible to estimate the age of orange roughy on the basis of radiocarbon dating of otolith carbon. Radiocarbon age decreased from the otolith core to the otolith edge and radiocarbon decay was detected in individual orange roughy otoliths. Radiocarbon dating of individual orange roughy otoliths is feasible. Measurements of $\Delta^{14}C$ made at the otolith edge are not significantly different from $\Delta^{14}C$ measured in seawater dissolved inorganic carbon at orange roughy depths near the time of sample collection. High precision radiocarbon dating of orange roughy otoliths suggests that these fish are long-lived with maximum ages of 100 years or more Two otoliths that were free of any analytical or contamination problems yielded age estimates of 165 ± 118 years and 192 ± 96 years. Age estimates from increment counts for these fish were 138 years and 154 years, respectively. Reservoir corrected ages ranged from 22 to 160 years. However, reservoir corrected age estimates are difficult to interpret due to variability in the radiocarbon reservoir in different orange roughy habitats. The age estimates are consistent with estimates based on increment counts in thin sections and with estimates of age from radiometric techniques in that they support the hypothesis that orange roughy are very long-lived. Samples collected from orange roughy living at depths of about 1000 m and as long ago as 1985 can be contaminated by bomb radiocarbon and this makes it extremely difficult to determine the absolute age of individual fish. It is unlikely that it will be possible to overcome this problem as orange roughy were not collected with any regularity prior to the early 1980s.

Red snappers and jobfish (Lutjanus spp.)

Lutjanids are important recreational and commercial fish species in tropical Australia. Contrasting results from biological studies have produced uncertainty in regard to the longevity and associated demographic parameters of these species and hence the potential for development in Australia's northern demersal fisheries. The use of the bomb radiocarbon chronometer in this study provided an independent and supplementary source of validation of the method of age estimation based on thin sections of otoliths of the four economically most important Lutjanus species from the central Great Barrier Reef. Measurement of radiocarbon in the cores of selected lutjanid otoliths supported longevities of at least 20 — 32 years estimated for these species in recent studies. Otolith $\Delta^{14}C$ varied from initially anticipated values based on a time series of $\Delta^{14}C$ derived from Great Barrier Reef corals with many results yielding $\Delta^{14}C$ considerably higher than that measured in corals. The variation in $\Delta^{14}C$ could be explained by existing knowledge of the early life history and ontogenetic habitat shifts of the species. Juveniles less than one year of age are found, to varying degrees, in coastal habitats that are influenced by freshwater runoff. Shallow freshwater habitats, notably lotic environments, display $\Delta^{14}C$ levels that are equivalent to atmospheric levels and, therefore, higher than that measured in marine environments. Varying degrees of mixing in nearshore habitats explains the $\Delta^{14}C$ series yielded from the otolith cores from the different lutjanid species. Variability in $\Delta^{14}C$ series both within and among species is attributable, in part, to marked variation in freshwater runoff prevailing during the first year of life of each specimen examined and differences in the habitats of individual fish. In addition to providing a validation of the thin section method of age estimation, the radiocarbon data collected from the otoliths provides insight into habitat selection by a range of lutjanid species.

Jobfish (Pristipomoides spp.)

A pilot study was undertaken to determine the feasibility of validating age estimates based on otolith sections for sharptooth jobfish (*Pristipomoides typus*) and goldbanded jobfish (*Pristipomoides multidens*) based on the bomb radiocarbon chronomteter. Analysis of radiocarbon in otolith cores from a small number of samples of these two species was unable to provide a validation of the age estimation method. Relatively low $\Delta^{14}C$ suggested influence of the South Equatorial Current on the habitats of juvenile jobfish or significant over estimates of fish age based on otolith sections. Complex oceanography in the region of the Arafura Sea and the potential influence of both the Indonesian Throughflow and the South Equatorial Current may make it difficult to validate age for these, and perhaps other, species from the Arafura Sea off the Northern Territory. $\Delta^{14}C$ is likely to vary seasonally and inter-annually in the region, based on the relative influence of these two water sources. Further research on the oceanography of the region and the early life history of these jobfish species (e.g. spawning season, juvenile habitats) would be required to resolve these issues.

Jack mackerel (*Trachurus declivis*)

This study demonstrates that the otolith 'break and burn' and thin section methods of age estimation provide reasonably accurate estimates of age for Australian and New Zealand jack mackerel. Jack mackerel can live to ages in excess of 20 years, however, New Zealand jack mackerel may be longer-lived than fish caught off south eastern Australia. Although this study demonstrates the effectiveness of the methods, further research may be warranted to provide a more detailed understanding of the accuracy of age estimation methods used for jack mackerel.

