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EVALUATING GEAR AND SEASON SPECIFIC AGE-LENGTH KEYS TO IMPROVE THE PRECISION OF STOCK ASSESSMENTS FOR PATAGONIAN TOOTHFISH AT HEARD ISLAND AND MCDONALD ISLANDS

FRDC TACTICAL RESEARCH FUND PROJECT 2008/046

FINAL REPORT

D. C. Welsford, G. B. Nowara, S. G. Candy, J. P. McKinlay, J.J. Verdouw and J.J. Hutchins

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2008/046 Evaluating gear and season specific age -length keys to improve the precision of stock assessments for Patagonian toothfish at Heard Island and McDonald Islands

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Objectives

1 Evaluation of the sensitivity of integrated assessment models for toothfish at Heard Island and McDonald Islands to the inclusion of gear and/or season specific age-length keys

2 Refinement of protocols for reliable and efficient large scale ageing of toothfish otoliths

3 Refinement of sampling design for future otolith collection and processing

Non-Technical Summary

The Australian Territory of Heard Island and McDonald Islands (HIMI) is located on the Kerguelen Plateau, southwest of the Australian continent in the Indian Ocean sector of the Southern Ocean. Australia claims a 200 nm Exclusive Economic Zone around HIMI, which also falls within the area of the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR), to which Australia is a signatory. Like many of the other sub-Antarctic islands in the Southern Ocean, including South Georgia, Kerguelen and Macquarie Island, HIMI supports a Patagonian toothfish fishery.

Assessment of the Patagonian toothfish stock at the Heard Island and McDonald Islands (HIMI) began in the early 1990s, with the series of research surveys conducted by the Australian Antarctic Division. Trawling by the Australian fishing industry began in 1997 and since 2003 long -lining has also been an important gear type used in the fishery. All vessels carry at least two scientific observers to collect fisheries operational data and comprehensive biological data from the catch, as well as retaining otoliths and conducting a tagging program

and an annual research survey under the direction of the Australian Antarctic Division

Currently, stock assessments are conducted by AAD using CASAL (C++ Analytical Stock Assessment Laboratory), a Bayesian integrated assessment framework which is used by CCAMLR to conduct all its major toothfish assessments. The current HIMI assessment integrates commercial catch at length data, annual survey abundance at length data, catch per unit effort and tag recapture data. These datasets are subdivided into spatial and temporal sub fisheries, to reflect the fact that fishing has been concentrated in particular locations and times as a result of both the distribution of fish, research plans such as the annual survey and management measures such as a limited season for long lines to avoid bird capture.

Prior to this project, catch-at-age was estimated using a von Bertalanffy growth curve. This approach has some limitations as opposed to directly estimating catch-at age from age length keys, as if there is variation in this growth function between years or between fisheries such (as may result from density dependant processes, or temporal and spatial structuring of the population, as is evident in toothfish at HIMI), then the abundances at age may be falsely estimated resulting in biased estimates of stock status. Evaluating the impact of using season and gear specific age-length keys therefore represents the primary objective of this study.

An efficient high-throughput methodology for reliable ageing of toothfish from thin-sectioned otoliths has been successfully developed and documented. A reference collection including over 200 sections from across the entire size range and all gear types used in the HIMI fishery has also been developed. The capacity now exists to conduct routine otolith analyses for toothfish, and conduct robust comparison in otolith interpretation between readers and laboratories in Australia and in other countries were toothfish ageing and assessments are conducted.

A randomized sub sampling procedure was successfully developed to select un-aged toothfish otoliths from all of the season and gear type combinations available. Using the new methodology, over 2600 otolith have been processed and analysed, including many large fish and fish captured by long line and pots, for the first time.

A statistical method has been developed to represent ageing error in integrated assessments through the calculation of an ageing error matrix. The method is shown to have application for the HIMI assessment, but also represents an effective method generally for incorporating ageing error in integrated assessment using age length keys, were traditional methods of comparing reader performance, such as age-bias plots or index of average percent error, are of limited use.

Comparisons between the reads of the reference collection by AAD and the Central Ageing Facility, where otoliths collected from the HIMI fishery prior to 2003 have been analysed, has enabled the consolidation of over 6400 length at age estimates for fish, collected across the majority of seasons, gear types (commercial and survey trawl, long line and trap), length classes and fishing grounds, since the fishery started as well as from the surveys conducted prior to commercial fishing, with more than 5500 included in the stock assessment, including nearly all otoliths processed for this project.

The precision of critical parameters estimating rate of recruitment and the selectivity of different gear types were shown to be sensitive to the inclusion of length at age data in the form of season and gear specific age-length keys. Although toothfish at HIMI can be up to 25 years old when captured, the majority of the catch is between 3 and 14 years old. Hence the careful preparation and consistent interpretation of otolith sections is crucial, as even relatively small errors, of the order of ±1 year for more than 40% of reads within an age class, significantly reduce the power of age length keys to resolve cohorts in the assessment model.

A range of future work is recommended from this study, including expanding the AAD reference collection, particularly with the inclusion of more old and young fish, and interlaboratory comparison with international research groups. The large dataset collected in this project also provides an opportunity to refine the current von Bertalanffy growth function used for this fishery, as well as using ancillary data such as sex and otolith weight to refine prediction of age class. Otolith collection should continue for this fishery, with observers continuing the current method of 'length bin' sampling, whereby a fixed number of otoliths are collected from all the size classes present in the catch.

The results of this project provide a firm basis for stock assessments scientists, fishers and resource managers to evaluate the benefit of including season and gear specific age-length keys, as well as providing increased confidence in the plausibility and robustness of the current assessment framework for toothfish, at an early stage of its use to provide managment advice at HIMI. The statistical methods developed, particularly the methods for incorporating reader error in age length keys has application for all stock assessment that use such data.

Outcomes Achieved

The primary outcome of this project has been the successful development of statistical methods for the sensitivity testing of the HIMI integrated assessment model to the inclusion of length-at-age estimates from over 5500 individual toothfish, in the form of gear and season specific age-length keys.

The results of this project provide a firm basis for stock assessments scientists, fishers and resource managers to evaluate the costs and benefits of including season and gear specific age-length keys in the HIMI toothfish assessment in future, providing increased confidence in the current stock assessment approach, as well as providing statistical tools that can be readily applied in other fisheries were age length keys and/or integrated assessments are used.

This project highlights the value of age at length data, drawn from 2007 back to when the fishery commenced, and all gear types used in the fishery to date, in producing precise and plausible results under the assessment framework used for the HIMI toothfish fishery. The inclusion of age-length data results in significant refinements to the estimates of several key parameters in the assessment. These include more precise estimates of the level and variability of recruitment of juvenile toothfish to the stock than when size at age is estimated only using a von Bertalanffy growth function.

Using the methodologies developed in this project, high-quality, high-throughout ageing of toothfish otoliths is shown to be feasible, such that season and gear specific age-length keys could be routinely incorporated into the HIMI assessment with less than one year lag. It is clear that each gear type samples a different part of the population, and so provides insight into the toothfish stock over the broadest range of age classes. It is recommended that otolith collection should continue across all gear types in the HIMI toothfish fishery, including long lines, trawls and survey trawls, sampling across all length classes present in the catch.

Keywords

Patagonian toothfish, *Dissostichus eleginoides*, age-length keys, otoliths, multi-gear fishery, Heard Island and McDonald Islands, integrated assessment, age-based assessment, sub-sampling, effective sample size, reader error.

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Our sincere thanks also, to the Australian Fisheries Management Authority scientific observers and Data Collection Officers from Fisheries Audit Services, for collecting all of the otoliths used in this project. Many thanks also to Dick Williams for establishing the original otolith collection program, Tim Lamb for database management and Andrew Constable for assistance in developing the project and the integrated assessment framework.

We also gratefully acknowledge Kyne Krusic-Golub and the Central Ageing Facility, Queenscliff for conducting inter-laboratory ageing comparisons.

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Background

The Heard Island and McDonald Islands toothfish fishery

Heard Island and the McDonald Islands (HIMI) lie in the Indian Ocean sector of the Southern Ocean about 4000 km south-west of Western Australia and 1700 km north of the continent of Antarctica. Along with the îles Kerguelen, HIMI are the only emergent parts of the Kerguelen Plateau, the largest submarine plateau in the Southern Hemisphere.

Unregulated fishing by former Soviet and Eastern Bloc fleets are known to have occurred on the Kerguelen Plateau during the 1970s, resulting in over fishing of demersal fish species such as the marbled rock cod *Notothenia rossii*, and the grey rock cod *Lepidonotothen squamifrons* (Candy *et al.* 2009; Duhamel *et al.* 2005). Regulation of fishing began in 1978, with the French declaration of the 200 nm exclusive economic zone (EEZ) around Îles Kerguelen. Australia made an equivalent declaration for the HIMI in 1979, and commercial fishing ceased.

Exploratory surveys were conducted by the Australian Antarctic Division from aboard the RV *Aurora Australis* in May/June 1990, February 1992 and September 1993. These surveys were designed to sample the overall distribution and abundance of major species and to indicate regions of high abundance. Results indicated that Patagonian toothfish were one of the most abundant species, and preliminary yield models, implementing decision rules based on the CCAMLR rational use principles, indicated that the population at HIMI could support a commercial fishery of the order of 100s of tonnes per year (Williams and de la Mare 1995).

Commercial fishing first occurred in the Australian EEZ around HIMI in April 1997, when trawling for Patagonian toothfish (*Dissostichus eleginoides*) and icefish (*Champsocephalus gunnari*) commenced under an AFMA license issued to an Australian fishing company (Meyer *et al.* 2000).

In 2003, an exploratory fishing license was granted for a long-liner, with a season restricted to the winter months to minimize risk of bird by-catch. Both trawling and long-lining has occurred in every season since. In 2006, a vessel was equipped to trap for toothfish, and fished for a single season.

As a result of the different timing of fishing between long lining and other methods, and the limited available of fishable grounds for trawling, catch and effort is highly structured in space and time at

HIMI. The RSTS is conducted across the entire plateau, in waters generally less than 750 m, and occurs in the middle of the year between April and July. Commercial trawling generally occurs in two main grounds, near the edge of the plateau in water less than 1000m, and year round, while long lining is confined to the months of May-October and occurs around the deeper slope of the plateau between 1000 and 2000m. As a result of the movement of toothfish, which tend to live in deeper water as they grow (Agnew *et al.* 1999), the RSTS tends to catch small juveniles, while the commercial trawl fishery catch older juveniles and the long-line fishery catches larger adolescent and mature fish.

Data collection and stock assessment approaches

From the first fishing season in 1997, Australian fishery operators have conducted an annual random stratified trawl survey (RSTS) across the entire HIMI plateau, to collect data on the abundance of juvenile toothfish and/or icefish prior to their recruitment to the fishery. All vessels have also been required to collect operational and biological data. This is facilitated by all vessels carrying at least two scientific observers at all times. These observers tasked with collecting catch and effort data, biological data (size, weight and sex) on catch and by-catch, as well as otoliths.

The Heard Island and McDonald Islands (HIMI) EEZ lies within the Convention for the Conservation of Antarctic Marine Living Resources, which was established in 1982, over concerns that over fishing was threatening ecosystem processes in the Southern Ocean. As a claimant State in Antarctica, and a founding signatory of the Antarctic Treaty and CCAMLR, Australia is committed to maintaining the Antarctic Treaty system, including CCAMLR and to managing its fishing activities in the Convention Area in accordance with the objectives of CCAMLR.

The objective of the Convention is the conservation of Antarctic marine living resources, with conservation being defined as including rational use. Such rational use takes into account three principles:

- to ensure the stable recruitment of harvested species;
- the maintenance of the ecological relationships between harvested and other species; and
- to ensure that any changes made are potentially reversible within two or three decades.

The first assessments of long-term yields for toothfish at HIMI, implementing the principles outlined above, were undertaken in 1996, following discussion on best approaches by the CCAMLR Working

Group on Fish Stock Assessment (WG-FSA) in 1995. This approach used the abundance-at-length data collected during the RSTS, decomposed into age classes using CMIX (de la Mare 1994; de la Mare *et al.* 2002), to generate cohorts strengths. These estimates were then combined with estimates of growth, fishing and natural mortality and maturity rates, and projected forward over a 35 year time frame using the Generalised Yield Model (GYM) (Constable and de la Mare 1996). The GYM was used to set catch limits for the HIMI toothfish fishery until 2005, however by this stage alternative 'integrated assessment' models (i.e. using maximum likelihood and Bayesian statistical tools to incorporate diverse datasets (e.g.Butterworth *et al.* 2003; Hillary *et al.* 2006; Maunder 2003)) were identified by WG-FSA as alternatives to replace the GYM. These models had the potential advantage of using all commercial and research catch-at -length data to directly estimate the selectivity of the various fishing gear types used in a fishery, as well as changes through time, and incorporate catch-per-unit effort data and tag recapture data. This feature of integrated assessments is particularly useful in understanding the HIMI fishery where catches and gear type used are structured in space and time in a way that was difficult to adequately represent using the GYM.

In 2004 a preliminary integrated assessment of Antarctic toothfish in the Ross Sea was presented at WG-FSA using the C++ Algorithmic Stock Assessment Laboratory (CASAL) (Bull *et al.* 2005; Dunn *et al.* 2004). CASAL subsequently became the modeling framework to perform all of the major CCAMLR toothfish assessments; in South Georgia (Hillary *et al.* 2006), the Ross Sea (Dunn and Hanchet 2007) and HIMI (Candy and Constable 2008).

The data used in these assessments do differ in some key respects. The Ross Sea and South Georgia assessments routinely use tag recapture data to provide an index absolute abundance, while the HIMI assessment uses both tag recapture data and the results of the RSTS as abundance estimates. The HIMI fishery assessment incorporates a range of sub-fisheries to reflect the different gear types, fishing seasons and spatial separation of the main fishing grounds. Hwoever, while the other CCAMLR toothfish assessments may include spatial segregation (as in the Ross Sea assessment) they only include long line fishing as there is no trawling for toothfish outside of the HIMI fishery. The Ross Sea and South Georgia assessments incorporate age-length keys based on batches of otoliths randomly sampled and aged from across the fishery each year, while the HIMI assessment uses a growth vector based on a von Bertalanffy growth curve (Candy *et al.* 2007). One of the key recommendations out of the WG-FSA in recent years has been for the investigation of ways of incorporating age data into the HIMI assessment (SC-CAMLR 2007, paragraph 12.3), which in part has lead to the development of this project (see *Need* below).

Validating ageing for Patagonian toothfish

The success any ageing program should be predicated by the determination of a methodology that is both valid (that is the age determined reflects the absolute age of any individual, not just an index of relative age) (Campana 2001). It is rare in fisheries, with the exception of semelparous species, or those that have been kept in captivity, for the absolute age of fish to be known. Hence proxies for absolute age are employed, such as decomposition of length frequency data into age classes (de la Mare 1994; Fournier *et al.* 1990), or use of growth rings in hard parts such as otoliths (Campana 2001; Francis *et al.* 1992). In these instances the prerequisites for a valid ageing protocol are:

1. the presence of periodic structure in the otolith;
2. confirmation that the periodic structures reflect an external time scale (e.g. rings are laid down annually) through out the life of the fish; and
3. determination of when the periodic structures begin forming

The repeatability of age readings is another important step in the age determination process. This can be achieved by consistent and well documented preparation processes, and by the use of a reference collection of otoliths, to test the similarity of age estimates between readers and, with regular re-reading, to test repeatability in readings by the same readers over time (Campana 2001; Campana *et al.* 1995). However, in some studies toothfish, the otolith preparation process is not well described, and the use of a reference collection, or statistics showing the repeatability of reads are not reported, leading to difficulty in determining the precision of age estimates reported and contradictory results between some research groups aging toothfish as fisheries for the species developed around the Southern Ocean in the 1990s.

Patagonian toothfish sagittal otoliths in section consist of a central dark core surrounded by alternating bands of opaque and translucent material (rings), and rings are also visible on their scales, satisfying the first validation prerequisite above. Early age determinations for toothfish were carried out using scales (e.g. Cassia 1998) due to the ease of collection and preparation, but it has been shown that readings from scales consistently under age and show greater variation when compared to otolith readings (Ashford *et al.* 2001; Cassia *et al.* 2004), confirming studies in other species have shown that scales underestimate age in older fish (Francis *et al.* 1992;

McFarlane and Beamish 1995). Hence most researchers have subsequently used thin sections for the purposes of attempted to infer ages for toothfish.

Kalish *et al.* (2000) attempted the first validation of the ages of toothfish derived from otoliths using bomb radiocarbon analysis to corroborate ages imputed from counts of otolith rings, using material collected from many areas of the species geographic distribution. The reading of rings for comparison in this study showed the variability of age estimates between readers to be relatively high, but still smaller than could be resolved by the radiocarbon dating. However, radiocarbon dating was judged to be suitable for the estimation of ages to within 5 to 10 years of the actual age, and confirmed that Patagonian toothfish were relatively long-lived with maximum ages of 30-40 years.

In 2001 a workshop was organised to compare methods being used in the three main laboratories engaged in age determination of toothfish at the time (Central Ageing Facility, Australia; Centre for Quantitative Fisheries Ecology, USA; and National Institute of Water and Atmospheric Research, New Zealand), and to establish a consistent protocol for the reading of toothfish otoliths (SC-CAMLR 2001). All three laboratories used thin sections through the primordium of the otolith for ageing, and although there were differences in otolith preparation protocols, all were considered suitable for toothfish age determination and gave similar estimates of age. It was recognised that it is very difficult to prepare a clear section displaying all annuli without artefacts, due to the sometimes complex growth pattern of toothfish otoliths (SC-CAMLR 2001).

Some of conclusions of this workshop with regard to interpretation of otolith sections include:

- Definitions of the features of sectioned otoliths to provide a common language for discussions;
- Identifying the occurrence of split zones or checks on the otoliths, defined as being consistent between the dorsal and ventral sides of the section;
- A definition for determining whether an area of predominantly translucent material consists of a single split or two distinct annuli. It was to be considered a single annulus if occurring in the first 8 years, otherwise it was to be considered as two;
- The birth date to be used for toothfish is 1st July; and
- The importance of the use of reference collections.

