Ministry for Primary Industries
Manatū Ahu Matua

# Age determination of frostfish (*Lepidopus caudatus*) off west coast South Island

New Zealand Fisheries Assessment Report 2013/21

P.L. Horn

ISSN 1179-5352 (online)
ISBN 978-0-478-40595-8 (online)

April 2013

New Zealand Government

Growing and Protecting New Zealand

Requests for further copies should be directed to:

Publications Logistics Officer
Ministry for Primary Industries
PO Box 2526
WELLINGTON 6140

Email: brand@mpi.govt.nz
Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries websites at:
http://www.mpi.govt.nz/news-resources/publications.aspx
http://fs.fish.govt.nz go to Document library/Research reports

# Table of Contents

## EXECUTIVE SUMMARY

**Horn, P.L. (2013). Age determination of frostfish (*Lepidopus caudatus*) off west coast South Island.**

***New Zealand Fisheries Assessment Report 2013/21*. 24 p.**

An examination of length-frequency distributions and otoliths of frostfish (*Lepidopus caudatus*) off the west coast of South Island has enabled the estimation of von Bertalanffy growth parameters, separately by sex. The species can be aged by counting zones in whole otoliths, and although the otoliths are quite difficult to interpret, the ageing method has been verified up to age 4 years using distinct juvenile modes in length-frequency distributions. Frostfish are fast-growing and short-lived; most are more than 100 cm long by the end of their third year, and only about 1% of fish live longer than 7–8 years. This indicates an instantaneous natural mortality rate of about 0.6 $yr^{-1}$. Maximum estimated age for both sexes was 10.6 years. Females reach a larger size (about 150 cm) than males (about 135 cm).

A similar fast-growing and short-lived life history has been concluded for the same species in the Mediterranean Sea and central Atlantic Ocean, with a maximum age of 8 years recorded in those areas using the same whole otolith ageing method. However, it appears likely that northern hemisphere fish reach a larger asymptotic length than those in New Zealand waters.

A sample of 400 aged fish is likely to be adequate to estimate a precise commercial catch-at-age distribution (i.e., with a mean weighted c.v. across all age classes lower than 30%). The mean weighted c.v. for the FRO 7 fishery in 2007–08 is 13% (using 444 aged fish). The WCSI winter fishery catch data can be satisfactorily stratified into three depth strata. Any analyses of catch-at-age for the entire west coast New Zealand frostfish biological stock will probably need to include a time stratum to separate the distinct summer and winter fisheries.

# 1. INTRODUCTION

Frostfish (*Lepidopus caudatus*) is a benthopelagic trichiurid (Cutlassfish) occurring in the Eastern Atlantic, Indian Ocean and Southwest Pacific in depths of 50–1000 m (Nakamura & Parin 1993). In New Zealand, frostfish are found between latitudes 33°S and 49°S, but are most common off the west coasts of North and South Island between latitudes 37°S and 44°S in depths of 50–400 m (Anderson et al. 1998). Trichiuridae are represented by 32 species in 9 genera worldwide, but there are only three genera in New Zealand waters: *Lepidopus*, *Benthodesmus*, and *Aphanopus* (Paulin et al. 1989). *Benthodesmus* and some species of the related family Gempylidae, especially the false frostfish (*Paradiplospinosus gracilis*), are sometimes misidentified as frostfish. Worldwide, there are six species in the genus *Lepidopus*, but only *L. caudatus* is considered an important commercial fish species (Nakamura & Parin 1993).

Frostfish were introduced into the Quota Management System from 1 October 1998. The current TACC for all areas combined currently stands at just over 4000 t, but with most allocated to FMAs 7 (Fishstock FRO 7, 2623 t) and 8 (Fishstock FRO 8, 649 t). Landings have not exceeded 3000 t annually since 1998, although they did peak at 4400 t in 1991. Frostfish are predominantly taken as bycatch in target trawl fisheries for jack mackerel and to a lesser extent, hoki and barracouta (Bentley et al. in prep.).

Little research has been conducted on frostfish in New Zealand waters. Robertson (1980) identified spawning areas from eggs taken in plankton tows around New Zealand, and concluded that the adults probably congregate in late spring, and spawn during the summer and autumn over the mid to outer shelf. Bagley et al. (1998) summarised landings and biological information, and proposed four stocks for management purposes based on fish distribution and known spawning areas. A determination of age and growth by Knuckey (2001), based on zone counts in otolith thin sections, produced a maximum age estimate of 14 years, but a growth curve that was markedly different to those produced for the same species in the northern hemisphere (e.g., Demestre et al. 1993). The ageing method could not be validated. A comprehensive fishery characterisation and catch-per-unit-effort analysis, examining data from 1989–90 to 2009–10, was produced by Bentley et al. (in prep.).

Productivity parameters for New Zealand frostfish are uncertain (owing to the differences in estimated growth between New Zealand and northern hemisphere fish) and catch-at-age distributions have not been estimated. The work reported here re-investigates the age and growth of frostfish by examining otoliths and the series of length-frequency data, and estimates fishery catch-at-age for one year off the west coast of South Island. This report fulfils the reporting requirements for Objective 8 of Project MID201001B, "Routine age determination of hoki and middle depth species from commercial fisheries and trawl surveys", funded by the Ministry for Primary Industries. That objective is: "To age other species as required for validation of the ageing technique or for targeted studies to meet specific research requirements".

# 2. REVIEW OF AGE-RELATED STUDIES

Previous studies on the age or growth rates of frostfish have examined fish from the Mediterranean Sea (Molí et al. 1990, Demestre et al. 1993, D'Onghia et al. 2000), the eastern-central Atlantic Ocean (Tuset et al. 2006), and off the west coast of South Island (WCSI), New Zealand (Knuckey 2001). Von Bertalanffy parameters from all these studies are listed in Table 1.

The first published study of frostfish growth was a short report by Molí et al. (1990) based on fish caught by commercial trawl and longline off the northern Mediterranean coast of Spain. Note that Molí et al. (1990) measured total length (TL) rather than fork length (FL); Tuset et al. (2006) showed that, across all lengths, fork length can be converted to total length using a factor of 1.039. Otoliths were read whole against a black background and immersed in glycerol, using a compound microscope. Each otolith was read twice by different readers, and only readings in agreement (86%) were used in the subsequent analysis. The maximum recorded age was 8 years. Von Bertalanffy parameters were produced for the sexes separately, and for all fish combined. Molí et al. (1990) attempted to validate their ageing method by analysing the marginal state of otoliths over a year. They noted that the narrowness of the otolith margin often made it difficult to assess the presence of an opaque edge, and provided no marginal state data. However, they stated that “it seems that hyaline [translucent] rings are formed annually with a peak in October”.

Demestre et al. (1993) sampled trawl and longline fishery catches from the northern Mediterranean coast of Spain. Fish were measured to total length. Using a compound microscope, the whole otoliths were examined against a dark background, illuminated by reflected light, after being soaked in seawater for 24 h. Both the medial and distal faces of the otoliths were read, although the annual zones were found to be most easily distinguished on the distal surface. [Note: Demestre et al. (1993) referred to the medial and *caudal* sides of the otoliths. It is assumed here that *caudal* was reported in error, and that *lateral*, being the antonym of medial, was actually meant.] Two readers were used and the analysis included only data where there was complete agreement between readers (i.e., 79% of readings). Von Bertalanffy parameters were produced for the sexes separately, and for all fish combined. Females grew to a significantly larger size than males, but maximum estimated age (8 years) was the same for both sexes. Demestre et al. (1993) also estimated growth parameters based on length frequencies sampled over a 2-year period (using the ELEFAN method). They concluded that because estimates of growth based on otoliths and length frequencies were similar, then both methods were likely to be valid.

Frostfish from trawl and longline survey catches in the Ionian Sea off southern Italy were sampled by D’Onghia et al. (2000). Fish were measured to total length. The otoliths were immersed in a 30:70 glycerin-alcohol mix in a black dish, and examined using a stereo-microscope, with illumination by reflected light. Otoliths from fish shorter than 110 cm TL were examined untreated, while those from larger fish were ground with sandpaper (presumably on the medial surface to thin the otolith, though this is not stated). Otoliths were read twice independently by two readers, and agreement was reached on 85% of readings. D’Onghia et al. (2000) described the otoliths as having a large opaque nucleus, surrounded by annual rings, and illustrated this with a photograph of the presumed annual zones on the postrostrum of one otolith. Von Bertalanffy parameters were produced for the sexes separately, and growth was described as being ‘rather fast’ (i.e., greater than 40 cm per year) in each of the first two years. Maximum estimated ages were 7 years for females and 5 years for males, and females grew to a larger size than males.

Tuset et al. (2006) determined the growth of frostfish from off the Canary Islands in the eastern-central Atlantic Ocean, sampling commercial longline catches. Fish were measured to fork length. To find the best method for age determination using otoliths, several experienced readers interpreted growth rings from whole otoliths and from sections through the otoliths from a random sample of 20 individual fish. The between-method results were not presented, but it was stated that there were no differences between both reading methods, so age was subsequently estimated from whole otoliths. Otoliths were placed in a black dish with 70% alcohol and examined using a compound microscope, with illumination by reflected light. Two readers independently counted opaque zones in each otolith, and only agreed readings were accepted into the analyses (i.e., 79% of readings). Fish from age 1 to 7 years were found for females and from 2 to 5 years for males. Von Bertalanffy growth parameters were estimated for females (but not males, owing to a small sample size) and for both sexes combined.

Frostfish otoliths sampled from commercial trawl catches off the north-west coast of South Island, New Zealand, were examined by a reader at the Central Ageing Facility (CAF), Queenscliff, Australia (Knuckey 2001). Fish were measured to fork length. Otoliths were embedded in rows of 5 in blocks of polyester resin and three or four sections approximately 0.3 mm thick were cut through their centres with a rotary saw, using a blade 0.25 mm thick. Sections were mounted on microscope slides under cover slips with polyester resin. Sections were then viewed with transmitted light at ×32 magnification, and the section closest to the primordium was used for ageing. Knuckey (2001) described the observed growth zones as being relatively diffuse and difficult to interpret.

Consequently, all otolith sections were examined three times to determine a consistent interpretation of the structures presumed to be annual. Age estimates were obtained from 97% of the preparations. Estimated ages ranged from 1 to 14 years, with a mode at 6 years. Von Bertalanffy parameters were produced for the sexes separately, and for all fish combined.

A comparison of the available growth curves shows that the results from three of the four northern hemisphere studies are virtually identical (Figure 1). The remaining northern study (Molí et al. 1990) differs from the others only at the young end of the growth curve. The southern hemisphere study (Knuckey 2001) differs markedly from the other four in terms of both maximum age and the shape of the curve. Either frostfish growth differs markedly between the northern and southern hemispheres, or the otolith zones from these two areas have been interpreted differently.