Silver trevally (*Pseudocaranx dentex*)

Estimates of silver trevally *Pseudocaranx dentex* age off southeast Australia were determined from transverse sections of 207 otoliths. Age estimates were repeatable and there was a high level of agreement between otolith readers. Silver trevally are relatively slow growing with no detectable difference in growth rate between males and females. Von Bertalanffy growth parameters for both sexes combined are $K=0.145$, $L\infty=50.7$ and $t0=-1.823$. Growth rates are slower for silver trevally in Australia when compared with New Zealand trevally. Bomb radiocarbon validation of the age estimation procedure based on thin sections of silver trevally otoliths determined that this method of age estimation is accurate. The high level of agreement between radiocarbon data from silver trevally and redfish (*Centroberyx affinis*) otoliths provides a more precise characterisation of radiocarbon levels off eastern Australia during the period 1975 to 1990 and increases the value of the bomb radiocarbon chronometer for age validation of younger fish with birth dates after peak radiocarbon levels. The standard length at 50% maturity was 20.9 cm for male and 20.3 cm for female trevally, considerably smaller than New Zealand trevally.

Banded morwong and jackass morwong (Cheilodactyladae)

High precision analyses of radiocarbon in small samples of otoliths from two morwong species provide good evidence to support the conclusion that age estimates based on quantification of presumed annual increments in otolith thin sections are accurate. This conclusion is well supported for banded morwong despite the small number of samples analysed and less so for jackass morwong. Further analyses are warranted to determine the accuracy of the routine age estimation method for these species, but emphasis should be placed on research that considers jackass morwong.

Broadbill swordfish (*Xiphias gladius*)

The ability to use measurements of radiocarbon in swordfish vertebrae to determine fish age appears to be limited due to the likelihood that swordfish vertebrae undergo significant reworking during development and growth. This result is very different from measurements of radiocarbon in the vertebrae of school shark which provided evidence that vertebral collagen is not reworked to any great extent, if at all (Chapter 10). The different results for swordfish and school shark suggest dramatic differences in the metabolism of bone in these distantly related fish species. There is a significant increase in the density of swordfish vertebrae during growth and this provides clear evidence that the vertebrae are going through a process of reworking during growth. As a result it seems likely that the vertebrae of swordfish violate a key assumption required for the successful application of the bomb radiocarbon chronometer, specifically that the material (carbonate or collagen) deposited in early life is not subject to resorption or reworking. The difference between $\delta^{13}C$ from carbonate and collagen in swordfish vertebrae clearly identifies a dominant metabolic source for carbon destined for collagen and an inorganic source for carbon with collagen more depleted in the heavier stable carbon isotope, $^{13}C$, than the carbonate fraction.

Bight redfish (*Centroberyx gerrardi*)

Measurements of radiocarbon in the cores of otoliths from presumably old Bight redfish confirm that this species lives to a maximum age of at least 35 years. Samples analysed were not suitable for a more detailed analysis of the accuracy of the otolith section method of age estimation. However, the appearance of opaque and translucent zones in thin sections of Bight redfish otoliths are very similar to those seen in redfish (*Centroberyx affinis*) otoliths. Therefore, it is extremely likely that accuracy similar to that for reading C. affinis will also be achieved for Bight redfish.