A further attempt at validation of the annual periodicity of otolith increments was made with tag recapture samples from toothfish which had been marked with strontium chloride on release at the HIMI and Macquarie Island fisheries (Krusic-Golub and Williams 2005). In this study 139 fish were examined, and showed that the observed number of increments was the same as the expected number of increments, based on time at liberty, in 88% of cases. Most of the remainder had a difference of ± 1 year. This study successfully validated that one opaque ring was laid down annually for fish of ages 5 to 18, and hence showed that toothfish otoliths satisfied the second prerequisite for a validated ageing protocol.

The final prerequisite, determination of the position of the first annual ring, has also recently been resolved. In contrast to the outcomes of the 2001 workshop, Ashford *et al.* (2002) proposed that the first year of growth was represented by the second (rather than the first) opaque zone outside of the nucleus in otoliths of fish from South Georgia. This lead to very rapid growth estimates for the early growth of toothfish, and the authors acknowledged that there were other data in conflict with this interpretation, such as the presence of late larvae in the water column simultaneously with large post-settlement fish estimated to be in the 0+ age class on the basis of their otoliths. Hence they conceded that it was possible that their ages were underestimated by one year, but concluded that further research was needed to determine when spawning and hatching occur, when opaque zones are formed and the period to which the nucleus corresponds.

The alternative interpretation noted by Ashford *et al.* (2002) was largely confirmed by a study of increments in the core of the otolith made by Krusic-Golub *et al.* (2005) from samples collected at HIMI. The results of increment counts (assumed to be daily) of seven otoliths from small juveniles (<25 cm) showed an average of 229 days from the hatch mark to the outer edge of the opaque nucleus, and, although it is yet to be validated that the rings observed form on a daily basis, the number of rings observed to the putative first annual increment was consistent with what is known about the time of spawning and settlement of toothfish at HIMI. It was therefore assumed that the zone between the primordium or core of the otolith and the outer edge of the first translucent zone (or ring) beyond the core corresponded to one year's growth. The mean distance from the primordium to the outer edge of the first translucent zone was measured to be 0.63mm (Krusic-Golub *et al.* 2005).

A similar conclusion about the age when the first ring was deposited was drawn by Horn (2002) examining otoliths from Patagonian toothfish coming from the Ross Sea. The first translucent zone was considered to represent a fish that was just over one year old on the basis that spawning is believed to occur between July and September and that the translucent zone appears to be deposited between June and October.

Toothfish ageing in Australian fisheries

Prior to the current project, most of the ageing of toothfish from the Australian fishing zones at HIMI and at MI was carried out by the Central Ageing Facility at Queenscliff in Victoria, following a protocol consistent with other laboratories around the world as noted above. Around 4,300 otoliths were aged from fish captured in research trawling by the RV *Aurora Australis* (in the early 1990s) and from the commencement of the commercial trawl fisheries at Macquarie Island (in 1995) and HIMI (in 1997) up to 2003 (Krusic-Golub and Ackerman 2003; Krusic-Golub *et al.* 2000). These otolith were chosen primarily to determine the growth rate of toothfish(Candy *et al.* 2007), and models based on these data were used in the GYM and CASAL assessments at HIMI (Candy and Constable 2008; Welsford *et al.* 2006).

Recently, facilities for the preparation and ageing of Patagonian toothfish otoliths have been established at the Australian Antarctic Division by staff experienced in the ageing of finfish otoliths. The sample preparation protocol is based on recommendations from the workshop on estimation of age in Patagonian toothfish (SC-CAMLR 2001) and further refinements developed from experience, such as the practice of setting only one otolith per resin block and carefully measuring the cutting angle to provide an optimal display of the rings. The rings are counted commencing from the first opaque band after the opaque core region, which corresponds to the first year of growth as shown by Krusic-Golub *et al.* (2005), and taking into account the published knowledge on structure of toothfish otolith thin sections. The AAD has also developed a reference collection which can be used to teach novice readers and also be re-read to determine the extent of any drift in the interpretation of otoliths over time. A manual developed by AAD for toothfish otolith preparation is presented in Appendix 3.

Need

Accurate estimates of size-at-age and recruitment variability, as well as fishery specific catch-at-age and gear selectivity are critical to the integrated stock assessments for toothfish in the Heard Island and McDonald Islands toothfish fishery (Candy and Constable 2008). Otolith analysis represents a powerful method for improving these estimates.

Currently, a growth model based on fish aged from the trawl fishery between 1997 and 2003 (Candy *et al.* 2007) is used to predict catch-at-age for trawl and long line catches and abundance-at-age from trawl surveys. This is done by using the growth model to partition numbers at length into age classes. However if there is variation in this relationship between years or between fisheries then the abundances at age may be falsely estimated resulting in poor estimation of stock status. This is an important potential source of bias in current models and should be addressed by developing age-length keys.

Unbiased age-length keys require analysing sufficient otoliths, collected from a representative sample of size classes captured by the fishery and survey, such that the age composition of the catch, age-based selectivity of fishing gears and the age structure of the stock can be better estimated.

The otoliths aged in 1997-2003 were sampled primarily to develop a growth model, and are not suitable for evaluating season and gear-specific age-length keys. The majority of age-length estimates available prior to this study resulted from analysis of otoliths collected before the long line fishery (which catches larger fish than the trawl fishery) commenced, so very few otoliths from larger fish or from long line grounds have been analysed. Furthermore, much of the ageing performed in the past occurred before the latest validation data (e.g. Krusic-Golub *et al.* 2005; Krusic-Golub and Williams 2005) for toothfish was available. Hence there is a need to construct age-length keys across gear types and seasons, and conduct sensitivity tests to evaluate the impact of gear and inter-seasonal variability in age structure in assessments.

Otolith-based ageing of the Patagonian toothfish (*Dissostichus eleginoides*) for the Heard Island and McDonald Islands: modelling fixed and random reader error using multiple readings of a reference collection

Steven G. Candy, G. B. Nowara, Dirk Welsford, John McKinlay

Integrated assessments that use catch-at-age or abundance-at-age data for model calibration require an ageing error matrix as input in order to adequately account for uncertainty in the data resulting from the imprecision of age determination using annual ring counts from otoliths. This paper describes the methods and results used to provide an ageing error matrix to the HIMI toothfish integrated assessment using repeat readings by 4 readers of a set of 203 reference otoliths sampled from the HIMI fishery. The methods of sampling, preparing, reading, and modelling random reader error for this reference set of otoliths is described. A total of 933 readings were taken and errors were defined as the nearest integer (NI) value deviations, denoted as integer errors (IE), from the mean age for an individual otolith. Since the true age of the fish is unknown, only imprecision and relative differences between readers could be quantified. Linear mixed model analyses indicated that the mean IE ranged between readers only slightly (± 0.27 yr) whereas frequencies of random IEs, treated as classes, between readings were relatively high for ±1 yr relative to the zero IE frequency, and less so for the ±2 yr and greater classes. These frequencies depend on the readability score of the otolith and its average age and were modelled in two stages. In the first stage the frequency of the absolute value of IE, the AIE, considered as 0, 1, 2, 3, 4, 5 yr and greater, classes for each of the 4 readability classes and 7 aggregate age classes were modelled using continuation ratios and predicted proportions in each AIE class obtained for a given readability score and age. Proportions of the AIE 1 yr error class decreased relative to the AIE 0 class as readability improved while, in general, it increased as age increased. To model any degree of asymmetry in IEs, a binomial/logistic model of the proportion of non-zero IEs that were negative was fitted for given readability and age. This probability decreased from around 0.7 to 0.4 for ages 5 and 21 yr respectively, but did not depend on readability. The construction of the ageing error matrix is described and combines the modelled probabilities for AIE and negative IE while taking into account logical constraints. This two stage approach makes efficient use of the data since only half the number of combinations of error class by readability by age class is required compared to modelling IE classes directly. This approach differs from other studies of ageing error in that it takes into account the otolith readability score and the integer nature of ring count data.

Introduction

Age estimation for the sub-sample of fish aged to provide this data involves specialist readers counting the number of annual growth rings on otoliths extracted from sampled fish. This counting process is subject to error since it requires subjective assessment by a reader of whether ambiguous markings on an otolith should be interpreted as true annual rings.

We assume that increments between rings formed on the otolith represent annual growth, following the interpretation of increments of Krusic-Golub and Williams (2005) and SC-CAMLR (2001) (see also Appendix 3) and that the error in age determination arises from the above subjective assessment by a reader. This ability to distinguish true from false rings may vary from reader to reader and from otolith to otolith. The readability score is a useful measure of how clearly distinguishable the nominal annual rings are for a given otolith. It is a subjective score assigned by the reader at the time of reading and, under the AAD protocol, ranges in integer value from 1 (unreadable) to 5 (excellent readability). Only read ages of readability scores 2 (just readable) to 5 were used for this study. The median readability for read otoliths used for determining catch-at-age proportions described in Candy (2009b) was 3.

Since the true age is not known for each fish, only the precision of age estimates between and within readers and between and within otoliths can be directly quantified. The average deviation, across otoliths, between readers in the read age and the average for the otolith for multiple reads of the same otolith, is quantified but bias in terms of the difference between the average read age and the true age for an individual otolith is assumed to be zero. There were insufficient data to model within-reader-within-otolith variation (i.e. repeat readings of the same otolith by the same reader) separately to between-reader-within-otolith variation so these two sources of variation are not distinguished in the models describing the precision of read ages.

After application of an age length key to catch-at-length data to derive a catch proportions by ages (Candy 2009a; Candy 2009b), these proportions are input as observations to the integrated assessment carried out using CASAL (Bull *et al.* 2005). CASAL allows as input an ageing error matrix, **E**, which is specified as the probability that a sampled fish of true age class a $(a=1,\ldots,r)$ (where r is a 'plus-class' for accumulating ages of r and above) is assigned to observed class a' $(a=1,\ldots,r)$ in the above input data, so that the $(a,a')^{\text{th}}$ element of **E** is given by

$$\mathrm{E}_{a,a'} = \Pr\left(A' = a' \middle| A = a\right)$$

where $\sum_{a'=1}^{r} \mathrm{E}_{a,a'} \equiv 1$.

If p_a is the observed proportion in the input data and Q_a is the CASAL model-based expected proportion of the catch in true age class a, then CASAL calculates the predicted proportion in age class a, $\hat{P}_a$ to compare with the observed value p_a, and thus calculates the likelihood contribution of that observed/predicted pair by applying the ageing error matrix to Q_a so that

$$\hat{P}_a = \sum_{i=1}^{r} E_{a,i} Q_i .$$

If the observed proportions could be determined without ageing error, given by q_a, then $p_a = \sum_{i=1}^{r} E_{a,i} q_i = \sum_{i=1}^{r} E_{a,i} \left(Q_i + \delta_i\right)$ where δ_i is a random error that has zero expectation given constraints that $\sum_{i=1}^{r} q_i \equiv \sum_{i=1}^{r} Q_i \equiv \sum_{i=1}^{r} P_i \equiv \sum_{i=1}^{r} p_i \equiv 1$. Therefore, the ageing error matrix affects not only the expected value of p_a but also its variance via the term $E_{a,i}\delta_i$ in the above summation. Since a multinomial likelihood for catch-at-age data with nominal sample size *N* is assumed for CASAL estimation it is possible to express the effect of a fixed reader error matrix **E**, random ageing error (i.e. draw random samples from a multinomial with proportions $E_{a,i}$ ($i = 1,\ldots,r$) separately for each *a*), and sub-sampling of length frequency samples for age determination (subsequently used to construct ALKs) using an 'effective sample size' (ESS) assigned to *N*. The method of determining ESSs for the CASAL assessment is described in Candy (Candy 2009a).

In this paper the method of determining the ageing error matrix **E** is described. It uses the reference collection of multiple reads of the same set of otoliths by the CAF and three in-house readers. The calculation of the matrix **E** takes into account the readability score of otoliths. However, application of **E** in the integrated CASAL assessment must assume an average readability (converted to the nearest integer) for all age samples used for the assessment since CASAL allows only a single overall ageing error matrix. For the calculation of ESS, carried out prior to estimation of age-structured model parameters by CASAL, the average readability of otoliths within specific fishery and year combinations was used to determine **E**. Each row of this matrix was then used to give the expected values for random multinomial sample draws in order to construct a randomly sampled **E** matrix (Candy 2009a; Candy 2009b).

Since read ages based on ring counts are integers, and given the availability of readability scores under the AAD protocol for ageing toothfish, a different approach is taken to that described by Punt *et al.* (2008) who did not allow for different levels of readability and assumed read ages are continuous values via their use of the Gaussian distribution for errors. Integer error (IE) (i.e. the integer value of the difference between a read age and the nearest integer to the average age across reads of the same otolith) and absolute integer error (AIE) (i.e. the absolute value of IE) were calculated and the frequency of AIE values of 0, 1, 2, 3, 4 and 5 and greater were tabulated by age class and readability class.

Age classes of more than a single year were employed to reduce the number of zero frequencies. The proportion of read ages in each AIE class was modelled using continuation ratios (Candy 1991; Candy 2003; Fienberg 1980) constructed as a sequence of conditional binomials for AIE classes 0 to 4 with expectation given by the inverse logit of a linear predictor consisting of the readability factor plus a linear term in the mean age of each age class. Therefore a set of parameter estimates were obtained for each of AIE classes 0 to 4. The binomial/logit models for each AIE class were fit as a Generalized Linear Model (GLM) (McCullagh and Nelder 1989). Predicted proportions in each AIE class as a function of age and readability were recovered, with the 5 yr or greater absolute error class obtained by difference between the total for classes 0 to 4 and unity (Candy 2003). To determine if errors are symmetric (i.e. an equal probability of an under-estimate by 1 year to that of an over-estimate by 1 year, and similarly for a 2 year error and so on) a binomial/logistic GLM was fitted to the frequency of negative errors as a proportion of all non-zero errors as a function of readability class and age class. This probability can then be applied to predicted proportions in each non-zero AIE class to give the proportion of negative errors and conversely to give the proportion of positive errors. This two stage approach is more efficient in its use of the data since it requires only half the number of combinations of error class by readability class by age class compared to modelling integer error (IE) classes which take the sign of the error directly into account.

The above approach does not consider readers as fixed effects (i.e. to be included in predictions of **E**) and considers that the variability in error is largely between reads of the same otolith. These assumptions were tested using linear and linear mixed models of integer error (IE) and absolute relative error (i.e. AIE divided by age).

The matrix **E** can then be calculated given the average readability (to the nearest integer) of the relevant set of otoliths using these predicted proportions in each AIE class. The diagonal of **E** is given by the AIE 0 class, and lower diagonal off-diagonal elements are given by the probability of negative errors multiplied by the probability of an error being in each of the 1 to 5+ AIE classes except where logical constraints are imposed. For upper diagonal off-diagonal elements the same calculation is used except that the complement of the probability of a negative error is used and again logical constraints are imposed. Further details and an example of the calculated **E** matrix are given later.

The advantage of considering errors as integers rather than as continuous values (i.e. incorporating fractions of a year) as in Punt *et al.* (2008) is that if the readers are, on average across readers, unbiased then the true age will be the nearest integer to the mean age of the sample of read ages for an individual otolith. In addition, modelling errors as integers is consistent with the integer-age valued error matrix and also allows logical constraints on construction of the ageing error matrix for ages near the minimum and maximum of their range used in the integrated assessment (i.e. age 1 and 35 for the HIMI integrated assessment, Candy and Constable, 2008) to be easily incorporated. Using a Gaussian distribution for errors considered as continuous values (Punt *et al.* 2008) makes imposing these constraints more difficult since a further constraint is that the proportions of the error matrix should sum across columns to 1 for each row; the question is then how to scale the predicted proportions from a Gaussian distribution when the tails of this distribution violate logical constraints? One way this could be approached is to integrate the Gaussian distribution between integer values to give probabilities for each integer error class but this does not appear to have been incorporated in Punt *et al.* (2008) (i.e. see their equations 1 and 2). We prefer to model the integer errors directly since ring counts are by nature integers.

Methods

Selection, preparation, and reading of otoliths

A collection of over 21,000 otoliths have been collected from the HIMI fishery since it began (Welsford and Nowara 2007), necessitating a subsampling program to select unaged otoliths for processing and reading. A routine was developed to randomly select unaged otoliths, using aged otoliths from the HIMI region aged to develop a von Bertalanffy growth curve (Candy *et al.* 2007). The routine was designed to subsample the HIMI otolith collection such that the minimum otoliths would be selected for processing, from each length class present in any length 50 mm length class × season × subfishery combination used in the HIMI integrated assessment (Candy and Constable 2008), while maximizing the number of age classes likely to be detected in the subsample. The subsampling routine is described in detail in Appendix 4.

Each otolith was cleaned, if necessary, and then weighed to four decimal places on a Mettler Toledo balance. One otolith of each pair, chosen at random, was set in a polyester resin block and three to five 0.35mm sections were cut around the primordium using either a Buehler Isomet low speed saw or a Gemmasta high speed saw, both with diamond impregnated blades. The sections were rinsed in water and ethanol before being mounted on a slide. The three to five sections from one otolith were placed in order of cutting onto a single slide and covered in clear casting resin under a cover slip. Images were taken of the best section and the number of rings was read from these images and entered into the database along with a readability index. Each otolith was rated for readability, using a five point scale from 1 (unreadable) to 5 (very good). The preparation method is described in detail in Appendix 3.

A reference collection of 203 otoliths was created. Campana (2001) suggests that a reference collection should include otoliths that are representative of the entire length range, age, sex, season, geographic range, method of capture and collection years. Otoliths were selected on a random basis from within these categories (Appendix 4), with the exception that some otoliths with average readability were substituted with better quality specimens having equal values on other covariates. This was done because sections of average quality were well represented and it was important to have a reasonable number of good quality sections for training purposes in reading of otoliths. Substitution in this manner would affect sample sizes for subsequent analyses of reader error, but would not be expected to bias results since otolith readability was included as a covariate. In summary, the reference collection contains otoliths of fish from across the size range, years and the three gear types (trawl, trap and long line) used in the HIMI toothfish fishery.