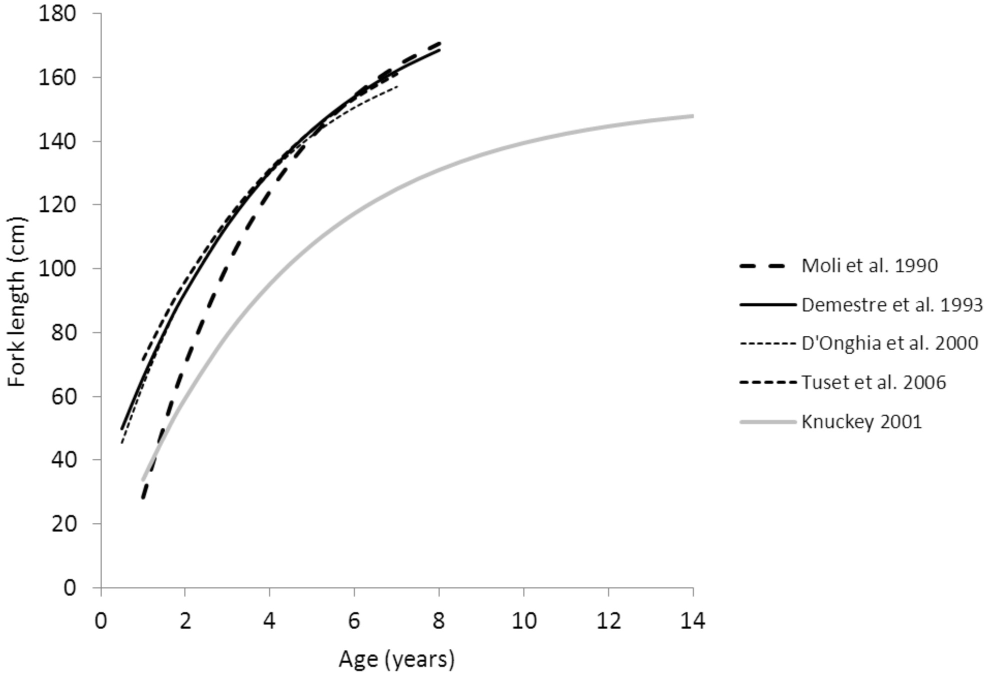

**Figure 1: Estimated otolith-based von Bertalanffy curves available in the literature. All curves are for both sexes combined, except for D'Onghia et al. (2000) where the female curve is presented because a combined curve is not available. (See Table 1 for curve parameters.)**

Knowledge of the spawning time is useful to help establish actual age at the time of formation of the first zone apparent in otoliths. In north-eastern New Zealand waters, Robertson (1980) concluded that adults probably congregate in late spring, and spawn during summer and autumn over the mid to outer shelf, although spawning began in spring in the Bay of Islands. Spawning has also been recorded off WCSI in March (Bagley et al. 1998). An analysis of data on female gonad stages from the scientific observer programme (Bentley et al. in prep.) suggests that frostfish off the west coast of both the North and South Islands have a protracted spawning period starting in mid-winter with a peak from summer to early autumn. Based on these data it is suggested that setting the 'birthday' for frostfish in New Zealand at 1 January would be appropriate. Northern hemisphere studies have also indicated that this species spawns during much of the year, but with peaks more prominent between spring and autumn (Demestre et al. 1993, D'Onghia et al. 2000, Tuset et al. 2006).

**Table 1: Available von Bertalanffy growth parameters for frostfish cited in the literature and from the current study, by sex and for sexes combined. Where necessary, the $L_\infty$ values have been adjusted so that all parameter sets in this table refer to fork length. Ages, age range of fish used to calculate the listed parameters; Lengths, length range (FL) of all fish aged; –, data not available. *, parameters derived from length-frequency analysis (all other lines of data are from age estimates derived from otolith zone counts).**

| Area | Male | | | | Female | | | | Sexes combined | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $L_\infty$ | $k$ | $t_0$ | Ages | $L_\infty$ | $k$ | $t_0$ | Ages | $L_\infty$ | $k$ | $t_0$ | Ages | Lengths | |
| NW Mediterranean | 178.2 | 0.333 | 0.34 | – | 188.1 | 0.207 | 0.32 | – | 190.8 | 0.298 | 0.46 | 1–8 | 25–179 | Molí et al. 1990 |
| NW Mediterranean | 194.2 | 0.226 | -0.92 | 1–8 | 234.7 | 0.142 | -1.63 | 1–8 | 192.5 | 0.238 | -0.76 | 0–8 | 25–189 | Demestre et al. 1993 |
| NW Mediterranean* | – | – | – | – | – | – | – | – | 238.0 | 0.28 | – | – | – | Demestre et al. 1993 |
| Central Mediterranean | 167.7 | 0.31 | -0.53 | 0–5 | 175.6 | 0.30 | -0.50 | 0–7 | – | – | – | – | 18–157 | D'Onghia et al. 2000 |
| Canary Islands | – | – | – | 2–5 | 186.9 | 0.23 | -1.23 | 1–7 | 191.2 | 0.23 | -1.04 | 1–7 | 82–188 | Tuset et al. 2006 |
| WCSI, New Zealand | 137.4 | 0.30 | -0.01 | 2–14 | 158.4 | 0.244 | 0.24 | 2–14 | 153.1 | 0.241 | -0.04 | 1–14 | 42–179 | Knuckey 2001 |
| WCSI, New Zealand | 129.2 | 0.560 | 0.08 | 0–10 | 143.5 | 0.457 | 0.04 | 0–10 | 137.0 | 0.505 | 0.07 | 0–10 | 17–179 | Current study |

# 3. METHODS

## 3.1 Analysis of length-frequency data

Given that most of the available ageing studies of frostfish indicate that the species has a fast rate of growth it was considered logical to examine series of comparable length-frequency distributions for consistent modes indicative of consecutive age classes. Based on the descriptive analysis of Bentley et al. (in prep.), it was apparent that two series could provide sufficient and consistent data: the inshore trawl survey series of WCSI and Tasman and Golden Bays carried out by R.V. *Kaharoa* during March–April, and the observer series from the WCSI trawl fishery where samples were taken predominantly from July to September (i.e., during the spawning hoki fishery).

Frostfish length data from each of seven surveys in the trawl survey series were grouped into 2 cm bins, by sex. Data from small (i.e., less than 80 cm FL) unsexed fish were included in both distributions. Unscaled raw data are presented. Distributions of data, by sex, from all surveys combined are also presented.

Length data collected by observers off WCSI were, for each year from 1999 to 2010, scaled to the total commercial catch as described by Bentley et al. (in prep.). However, the data presented here were grouped into 2 cm bins, by sex. Data from small unsexed fish were included in both distributions. Distributions of data, by sex, from all sampled years combined are also presented.

## 3.2 Otolith-based ageing

Otoliths collected from FRO 7 during the winter trawl fisheries for hoki, jack mackerels, and barracouta were used in this study. Otoliths from the most comprehensively sampled year (1999–2000) had already been aged (Knuckey 2001). An examination of the temporal distribution of the observer length and otolith samples showed that there were two other years (2002–03 and 2007–08) when more than 400 otoliths were available from June–September. The length ranges from these two years were 74–165 cm and 72–167 cm, respectively. Only the (previously prepared) 1999–2000 sample includes fish shorter than 70 cm (i.e., to 42 cm), and it also held fish up to 179 cm. We chose to age about 400 FRO 7 otoliths from June–September 2008 (i.e., the 2007–08 fishing year), plus an additional 62 otoliths from short and long fish 1999–2000 (‘sister’ otoliths to some of those aged previously by Knuckey (2001), and some otoliths from small fish sampled from the *Kaharoa* survey (kah0004)) to extend the available length range.

This selection of otoliths was intended to:

- Provide sufficient age data to produce comprehensive growth curves, by sex, using the full length range of the species in New Zealand waters,
- Allow some direct between-reader comparison with the data from Knuckey (2001),
- Enable the estimation of the 2007–08 FRO 7 commercial catch-at-age, and determine whether a sample of approximately 400 ages produces a suitably precise estimated age distribution.

All otoliths were immersed in water for at least 10 minutes in a black dish, and examined using a stereo-microscope at ×30 magnification, with illumination by reflected light. Large otoliths (i.e., those from fish longer than about 120 cm) sometimes required up to a 30 minute soak time to optimally clarify the zonation pattern. [It was later found that immersing the otoliths in paraffin oil rather than water markedly reduces the soak time necessary to clarify the zonation pattern.] Counts of translucent zones (that appeared dark under the described illumination regime) were made on the distal surface of the otolith. The zonation pattern was generally clearest on the ventral edge of the otolith (particularly for fish older than three years), but was also frequently clear on the postrostral (posterior) tip, and sometimes on the rostral tip. Radial measurements from the nucleus to the outer edge of each of the translucent zones were made, when the zones were clear, along the posterior axis of each aged otolith.

Widths of the complete first and second zones were also recorded transversely across the nucleus in some whole otoliths.

A subsample of 50 otoliths covering the full length range was selected for re-reading by the same reader. The index of average percentage error (IAPE, Beamish & Fournier 1981) between the two sets of readings was calculated and age-bias plots (Campana et al. 1995) were prepared.

In addition, 50 ‘sister’ otoliths from the 2007–08 observer sample were selected and prepared for reading as thin sections. These otoliths were embedded untreated in epoxy resin, and a section about 300 µm thick was taken transversely across the otolith through the nucleus using a diamond-edged rotary saw. The sections were mounted on microscope slides using epoxy resin, and were ground on a carborundum wheel until they were about 250 µm thick. The sections were then coated with paraffin oil and examined under a stereo-microscope at ×30–40 magnification, with illumination by transmitted light. These readings were compared with those obtained from the whole sister otoliths. An IAPE between these two sets of readings was also calculated.

The whole otolith readings derived from the sister otoliths that had been sectioned and aged by the CAF reader (Knuckey 2001) were also compared.

The length-at-age data were fitted using the von Bertalanffy growth model:

$$L_t = L_\infty \, [1 - \exp(-K*(t-t_0))]$$

where $L_t$ is the expected length at age $t$ years, $L_\infty$ is the asymptotic maximum length, $K$ is the von Bertalanffy growth constant, and $t_0$ is the theoretical age at zero length. Growth curves were fitted separately for males, females, and both sexes combined using the nonlinear least squares procedure in the R statistical package (R Development Core Team 2008), and assuming a lognormal error structure of age at length.

## 3.3 Estimating catch-at-age

The FRO 7 fishery was stratified using observer sampled catch data from fishing years 1999–2000 to 2010–11. An additional analysis using data from the FRO 7, 8 and 9 fisheries combined was also completed because Bentley et al. (in prep.) concluded that these three FMAs encompassed a single biological stock. Essentially, the mean length of the frostfish measured in a sampled tow was calculated, and then each tow (as defined by mean fish length) was allocated to a stratum using the following covariates that are generally available in both the observer and catch effort data:

| | |
|---|---|
| month | a `factor` variable |
| statistical area | a `factor` variable |
| target species | a `factor` variable |
| latitude | |
| longitude | |
| bottom depth | |

The classification tree method (Breiman et al. 1984) was used to derive a classification tree that is defined by suitable splitting variables and whose leaves have similar proportions within each stratum and diverse proportions between strata. A classification tree was fitted to the observer sampled catch data using the `rpart` package in `R` (R Development Core Team 2008). The splitting criterion (impurity measure) at any node of the tree was the Gini index, which is the default setting for fitting a classification tree.

Catch-effort data for frostfish from the west coast fishery in 2007–08 were obtained from the Ministry for Primary Industries (extract #8570), and each fishing event was allocated to one of the strata defined as described above. Observed and total (from catch-effort data) catches were examined for spatial and temporal variability to determine whether the sampling adequately represented the fishery. The observed data, which had already been stratified, were scaled up to the total catch for each stratum using the NIWA catch-at-age software (Bull & Dunn 2002). The age data from 2007–08 only were used to construct an age-length key which in turn was used to scale the weighted length composition of the catch up to catch-at-age by sex using the NIWA catch-at-age software (Bull & Dunn 2002). This software also provides estimates of c.v.s-at-age using a bootstrap procedure.