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| Scientific name | Common name | Validation status | Comments | Collaborators |
|---|---|---|---|---|
| *Galeorhinus galeus* | school shark | Not validated | Analysis of vertebral collagen provided conclusive evidence that age estimates based on counts of annuli in vertebrae greatly underestimated true fish age. | ANU, MAFRI |
| *Macruronus novaezelandiae* | blue grenadier | Validated | Thin section method of age estimation validated. $\Delta^{14}C$ data provide evidence that juvenile habitat is below the surface mixed layer. | ANU, MAFRI |
| *Genypterus blacodes* | pink ling | Validated | Thin section method of age estimation validated. | ANU, MAFRI |
| *Hoplostethus atlanticus* | orange roughy | Longevity supported | Radiocarbon dating method used, rather than bomb radiocarbon chronometer. Results support estimates of extreme longevity (>100 years). $\Delta^{14}C$ data show evidence of early penetration of radiocarbon to deepwater habitats. | ANU, MAFRI, NZ NIWA, NZ MOF, NZ FIB |
| *Centroberyx affinis* | redfish | Validated | Thin section method of age estimation validated. | ANU, MAFRI |
| *Centroberyx gerrardi* | Bight redfish | Longevity supported | Inadequate samples analysed for detailed validation, but maximum ages in excess of 35 years validated. | ANU, MAFRI |
| *Cyttus traversi* | king dory | Validated | Thin section method of age estimation validated. | ANU, MAFRI |
| *Allocyttus niger* | black oreo | Validated | Thin section method of age estimation validated. Extreme longevity of species demonstrated. Further research to determine accuracy of age estimation method is warranted. $\Delta^{14}C$ data provide evidence of juvenile habitat in surface waters of the Southern Ocean and south of adult habitat. | ANU, MAFRI, NZ NIWA, NZ MOF, |
| *Allocyttus verrucosus* | warty oreo | Validated | Thin section method of age estimation validated. Extreme longevity of species demonstrated. Further research to determine accuracy of age estimation method is warranted. | ANU, MAFRI |

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| *Neocyttus rhomboidalis* | spikey oreo | Validated | Thin section method of age estimation validated. Extreme longevity of species demonstrated. Further research to determine accuracy of age estimation method is warranted. | ANU, MAFRI |
| *Pseudocyttus maculatus* | smooth oreo | Validated | Thin section method of age estimation validated. Extreme longevity of species demonstrated. Further research to determine accuracy of age estimation method is warranted. $\Delta^{14}C$ data provide strong evidence of juvenile habitat in surface waters of the Southern Ocean (latitudes >60°S) and south of adult habitat. | ANU, MAFRI, NZ NIWA, NZ MOF, |
| *Epinephelus octofasciatus* | eight barred rockcod | Validated | Thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. | ANU, WA Fisheries |
| *Pseudocaranx dentex* | silver trevally | Validated | Thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. | ANU, NSW Fisheries |
| *Trachurus declivis* | jack mackerel | Validated | 'Break and burn' and thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. | ANU, Tasmanian DPIWE |
| *Etelis carbunculus* | ruby snapper | Validated | Thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. | ANU, WA Fisheries |
| *Lutjanus erythropterus* | crimson seaperch | Validated | Thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. $\Delta^{14}C$ data provide strong evidence of estuarine juvenile habitat with significant freshwater influence. | ANU, AIMS |
| *Lutjanus johnii* | fingermark seaperch | Validated | Thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. $\Delta^{14}C$ data provide strong evidence of estuarine juvenile habitat with | ANU, AIMS |

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| | | | significant freshwater influence. | |
| *Lutjanus malabaricus* | saddletail seaperch | Validated | Thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. $\Delta^{14}C$ data provide strong evidence of estuarine juvenile habitat with significant freshwater influence. | ANU, AIMS |
| *Lutjanus sebae* | red emperor | Validated | Thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. $\Delta^{14}C$ data provide strong evidence of estuarine juvenile habitat with significant freshwater influence. | ANU, AIMS |
| *Pristipomoides multidens* | goldband jobfish | Not validated | Provisional validation indicated thin section age estimates are not grossly over-estimated. Species unlikely to be long-lived (<25 years). Inadequate samples analysed for validation. Further research to determine accuracy of age estimation method is warranted. . | ANU, NT Fisheries |
| *Pristipomoides typus* | sharptooth jobfish | Not validated | Provisional validation indicated thin section age estimates are not grossly over-estimated. Species unlikely to be long-lived (<25 years). Inadequate samples analysed for validation. Further research to determine accuracy of age estimation method is warranted. . | ANU, NT Fisheries |
| *Lethrinus nebulosus* | spangled emperor | Validated | Thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. | ANU, WA Fisheries |
| *Cheilodactylus spectabilis* | banded morwong | Validated | Thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. Longevity (ages >40 years) confirmed. | ANU, MAFRI |
| *Nemadactylus macropterus* | jackass morwong | Validated | Provisional validation of thin section method of age estimation. Inadequate samples analysed for | ANU, MAFRI |