The reference collection was read by four readers, with one of them being the reader from the CAF in Victoria who had read most of the previously aged otoliths up to 2003. The reference collection was read by the principal reader several times during the project to assess possible drift in age estimates over time by this reader. The complete selection of otoliths was read by the principal reader, with a sub-sample read by another two readers.

A total of 203 otoliths were used for the reference collection with 933 readings taken across readers. Most otoliths were read by each of the 4 readers, denoted CAF, Read1, Read2, Read3, where the last three readers were AAD readers. A total of 259 readings were multiple readings of an otolith by the same reader while 9 otoliths were read by fewer than the 4 readers. Figure 1 shows a histogram of the number of readings per otolith. Figure 2 shows a histogram of the average reliability score calculated for each of the 203 otoliths. Figure 3 shows a histogram of the NI average estimated age of otoliths.

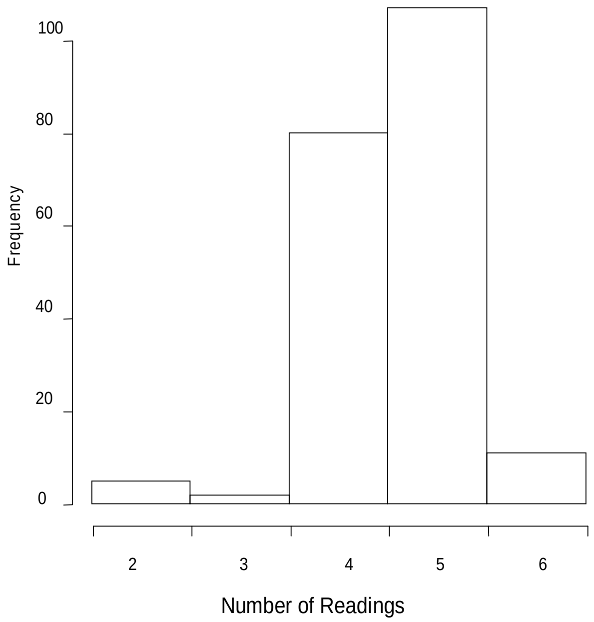

Figure 1. Histogram of the number of readings per otolith in the reference collection.

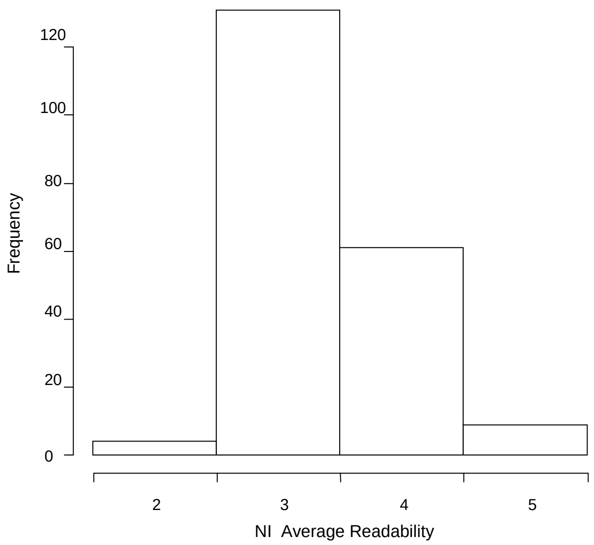

Figure 2. Histogram of the average reliability score calculated for each of the 203 otoliths in the reference collection (NI = nearest integer)..

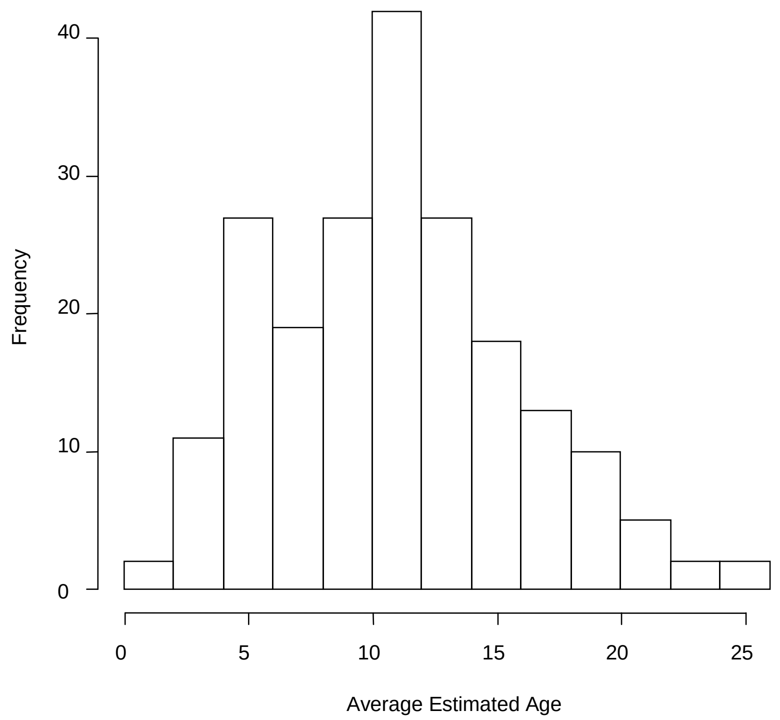

Figure 3. Histogram of the nearest integer average estimated age of otoliths.

Statistical methods and models

Three measures of reader error were investigated where the 'true' age was taken to be the mean of the age estimated for a given otolith across readers. This mean may have more than a single read contributed for a given reader (see Table 1). The simple integer error (IE), was calculated as, $e_{ijk} = a_{ijk} - \bar{a}_i$; $j = 1,...,n_i$, $k = 1,...,r_{ij}$, where $\bar{a}_i$ is the mean age over all reads of otolith *i* rounded to the nearest integer (NI), and a_{ijk} is the age given by read *k* of reader *j*. Absolute integer error (AIE) as $\varepsilon_{ijk} = \left| a_{ijk} - \bar{a}_i \right|$ while absolute relative error (ARE) was given as $\xi_{ijk} = \left| a_{ijk} - \bar{a}_i \right| / a_{ijk}$. The frequency of AIEs of 0,1,2,3,4, and 5 or greater years was calculated for each combination of readability class (2,3,4,5) and age classes of (1,2),(3-5),(6-8),(9-11),(12-14),(15-17), and >17 years.

Integer error (IE) and absolute relative error (ARE) were modelled as linear models (LM) and linear mixed models (LMM), respectively, as a function of age, readability, and reader, with these last two variables each represented as 4-level factors. The random term in the LMM for ARE was otolith identifier represented as a 203-level factor. A similar random term could not be used in a LMM for IE since the nature of the calculation IE (i.e. as an otolith-level deviate) results in a close to zero estimate for all otolith-level random effect estimates for this analysis.

Continuation-ratio models of absolute integer error class frequency

The proportion of read ages in each AIE class was modelled using continuation ratios constructed as a sequence of conditional binomials for AIE classes 0 to 4 with expectation the inverse logit of a linear predictor consisting of the readability factor plus a linear term in the mean age of each age class.

Taking AIE class in turn, from 0 to 4 yr, the probability of an age reading being in class *j*, *j*=1…*s*-1, conditional on being in class *j* or higher is given by

$$\begin{aligned} p_{ikj} = & \ \Pr(\text{in class } j \text{ for age } a_i \text{ and readability } r_k \mid \text{in class } j \text{ or later}) \\ = & \ h^{-1}\left(\eta_{ikj}\right) \end{aligned}$$

where $h(.)$ is a link function (McCullagh and Nelder 1989),

$\eta_{ikj} = \beta_{0j} + \beta_{1j} I_{k,3} + \beta_{2j} I_{k,4} + \beta_{3j} I_{k,5} + \beta_{4j} a_i$ is the linear predictor,

and $\left(\beta_{0j}, \beta_{1j}, \beta_{2j}, \beta_{3j}, \beta_{4j}\right)$ the regression parameters for the j^{th} AIE class, and $I_{kr_{k'}}$, (k'=2,3,4) is a set of dummy variables specifying readability class as a 'factor' so that $I_{kr_{k'}} = 1$ if $r_k = r_{k'}$ and zero otherwise. No model is required for the final class, *s*, as explained later. Following Candy (1991), the frequency of each AIE class by age class by readability class, n_{ikj} is modelled as binomially distributed conditional on N^*_{ikj} with expected value $N^*_{ikj} p_{ikj}$ where

$$\begin{aligned} N^*_{ikj} &= N_{ik} , & j &= 1 \\ &= N_{ik} - \sum_{h=1}^{j-1} n_{ikh} , & j &= 2, \ldots, s-1 \end{aligned} ,$$

and $N_{ik} = \sum_{j=1}^{s} n_{ikj}$ is simply the sum of the number of errors across all error classes for age class *i* and readability class *k*.

The logit link function was used so that $h\left(p_{ijk}\right) = \ln\left\{p_{ijk} / \left(1 - p_{ijk}\right)\right\}$.

The continuation ratios (CR), c_{ikj}, (Fienberg 1980) are the odds $c_{ikj} = p_{ikj}\left(1 - p_{ikj}\right)^{-1}$ with sample values $n_{ikj}\left(\sum_{h>j}^{s} n_{ikh}\right)^{-1}$ so that the logarithm of the continuation ratio is equivalent to the logit of the conditional probability, p_{ikj}.

An estimate of the unconditional probability of being AIE class *j* for age a_i and readability class r_k, can be recovered from the fitted model (1) (Candy 1991; Candy 2003) as

$$
\begin{aligned}
\hat{q}_{ikj} &= h^{-1}\left(\hat{\eta}_{ikj}\right) & j &= 1 \\
&= \left(1 - \sum_{h=1}^{j-1} \hat{q}_{ikh}\right) h^{-1}\left(\hat{\eta}_{ikj}\right) & j &= 2,\ldots,s-1 \\
&= \left(1 - \sum_{h=1}^{s-1} \hat{q}_{ikh}\right) & j &= s
\end{aligned}
$$

So although the (*s*-1) models are fitted to the observed conditional probabilities, n_{ikj} / N_{ikj}^{*}, it is the predicted unconditional probabilities, $\hat{q}_{ikj}$, that are the required proportions of each AIE class for age class. No model is required for the final stage since the unconditional probability for this stage is obtained by difference as seen above.

Therefore a set of parameter estimates were obtained for each of AIE classes 0 to 4. The binomial/logit models for each AIE class were fit as a Generalized Linear Model (GLM). For some combinations of readability factor and age class there were no otolith ages so binomial totals for these combinations were set to missing values in the fit of the conditional binomial. Also for the AIE classes 3 and 4 no read ages were available for readability class 5 and for AIE class 4 no read ages were available for readability class 4. This is because large reading errors are unlikely when otoliths have very good readability. Therefore for AIE classes 3 and 4 a simpler conditional binomial model was used which depended only on age and not readability. Predicted proportions in each AIE class as a function of age and readability were then recovered with the 5 yr or greater absolute error class obtained by difference between the total for class 0 to 4 and unity (Candy 1991; Candy 2003).

Analysis of AIE assumes symmetry of errors around zero. To investigate this the number of negative and positive IE values (i.e. ignoring IE values of zero) were tabulated by readability class and age class (using the same age classes as used to tabulate AIE), with the exception that the age 1-2 class was dropped since logical constraints preclude negative errors of either 1 or 2 years for this class. The proportion of negative errors was modelled using a binomial GLM with logit link function as a function of readability class and age. This model is given by

$$\begin{aligned}\pi_{ik} &= \text{Pr(non-zero error is negative for age } a_i \text{ and readability } r_k\text{)}\\ &= h^{-1}\left(\beta_0 + \beta_1 I_{k,3} + \beta_2 I_{k,4} + \beta_3 I_{k,5} + \beta_4 a_i\right)\end{aligned}$$

where a_i is the average age for age class *i*, $h(\pi_{ik}) = \ln\{\pi_{ik}/(1-\pi_{ik})\}$ and the frequency corresponding to π_{ik} is assumed binomial conditional on the total number of non-zero errors in the corresponding age class by readability class combination.

Calculation of the ageing error matrix, E

The CASAL software (Bull *et al.* 2005), and calculation of effective sample size (ESS) for age data described in Candy (2009a), require calculation of an ageing error matrix (AEM). Each row of the AEM represents the true age (without reader error) and rows ranging from 1 to 35 represent ages 1 to 35 in the HIMI integrated toothfish assessment. The body of the matrix represents the proportions of observed ages (i.e. read ages) for a given true age. The diagonal of this matrix is given by the proportion predicted given true age that has an AIE of 0 given average readability of the set of otoliths aged.

The matrix **E** can then be calculated given the average readability (to the nearest integer) of the relevant set of otoliths using these predicted proportions in each AIE class. The diagonal of **E** is given by the AIE 0 class, and upper off-diagonal elements are given $(1-\pi_{ik})q_{ik}$ except where logical constraints are imposed (e.g. a true age 1 fish can only have a +1 or greater read age so q_{ik} rather than $(1-\pi_{ik})q_{ik}$ probabilities were employed for this true age class and similarly for true age 2 class and +2 and greater errors). Note that the proportion of AIE class reads that were 5 years or greater was negligible for all readability classes and age classes so the logical constraints were only imposed up to an absolute error of 5 yr. Similarly, lower off-diagonal elements of **E** are given $\pi_{ik}q_{ik}$ except where logical constraints are imposed for age *r*, *r*-1, …*r*-5 fish.

Results

Table 1 shows the frequency of IE, AIE, ARE across all age and readability classes.

Figure 4 shows the read age versus the nearest integer (NI) Mean age for the otolith with circles proportional to the frequency in the combination of the two ages expressed as integer factors. The frequencies used in Figure 4 were summed across otoliths and readers for each combination.

Table 1. Frequency of integer error (IE), absolute integer error (AIE) and absolute relative error (ARE) values aggregated across all age and readability classes

Variable	Integer value/class									
IE (yrs)	-5^-	-4	-3	-2	-1	0	1	2	3	4	5^+
Frequency	3	2	13	55	237	382	169	48	16	5	3
AIE (yrs)	0	1	2	3	4	5^+					
Frequency	382	406	103	29	7	6					
ARE	0	0.1	0.2	0.3	0.4	0.5	0.6^+				
Frequency	382	254	204	38	27	17	11				

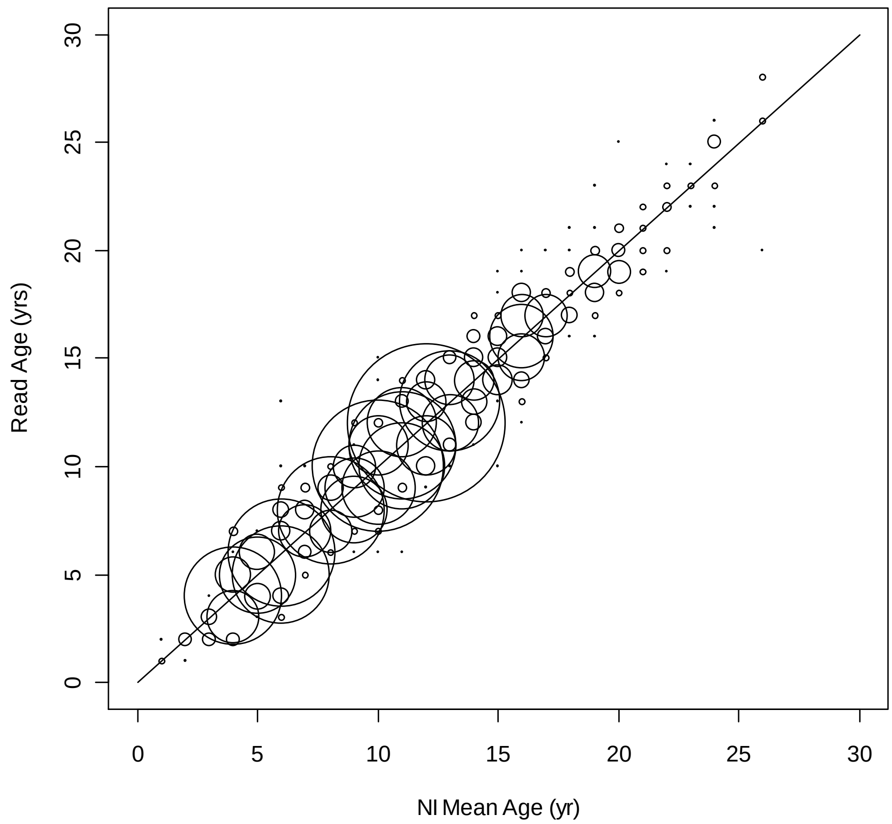

Figure 4. Read age versus nearest integer (NI) mean age with circle size proportional to the frequency in the combination of the two ages expressed as integer factors. The frequency is summed across otoliths and readers for each combination. The 1:1 line is shown.

Table 1 indicates that there is a preponderance of negative 1 year IE values compared to positive 1 year IE values (chi square statistic 10.4, 1 df P<0.001) and this is also the case for 2 year IE values but the difference is not statistically significant (chi square statistic 0.5, 1 df P= 0.5). To investigate this further the number of negative and positive IE values (i.e. ignoring IE values of zero) were tabulated by readability class and age class (using the same age classes as used to tabulate AIE) with the exception that the age 1-2 class was dropped since logical constraints preclude negative errors of either 1 or 2 years for this class. The proportion of negative errors was modelled using a binomial GLM with logit link as a function of readability and age class main effects (i.e. replacing continuous age class mean values with age class as a factor). Readability class was found to be non-significant (P>0.05) but age class was highly significant (P<0.001). Figure 5 shows the estimated values of proportion negative errors (± SE) along with fitted curves for the quadratic GLM (i.e. age as a factor replaced by linear and squared terms in mean age for each age class) and the spline fit for the binomial/logit Generalized Additive Model (GAM) (Wood 2006). The quadratic GLM model is given by

$$\begin{aligned}\pi_{ik} &= \text{Pr(non-zero error is negative for age } a_i\text{)} \\ &= h^{-1}\left(\beta_{0j} + \beta_1 a_i + \beta_2 a_i^2\right)\end{aligned}$$

where estimates of $(\beta_0, \beta_1, \beta_2)$ were 1.858485 (SE=0.596440), -0.194970 (SE=0.098300), and 0.003770 (SE=0.003735), respectively. The residual deviance was 30.3 for 21 degrees of freedom while the corresponding values for the GLM with age included as a factor were 27.8 and 18 degrees of freedom. This indicates that the lack-of-fit of the quadratic model is non-significant (chi square statistic of 2.5 on 3 degrees of freedom, P=0.5).