# 4. RESULTS

## 4.1 Analysis of length-frequency data

The length distributions from the March–April WCSI inshore trawl surveys varied quite markedly between surveys (Figure 2). However, some modes at relatively consistent lengths throughout the series were apparent, e.g., a mode between 10 and 30 cm, and another between 50 and 70 cm. The modes were most clearly depicted in the length distributions combining data from all surveys (Figure 2, bottom panel). For males, the modal peaks were at 17, 59, 93, and 109 cm; for females they were at 17, 59, 95, and 117 cm. The most likely explanation for these modes is that they represent consecutive age classes, and this is supported by the decreasing difference between the modes as length increases (as occurs in fish growth). The difference between the first and second modes (59–17 = 42 cm) is the average amount of growth that a fish starting at 17 cm FL would experience in one year. Clearly, given an annual growth of 42 cm, the mode at about 17 cm comprised fish less than 1 year old. Taking the assumed birthday for frostfish (1 January) and the mid-time of the survey series (about 31 March), it was deduced that frostfish are about 17 cm FL at an age of 0.3 years. The subsequent length modes are each one year later, i.e., for males, 59 cm at 1.3 years, 93 cm at 2.3 years, and 109 cm at 3.3 years.

The length distributions for frostfish caught by commercial trawlers off WCSI primarily from July to September each year are shown for males (Figure 3) and females (Figure 4) separately. Consistent modes in yearly samples were not as apparent as they are for the trawl survey series, but when data from all years are combined a clear pattern emerges. For males, there were clear modes at 72 and 98 cm, with weaker subsequent modes at 114 and 123 cm. For females, the modes were at 71, 103, 114, and 128 cm. Given that the samples and the fishery are centred around August, these modes will represent mean lengths-at-age about 0.6 years after the assumed birthday. The modal length of 71 cm is between the lengths of 59 cm and 93–95 cm that were deduced from the trawl survey series to comprise 1.3 and 2.3 year old fish. It is concluded, therefore, that the 71 cm mode comprises fish about 1.6 years old, and that the subsequent modes in the commercial fishery data are each one year later, i.e., for males, 98 cm at 2.6 years, 114 cm at 3.6 years, and 123 cm at 4.6 years.

The length mode analysis is clearly indicative of species with fast growth, at least for the first four years.

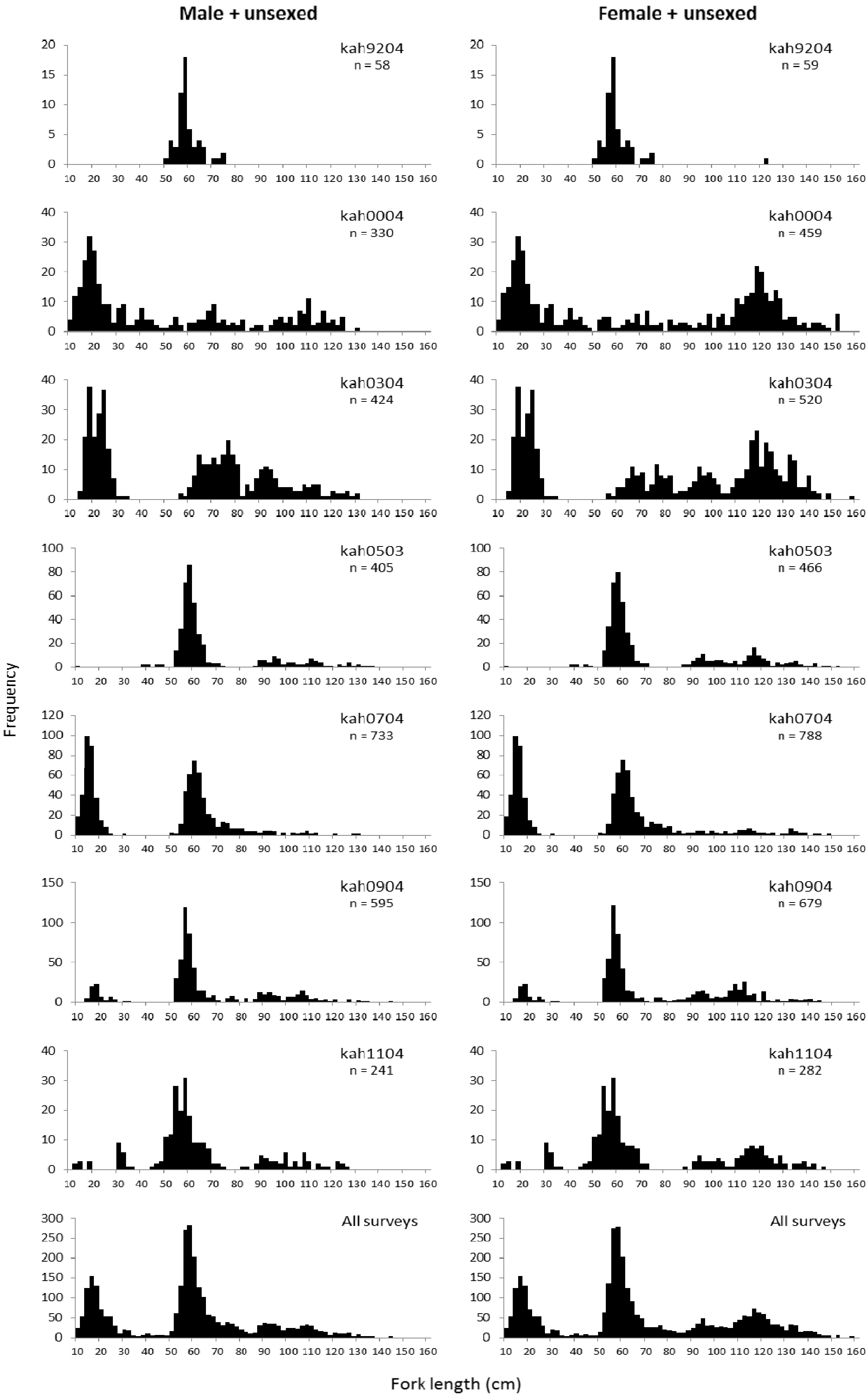

**Figure 2: Unscaled length frequencies of measured frostfish (male plus unsexed, female plus unsexed) from the west coast South Island *Kaharoa* trawl surveys from 1992 to 2011. n, number of fish measured.**

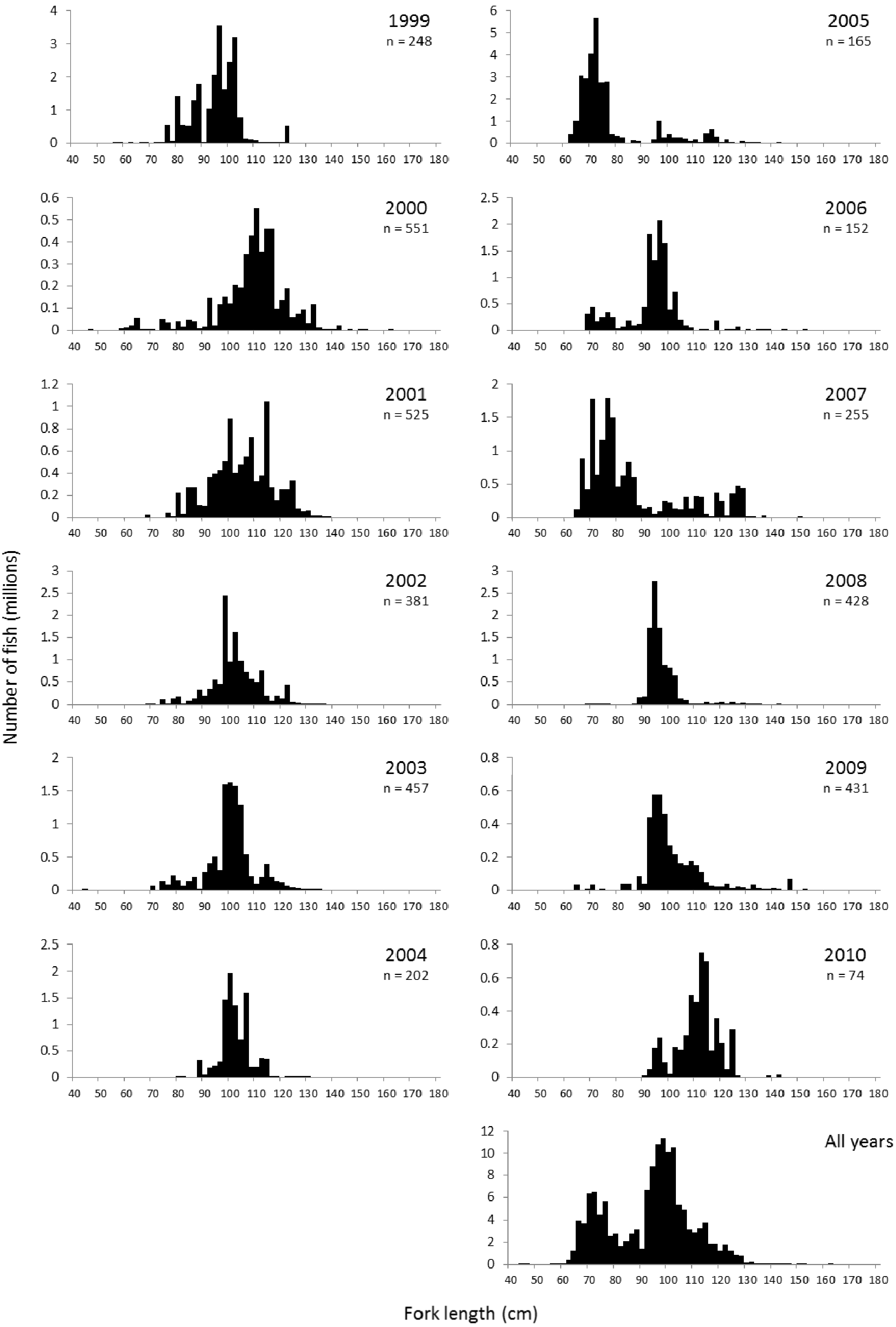

**Figure 3: Scaled length frequencies of frostfish (male plus unsexed) sampled by observers in commercial catches from off the west coast South Island (primarily during July–September) from 1999 to 2010. n, number of fish measured.**