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| | | | validation. | |
| *Dissostichus eleginoides* | Patagonian toothfish | Validated | Thin section method of age estimation validated. Further research to determine accuracy of age estimation method is warranted. $\Delta^{14}C$ data provide unique results relevant to research on carbon flux in the Southern Ocean. | ANU, AAD, ANU, AFMA, Austral Fisheries, BAS, MAFRI, National Museum Paris |
| *Thunnus maccoyii* | southern bluefin tuna | Validated | Thin section method of age estimation validated. | ANU, CSIRO |
| *Xiphias gladius* | swordfish | Not validated | Analysis of vertebral collagen and carbonate provide provisional evidence for moderate longevity (<20years). Inadequate samples analysed for strong conclusions. Strong evidence for reworking of vertebral tissues during development. Further research required. Differential sources of carbon for vertebral collagen and carbonate determined from $\delta13C$ and $\Delta^{14}C$ data. | ANU, US NMFS |
| *Hyperoglyphe antarctica* | blue-eye trevalla | Not validated | Evidence of systematic underestimation of age based on otolith thin sections. Maximum ages significantly underestimated. $\Delta^{14}C$ data suggest a habitat below the surface mixed layer or to the south of adult habitat for fish less than 1 year of age. Further research on age estimation and life history required. | ANU, MAFRI |

# Chapter 26 Benefits

The outcomes of this research are of direct benefit to a number of State/Territory and Commonwealth fisheries. The species analysed in this research were from the Southern Tuna Fishery (1 species; Commonwealth), Eastern Tuna and Billfish Fishery and Southern and Western Tuna and Billfish Fishery (1 species) South East Fishery (12 species; Commonwealth), Southern Shark Fishery (1 species; Commonwealth), Heard and McDonald Islands Fishery and Macquarie Island Fishery (1 species; Commonwealth), Jack Mackerel Fishery (1 species; Commonwealth), Great Australian Bight Fishery (1 species; Commonwealth), Northern Territory fisheries (2 species), Queensland State fisheries (4 species); Tasmanian State fisheries (1 species)Western Australia State fisheries (3 species); The benefits to this diverse array of fisheries are derived from reduction in the uncertainties associated with stock assessments for the relevant species. In the majority of cases investigated the research program on radiocarbon in calcified tissues has provided conclusive validation of age estimation methods that are critical for the collection of age data from most fisheries. In several instances the research has provided clear evidence of shortcomings with current age estimation procedures and highlighted the need for refinement of these methods or identification of more appropriate approaches. Ultimately, these results will ensure more effective management of these fisheries.

In addition to these benefits, the research has provided new insight into the biology and ecology of many of the fish species under investigation. Information on life history, migration and movement and physiology have been gained from this research and these findings will serve to develop further avenues for research on these commercially important species.

Finally, this research will be of significant benefit to research on global change and prediction. Ocean radiocarbon data is an excellent indicator of ocean circulation with the radiocarbon 'injected' into the atmosphere from testing of atomic weapons acting as a tracer of both atmospheric and ocean circulation. The rate of deep ocean mixing is slow with deepwaters showing easily detected radioactive decay. The augmentation of surface ocean radiocarbon by atmospheric inputs from atomic weapons testing has accentuated the difference between surface ocean and deep ocean radiocarbon. This increased contrast allows for clearer delineation of the details of global ocean ventilation. A significant impediment to the application of this feature is the difficulty and expense associated with collection of time series of radiocarbon from a range of locations in the world's oceans. The radiocarbon data collected from this study will be provided to the Carbon Dioxide Information Analysis Center (CDIAC), a repository for carbon flux related data maintained by Oak Ridge National Laboratory (U.S. Department of Energy) and part of the Global Change and Data Information System (GCDIS). This will make the data readily available to other researchers involved in the development of models of ocean circulation and general circulation models (GCMs).

# Chapter 27 Further Developments

The results of this research have been reported to the relevant agencies and individuals involved with the broad range of species investigated. Interaction with those individuals interested in further application of the technique in Australia and overseas is ongoing. In addition, studies are underway to use radiocarbon data from known age fish to further our understanding of radiocarbon flux in the ocean and atmosphere. One study of this type was completed recently (Kalish et al. 2001) and this research will further our understanding of carbon flux and ocean circulation in Arctic seas. A large study entitled 'Bomb-Dated Growth Dynamics of Arctic Fishes' based on findings from this research has recently received support from the U.S. National Science Foundation and will increase our knowledge of polar environments. Several other projects, using the methods described and developed further here, are underway in North America and Europe.