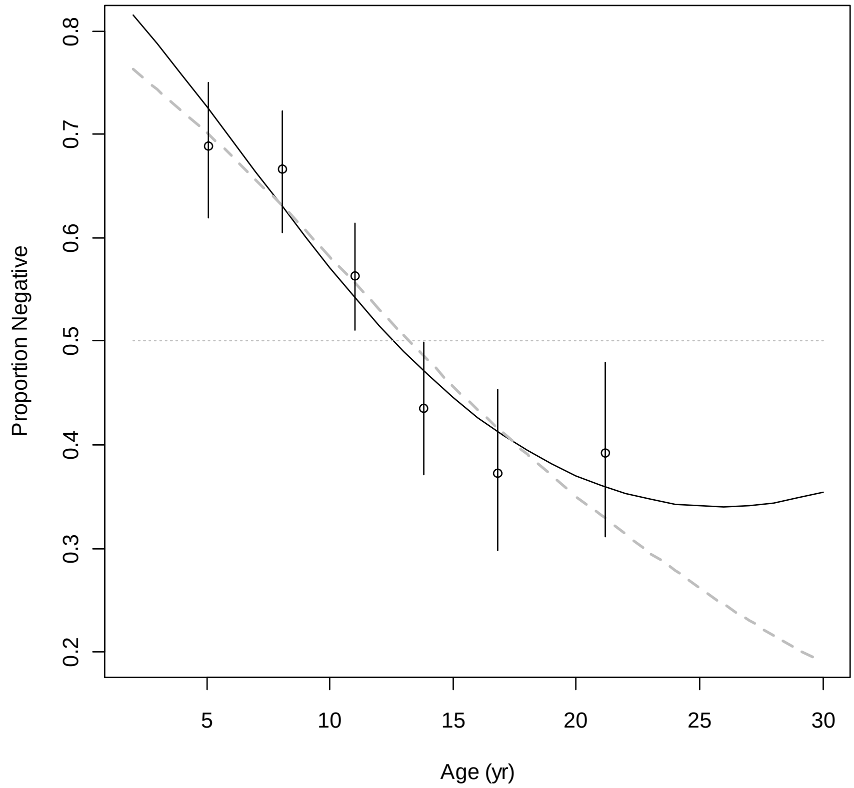

Figure 5. Proportion of errors (integer error) that are negative as a function of age. Points are fitted values from the binomial GLM with age class as a factor (standard error bars shown), the solid line is the prediction from the fit of the binomial GLM with linear and quadratic terms in age-class mean age, and the dashed line is the prediction from the binomial GAM.

Table 2 shows the mean values of IE for each reader from the fit of a linear model (LM). The corresponding ANOVA showed that there is a significant difference between readers in the mean IE but these differences are small (ranging from -0.28 to + 0.26 yr) relative to random errors within and between readers given by the residual standard error for the LM of 1.2 yr and compared to the high frequency of IE values of ±1, ±2 years shown in Table 1.

Table 2. Means from linear model fit to integer error with readers as fixed effects

	Reader (Standard Error)			
	CAF	**Reader1**	**Reader2**	**Reader3**
Mean IE	0.025 (0.085)	0.275	-0.190	-0.258
Difference (Reader#-CAF)		0.250 (0.120)	-0.215 (0.114)	-0.283 (0.111)

The LMM fitted to ARE values used fixed effects of age, the square of age, and readability class, with random effects for each otolith and reader. When reader was included in the LMM as a fixed effect this term was not statistically significant (*P*>0.2). Wald statistics indicated that readability class and age terms were highly significant. Average ARE (adjusting for age) decreased consistently with increasing readability, with the age adjusted difference between the reference score of 2 and scores of 3, 4, and 5 of 0.026 (SE=0.015), 0.046 (SE=0.016), and 0.065 (SE=0.022), respectively. ARE declined with age initially but the decrease slowed with the quadratic trend approaching its lower limit by age 32. Most of the variability in ARE was between reads within otoliths with an estimated variance of 0.012 while between otolith and between reader variances were 0.003 and 0.00003, respectively.

Coefficient-ratio models

Table 3 gives the coefficients for each continuation-ratio model for AIE values of 0,1, 2, 3, 4 yrs. Figure 6 shows predicted values of probabilities, $\hat{q}_{ikj}$, for AIE classes 0, 1, 2, 3, 4, and 5+ for each readability class and each age class. Figure 7 also shows the predicted probabilities but in addition overlays the observed frequencies expressed as proportions along with the SE bars determined for the multinomial distribution with sample size given by the total number of AIE values in each readability class by age class combination.

Appendix 5 gives predicted error matrices, **E**, for ages 1 to 35 for each of readability scores of 3,4 and 5.

Table 3. Continuation-ratio model regression parameter estimate for absolute integer error (AIE) values.

AIE value	Regression parameter estimate (SE)				
	Readability				Age
	2 (Intercept)	3	4	5	
0	-0.26345 (0.28463)	0.17489 (0.26997)	0.52351 (0.28171)	1.39572 (0.36558)	-0.04160 (0.01428)
1	0.97447 (0.36529)	0.83984 (0.32927)	1.54899 (0.37515)	3.19698 (1.07455)	-0.08001 (0.02036)
2	1.00638 (0.61912)	0.53980 (0.51015)	0.69393 (0.64261)	16.52525 (2399.5)	-0.04566 (0.03642)
3	1.67913 (0.91421)	-	-	-	-0.06715 (0.06075)
4	-0.09559 (1.5106)	-	-	-	0.01708 (0.09614)

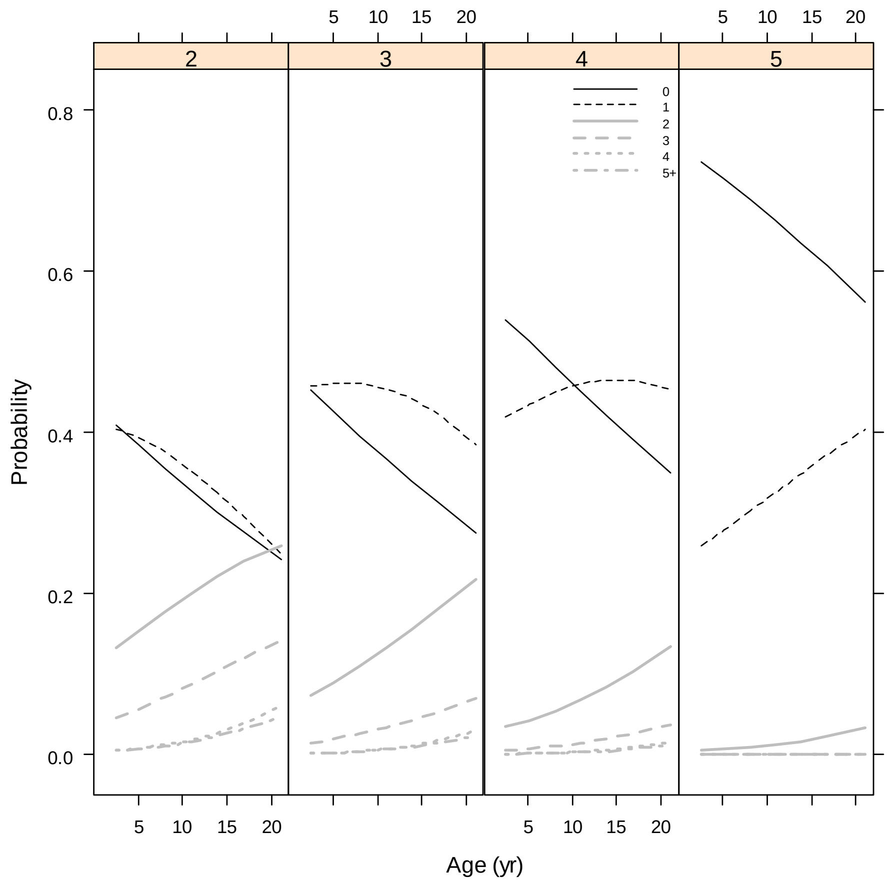

Figure 6. Predicted values of probabilities, $\hat{q}_{ikj}$, for absolute integer error (AIE) classes 0, 1, 2, 3, 4, and 5+ for each readability class and each age class.

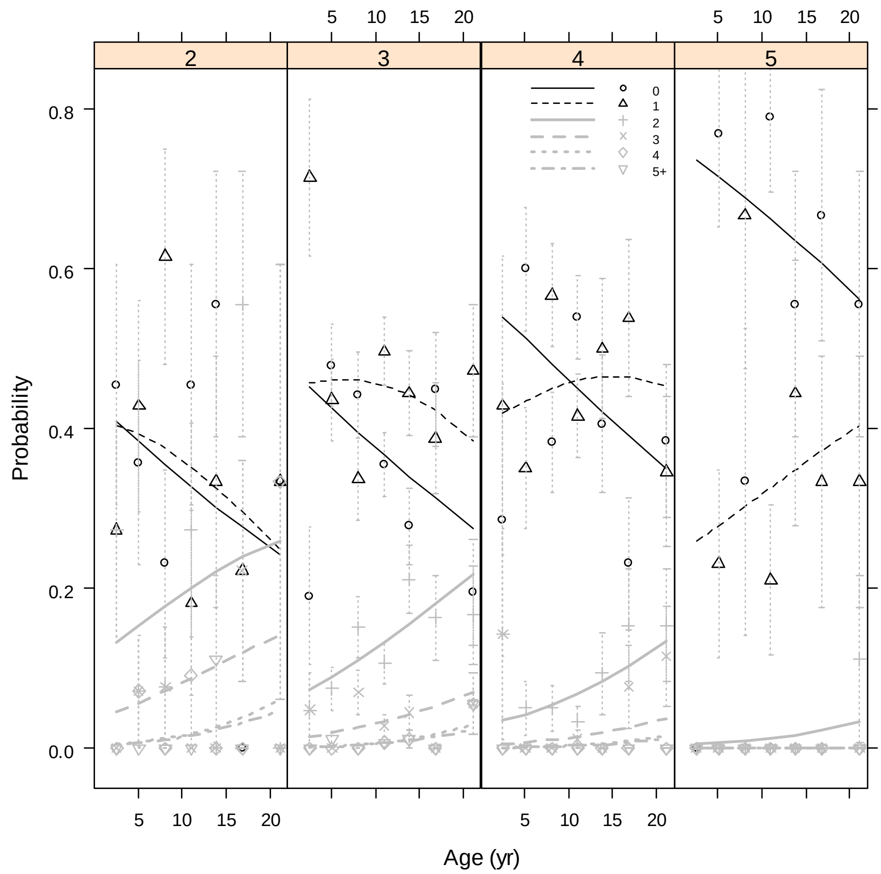

Figure 7. Predicted values of probabilities, $\hat{q}_{ikj}$, and observed proportions showing SE bars for AIE classes 0, 1, 2, 3, 4, and 5+ for each readability class and each age class.

Discussion

This study has, for the first time, incorporated readability scores of otoliths in estimation of reader error and Figure 6 demonstrates that it is an important factor in accurately quantifying reader error, even when the readability scores of 2 and 5 were not well represented in the sample. Overall, the low sample size of otoliths in the reference collection and total number of repeat readings has limited the ability to model reader error more precisely. This is reflected in the large standard errors of observed proportions seen in Figures 5 and 7 and indicates that the sample size of 203 otoliths with 933 readings in total should be increased if more precise predictions are required. Given the sensitivity of the results of the integrated assessment, in particular the key parameter estimate of the recruitment coefficient of variation (Candy 2009b), to the estimated ageing error matrix, increasing the number of otoliths in the reference collection is recommended. In sampling extra otoliths a priority should be to increase the number in the poorly represented readability classes 2 and 5, and in the tails of the age distribution (i.e. above age 18 and below age 5, Figure 3).

This study has also emphasised the advantage of considering errors as integers rather than as continuous values (i.e. incorporating fractions of a year) as in Punt *et al.* (2008). Modelling errors as integers is consistent with the integer-age valued error matrix and also allows logical constraints on construction of the ageing error matrix for ages near the minimum and maximum of their range used in the integrated assessment (i.e. age 1 and 35 for the HIMI integrated assessment,Candy and Constable 2008) to be easily incorporated. It also is theoretically more appropriate to model errors as integers directly since ring counts are by nature integers.

Incorporating sample variation and random reader error into effective sample size calculation in the application of age length keys and catch-at-age data to integrated assessments for *Dissostichus eleginoides*

Steven G. Candy

Catch-at-age proportions are generally incorporated into an integrated assessment as observations that contribute to the objective function via a multinomial likelihood. The multinomial likelihood requires a nominal sample size for each fishery and year combination. A method is described for estimating an effective sample size (ESS) that can be used as the nominal multinomial sample size. The method accounts for both the variation associated with the sub-sampling of the random length frequency (LF) sample for ageing, and random reader error when ageing fish. The catch-at-age ESS is estimated by dividing the ESS for the LF sample, where this ESS is obtained from the haul-level LF data, by an over-dispersion parameter estimate obtained from simulated samples of age frequency data. These samples are obtained using Monte Carlo multinomial replicates of the observed age length key (ALK) with each ALK used to generate a replicate age frequency sample. For each replicate a random draw of the ageing error matrix was taken and applied to the age frequency sample, thus combining both sources of variation. The over-dispersion parameter was estimated from the fit of a log-linear Poisson generalized linear model to the replicated length frequency data. Using simulated data to include only sampling error, the over-dispersion parameter declined from a maximum of around 6 to 8 down to close to 1 as the aged sample fraction increased from 1% to 10%. When random ageing error was combined with sampling error the corresponding values were lower, with a corresponding range of around 4 down to 1. This reduction is due to the way the ageing error matrix ‘smooths-out’ peaks in the true (i.e. without ageing error) age frequency data.

Introduction

The integrated assessment for Patagonian toothfish *Dissostichus eleginoides* for Heard Island and McDonald Islands (CCAMLR Statistcial Division 58.5.2) (Candy and Constable 2007; Candy and Constable 2008) uses the CASAL software (Bull *et al.* 2005) and catch-at-length data from commercial catches and abundance-at-length data from annual Random Stratified Trawl Surveys (RSTS), along with other observational data and input parameters. This assessment has been revised (Candy 2009b) to incorporate catch-at-age and abundance-at-age data using age length keys constructed where there exist adequate samples of aged otoliths from annual RSTS and commercial fisheries (i.e. combinations of fishing method, ground and year) including additional ageing of 2535 otoliths collected since the long line fishery commenced as part of this project.

To carry out the CASAL assessment using commercial catch-at-age frequency data, a multinomial distribution is assumed involving proportion-at-age observations for the catch with a nominal multinomial sample size. For catch-at-length and catch-at-age data, Candy (2008), using simulated data, compared methods of assigning an 'effective sample size' (ESS) to the nominal sample size to account for between-haul heterogeneity in frequencies using haul-level data and process error in predicted frequencies from CASAL's age structured population/fishery model. The method adopted for the integrated assessment described in Candy and Constable (2008) used the recommended methods from Candy (2008) for assigning effective sample size to catch-at-length frequencies. However, the simulation study of Candy (2008) for catch-at-age data only considered the case when all fish sampled from the catch were aged and that ages were observed without error. In practice, a sample of the catch is measured for length (i.e. the length frequency (LF) sample), then a usually small sub-sample of fish have their otoliths removed and a further reduced sample of these otoliths are prepared and read for age determination. The catch-at-age is then estimated by combining an age length key (ALK) with catch-at-length proportions. The sub-sampling of LF sample for ageing introduces greater uncertainty in the proportions-at-age in the catch compared to proportions-at-length from the much larger LF sample. In addition, estimation of age by counting growth rings on prepared otoliths is subject to error due to the difficulty in same cases of distinguishing true from false annual rings. Candy *et al.* (2009) described the method used to quantify reader error using a reference collection of otoliths with multiple readings of the otoliths by 4 readers, and also describes the construction of the ageing error matrix used in the CASAL assessment given in Candy (2009b).

This paper describes a method of assigning ESS to catch-at-age data which takes the ESS derived for the catch-at-length frequencies and reduces this ESS to give an ESS that is appropriate for the catch-at-age data. The catch-at-age proportions are obtained by applying an ALK to the catch-at-length proportions which requires the joint sample frequencies for length bins by age classes from the aged sample for the particular fishery and year. A method by which the catch-at-age ESS takes into account uncertainty due to both the sampling fraction of the LF sample that was aged and random ageing error is described. This method has some similarity to that described by Candy (2008) for dealing with 'process error', where a log-linear Poisson generalized linear model (GLM) is fitted to catch-at-length frequencies and the overdispersion parameter estimate from this fit (when greater than 1) used to scale down the catch-at-length ESS (i.e. Finney's correction,Finney 1971). However, there is an important difference between the two approaches. The 'process error' approach is carried out iteratively using two steps: (i) a CASAL fit and (ii) a re-estimation of the dispersion parameter and ESS with step (ii) using CASAL's predicted frequencies as expected values in the log-linear frequency model (Candy 2008). The method used here to estimate the over-dispersion parameter for catch-at-length data is carried out prior to the CASAL fit and fits the log-linear frequency model to a set of Monte Carlo samples of the length by age frequency data (i.e. as a multinomial sample), and for each sample applies the sample's ALK, then draws a random ageing error matrix (using a single Monte Carlo sample draw from a multinomial distribution) with expected value an estimated fixed ageing error matrix as described by Candy *et al.* (2009, and see Appendix 5).