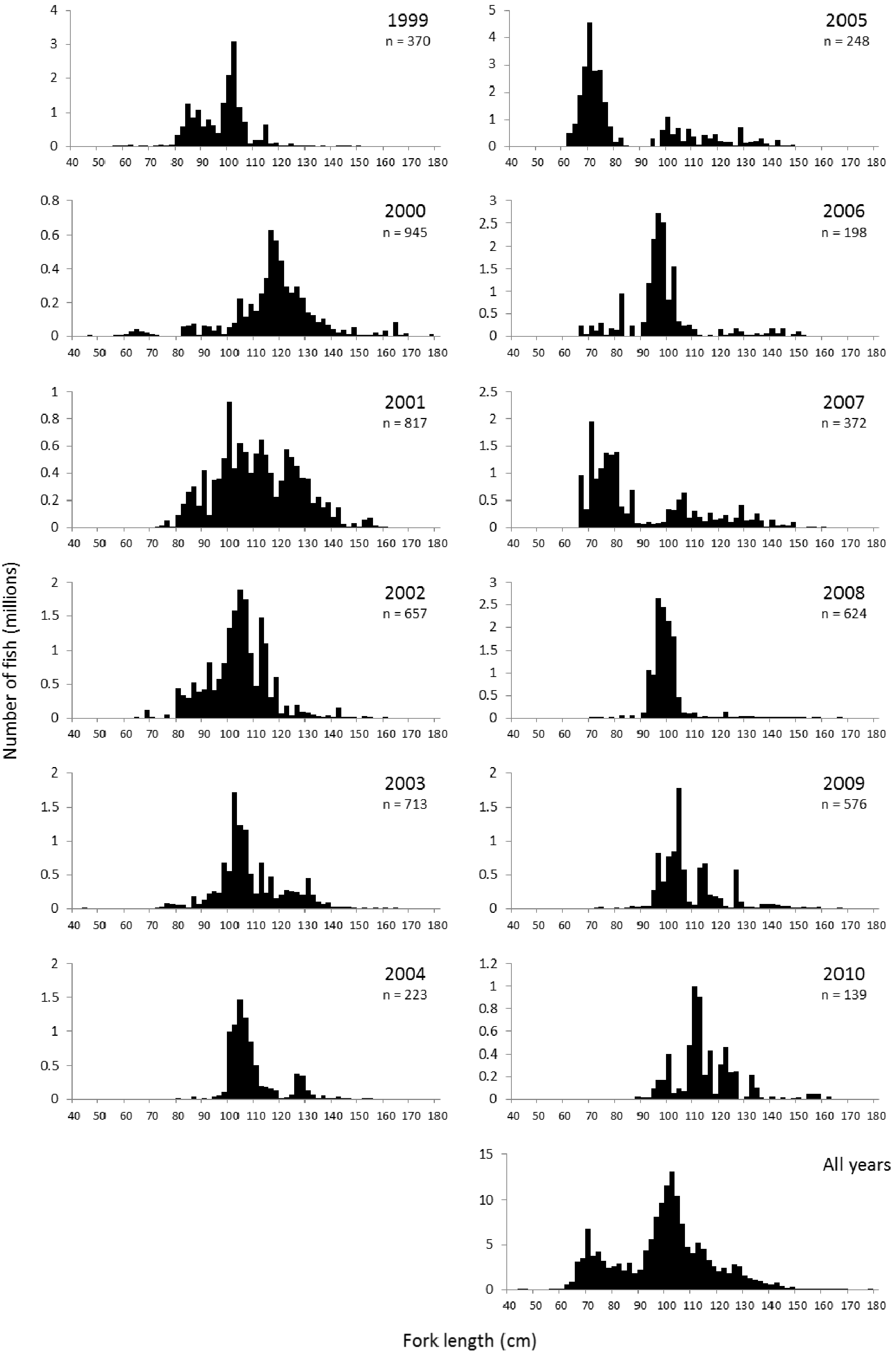

**Figure 4: Scaled length frequencies of frostfish (female plus unsexed) sampled by observers in commercial catches from off the west coast South Island (primarily during July–September) from 1999 to 2010. n, number of fish measured.**

## 4.2 Otolith interpretation

Frostfish otoliths are not easy to interpret. Some sample otolith images are presented in Appendix A. Growth in fish length is very rapid in the first two years, and the concurrent otolith growth is also fast. The otolith material formed at this time comprises a complex pattern of multiple light and dark zones, with the 'true' annual zones often being difficult to clearly distinguish (Figures A1 and A2). However, the analysis of length-frequency data described above had identified length modes for which ages could be reliably deduced. Otoliths from fish in the 0+, 1+, and 2+ modes were examined (with the reader aware of the likely age of the fish) to help determine the correct interpretation of the zonation pattern in the first two years of growth. Some preliminary estimates of the longitudinal and transverse distances to the outer edges of the first and second translucent annual zones were also made at this stage. Zones from age 3 onwards were generally much more distinct and less complex than the first two annual zones (Figures A3 and A4).

Otoliths were subsequently examined by a single reader who read all the whole otoliths first, then the 50 transverse sections, then 65 whole otolith repeat readings. Age bias plots depicting comparisons of paired age estimates are shown in Figure 5. The estimate of within-reader average percentage error for whole otolith readings was low (2.8%), and there was no bias in the reading differences, indicating that the interpretation method was consistent between readings. The comparison of readings from whole otoliths with those from transverse sections also had a low IAPE (3.0%) with unbiased differences, so it is likely that whole otoliths of frostfish can be used to produce satisfactory age estimates, at least to age 8 (see Figures A4 and A5).

The comparison between the whole otolith method described here and the transverse section method used by CAF (Knuckey 2001) is not comprehensive in that only the readings from very small and very large fish were compared, and 36 of the 46 compared fish were aged 1 by the NIWA reader. However, it is clear that the two interpretation methods are different, with the CAF method producing higher (often much higher) ages than the NIWA method.

Measurements to the outer edge of the first six presumed annual zones on the longitudinal posterior radius are listed in Table 2. The relationship between fish length (FL, cm) and posterior otolith radius (R, mm) is: $R = 0.0235\ FL + 0.427$ ($r^2 = 0.89$, $n = 468$, length range = 17–179 cm).

**Table 2: Mean measurements (mm) along the longitudinal posterior radius (nucleus to the outer edge of the translucent zone) and across the transverse diameter (through the nucleus to the outer edges of the translucent zone) of frostfish otoliths. *n*, number of measurements; s.d., standard deviation.**

| | Annual zone | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Longitudinal radius (mm) | 1.71 | 2.45 | 2.84 | 3.14 | 3.34 | 3.48 |
| *N* | 393 | 369 | 82 | 33 | 14 | 6 |
| s.d. | 0.10 | 0.12 | 0.13 | 0.11 | 0.14 | 0.08 |
| Transverse diameter (mm) | 1.21 | 1.67 | | | | |
| *N* | 87 | 36 | | | | |
| s.d. | 0.10 | 0.10 | | | | |

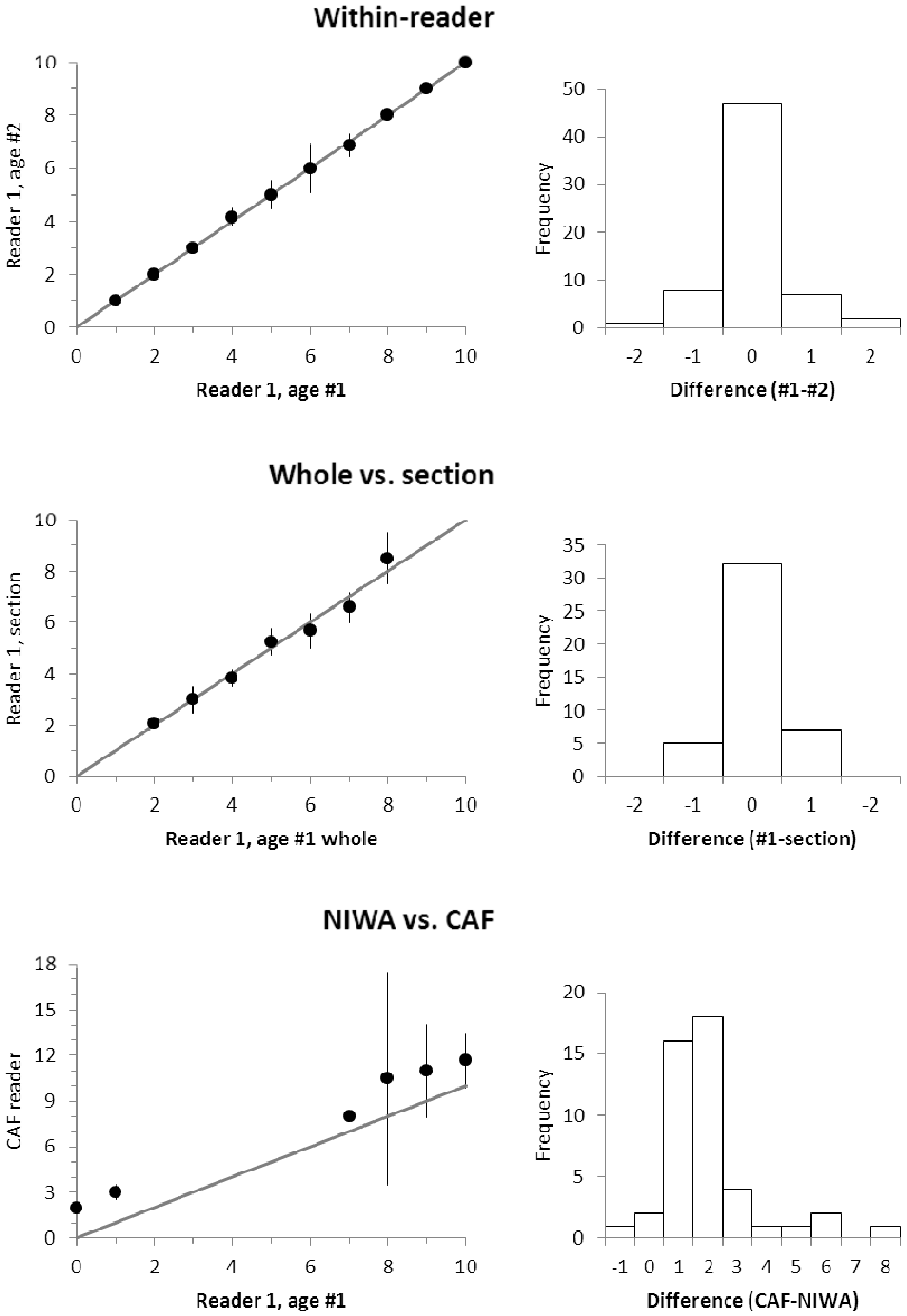

**Figure 5: Results of reading comparison tests: (left column) differences between ages estimated during each reading relative to the result of Reader 1's first readings; and (right column) histogram of differences between the ages estimated during each comparison. The error bars on the plots are 95% confidence intervals about the mean age produced during the second set of readings for a given age produced during the first set of readings. Comparisons are: (top panel) first and second readings from whole otoliths (*n* = 65); (middle panel) readings from whole otoliths and cross-sectioned sister otoliths (*n* = 44); (bottom panel), Reader 1's readings from whole otoliths and CAF readings from sectioned sister otoliths (*n* = 46).**

## 4.3 Age-length data and growth estimation

All of the raw age-length data derived from the first reading of whole otoliths are plotted in Figure 6. The maximum estimated age was 10.6 years for both males and females, although few fish were older than 8 years. The commercial catch off WCSI in winter comprises fish aged mainly from 1.6 to 4.6 years old. The estimated ages of the length modes derived as described above are also plotted on Figure 6 and appear to match well with the zones counts obtained from whole otoliths.