The analysis of radiocarbon in fish otoliths provides further evidence of the value of these structures to a range of investigations of fish biology, ecology and environmental studies. These and other applications have provided further impetus for studies of otoliths to the extent that these structures are now used in research on fisheries, oceanography, global change, palaeoclimate, archaeology and palaeontology (e.g. Fossum et al. 2000).

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| **Species name** | **Collaboratoring organisations** | **Collaborators** | **Funding bodies** |
|---|---|---|---|
| *Galeorhinus galeus* | MAFRI | Terry Walker, Russell Hudson | ANU, FRDC |
| *Macruronus novaezelandiae* | MAFRI | Sandy Morison, Simon Robertson, David Smith | ANU, FRDC |
| *Genypterus blacodes* | MAFRI | Sandy Morison, Simon Robertson, David Smith | ANU, FRDC |
| *Hoplostethus atlanticus* | MAFRI, NZ NIWA, NZ MOF, NZ FIB | Sandy Morison, Simon Robertson, David Smith, Di Tracey | ANU, FRDC, NZ FIB, NZ MOF |
| *Centroberyx affinis* | MAFRI | Sandy Morison, Simon Robertson, David Smith | ANU, FRDC |

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|---|---|---|---|
| *Centroberyx gerrardi* | MAFRI | Sandy Morison, Simon Robertson, David Smith | ANU, FRDC |
| *Cyttus traversi* | MAFRI | Sandy Morison, Simon Robertson, David Smith | ANU, FRDC |
| *Allocyttus niger* | MAFRI, NZ NIWA, NZ MOF, | Sandy Morison, Simon Robertson, David Smith, Corey Green, Peter McMillan, Di Tracey | ANU, FRDC, NZ MOF |
| *Allocyttus verrucosus* | MAFRI | Sandy Morison, Simon Robertson, David Smith, Corey Green | ANU, FRDC, NZ MOF |
| *Neocyttus rhomboidalis* | MAFRI | Sandy Morison, Simon Robertson, David Smith, Corey Green | ANU, FRDC, NZ MOF |
| *Pseuodocyttus maculatus* | MAFRI, NZ NIWA, NZ MOF, | Sandy Morison, Simon Robertson, David Smith, Corey Green, Peter McMillan, Di Tracey | ANU, FRDC, NZ MOF |
| *Epinephelus octofasciatus* | WA Fisheries | Stephen Newman | ANU, FRDC |
| *Pseudocaranx dentex* | NSW Fisheries, NZ NIWA | Alastair Graham | ANU, FRDC |
| *Trachurus declivis* | Tasmanian DPIWE, MAFRI, NZ NIWA | Jeremy Lyle, Kyne Krusic-Golub, Sandy Morison, Peter Horn | ANU, FRDC |
| *Etelis carbunculus* | WA Fisheries | Stephen Newman | ANU, FRDC |
| *Lutjanus erythropterus* | AIMS | Mike Cappo, Stephan Newman | ANU, FRDC |
| *Lutjanus johnii* | AIMS | Mike Cappo, Stephan Newman | ANU, FRDC |
| *Lutjanus malabaricus* | AIMS | Mike Cappo, Stephan Newman | ANU, FRDC |
| *Lutjanus sebae* | AIMS | Mike Cappo, Stephan Newman | ANU, FRDC |
| *Pristipomoides multidens* | NT Fisheries | Julie Lloyd | ANU, FRDC |
| *Pristipomoides typus* | NT Fisheries | Julie Lloyd | ANU, FRDC |
| *Lethrinus nebulosus* | WA Fisheries | Stephen Newman | ANU, FRDC |

| | | | |
|---|---|---|---|
| *Cheilodactylus spectabilis* | Tasmanian DPIWE | Jeremy Lyle, Ray Murphy | ANU, FRDC |
| *Nemadactylus macropterus* | MAFRI | Sandy Morison, Simon Robertson, David Smith | ANU, FRDC |
| *Dissostichus eleginoides* | AAD, AFMA, Austral Fisheries, BAS, MAFRI, National Museum Paris | Dick Williams, Tim Lamb (AAD), Martin Scott, Stephanie Kalish (AFMA), Murray France (Austral Fisheries), Julian Ashford (BAS), Corey Green (MAFRI), Guy Duhamel (Paris) | ANU, AAD, FRDC |
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| *Xiphias gladius* | US NMFS | Edward de Martini | ANU, FRDC, US NMFS |
| *Hyperoglyphe antarctica* | MAFRI | Sandy Morison, Simon Robertson, David Smith | ANU, FRDC |

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