The advantage of replacing catch-at-length data with catch-at-age data in an integrated assessment, despite the greater uncertainty in the the catch-at-age proportions compared to that for the corresponding catch-at-length proportions due to the reduction in ESS, is that catch-at-age observations relate directly to the age-structured model in CASAL whereas catch-at-length proportions must be converted to catch-at-age proportions using a single mean length-at-age model or vector and a single value for the coefficient of variation (CV) of length given age (Candy and Constable 2008). Therefore, the application of age length keys (ALKs) that are specific to each combination of fishery and year should be superior in terms of accuracy of CASAL model parameter estimates compared to models fitted to catch-at-length proportions which require the above assumption of a single mean length-at-age vector and CV.

For abundance-at-age data the same approach is taken to estimate the ESS for the corresponding proportions-at-age. Proportions-at-age are obtained by applying the survey-year specific ALK to the stratum-area-weighted estimates of proportion-at-length. However since these proportions-at-age must be multiplied by the estimated total population size vulnerable to the survey to give abundance-at-age, the variance of abundance-at-age estimates can be obtained using the variance of the proportions-at-age (assuming multinomial distribution with the proportions-at-age ESS as nominal sample size) and the variance of estimated total population size. This method of calculating the variances of abundance-at-age estimates is described in detail in Candy (2009) but is not investigated further here.

This paper describes the above methods in terms of mathematical formulae and gives an example of their application for the calculation of the ESS for catch-at-age proportions using simulated data. The simulated data is used to investigate the effect on the over-dispersion parameter estimate of (i) the sampling fraction of the LF sample that is aged, (ii) combining (i) with a fixed ageing error matrix, and (iii) combining (i) with a random ageing error matrix. It is argued that it is method (iii) that is appropriate for application to the HIMI toothfish assessment and is the method applied in Candy (2009).

Methods

Application of age length keys to obtain catch-at-age proportions

To calculate proportions-at-age from a sample of aged fish with corresponding measured lengths, the aged sample is used to obtain a frequency table cross-classified by age classes, usually consisting of individual years (i.e. ages 1 to 35 in Candy, 2009a), and length bins. The non-parametric ALK approach (Quinn and Deriso, 1999) is to estimate the proportion of fish in the catch at age *a*, p_a, from the frequencies $n_{a,j}$ for age *a* and length bin B_j and LF total sample size of *M* with length bin B_j frequency of m_j as

$$p_a = \sum_{j=1}^{S} \left(m_j / M \right) n_{a,j} / n_{.j} \qquad (1)$$

where $n_{.j} = \sum_{a=1}^{R} n_{aj}$, $N = \sum_{a=1}^{R} \sum_{j=1}^{S} n_{a,j}$,

(*a*=1,…,*R*), and length bins are given by B_j, (*j*=1,…,*S*) with bin upper limits of $K_1 < .. < K_j < ... < K_{S-1}$ so that a fish of length *L* is allocated to bin *j* (i.e. $L \in B_j$) if $K_{j-1} \le L < K_j$ with endpoints defined as $K_0 = 0,\ K_S = +\infty$. The ALK is given by the set of proportions $n_{a,j} / n_{.j}$ for (*a*=1,…,*R*), and (*j*=1,…,*S*).

The estimate p_a is the sample equivalent to theoretically calculating the marginal distribution of age in the population, $f_A(a)$ [i.e if A is considered continuous rather than integer-valued then strictly p_a is an estimate of $\int_{u=a-\frac{1}{2}}^{a+\frac{1}{2}} f_A(u)du$]. This can be seen by considering the conditional distribution for age given length, $f_{A,L}(a|l)$ if fishing selectivity is excluded for the moment (i.e. selectivity is accounted for in the CASAL age-structured population/fishery model, Candy 2009a). It can be shown (see Appendix 7) that $f_A(a)$ can be approximated by $f_A^*(a)$, as follows

$$f_A^*(a)=\sum_{j=1}^{s} P_L(l\in B_j)\left\{\int_{l=K_{j-1}}^{K_j} f_{A,L}(a|l)dl\right\} . \tag{2}$$

Therefore p_a is an unbiased estimate of $f_A^*(a)$, if $Cov(m_j/M, n_{a,j}/n_{.j})\equiv 0$, since m_j/M is an unbiased estimate of $P_L(l\in B_j)$ and $n_{a,j}/n_{.j}$ is an unbiased estimate of $\int_{l=K_{j-1}}^{K_j} f_{A,L}(a|l)dl$ (Quinn and Deriso,1999, see their equation 8.14a) .

Note that length-bin random sampling (i.e. fixed allocation, Quinn and Deriso, 1999) has been used in this fishery for sampling otoliths where this sampling method over-represents the tails of the LF distribution in order to obtain a greater representation of older fish (and very young fish) for ageing than would occur under simple random sampling (Candy *et al.*, 2007). However, the unbiasedness of $n_{a,j}/n_{.j}$ holds true under length bin sampling as is the case for simple random sampling, where in this last case the expected proportion of aged fish in length bins is the same as that for the LF sample, since equation (2) uses the conditional distribution of age given length and not length given age. Modelling the conditional distribution of length given age requires sampling probabilities determined from the length-bin sampling protocol to be incorporated in estimation (Candy *et al.* 2007).

The effect of ageing error on predictions in CASAL and on effective sample size

After application of an age length key to catch-at-length data to derive catch proportions by age using equation (1) these proportions are input as observations to the integrated assessment carried out using CASAL (Candy 2009b). CASAL allows the input of an ageing error matrix, **E**, which is specified as the probability that a sampled fish of true age class a $(a = 1,...,R)$ (where R is a 'plus-class' for accumulating ages of R and above) is assigned to observed class a' $(a' = 1,...,R)$ in the above input data, so that the $(a,a')^{\text{th}}$ element of **E** is given by

$$\mathrm{E}_{a,a'} = \Pr\left(A' = a' \middle| A = a\right)$$

where $\sum_{a'=1}^{R} \mathrm{E}_{a,a'} \equiv 1$.

If p_a is the observed proportion in the input data and Q_a is the CASAL model-based expected proportion of the catch in true age class a, then CASAL calculates the predicted proportion in age class a, $\hat{P}_a$ to compare with the observed value p_a, and thus calculate the likelihood contribution of that observed/predicted pair, by applying the ageing error matrix to Q_a so that

$$\hat{P}_a = \sum_{i=1}^{R} E_{a,i} Q_i .$$

If the observed proportions could be determined without ageing error, given by q_a, then $p_a = \sum_{i=1}^{R} E_{a,i} q_i = \sum_{i=1}^{R} E_{a,i} \left(Q_i + \delta_i\right)$ where δ_i is a random error that has zero expectation given constraints that $\sum_{i=1}^{R} q_i \equiv \sum_{i=1}^{R} Q_i \equiv \sum_{i=1}^{R} P_i \equiv \sum_{i=1}^{R} p_i \equiv 1$. Therefore, the ageing error matrix affects not only the expected value of p_a but also its variance via the term $E_{a,i}\delta_i$ in the above summation. Since a multinomial likelihood for catch-at-age data with nominal sample size N is assumed for CASAL estimation, it is possible to express the effect of a fixed reader error matrix **E**, random ageing error (i.e. draw random samples from a multinomial with proportions $E_{a,i}$ $(i = 1,...,R)$ separately for each a), and sub-sampling of length frequency samples for age determination (subsequently used to construct ALKs) using an 'effective sample size' (ESS) assigned to N. The method of determining ESSs for the CASAL assessment is described below. The fixed ageing error matrix, **E**, was calculated as described in Candy *et al.* (2009) using a readability of 4.

Monte Carlo sampling of age length keys and drawing a random ageing error matrix

For a given ALK, with frequencies n_{aj}, a set of F-replicate ALKs with frequencies n_{ajf} were generated by carrying out f=1…F draws from a multinomial distribution with R by S classes with expected proportions given by $\rho_{aj} = n_{aj} / N$ and sample size N where

$N=\sum_{a=1}^{R}\sum_{j=1}^{S}n_{a,j}$. An F-size replicate set of catch-at-age proportions were obtained by applying equation (1) with the same set of catch-at-length proportions, $p_j^{(L)}=m_j/M$, used for each replicate and its ALK to give a set of catch-at-age proportions, p_{af} , with nominal sample size for all replicates of M' , where M' is the estimated effective sample size for the LF data obtained using the gamma GLM method described by Candy (2008) for haul-level heterogeneity in catch-at-length proportions. This Monte Carlo sampling therefore provides replicate frequencies in order to estimate overdispersion in catch-at-age proportions due to sub-sampling the LF data for fish to be aged.

An additional step was applied for each replicate to account for random reader error. After generating a replicate vector of catch-at-age proportions, p_{af} , for each row of the error matrix **E**, a multinomial sample was drawn with probabilities for row a, of $\mathrm{E}_{a,a'}$ $(a'=1,\ldots,R)$ and sample size $n_{a.}$ where $n_{a.}=\sum_{j=1}^{S}n_{aj}$. This random ageing error matrix $\mathbf{E}_f$ was then applied to p_{af} to give the 'observed-with-ageing error' catch-at-age vector of proportions $\mathbf{p}'_f=\mathbf{E}_f\mathbf{p}_f$. The corresponding catch-at-age vector of frequencies, n'_{af} , was obtained by multiplying $\mathbf{p}'_f$ by M' .

Log-linear modelling of replicated catch-at-age frequencies

A Poisson (log-linear) generalized linear model (GLM) (McCullagh and Nelder 1989) was fitted to the replicated catch-at-age vector of frequencies, n'_{af} , with main effects of age and replicate (as categorical factors) in order for the Poisson model to be constrained to give a log-likelihood equivalent to that of the multinomial. This model is described by

$$\log_e\left\{E\left(n'_{af}\right)\right\}=\beta_1+\sum_{r=2}^{R}\beta_r I_{ar}+\sum_{h=2}^{F}\beta_{R+h-1}J_{fh} \qquad (3)$$

where a Poisson distribution is assumed for the response variable n'_{af} , I_{ar} is a set of dummy variables specifying age as a 'factor' so that $I_{ar}=1$ if r=a and zero otherwise, similarly J_{fh} is a set of dummy variables specifying replicates as a 'factor' so that $J_{fh}=1$ if f=h and zero otherwise, and the β's are parameters to be estimated. Note that since age class and replicate

are included as main effects the Poisson deviance for this model is the same as that for n'_{af} considered as multinomial conditional on the M' (McCullagh and Nelder 1989).

The ESS for the catch-at-age proportions due to sub-sampling and random ageing error was obtained by dividing the catch-at-length ESS, M', by the dispersion parameter which was estimated as the residual mean deviance (McCullagh and Nelder, 1989) from the fit of the GLM defined by equation (3). Therefore if the dispersion parameter estimate, $\hat{\Phi}$, is obtained as the residual mean deviance, then the catch-at-age ESS is given by $\hat{n}' = M'/\hat{\Phi}$. This adjustment is based on Finney's heterogeneity factor (Finney 1971; McCullagh and Nelder 1989). Fitting only main effects in a log-linear Poisson model as in model (3) corresponds to an assumption that there is no significant interaction, while significant interaction (i.e. overdispersion under the main effects model) is indicated if the residual deviance is significantly larger than its degrees of freedom assuming this deviance has a chi-square distribution.

Simulation model

The simulation model used is described in terms of generating catch-at-length frequencies in Candy (2008). For a single fishery and year a sample of 100 hauls, each with a sample of 150 fish measured for length, were drawn from haul-level multinomial distributions where each multinomial had expected values drawn from a set of scaled gamma distributions which, when aggregated across hauls, corresponds to a Dirichlet-multinomial (D-M) distribution (Candy 2008). This simulation model used here is given as Model 1 in Candy (2008) with haul-level sample size set to 150 and D-M over-dispersion parameter set to 10. For each sample fraction ranging from 1% to 10% in 1% increments, an aged sample was drawn from the 15000 length samples, using length bins of 20 mm between 200 and 1300 mm, and 100 mm bins from 1300 to 2200 mm, and ages of 1 to 35 yr. The frequencies for length bins by age class were obtained by multiplying the sample size of aged fish, by the probability for each combination of age class and length bin to allow an ALK to be constructed. These probabilities (i.e. the expected values ρ_{aj}

described earlier) were obtained from the joint distribution of length and age using the von Bertalanffy model, coefficient of variation of length given age, and length-at-age lognormal distributions described in Candy (2008). The ESS for the length frequency data was calculated using the gamma GLM method as described in Candy

(2008) and the LF sample was taken to be the full sample of 15000 fish.

Monte Carlo sampling using 100 replicates with multinomial sampling using the above age by length bin probabilities and nominal sample size given by the particular aged sample size, both with and without random reader error matrix (**E**$_f$), were obtained for each sampling fraction. In an alternative simulation, the above Monte Carlo samples were combined with the fixed reader error matrix, **E**, rather random values. The fixed ageing error matrix was calculated as described in Candy *et al.* (2009) using a readability of 4.

Results

Figure 7 gives an example of an estimated age frequency vector using an ALK for a 5% sample of the 15000 length samples. Figure 1 compares these estimated frequencies to those obtained for the same ALK and aged sample after applying the fixed ageing error matrix (**E**).

Figure 8 compares estimates (points) of the dispersion parameter estimate, $\hat{\Phi}$, from the fit of the log-linear GLM (3) to replicated age frequencies for a given fraction of length samples that were aged for each of the three error models, (i) sampling error (SE) (i.e. due to sub-sampling the LF data) alone, (ii) SE with the application of a fixed ageing error matrix (**E**), and (iii) SE with the application of a random ageing error matrix ($\mathbf{E}_f$). The lines in Figure 8 are exponential decay regressions fitted to the points given by the dispersion parameter estimates.

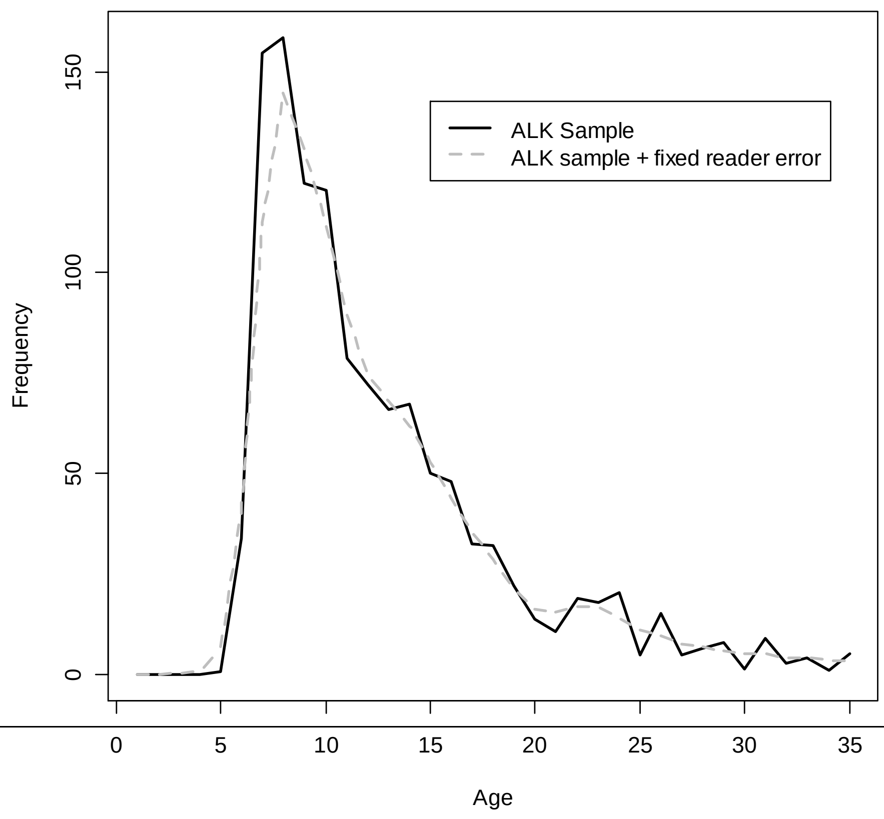

Figure 7. Example of estimated age frequency using an age length key (ALK) from a 5% sample of the 15000 length samples (solid line) and the estimated age frequency for the same ALK and aged sample after applying the fixed ageing error matrix (E).

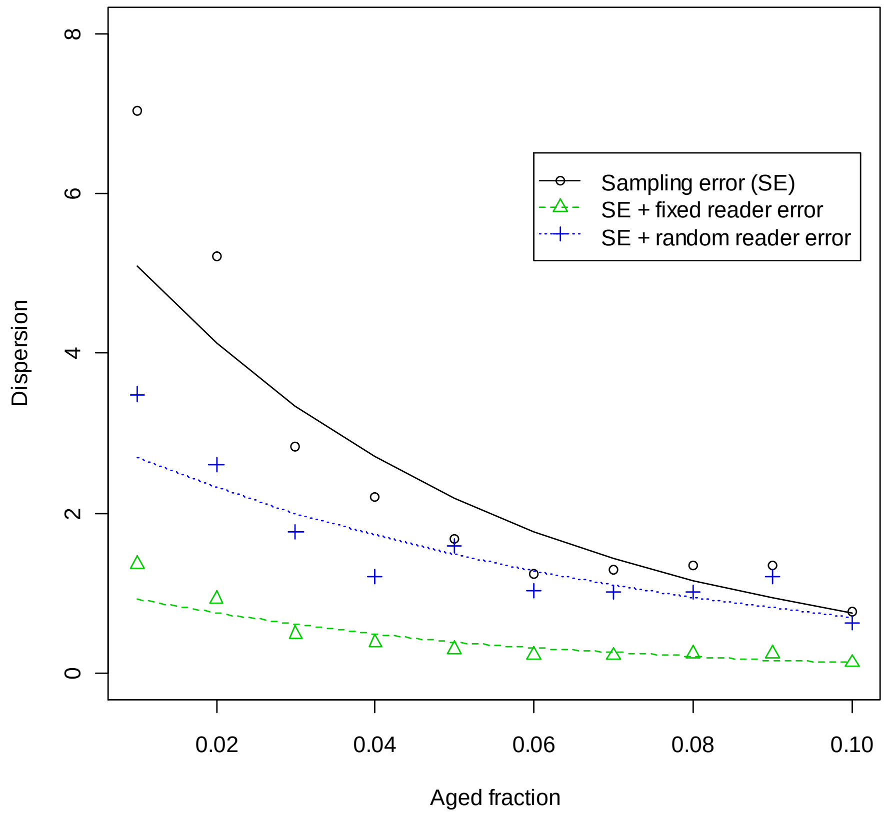

Figure 8. Dispersion parameter estimate, $\hat{\Phi}$, from the fit of the log-linear generalised linear model to replicated age frequencies for a given fraction of length samples that were aged and sampling error (SE) only (solid line, circles), SE with the fixed ageing error matrix (E) applied (dashed line, triangles), and SE with a random draw of the ageing error matrix for each replicate E_f (dotted line, crosses).