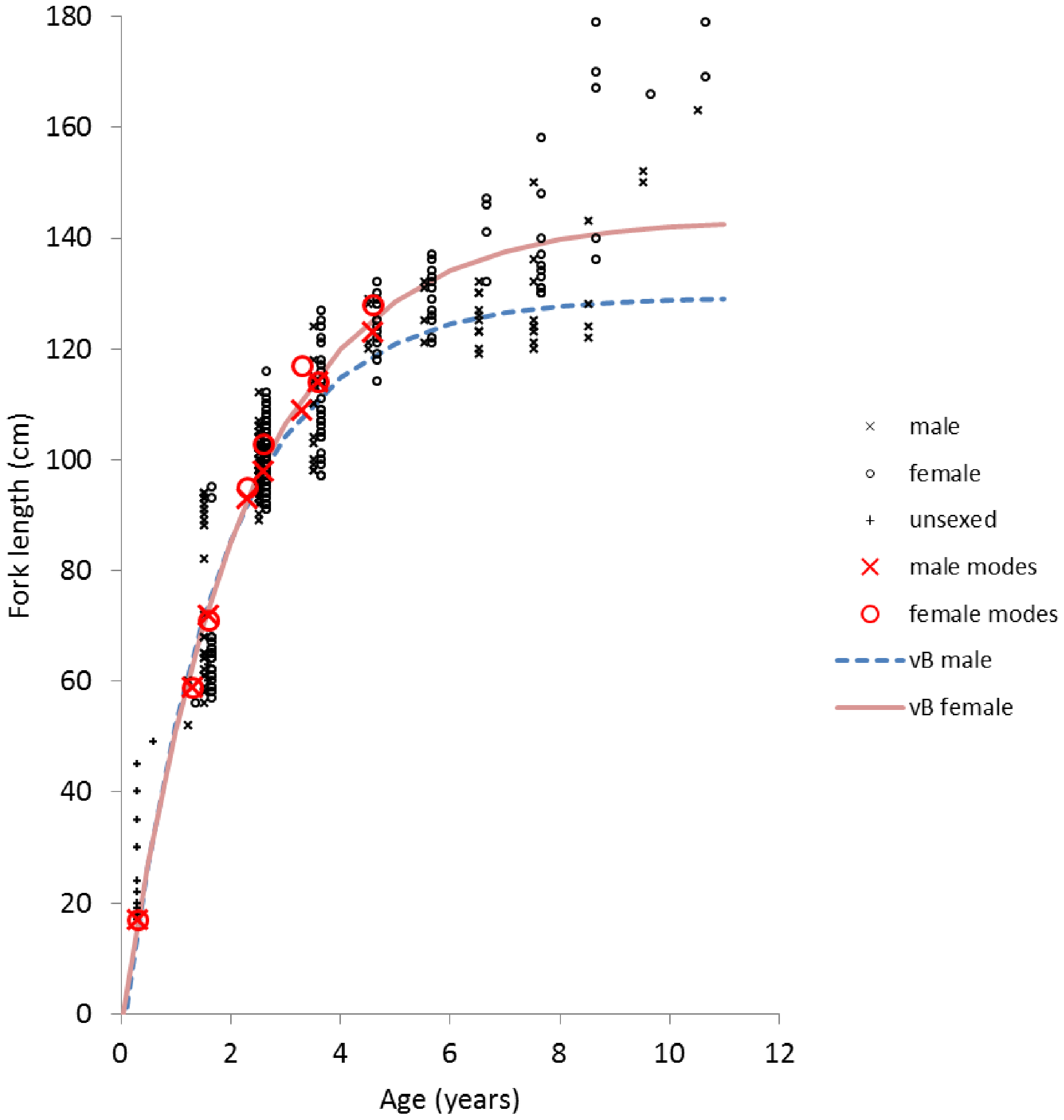

**Figure 6: Raw age-length data (small black symbols; male and female points have been adjusted horizontally to allow visual differentiation), length modes derived from section 4.1 (large red symbols), and estimated von Bertalanffy curves.**

The raw data points do not represent a random sample from the population or the fishery. The sample of otoliths from the 2008 fishery was chosen to represent all length classes, resulting in over-sampling of the smallest and largest fish. The fish aged from the 2000 fishery sample were only the smallest and largest individuals. The sample of juveniles from the kah0004 survey was chosen to comprehensively cover the length range of fish shorter than 60 cm FL. Consequently, if the von Bertalanffy model was fitted to the raw data, the over-sampling of large fish would produce $L_\infty$ values that are biased high (and therefore, inappropriate $K$ values as well).

Age-length data more appropriate to define a growth curve applicable to the population were derived in the following way. The scaled length-frequency distributions from the commercial fishery in all sampled years combined (i.e., the bottom distributions in each of Figures 3 and 4) were converted into estimated numbers at age and length (in 2 cm length bins), by sex, using sex-specific age-length keys derived from the first reading of all otoliths from 2000 and 2008. Sex-specific von Bertalanffy curves were then fitted to the resulting mean lengths at age (weighted by fish number) for all fish aged 4.6 years or older, plus the seven aged length modes per sex from ages 0.3 to 3.6 years (as derived in section 4.1). The curves are plotted in Figure 6 (with the parameters listed in Table 1) and appear to fit the available data well (with the exception of the non-random data from the very large fish).

A comparison of the available growth models derived from northern and southern hemisphere studies is depicted in Figure 7. The curve derived from the current work differs markedly from that estimated by Knuckey (2001) in the previous analysis of New Zealand frostfish. However, it is similar (up to about age 4) to curves obtained from fish in the Mediterranean Sea and eastern Atlantic Ocean.

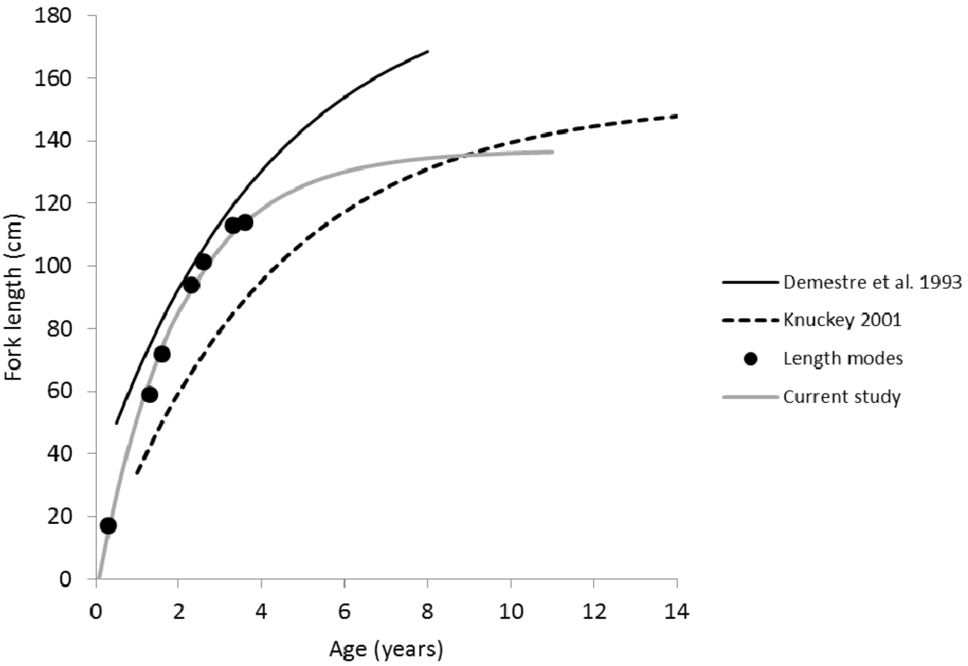

**Figure 7: Estimated von Bertalanffy curves (sexes combined) for frostfish from the Mediterranean (Demestre et al. 1993), and New Zealand (Knuckey 2001, and the current study). The seven length-frequency modes (average of males and females) derived from the current analysis are also plotted. (See Table 1 for curve parameters.)**

## 4.4 Catch sampling

The classification tree produced for frostfish from FRO 7 is shown in Figure 8. The analysis used data from 932 observed tows and 12 715 measured fish. Large fish predominated east of longitude 172.6°E (essentially those parts of Statistical Areas 037–040 in FMA 7), and fish west of that line were separated by depth at 401.5 m. When fish from the entire west coast biological stock were combined (i.e., FRO 7, 8, and 9, 1212 observed tows, 17 849 measured fish), the classification tree was very similar to that for FRO 7 alone (Figure 8, Table 3). The same depth stratification boundary was retained (401.5 m), but the previous longitudinal separation that had separated off the shallow waters in the South Taranaki Bight was replaced by a depth boundary at 147.2 m. The larger fish tended to occur in the shallower waters. Month, target species, or statistical area were not influential covariates. Consequently, the chosen stratification was the second example (i.e., FRO 7, 8, and 9 combined) as it returned essentially the same model as for FRO 7 alone, and yet would be applicable to any analyses that include length data from the entire biological stock (as might be used in any catch-at-age analyses for future stock assessments).

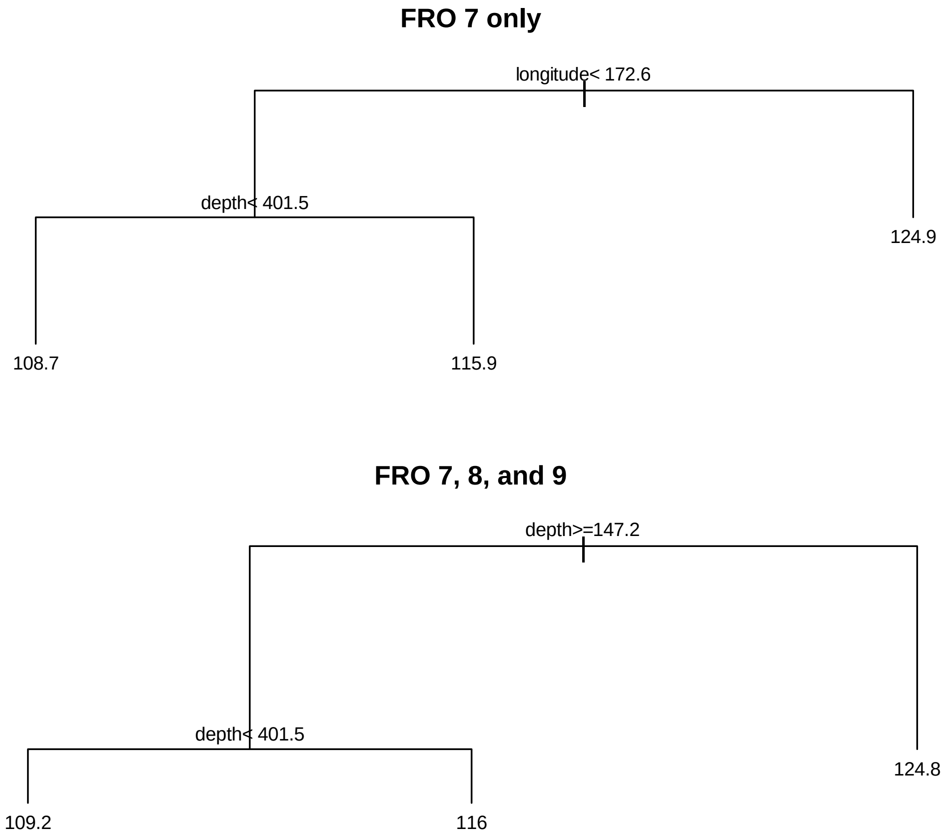

**Figure 8: Dendrogram of the classification trees used in the stratification of the FRO 7 only, and FRO 7, 8 and 9 trawl fisheries. Labels on the horizontal branches describe the situation at the left-hand end of the branch. The lengths of the vertical branches reflect the improvement in the Gini index. Numbers at the end of each branch represent mean fish length in that stratum.**

**Table 3: Node details for the classification tree for the FRO 7, 8, and 9 fisheries combined. Split, stratum definition; Node count, number of data rows in the node. Rows in bold with * indicate a leaf.**

| Node number | Split | Node count |
|---|---|---|
| 1 | root | 1212 |
| 2 | depth ≥ 147.25 | 836 |
| **3*** | **depth < 147.25** | 376 |
| **4*** | **depth = 147.3–401.45** | 390 |
| **5*** | **depth ≥ 401.5** | 446 |

The landings distribution from FRO 7, 8, and 9 in 2007–08 shows that there is a fishery from October to January concentrated in Statistical Areas 037, 040–042, and 801, followed by a secondary fishery from June to September concentrated off WCSI (Statistical Areas 034–036) but also taking catches from Areas 037 and 040–041 (Table 4). However, the current analysis is limited to the fishery off WCSI, and this clearly has a narrow temporal (June–August) and areal (Areas 034–036) distribution.