Discussion

Figure 7 demonstrates that applying an ageing error matrix to catch-at-age frequencies is effectively a smoothing process whereby peaks in the true length frequencies, due partly to sampling error, are 'smoothed out'. Some peaks can be due to strong year classes moving through the population but this is not the case in this simulation which assumes constant recruitment (Candy 2008). This smoothing process is due to the application of a row of the **E** matrix to true frequencies corresponding to a weighted moving average of these frequencies. Comparing **E** matrices predicted with readabilities of 3 and 5 given in Candy *et al.* (2009), it can be seen that lower readability gives lower values for diagonal elements of **E** and higher values either side of the diagonal. Therefore greater ageing error, as a result of lower readability, gives a greater degree of smoothing. This explains why in Figure 8, the estimate of the dispersion parameter for error model (ii) is much smaller than the other error models, 'over-powering' the overdispersion due to sampling error [i.e. error model (i)]. In fact, the estimate $\hat{\Phi}$ for this error model for sampling fractions above 2% is considerably less than the value that would be expected for true multinomial sampling for the replicated age frequencies (i.e. unity). When a random ageing error matrix was applied at each replicate in error model (iii), the overdispersion estimate recovered to closer to that of error model (i) but was still consistently lower.

Since the frequencies, n_{ajf}, are obtained by repeated sampling from a multinomial distribution with expected probabilities ρ_{aj}, a legitimate question is then 'how does over-dispersion, relative to a nominal multinomial for the age frequencies as quantified by $\hat{\Phi}$ values significantly larger than unity, occur?'. However, in attempting to answer this question it should be noted that the nominal multinomial distribution for resampled joint frequencies is not the same as that for the marginal age frequencies since these last frequencies are obtained from the ALKs by application of equation (1). If equation (1) is re-expressed as

$$p_a = \sum_{j=1}^{S} \left(m_j / M\right)\left(n_{a,j} / N\right) / \left(n_{.j} / N\right)$$

it can be seen that the ρ_{aj} are estimated by the sample values given by the middle term $n_{a,j} / N$. So while the proportions $n_{a,j} / N$ are derived from a multinomial sample, p_a is the weighted sum of these values across length bins with these weights involving a random term given by $n_{.j} / N$. This weighted sum with random weights could be expected to generate extra-multinomial heterogeneity in the values of p_a from the Monte Carlo simulation.

The estimates of $\hat{\Phi}$ are subject to sampling error which is not adequately quantified by the approximate distribution of (residual df)*$\hat{\Phi}$ as a chi square with degrees of freedom (DF) given by the residual DF from the fit of the GLM. This is because these degrees of freedom can be increased arbitrarily in order to lower the GLM estimates of error in $\hat{\Phi}$ to very low levels. More than 100 replicates could be used but, since the fit of the GLM requires the inversion of the Fisher scoring matrix (McCullagh and Nelder, 1989, pg 42) and the order of this matrix, given a fixed number of age classes, depends on the number of replicates, numerical problems in fitting the GLM may occur if the number of replicates is too large. An additional consideration is that the upper and lower tails of the empirical age frequency distribution may contain zero frequencies for some replicates and improvement in the way $\hat{\Phi}$ is calculated could be investigated in future refinements of this approach.

In practice for an integrated assessment, proportions of true ages falling outside their class in observed catch-at-age data (i.e. ± 1, ± 2,…etc yr errors) would be random about a vector of expected values, given by the corresponding row of **E**. Therefore drawing a random set of proportions for each row of **E**, corresponding to the row of $\mathbf{E}_f$ for random draw *f*, is appropriate for quantifying the effect of ageing error on the precision of observed catch-at-age proportions via the estimate of ESS using error model (iii). Also in an integrated assessment, unlike the simulation study here, the ρ_{aj} have already been subject to ageing error so it could be argued that by applying the Monte Carlo simulation approach to actual ALKs, the $\mathbf{E}_f$ is applied on top of the unknown ageing error matrix that was 'applied' when reading of otoliths was carried out. However, the estimation of the $\hat{\Phi}$ should not be very sensitive to smoothing of the true expected values, ρ_{aj}, as long as these smoothed values approximately reflect the true values (e.g. as in Figure 1). Since **E** is an estimated ageing error matrix, some allowance for estimation error could justifiably be incorporated in drawing the random matrix $\mathbf{E}_f$, however calculation of estimation error in this case is complex given the way the **E** matrix was estimated as described in Candy *et al.* (2009). Given an adequate calibration data set for estimation of **E**, the contribution of estimation compared to the multinomial error already incorporated in $\mathbf{E}_f$, as described earlier, should be relatively minor. This could be investigated in detail in future work.

A further step in estimation of ESS for catch-at-age data could be taken in adjusting the ESS for process error. This requires a two-step iterative procedure of fitting the age-structured assessment model and the calculating an over-dispersion parameter from the lack-of-fit of predictions. This was described in detail for catch-at-length proportions in Candy (2008) and Candy and Constable (2008) along with caveats on this approach related to ability to remove any systematic lack-of-fit from predictions in order to give unbiased estimates of overdispersion due to process error.

The method described here for obtaining ESS is not as formal as a full mixed effects multinomial model as described by the Gaussian-multinomial model of Hrafnkelsson and Stefánsson (2004). However, in their case the mixture distributions relate to spatial variation and survey catch-at-length proportions, and not sampling variability associated with ALKs and random reader error. The spatial variability is incorporated in the approach described here via the ESS calculated for catch-at-length data from haul-level LF data. Accounting for additional sources of variability in catch-at-age proportions of sampling variability associated with ALKs and random reader error is provided in a way that can easily be incorporated in integrated assessments via the ESS for the catch-at-age data. This is a more rigorous approach than arbitrarily assigning an ESS as a value somewhere between the number of aged fish and the number measured for length as in Maunder and Langley (2004).

Update of the integrated stock assessment for the Patagonian toothfish, *Dissostichus eleginoides*, for the Heard Island and McDonald Islands using CASAL with abundance-at-age and catch-at-age data.

Steven G. Candy

The integrated for Patagonian toothfish, *Dissostichus eleginoides*, for the Heard and McDonald Islands (Division 58.5.2) was updated by replacing catch-at-length proportions from commercial catches with catch-at-age using age length keys (ALKs) where the ALK for each combination of fishery and year had available a sufficient number of aged fish (assumed >50). For the trawl fisheries that were divided into periods within each year, the same ALK for the year was applied to the length frequency (LF) samples for each fishing period within that year. For years where insufficient fish were aged the catch-at-length proportions were retained but for a given fishery the same selectivity function and parameter values were logically applied to both types of data. For 2006 and 2007 random stratified trawl surveys, there were sufficient aged fish to convert abundance-at-length to abundance-at-age. Effective sample sizes for the commercial catch-at-age proportions, assuming a multinomial distribution, and the coefficient of variation (CV) for the abundance-at-age, assuming a lognormal distribution, each took into account uncertainty due to haul-level variability in catch-at-length proportions, ALK sampling error (sampling fraction of the LF samples that were aged ranged from 0.8% to 18%) and random ageing error. To ensure a realistic value of the CV for the length-at-age model, this parameter was not estimated but fixed to its value estimated from previous modelling of length-at-age data using the von Bertalanffy growth model. CASAL allows a single ageing error matrix to be defined and applies this matrix to predictions of numbers-at-age and proportions-at-age. In other work, this matrix was found to depend on the readability score of the otoliths used for ageing, and sensitivity of the assessment results to the assumed readability score was investigated for readability scores of moderate (3), good (4), and excellent (5). The median score for all aged fish was 3 but some fishery-by-year combinations had a higher value of 4. The output from the integrated assessment of most interest in this study is the CV of the estimated historical recruitment series, since this parameter strongly influences the effect of the depletion rule on the allowable catch. Compared to the assessment that did not incorporate catch-at-age or abundance-at-age data, the aged-based assessment dramatically lowered the CV for the recruitment series, from around 1.5 to 1.8 down to approximately 0.3 to 0.4, if a readability score 5 was assumed or if for a score of 4 the most stable subset (1986-2000) of the full historical series (1984-2006) was used to estimate the CV. There was no reduction in CV

for either series if a score of 3 was assumed. The difference between a readability of 3 and 4 in ageing error is that zero ageing errors are relatively less prevalent (e.g. for age 8 the percentage of errors that were zero was estimated from previous work at 40% for score 3 and 48% for score 4, the corresponding ± 1 yr errors had prevalence of 46% and 45%, respectively). A ± 1 year error may seem minor relative to the complete age range modelled of 1 to 35 yr, however most fish caught are in a more restricted, younger age range. For example, the upper age of fish in the main survey that have an upper selectivity greater than 0.2 was approximately 12 yr while the corresponding values for the trawl and longline fisheries were 15 and 20 yr, respectively. The results presented suggest that future ageing work would give a greater improvement to the integrated assessment if otoliths with readability score of at least 4 can be obtained in sufficient numbers to allow ALKs to be constructed only using ages obtained from these otoliths.

Introduction

The integrated stock assessment models for the Patagonian toothfish, *Dissostichus eleginoides*, for the Heard and McDonald Islands (CCAMLR Statistical Division 58.5.2) fitted using CASAL (Bull *et al.* 2005) were described in Candy and Constable (2007; 2008). The model denoted *a2-ess* in Candy and Constable (2007) was used to determine the allowable catch for 2008 and 2009 fishing seasons (SC-CAMLR 2007). In 2008 this model was updated using 2007-2008 season updates to the Random Stratified Trawl Surveys (RSTS) main survey group, the commercial catch-at-length data, and removals up to the end of July 2008. The 2006 pot fishery catch and catch-at-length data was also included in this update. This model is denoted the *a2-ess-2008* model/data set. These models and previous models rely almost entirely on abundance-at-length data from the main survey (i.e. annual RSTS for 2001 to 2007 and later, excluding 2003) and catch-at-length proportions for the main commercial fishing grounds and gear types (see Table 1 inCandy and Constable 2008). Catch-per-unit effort series and single year surveys that could not be considered comparable in coverage or methods to the main survey were found to have only minor influence on estimated parameters in the integrated assessment.

The output from the integrated assessment of most interest in this study is the coefficient of variation (CV) of the estimated historical recruitment series, since this parameter strongly influences the effect of the depletion rule on the allowable catch. In investigating the effect of this parameter on allowable catch under CCAMLR decision rules (Constable *et al.* 2000) using an informative prior, in previous unpublished work carried out by the author it was noted that allowable catch was strongly related to the mean of the prior lognormal distribution for recruitment CV. Because of the strong influence of the prior mean CV and the subjective nature of choosing such a prior, Candy and Constable (2007) used a non-informative prior and instead smoothed the historical recruitment series using a 2-year running mean of the annual recruitment series to give a CV of 0.86, which compares favourably to the very large estimate of 1.8 obtained from the series without smoothing. This approach, although less subjective that choosing an arbitrary prior distribution in the Bayesian approach, still suffers from uncertainty as to its efficacy in estimating a realistic CV since it could over- or under-smooth year estimates relative to the true series. Another suggestion to possibly improve the CV of estimated historical recruitment was to allow CASAL to estimate the CV_{VB} of the length-at-age distribution, since this parameter influences how LF data is allocated to age classes in

CASAL. The mean length-at-age is input as a fixed set of values in CASAL (or indirectly via the parameters of a von Bertalanffy (VB) model) and a CV_{VB} is also required. In Candy and Constable (2008) the CV_{VB} was estimated by CASAL at close to the value of 0.1 obtained by modelling length-at-age using a VB model with an early age adjustment (Candy *et al.* 2007) but did not improve the CV of the recruitment series relative to models that fixed CV_{VB} at 0.1.

The greatest improvement in modelling historical recruitment was thought most likely to occur when catch-at-age and abundance-at-age data could be provided as observations (i.e. data) to the integrated assessment. This was the rationale behind this project: that, where otoliths were available, the dataset of aged fish from the HIMI fishery would be added to in order to be able to construct fishery and year-specific ALKs. These ALKs could then be used to calculate commercial catch-at-age proportions and abundance-at-age from the surveys. This paper describes an update to the Candy and Constable (2008) integrated assessment using the ageing data to construct ALKs and then calculate catch-at-age proportions for the historical commercial catch. In addition, there were sufficient otoliths collected from the 2006 and 2007 RSTS to allow an ALK to be constructed for each of these years.

Effective sample sizes (ESS) for the commercial catch-at-age proportions, assuming a multinomial distribution, and the coefficient of variation (CV) for the abundance-at-age, assuming a lognormal distribution, each took into account uncertainty due to haul-level variability in catch-at-length proportions, ALK sampling error and random ageing error. Candy (2009) described the method of determining the ESS for commercial catch-at-age proportions and this method is also applied here to determine the CV for the abundance-at-age data by estimating the variance of the product of proportions-at-age in the survey and the stratified sample estimate of total population size for age classes that are vulnerable to the survey.

CASAL allows a single ageing error matrix to be defined and applies this matrix to predictions of numbers-at-age and proportions-at-age. The estimation of this matrix is described in Candy *et al.* (2009) and was found to depend on the readability score of the otoliths used for ageing. The sensitivity of the assessment results to the assumed readability score were investigated for readability scores of fair (3), good (4), and very good (5) (Appendix 3). Not all ages in an individual ALK, or the assessment in general, will be from otoliths with the same readability score therefore an average, rounded to the nearest integer, or alternatively the median score, is used to provide the single ageing error matrix that CASAL allows as input.

Methods

Data

A total of 2535 aged otoliths were added to the existing database to give 6429 ages of individual fish. Of the 6429 individual fish ages, 5455 were in fisheries covered by the assessment and all but 79 of the aged fish outside the area of interest of the assessment were from aging carried out prior to the current FRDC project. For the 5065 fish with a single age reading, 1007 had no recorded readability score and of these 1003 were for ages read prior to this project. The commercial catch-at-length and survey abundance-at-length data are described in Candy and Constable (2008) with the exceptions that data for 2008 were added for these two data types and the catch-at-length data for the single year of the pot fishery in 2006 were also added. Figure 9 shows a histogram of readability score of all otoliths used to construct ALKs. Appendix 6 shows the number of aged fish for each combination of fishery and year.

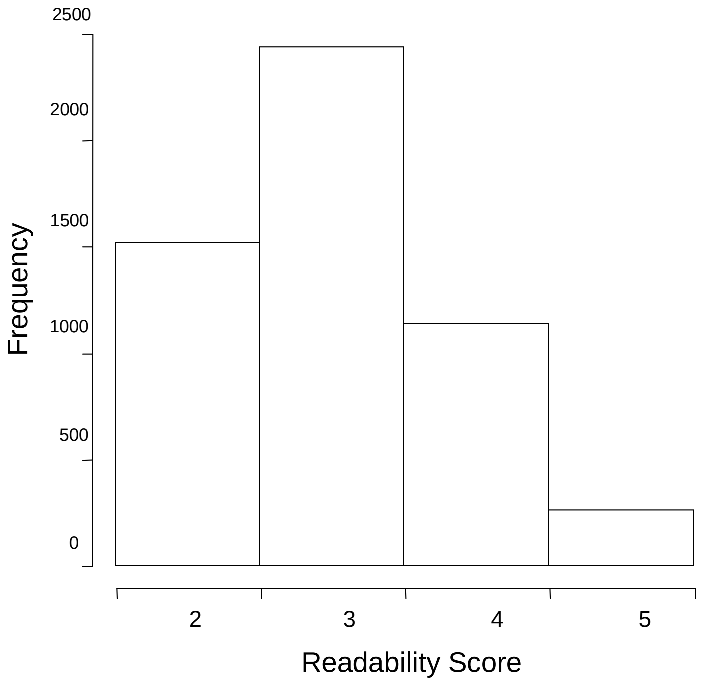

Figure 9. Histogram of readability score of all otoliths used to construct age length keys.

The median reliability score for all aged fish was 3 but some fishery year combinations had a higher value of 4. Appendix 6 also shows the median readability score for each combination of fishery and year and the number of length samples taken where there were at least 50 ages available for constructing the age length key (ALK). Appendix 6 shows that the sampling fraction of the length frequency (LF) samples that were aged ranged from 0.8% to 28%, which suggests that the over-dispersion of catch-at-age frequencies relative to a multinomial with nominal ESS corresponding to the catch-at-length proportions is likely to be an issue, as simulation studies (Candy 2009a) have shown that sampling fractions less than 10% give rise to dispersion estimates greater than 1.

CASAL assessment framework

The methods used and description of the CASAL models, gear types and grounds (except for the pot fishery, *f*10) are described in Candy and Constable Candy and Constable (2007; 2008) for catch-at-length and abundance-at-length observations. In this update of the integrated assessment, catch-at-age proportions as observations were obtained using ALKs where the ALK for each combination of fishery and year had available a sufficient number of aged fish (assumed >50). The method of calculating catch-at-age proportions using ALKs and proportions-at-length from LF data in his last case is described in Candy (2009a) along with a Monte Carlo sampling method for estimating effective sample size (ESS) for use as the nominal multinomial sample size. The catch-at-age ESS takes into account uncertainty due to haul-level variability in catch-at-length proportions (Candy 2008), ALK sampling error, and random ageing error. For the trawl fisheries that were divided into periods within each year, the same ALK for the year was applied to the LF samples for each fishing period within that year. For years where insufficient fish were aged the catch-at-length proportions were retained but for a given fishery the same selectivity function and parameter values were logically applied to both types of data.

For 2006 and 2007 random stratified trawl surveys, there were sufficient aged fish to convert abundance-at-length to abundance-at-age and the coefficient of variation (CV) for the abundance-at-age, assuming a lognormal distribution. To calculate the component of the CV of the abundance-at-age due multinomial variation in proportions-at-age, the ESS was calculated using the same method as that for the commercial catch-at-age proportions. Proportions-at-age were obtained by applying the survey-year specific ALK to the stratum-area weighted estimates of proportion-at-length. However, since these proportions-at-age must be multiplied by the estimated total population size vulnerable to the survey to give abundance-at-age, the variance of abundance-at-age estimates was obtained using the variance of the proportions-at-age and the variance of estimated total vulnerable population size (i.e. the variance for a stratified random sample, Cochran 1977). This method of calculating the variances of abundance-at-age estimates is described in Appendix 8.

For otoliths that had more than a single age reading, due to repeat readings by the same or different reader, one reading was selected at random. Because of this random selection combined with the Monte Carlo method of calculating the ESS, there is additional variability in the the extracted data associated with these random processes. To investigate the variation in estimates of the historical recruitment series due to the above random variation, an extra draw of the commercial catch-at-age and abundance-at-age data was carried out. Combined with this, for the original draw, the CASAL models were fitted with each of three ageing error matrices corresponding to readability scores of 3, 4, and 5 (as given in Appendix 5). These models are denoted *a2-2008-alkall-ir3*, *a2-2008-alkall-ir4*, and *a2-2008-alkall-ir5* for readability scores of 3, 4, and 5, respectively. The model combining the extra draw of the data and readability score of 4 is denoted *a2-2008-alkall-iir4*. To investigate the effect of using only the abundance-at-age data as the only aged data, model *a2-2008-alksg1-r4* was fitted (i.e. no commercial catch-at-age were used and instead all commercial catch-at-length data from model *a2-ess-2008* were retained). All the above "-alk" models used survey abundance-at-age data constructed from ALKs that were pooled across all fisheries for the specific year of survey (i.e. 2006 or 2007). This was done because of the small number of aged fish for the 2006 survey. The commercial catch-at-age proportions were constructed from ALKs that were specific to the year and fishery of the catch. For the survey data obtained using this approach of not pooling ALKs across fisheries, the CASAL model/dataset is denoted *a2-2008-alkallsg1-iir4* since it used the same catch-at-age data as model *a2-2008-alkall-iir4*. The final model fitted corresponds to *a2-2008-alkall-iir4* with the exception that the ESS for multi-year catch-at-length data was adjusted for process error using a single iteration of the two-step procedure of fitting CASAL and then estimating the over-dispersion parameter after adjustment for any systematic lack-of-fit (Candy 2008; Candy and Constable 2008). The only fisheries that had multi-year catch-at-length data in the assessment model after catch-at-age data were incorporated were fisheries f3 (trawl), f5 (longline) and f6 (longline). This CASAL model/dataset is denoted *a2-2008-alkall-iir4PE*. The definition and application of the ageing error matrix in CASAL is described in Bull *et al.* (2005) (see also Candy 2009a).

In CASAL projection trials, uncertainty surrounds the estimates of the parameters in the model as well as in how recruitment will vary in the future. In order to integrate across uncertainty in the parameters, sets of parameters were sampled from the results of the stock assessment in CASAL. The sampling method obtained independent multivariate normal (MVN) samples of the parameter set using the maximum posterior density (MPD) estimates of parameters and their estimated variance-covariance matrix.

Recruitment variability in each trial was modelled as a log-normal recruitment function. A random set of time series (1984 to 2006) of estimated number of age-1 recruits corresponding to estimates of year class strength (YCS) for 1983 to 2005 were obtained using the CASAL projection procedure (i.e. even though for this purpose there was no interest in the projected recruitments beyond 2006). To do this 1000, independent multivariate normal (MVN) samples of the parameter set were drawn using the parameter estimates and their approximate variance-covariance matrix, then used by CASAL to obtain 1000 samples of the recruitment time series. The samples of age-1 recruit numbers were then analysed in R (R Development Core Team 2006) using a linear mixed model (LMM). Random effects of year and, in a separate model, the addition of a 1st order autocorrelation parameter were obtained using the LMM fit to the logarithm of the number of recruits obtained using the *asreml* package (Butler *et al.* 2007) within R. The square root of the variance of the year random effects gives a robust estimate (i.e. based on a 1st order Taylor series expansion of the log transformation) of the CV_R required for the lognormal random recruitment facility in CASAL.

Results

Key parameters from the fit of each CASAL model/dataset are given in Table 4. Note that for the models incorporating aged data, the CV_{VB} parameter was set to 0.1 since when this parameter was estimated it resulted in instability in parameter estimates (i.e. the CV_{VB} parameter reached and stayed at its lower limit which was set to 0.05, while the B_0 parameter, representing the median pre-exploitation spawning stock biomass, showed larger variation than expected for replicated draws of the age data). Therefore the CV_{VB} parameter was not estimated but fixed at the independent estimate of 0.1 obtained by Candy *et al.* (2007). Appendix 6 shows the catch-at-length ESS and catch-at-age ESS for each combination of fishery and year where there were at least 50 aged fish available for constructing the ALK for one particular extraction of the catch-at-age data subject to random reader selection for multiple reads and calculation of the ESS using the Monte Carlo sampling procedure described in Candy (2009a).

Table 4. Results of assessments of stock status of *Dissostichus eleginoides* using CASAL. B_0 is the maximum posterior density (MPD) estimate of the pre-exploitation median spawning biomass, CV_{VB} is the coefficient of variation for length at age, SSB status 2008 is the ratio of the CASAL prediction of Spawning Stock Biomass in 2008 to B_0, and R_0 is the MPD estimate of mean Age 1 recruitment prior to exploitation (1981), and CV_R is the coefficient of variation of the annual recruitment series (1984-2006) with corresponding value for the series (1986-2000) given in brackets.

Model	Description	B_0 (tonnes) (SE)	CV_{VB} (SE)	SSB Status 2008	R_0 (mil.)	CV_R (1986-2000)
a2-ess	Model *a2-ess* Candy and Constable (2007,2008)	125 219 (5 806)	0.0977 (0.0008)	0.725[a]	4.538	1.822
a2-ess-2008	*a2-ess* + 2008 C-at-L and survey data	131 045 (5 918)	0.1221 (0.0009)	0.748	4.661	1.758 (1.574)
a2-2008-alksg1-ir4	*a2-ess-2008* + C-at-A SG1 Readability=4	123 577 (5 041)	0.1 (-)	0.706	4.471	2.591 (2.560)
a2-2008-alkall-ir3	*a2-ess-2008* + C-at-A all, Readability=3	135 003 (5 015)	0.1 (-)	0.730	4.885	2.531 (1.900)
a2-2008-alkall-ir4	*a2-ess-2008* + C-at-A all, Readability=4	110 286 (5 121)	0.1 (-)	0.620	3.990	1.767 (0.279)
a2-2008-alkall-ir5	*a2-ess-2008* + C-at-A all, Readability=5	119 866 (3 511)	0.1 (-)	0.687	4.337	0.367
a2-2008-alkall-iir4	*a2-ess-2008* + C-at-A all, Readability=4	117 938 (5 756)	0.1 (-)	0.658	4.267	1.632 (0.321)
a2-2008-alkallsg1-iir4	*a2-ess-2008* + C-at-A all, Readability=4	109 559 (4 276)	0.1 (-)	0.646	3.964	1.626 (0.330)
a2-2008-alkall-iir4PE	*a2-ess-2008* + C-at-A all+PE Readability=4	124 744 (4 088)	0.1 (-)	0.683	4.514	1.840 (0.311)

[a] SSB status at 2007

Figure 10 shows abundance-at-age observations from RSTS and CASAL predictions from model *a2-2008-alkall-iir4*. Figure 11 shows observed and predicted proportions-at-age for fishery *f2-s2* (main trawl ground in season 2, Candy and Constable, 2008) with predictions obtained from model *a2-2008-alkall-iir4*. Similarly, Figure 12 shows observed and predicted proportions-at-age for fishery *f5-s2* (a longline ground in season 2). Corresponding diagnostics for other fisheries with age data and catch-at-length for years with insufficient ages measured are available but not presented. Fits were similar across all 4 models that used all the age data available (i.e. '-*alkall*-' models). Figures 13 and 14 compare average length-at-age from the von Bertalanffy model used to convert catch-at-length and abundance-at-length to catch-at-age and abundance-at-age (Candy *et al.* 2007), respectively, to that obtained from the ALK for fishery *f2* and *f5* (noting that for fishery *f2* a single ALK was obtained for each year but not for each period within each year). The means and standard errors shown in Figures 5 and 6 were calculated from the ALK, conditioning on age class (i.e. using the length-bin frequencies), using frequency-weighted length-bin mid-points and have not accounted for the effect of length-bin sampling or, possible, length-based fishing selectivity as was done in construction the VB model (Candy *et al.* 2007).

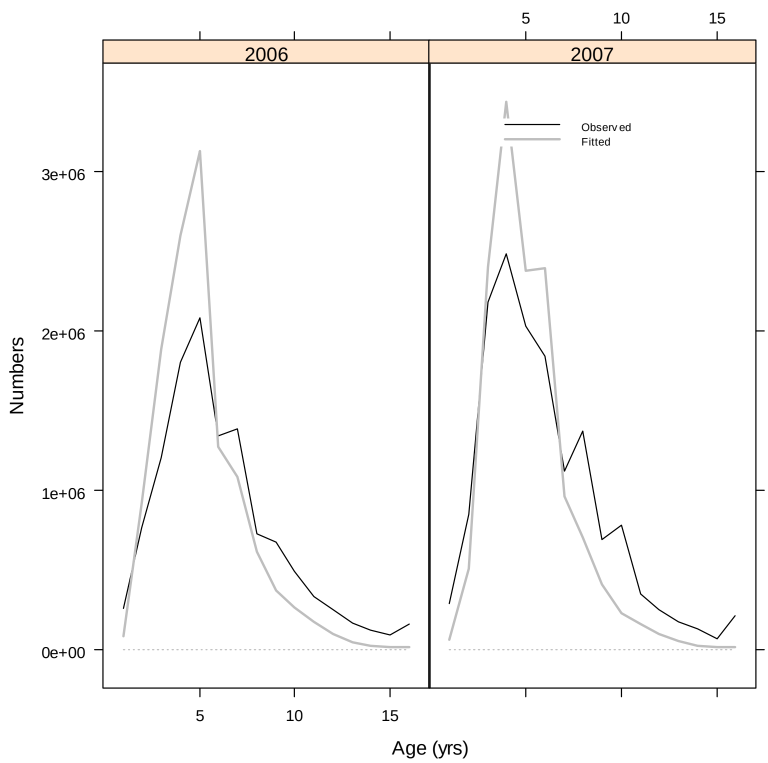

Figure 10. Abundance-at-age observations from annual survey and CASAL predictions model *a2-2008-alkall-iir4*.

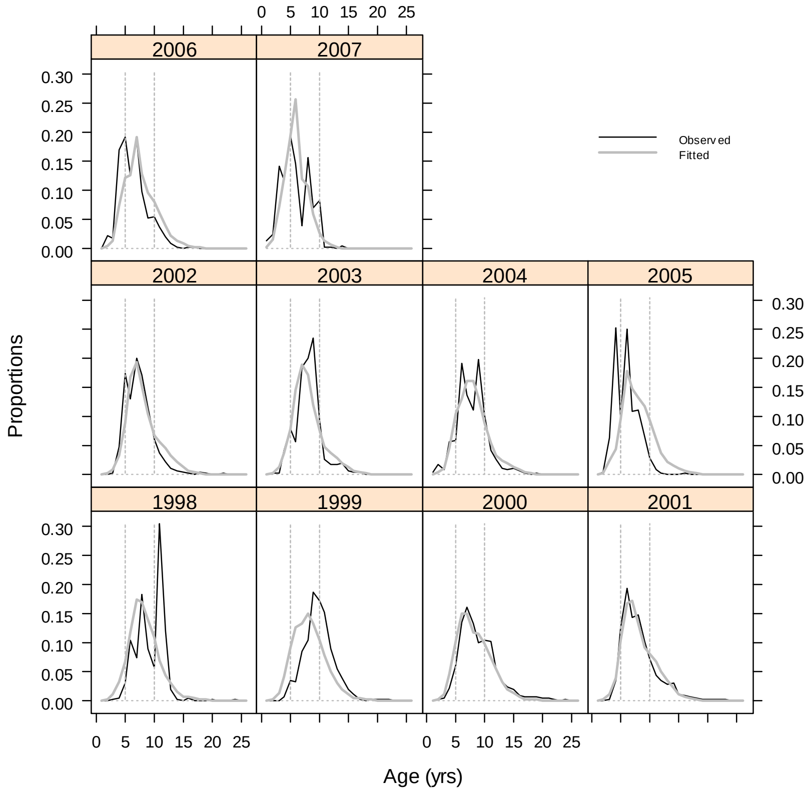

Figure 11. Observed and fitted proportions-at-age fishery f2-s2 and model a2-*2008-alkall-iir4* .

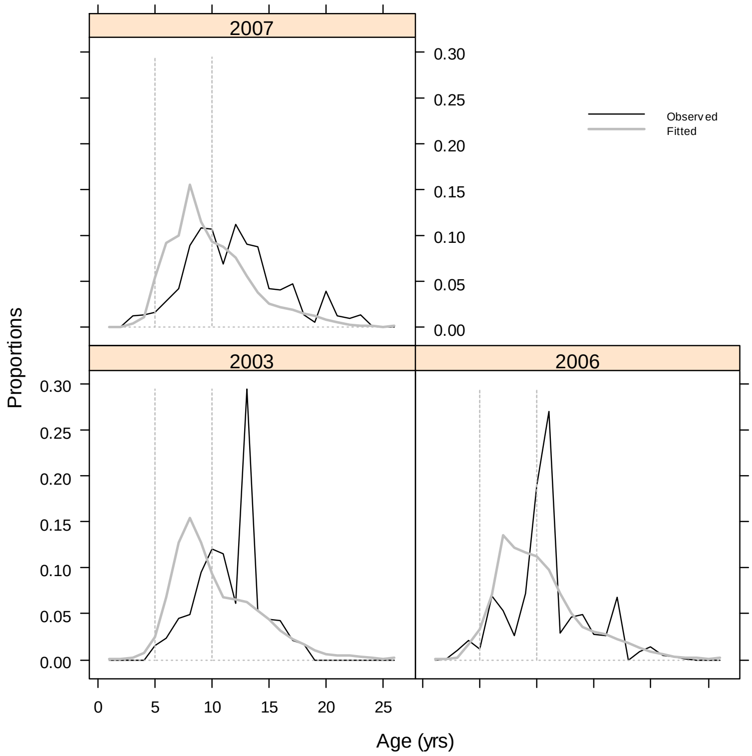

Figure 12. Observed and fitted proportions-at-age fishery f5-s2 model *a2-2008-alkall-iir4*.

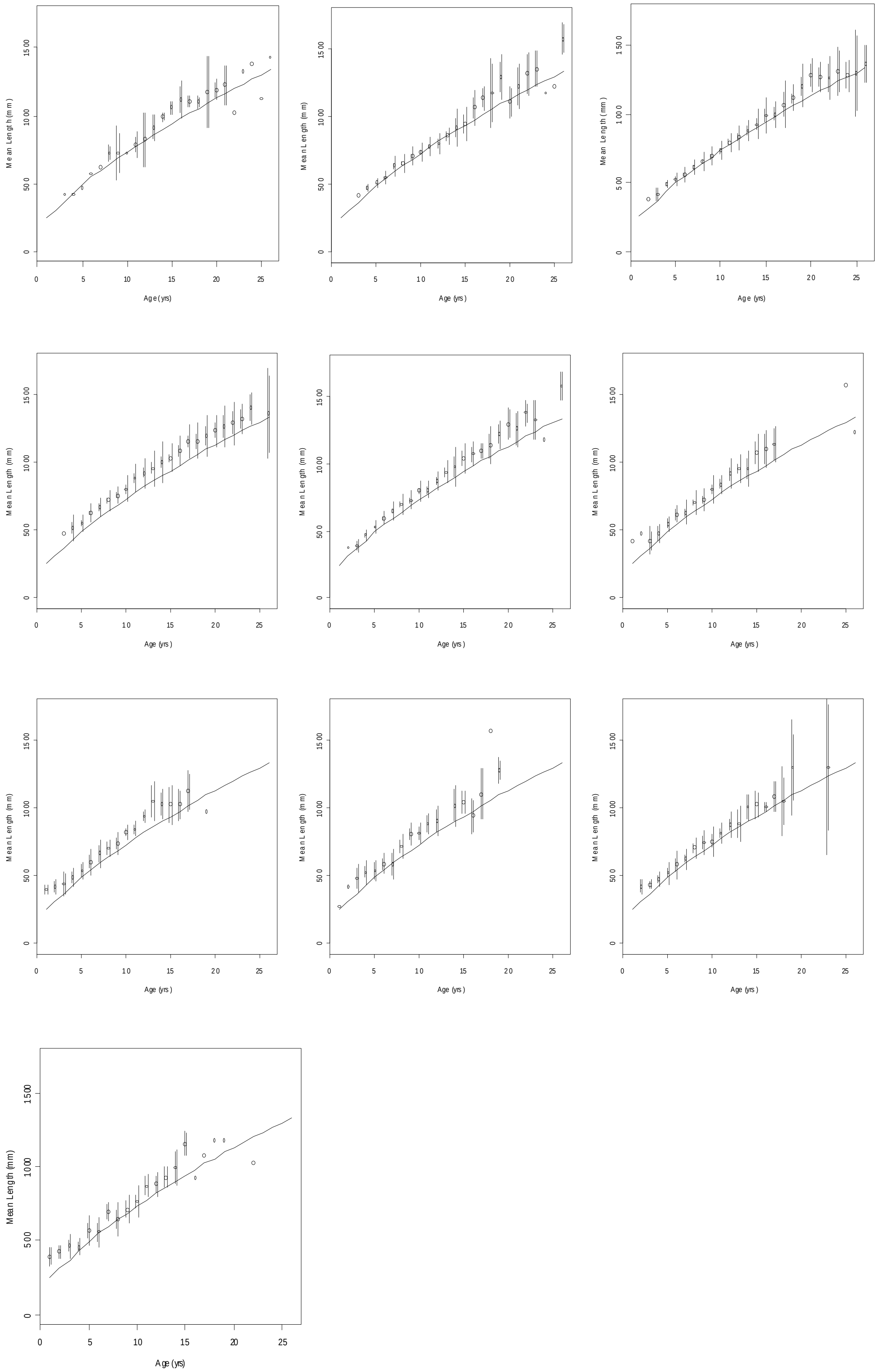

Figure 13. Comparison of average length-at-age from von Bertalanffy model used to convert catch-at-length and abundance-at-length to catch-at-age and abundance-at-age, respectively to that obtained from the age length keys for fishery *f2* for years 1998 to 2007 corresponding to reading panels left to right then top to bottom. Right-hand bar represents ±1 standard deviation (SD) while the left hand bar represents ±2SE of the mean (SE=SD/ $\sqrt{n}$ where *n* is the sample size). Bars are missing when *n*=1.