In 2007–08, about 17% by weight of the WCSI winter landings of frostfish was sampled by observers (Table 4). All month-statistical area combinations were well sampled except for Statistical Area 036 in July. Frostfish landings from the WCSI winter fishery were taken mainly as a bycatch of three target species, i.e., jack mackerels (51% of estimated frostfish landings), hoki (21%), and barracouta (20%). Most (75%) of the sampled tows had hoki as the reported target, with the remainder targeting jack mackerels, barracouta, or hake. However, it has been shown above that target species was not a variable that influenced the length composition of the frostfish catch. Consequently, it was concluded that sampling of the fishery was satisfactory to estimate the overall catch-at-age.

**Table 4: Distribution of estimated FRO 7, 8, and 9 total catch (t, rounded to the nearest tonne), by month and statistical area (Stat Area), in the 2007–08 fishing year. Values of 0 indicate landings from 1 to 499 kg; blank cells indicate zero landings. Landings from the WCSI fishery are highlighted in bold, and the sampled landing information (bottom panel of table) relates only to these months and areas.**

Estimated total catch (t), 2007–08

| Stat Area | | | | | | | | | | | | Month |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | All |
| 017 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 033 | 0 | 0 | 0 | | 1 | 1 | 0 | 0 | | | 0 | 0 | 2 |
| 034 | 0 | 0 | | 0 | | 0 | 1 | 0 | **4** | **55** | **78** | 1 | 139 |
| 035 | 0 | 0 | | | | | 0 | 3 | **35** | **83** | **191** | 0 | 313 |
| 036 | 9 | 0 | 2 | 8 | 0 | 0 | 0 | 4 | **143** | **52** | **11** | 3 | 233 |
| 037 | 41 | 0 | 34 | 26 | 0 | 0 | 1 | 3 | 14 | 3 | 0 | 25 | 147 |
| 038 | 0 | | | | | | | | 0 | 0 | | | 0 |
| 039 | 2 | | 0 | 0 | | 0 | 0 | | 0 | 0 | 1 | 0 | 4 |
| 040 | 35 | | 22 | 13 | | | 0 | 2 | 47 | 29 | 3 | 8 | 159 |
| 041 | 96 | 28 | 292 | 1 | | 0 | 0 | 0 | 23 | 17 | 0 | 6 | 463 |
| 042 | 10 | 3 | 34 | | 0 | 0 | 0 | 0 | 0 | 0 | | | 48 |
| 045 | 2 | | 4 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | | | 8 |
| 046 | | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | | | 0 | 1 |
| 047 | 0 | 1 | 1 | 1 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| 801 | 52 | 3 | 9 | 4 | | | | | 2 | | 2 | | 71 |
| All | 246 | 35 | 399 | 53 | 3 | 4 | 4 | 14 | 270 | 240 | 286 | 44 | 1 597 |

Sampled landings (% of estimated total catch)

| | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | All |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 034 | | | | | | | | | 8.8 | 10.3 | 24.4 | | 18.3 |
| 035 | | | | | | | | | 17.7 | 28.2 | 18.6 | | 21.1 |
| 036 | | | | | | | | | 11.5 | 1.6 | 27.3 | | 9.8 |
| All | | | | | | | | | 12.6 | 15.7 | 20.6 | | 17.0 |

## 4.5 Catch-at-age

No reported length-weight relationships were available for New Zealand frostfish. Such a relationship is necessary to allow the scaling of sampled length-frequency data to total catch. Data were available from three surveys conducted off WCSI (tan9911 in September 1999, kah0004 in March-April 2000, and tan0007 in July-August 2000). A power equation was fitted to data from all surveys to produce the following relationship where W is weight in g and L is fork length in cm:

$$W = 0.000407\ L^{3.155} \qquad [r^2 = 0.997,\ n = 965,\ \text{L range} = 10\text{–}164\ \text{cm}].$$

The details of the estimated catch-at-age distributions for trawl-caught frostfish from FRO 7 in the winter 2008 fishery are presented in Table 5. Note that some inferred data points were added to the age-length data file if there were lengths with no otoliths but where age could be confidently estimated, e.g., all fish 70–89 cm FL would be age 1.The mean weighted c.v.s across all age classes were well below the usual target value for middle depth species of 30%. The estimated distributions are plotted, by sex, in Figure 9. The catch is strongly dominated by 2 year old fish. The 4 year old age class appears to be relatively weak.

**Table 5: Calculated numbers-at-age, separately by sex, with c.v.s, for frostfish caught during commercial trawl operations in FRO 7 during the winter 2008 fishery. Summary statistics for the sample are also presented.**

| Age | Male | c.v. | Female | c.v. | Total | c.v. |
|---|---|---|---|---|---|---|
| 1 | 38 054 | 0.392 | 24 573 | 0.595 | 62 627 | 0.366 |
| 2 | 292 048 | 0.127 | 342 736 | 0.131 | 634 784 | 0.047 |
| 3 | 29 741 | 0.331 | 46 169 | 0.317 | 75 910 | 0.240 |
| 4 | 5 479 | 0.818 | 13 927 | 0.582 | 19 406 | 0.516 |
| 5 | 8 470 | 0.689 | 16 443 | 0.477 | 24 913 | 0.364 |
| 6 | 15 437 | 0.529 | 4 678 | 0.883 | 20 115 | 0.407 |
| 7 | 13 873 | 0.524 | 10 331 | 0.537 | 24 204 | 0.342 |
| 8 | 5 633 | 0.840 | 1 099 | 1.396 | 6 732 | 0.728 |
| | | | | | | |
| No. measured | | 562 | | 737 | | 1 299 |
| No. aged | | 199 | | 245 | | 444 |
| No. of tows sampled | | | | | | 72 |
| Mean weighted c.v. | | 22.6 | | 22.0 | | 12.8 |

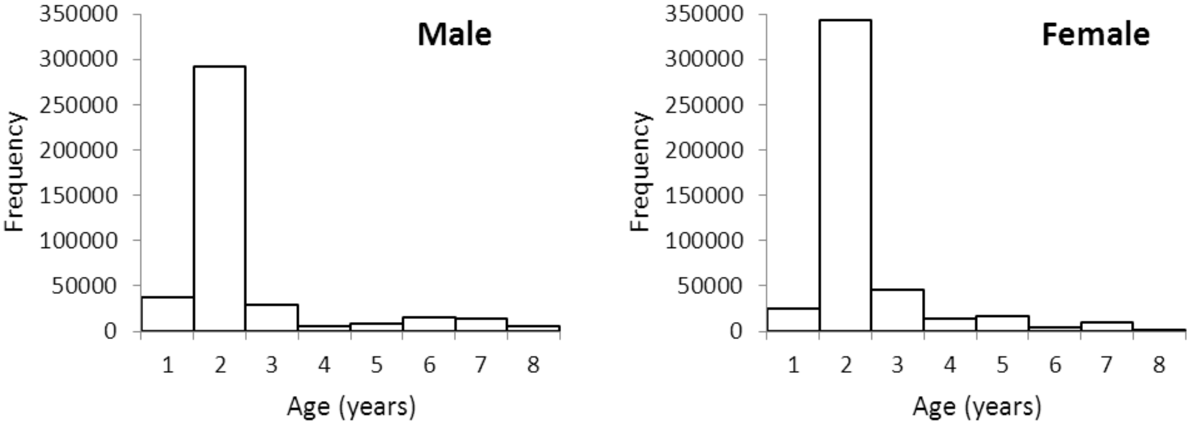

**Figure 9: Estimated commercial catch-at-age distribution, by sex, from FRO 7 in winter 2008.**

## 4.6 Mortality rate

Frostfish have a relatively short life span; it appears likely that the minimum age reached by the oldest 1% of fish is about 7–8 years. This age range was applied to Hoenig's (1983) estimator for instantaneous natural mortality ($M$), i.e.,

$$M = -\frac{\log_e(p)}{A}$$

where $p$ is the proportion of the population that reaches age $A$ or older, to produce a range for $M$ of 0.58–0.66, suggesting an $M$ of about 0.6 $\text{yr}^{-1}$.

## 5. DISCUSSION

Frostfish (*Lepidopus caudatus*) otoliths have been examined for growth zone patterns, and have been found to be quite difficult to interpret. Initial growth zones were found to be quite complex with much multiple banding and microstructure. Older growth zones can be very narrow. However, the species has very fast initial growth resulting in distinct modes in length-frequency distributions that have enabled a comprehensive interpretation of growth in the first four years independent of otoliths. An examination of the growth zones in otoliths of fish from these modes aided otolith zone interpretation, particularly up to age 2, resulting in an otolith-based ageing methodology that is validated up to age 4. Frostfish in New Zealand waters are a fast-growing, but short-lived species, as has been concluded for the same species in the Mediterranean Sea and central Atlantic Ocean (Demestre et al. 1993, Tuset et al. 2006).

The ageing method developed here used whole, untreated otoliths, and there is always a danger when using such a method that the age of older fish could be underestimated (e.g., Beamish 1979, Horn & Sutton 1996). This is owing to the tendency of otoliths of many fish species to become thicker, rather than longer or wider, as the asymptotic length is approached. Frostfish otoliths are relatively thin, and the zonation pattern at the margins of the otoliths of large fish was generally uncomplicated even though the zones were narrow (see Appendix A). It is also noted that validated ageing of some other closely related trichiurid and gempylid fishes has been achieved using zone counts in whole otoliths, e.g., black scabbard fish *Aphanopus carbo* (Morales-Nin & Sena-Carvalho 1996), gemfish *Rexea solandri* (Horn & Hurst 1999), barracouta *Thyrsites atun* (Horn 2002). Since it has been shown here that frostfish can be aged from zone counts on whole otoliths, using transverse sections is unnecessary as it would add time and expense to any ageing study.

The von Bertalanffy growth curves estimated from the current work are different to growth curves derived from all other ageing studies of this species. Knuckey (2001) clearly interpreted the otoliths differently to the current work, and generally incorrectly. His illustrations show that more than two zones (sometimes many more) were read in the section of the otolith that comprises two years of growth. It is concluded therefore that Knuckey's (2001) data cannot be used to estimate the catch-at-age distribution for the 2000 WCSI fishery. The data should probably be removed from the *age* database administered by NIWA for MPI, or at least marked with a comment noting their inaccuracy.

There are some marked similarities between the current work and the four northern hemisphere ageing studies of this species (Molí et al. 1990, Demestre et al. 1993, D'Onghia et al. 2000, Tuset et al. 2006). Estimated growth from all five studies is virtually identical up to about age four years, but after that age, the growth rate of New Zealand fish slows markedly to reach a length asymptote of about 140 cm, while the Mediterranean fish continue growing to an asymptote of about 190 cm. There are at least three possible reasons for this difference. First, there could be real differences in growth rate after about age four. This hypothesis is supported by length distributions (presumably from random commercial fishery samples) presented by Demestre et al. (1993), D'Onghia et al. (2000), and Tuset et al. (2006) which have their most dominant modes in the range 125 to 145 cm FL. New Zealand commercial fishery distributions have relatively few fish in this length range (see Figures 3 and 4). Second, the growth parameters calculated in the northern hemisphere studies may have used sets of age-length data that are not representative of the population. For example, if all the longest fish were aged and these data points were included in the analysis with no consideration given to weighting by the population length distribution then the estimated $L_\infty$ would be biased upwards relative to the true population value. Although not explicitly stated, it appears likely that all four northern hemisphere studies aged random samples from the commercial fishery, so the presented parameters are probably representative of the population as sampled by the fisheries. Third, the New Zealand fishery and surveys may not comprehensively sample the large fish in the population. The von Bertalanffy parameters estimated here fit the available data well up to age 8.6 for males and 7.6 for females. However, it is apparent that the mean lengths at age for fish older than this are higher than would be indicated from the growth curve (see Figure 6); all fish aged older than 8.6 years were longer than 150 cm. Because numbers of females longer than 150 cm and males longer than 135 cm are negligible in the commercial catch or survey samples, fish in these length ranges had little influence on the estimates of the von Bertalanffy parameters. However, the finding that all fish aged 9+ years were markedly longer than would be indicated by the estimated growth model raises the possibility that the fishery may not comprehensively sample very large frostfish. If this is so, then the true growth curve for the population would have higher $L_\infty$ values than those estimated here, and bring the curves closer to those estimated for the northern hemisphere samples. However, given the relatively intensive fishing off New Zealand's west coast, it seems unlikely that very large frostfish could be abundant but not caught. In conclusion, there is probably a real difference between New Zealand and the Mediterranean-Atlantic in the growth rate (and maximum size) of frostfish.

The length-frequency modes in the trawl survey and commercial fishery distributions provide a clear description of early frostfish growth. However, it was apparent (particularly for the modes aged 0.3, 1.3, and 1.6 years) that the distributions were skewed to the right and were weakly bimodal, e.g., the 0.3 year mode was dominated by fish around 17 cm FL but had a secondary mode at about 32 cm. This is indicative of a spawning season with two activity peaks. It appears most likely that the main peak (producing fish about 17 cm long in March-April) is in summer (Bentley et al. in prep.), with an earlier weaker (or less successful) spawning episode perhaps in late winter (producing the fish that were about 32 cm long in March-April).

Based on the analysis of the 2007–08 data, a sample of 400 aged fish appears to be adequate to estimate a precise commercial catch-at-age distribution (the mean weighted c.v. across all age classes was 13%). However, most of the fish sampled in that year had a relatively narrow length range resulting in a catch that was strongly dominated by 2-year-olds. In years when the length distribution is more widespread (e.g., 2001 and 2007), the same number of aged fish is likely to produce a higher c.v. Also, if future analyses aim to produce catch-at-age from the entire western New Zealand biological stock of frostfish (i.e., FRO 7–9), then there will probably be a need to add a time stratum owing to the occurrence of two distinct fisheries, i.e., a summer fishery primarily in the North and South Taranaki Bights, and a winter fishery primarily off WCSI (Bentley et al. in prep.).

## 6. ACKNOWLEDGMENTS

I thank the MPI Scientific Observers Programme for achieving good sampling coverage of the FRO 7 catch in 2007–08, Colin Sutton for preparing the otolith thin sections, Kris McKenzie for producing the images in Appendix A, and Malcolm Francis for a very helpful review of the manuscript. This work was funded by the Ministry for Primary Industries under project MID2010-01B.

## 7. REFERENCES

Anderson, O.F.; Bagley, N.W.; Hurst, R.J.; Francis, M.P.; Clark, M.R.; McMillan, P.J. (1998). Atlas of New Zealand fish and squid distributions from research bottom trawls. *NIWA Technical Report 42*. 303 p.

Bagley, N.W.; Schofield, K.A.; Colman, J.A. (1998). A summary of biology and commercial landings, and a stock assessment of the frostfish, *Lepidopus caudatus* Euphrasen, 1788 (Pisces: Trichiuridae), in New Zealand waters. New Zealand Fisheries Assessment Research Document 98/23. (Unpublished document available from NIWA library, Wellington.) 28 p.

Beamish, R.J. (1979). Differences in the age of Pacific hake (*Merluccius productus*) using whole otoliths and sections of otoliths. *Journal of the Fisheries Research Board, Canada 36*: 141–151.

Beamish, R.J.; Fournier, D.A. (1981). A method for comparing the precision of a set of age determinations. *Canadian Journal of Fisheries and Aquatic Sciences 39*: 277–287.

Bentley, N.; Kendrick, T.K.; McGibbon, D. (in prep.). Fishery characterisation and catch-per-unit-effort analyses for frostfish (*Lepidopus caudatus*), 1989–90 to 2009–10. *New Zealand Fisheries Assessment Report*.

Breiman, L.; Friedman, J.H.; Olshen, R.A.; Stone, C.J. (1984). *Classification and Regression Trees*. Belmont, CA: Wadsworth. 358 p.

Bull, B.; Dunn, A. (2002). Catch-at-age: User manual v1.06.2002/09/12. NIWA Internal Report 114. 23 p. (Unpublished report held in NIWA library, Wellington.)

Campana, S.E.; Annand, M.C.; McMillan, J.I. (1995). Graphical and statistical methods for determining the consistency of age determinations. *Transactions of the American Fisheries Society 124*: 131–138.

Demestre, M.; Molí, B.; Recasens, P.; Sánchez, P. (1993). Life history and fishery of *Lepidopus caudatus* (Pisces: Trichiuridae) in the Catalan Sea (Northwestern Mediterranean). *Marine Biology 115*: 23–32.

D'Onghia, G.; Mastrototaro, F.; Maiorano, P. (2000). Biology of silver scabbard fish, *Lepidopus caudatus* (Trichiuridae), from the Ionian Sea (Eastern-central Mediterranean). *Cybium 24*: 249–262.

Hoenig, J.M. (1983). Empirical use of longevity data to estimate mortality rates. *Fishery Bulletin 82*: 898–902.

Horn, P.L. (2002). Age estimation of barracouta (*Thyrsites atun*) off southern New Zealand. *Marine and Freshwater Research 53*: 1169–1178.

Horn, P.L.; Hurst, R.J. (1999). Age and stock structure of gemfish (*Rexea solandri*) in New Zealand waters. *Marine and Freshwater Research 50*: 103–115.

Horn, P.L.; Sutton, C.P. (1996). Validated ages, growth, and productivity parameters for silver warehou (*Seriolella punctata*) off the south and east coasts of South Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research 30*: 301–312.

Knuckey, I. (2001). Estimation of the age and growth of southern frostfish (*Lepidopus caudatus*). Final Research Report for Ministry of Fisheries Research Project FRO2000/01. (Unpublished report held by Ministry for Primary Industries, Wellington). 11 p.

Molí, B.; Lombarte, A.; Morales-Nin, B. (1990). Age and growth of *Lepidopus caudatus* on the Northwestern Mediterranean Sea. *Rapport de la Commission Internationale de la Mer Méditerranée 32(1)*: 269.

Morales-Nin, B.; Sena-Carvalho, D. (1996). Age and growth of the black scabbard fish (*Aphanopus carbo*) off Madeira. *Fisheries Research 25*: 239–251.

Nakamura, I.; Parin, N.V. (1993). FAO species catalogue vol. 15. Snake mackerels and cutlassfishes of the world. *FAO Fisheries Synopsis No. 125.* 136 p.

Paulin, C.; Stewart, A.; Roberts, C.; McMillan, P. (1989). New Zealand Fish: A complete guide. *National Museum of New Zealand Miscellaneous Series No. 19.* 279 p.

Robertson, D.A. (1980). Spawning of the frostfish, *Lepidopus caudatus* (Pisces: Trichiuridae), in New Zealand waters. *New Zealand Journal of Marine and Freshwater Research 14:* 129–136.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Tuset, V.M.; González, J.A.; Santana, J.I.; Moreno-López, A.; García-Díaz, M.M. (2006). Reproductive pattern and growth in *Lepidopus caudatus* (Osteichthyes, Trichiuridae) from the Canary Islands (Eastern-central Atlantic). *Electronic Journal of Ichthyology 2(1)*: 26–37.

## Appendix A: Otolith images

The images of whole untreated frostfish otoliths below all show the distal surface and are all orientated with the postrostral (posterior) tip to the left and the ventral edge down. Scale bar is 1 mm.

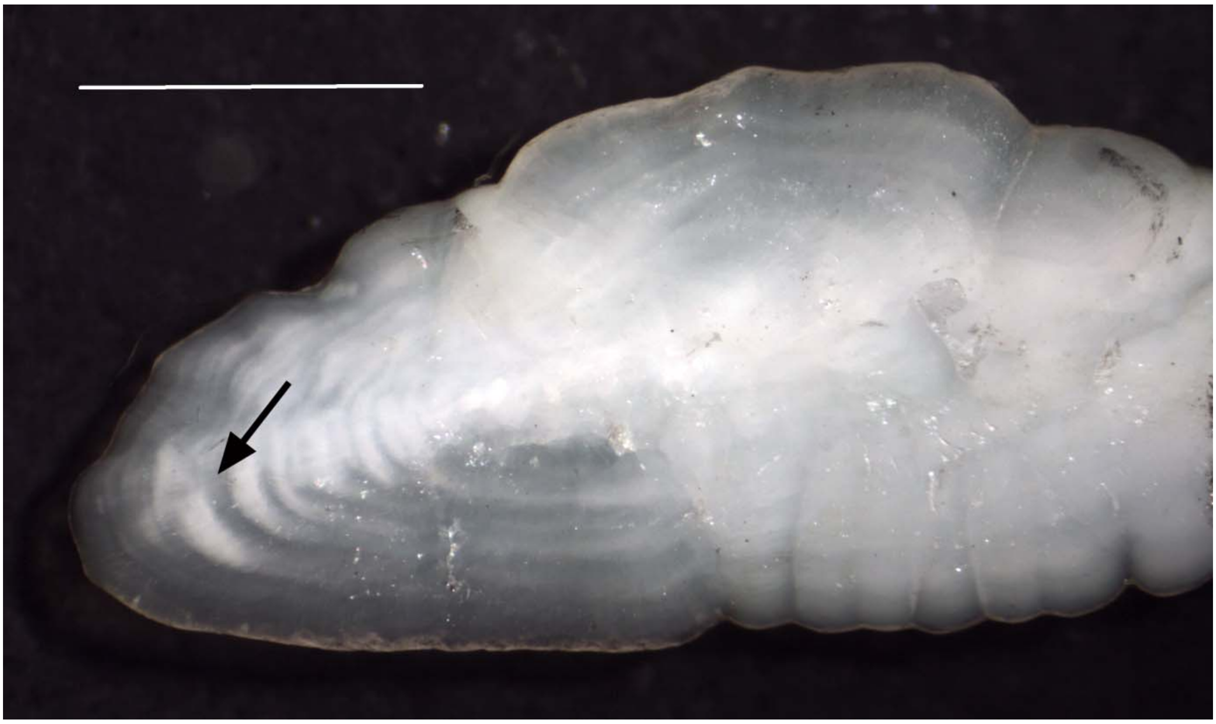

**Figure A1: Otolith from a 1.3 year old fish. The arrow indicates the likely position of the first true annual zone. Micro-zoning both inside and outside the annual zone is apparent.**

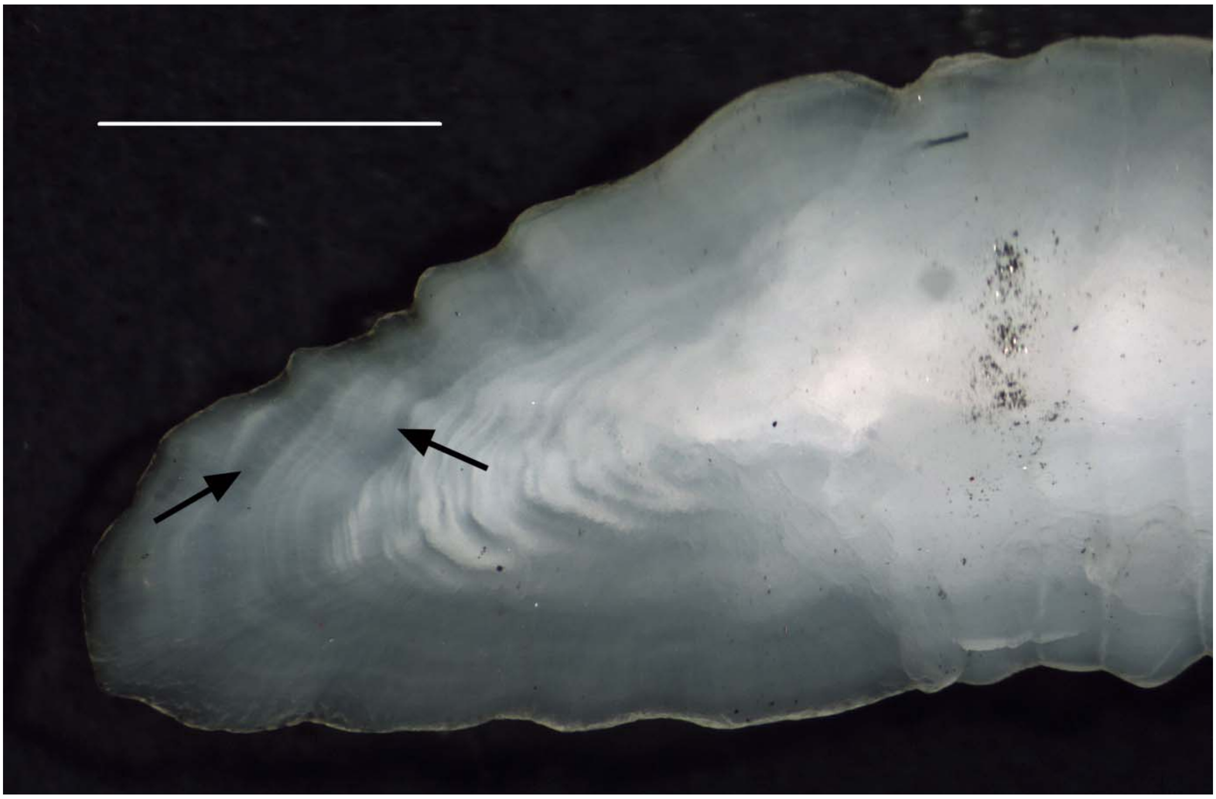

**Figure A2: Otolith from a 2.6 year old fish. The arrows indicate the likely positions of the first and second annual zones. Micro-zoning both inside and outside the annual zones is apparent.**

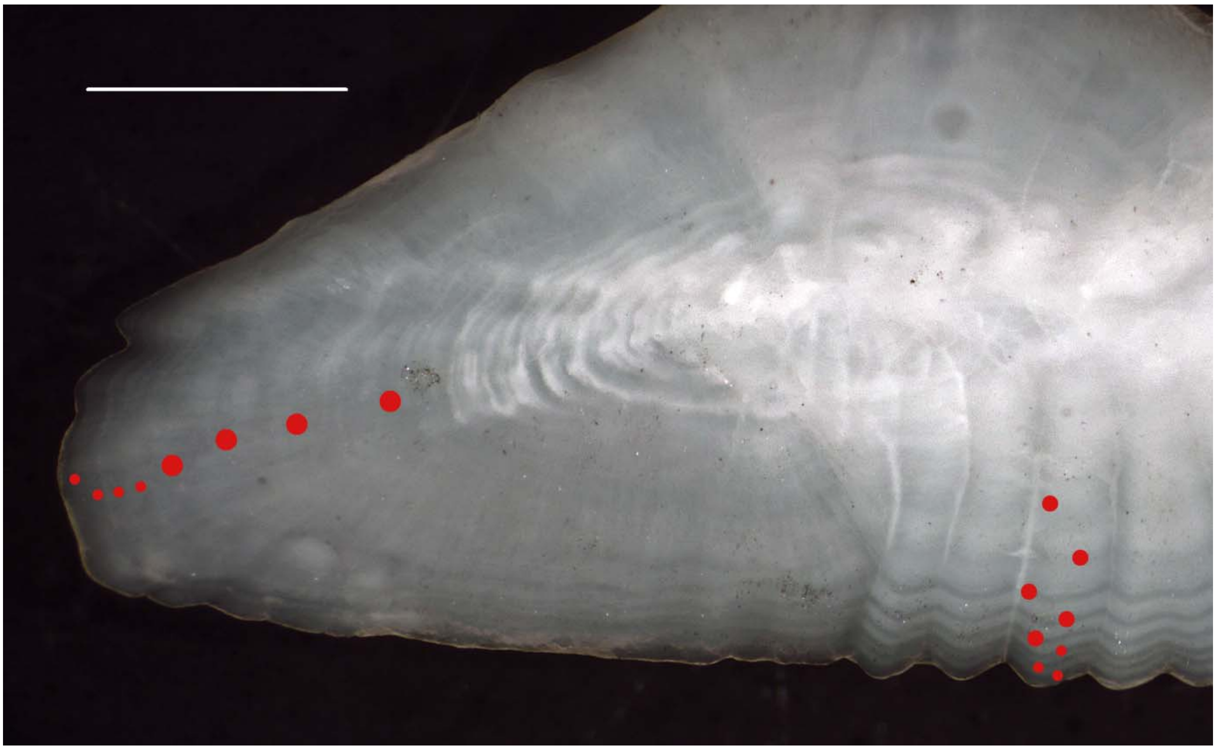

**Figure A3: Otolith from an 8.6 year old fish. The dots indicate the likely positions of the annual zones. The pattern of annual zones is very clear on the ventral edge of the otolith.**

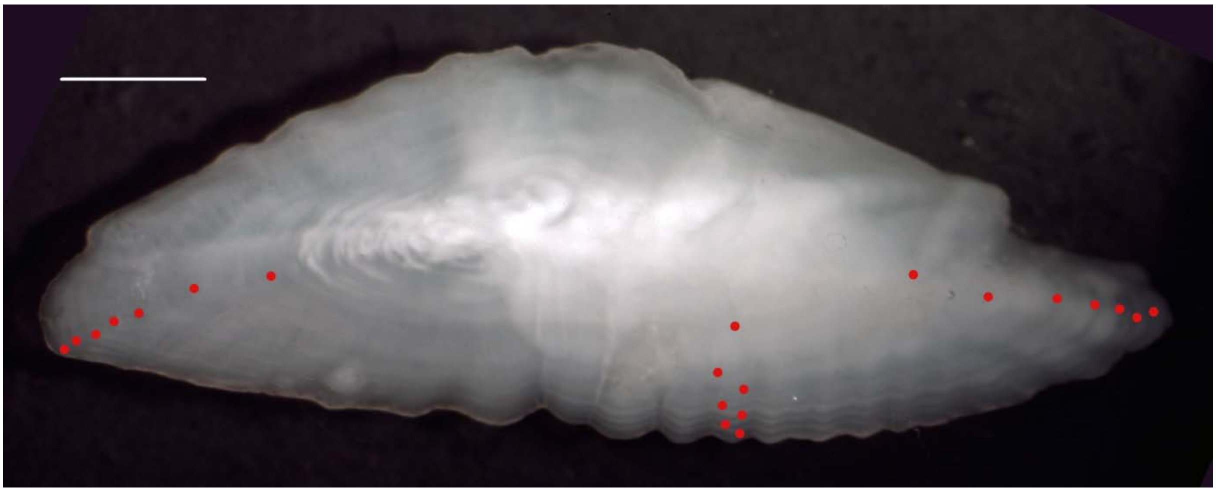

**Figure A4: Otolith from a 7.6 year old fish. The dots indicate the likely positions of the annual zones. The pattern of annual zones is apparent on the postrostral and rostral tips as well as on the ventral edge of the otolith.**

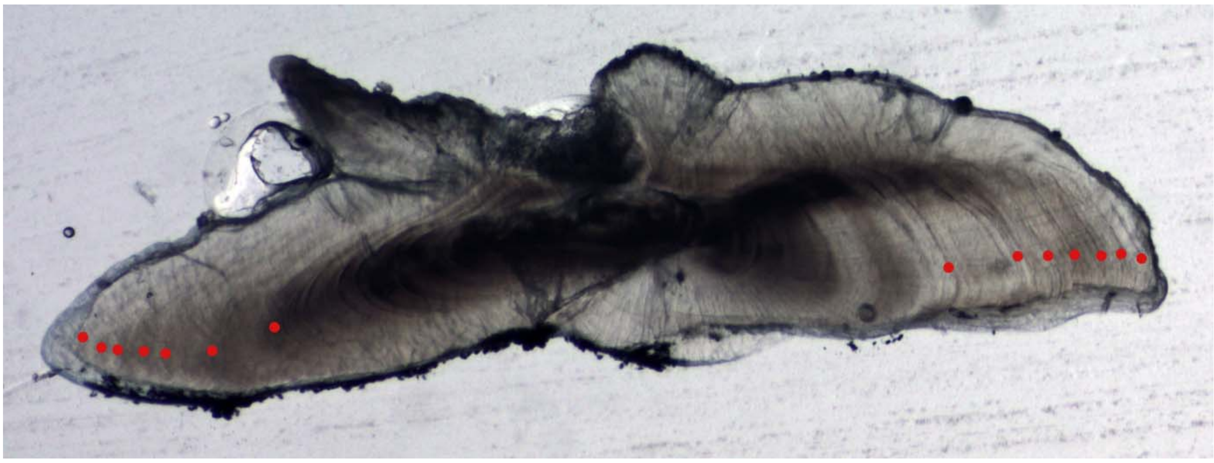

**Figure A5: Thin section through an otolith from a 7.6 year old fish (the sister otolith to that shown above in Figure A4). The dots indicate the likely positions of the annual zones. The ventral edge is to the left in this image.**