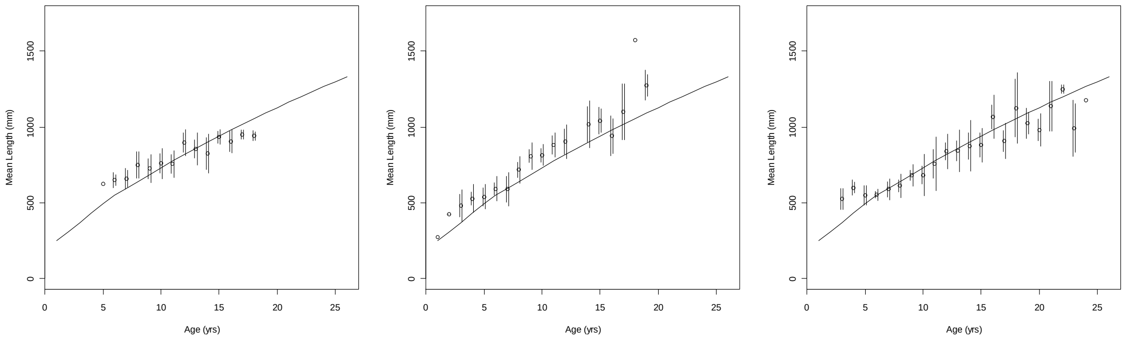

Figure 14. Comparison of average length-at-age from von Bertalanffy model used to convert catch-at-length and abundance-at-length to catch-at-age and abundance-at-age, respectively, to that obtained from the ALK for fishery f5 for years 2003, 2006, and 2007 corresponding to reading panels left to right. Right-hand bar represents ± 1 standard deviation (SD) while the left hand bar represents ± 2SE of the mean (SE=SD/ $\sqrt{n}$ where *n* is the sample size). Bars are missing when *n*=1.

Figure 15 shows the fitted selectivity functions for model *a2-2008-alkall-iir4*.

Figures 16 to 18 show the estimated time series of Year Class Strength (YCS) for combinations of models. Figure 19 compares YCS estimates for models *a2-2008-ess* and *a2-2008-alkall-ir4*.

Figure 20 compares Year Class Strength (YCS) estimates for models *a2-2008-alkall-ir4*, *a2-2008-alkall-iir4* and *a2-2008-alksg1-ir4* (abundance-at-age the only aged data) while Figure 10 shows these estimates for models *a2-2008-alkall-ir3*, *a2-2008-alkall-ir4* and *a2-2008-alkall-ir5* with each model incorporating all aged observations but with an ageing error matrix predicted for readability scores of 3, 4, and 5, respectively. Random MVN draws of YCS values based on the parameter estimates from CASAL combined with the estimate of B_0 (Table 1) and, additionally, parameters which determine the selectivity functions (Figure 7) combined with the Hessian matrix, are used to determine random recruitments from the product of YCS estimates and median pre-exploitation recruitment (R_0 ; Table 1) which is determined directly from B_0. Figure 20 compares Year Class Strength (YCS) estimates for models *a2-2008-alkall-ir4*, *a2-2008-alkall-iir4* and *a2-2008-alksg1-ir4* (abundance-at-age the only aged data) while Figure 10 shows these estimates for models *a2-2008-alkall-ir3*, *a2-2008-alkall-ir4* and *a2-2008-alkall-ir5* with each model incorporating all aged observations but with an ageing error matrix predicted for readability scores of 3, 4, and 5, respectively. Random MVN draws of YCS values based on the parameter estimates from CASAL combined with the estimate of B_0 (Table 1) and, additionally, parameters which determine the selectivity functions (Figure 7) combined with the Hessian matrix, are used to determine random recruitments from the product of YCS estimates and median pre-exploitation recruitment (R_0; Table 4) which is determined directly from B_0.

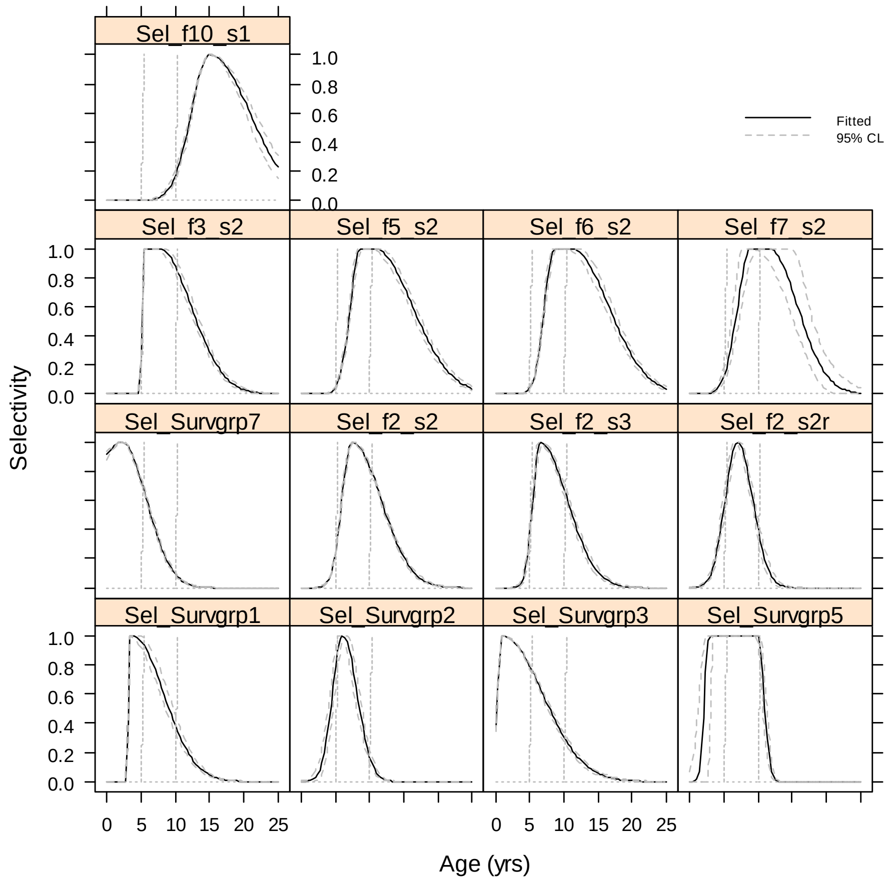

Figure 15. Double-normal-plateau (DNP) and double-normal (DN) fishing selectivity curves from fit of model *a2-2008-alkall-iir4* showing 95% confidence bounds obtained from the MVN sample. Panel headings: Survgrp1 (survey years 2001, 2002, 2004, 2005, 2006, 2007, 2008), Survgrp2 (survey year 1999), Survgrp3 (survey year 1990), Survgrp5 (survey year 1993), Survgrp7 (survey year 2003), f2_s2, f2_s3 (trawl fishery Ground B, seasons 1&2, season 3), f2_s2r (trawl fishery Ground B 2006, 2007 all seasons), f3_s2 (trawl fishery Ground C, all seasons), f5_s2 (longline fishery Ground D, season 2), f6_s2 (longline fishery Ground E, season 2), f10_s1 (pot fishery, season 1). Reference lines are shown at ages 5 and 10.

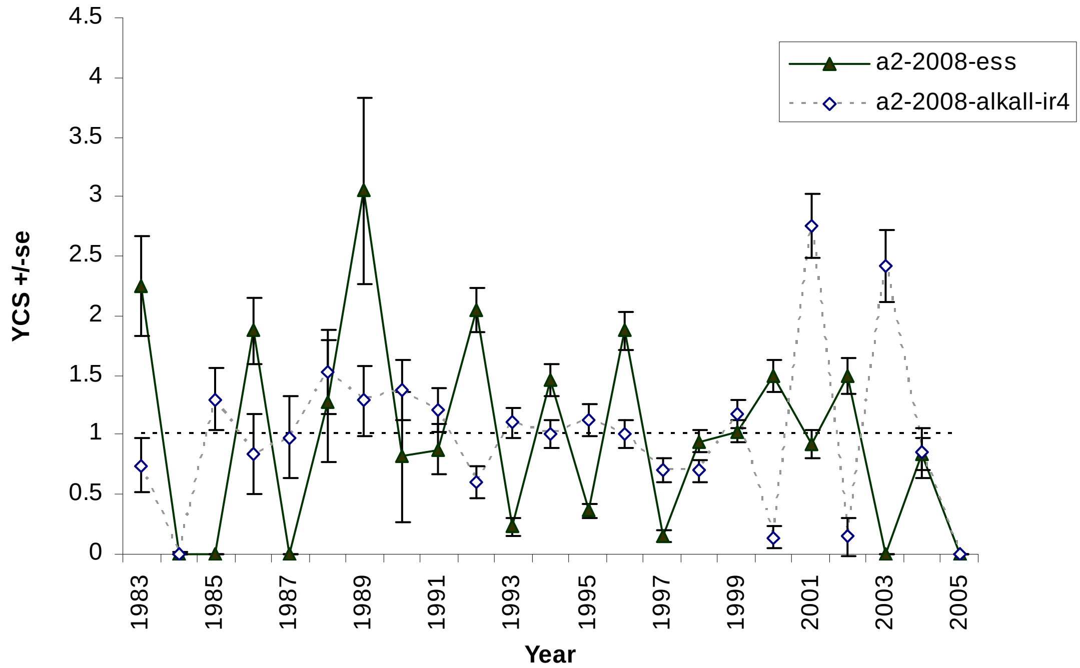

Figure 16. Comparison of year class strength (YCS) estimates (showing ±SE bars) for models *a2-2008-ess* (no aged observations) and *a2-2008-alkall-ir4*.

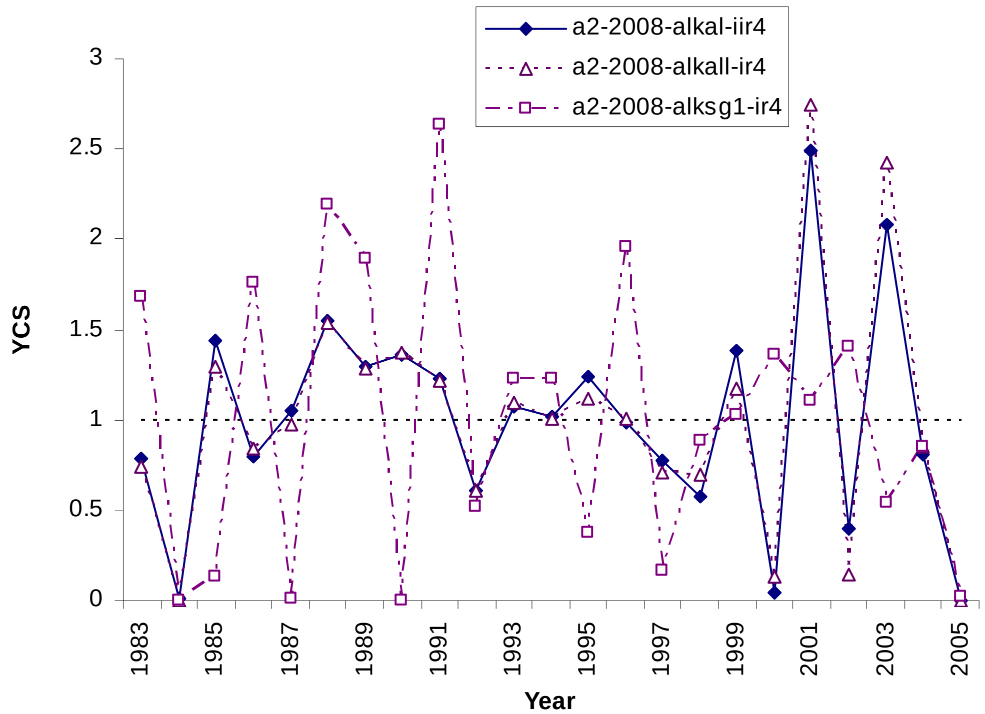

Figure 17. Comparison of year class strength (YCS) estimates for models *a2-2008-alkall-ir4*, *a2-2008-alkall-iir4* and *a2-2008-alksg1-ir4* (abundance-at-age the only aged data).

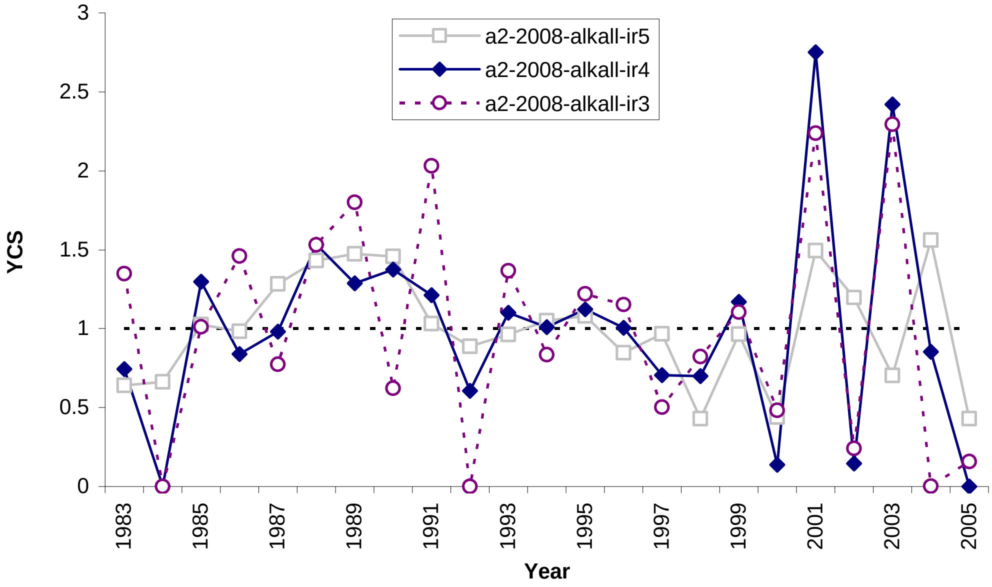

Figure 18. Comparison of Year Class Strength (YCS) estimates for models *a2-2008-alkall-ir3*, *a2-2008-alkall-ir4* and *a2-2008-alkall-ir5* with each model incorporating all aged observations but with an ageing error matrix predicted for readability scores of 3, 4, and 5, respectively.

Discussion

The results of fitting the different CASAL models/datasets indicate that the B_0 parameter is relatively stable ranging from 110 000 to 131 000 tonnes (Table 4). The difference in the estimate of B_0 between the two different draws of the aged data with the same ageing error matrix corresponding to a readability score of 4 (cf: *a2-2008-alkall-ir4* and *a2-2008-alkall-iir4*) was minor while the estimates of YCS were very close (Figure 17). In comparison, the model excluding the commercial catch-at-age data (*a2-2008-alksg1-ir4*) gave an appreciably different set of YCS estimates, with much more variability in the (1986-2000) segment of the series. In fact, the YCS estimates for *a2-2008-alksg1-ir4* are very similar to the estimates for the model without any aged observations of *a2-2008-ess* (Figure 17) which is not surprising since the only difference between these models (apart from a relatively minor difference in CV_{VB}) is the replacement of 2 years of abundance-at-length with abundance-at-age data. In contrast, the comparison series in Figure 17 for *a2-2008-alkall-ir4* shows much less variability in the 1986-2000 segment of the series. However, YCS estimates 'see-saw' quite dramatically after 2000 for this latter model compared to estimates from *a2-2008-ess*. It is expected that the recruitment series estimates would only be improved prior to 2001 using commercial catch-at-age data because of the generally high selectivity of fish greater than age 5 and less than age 12, so that backward-projections of age 6 and older cohorts, for a given year of catch, to year zero for

YCS and year-1 for recruitment would have a lag of 6 years until past recruitments are well represented in the year of the catch. Therefore commercial catch-at-age data would not be expected to contribute significantly to estimation of YCS in years 2000 to 2005. For these years it would be expected that the survey abundance-at-age data for 2006 and 2007 would contribute more to estimation of YCS due to the high selectivity of fish aged between 3 and 5. However, this only appears to occur for the age observation models when the ageing error matrix is based on a readability score of 5 (Figure 18). This is expected given that ± 1 year ageing error is more influential for the young survey-caught fish (e.g. ages 3 to 5) than the older commercially-caught fish.

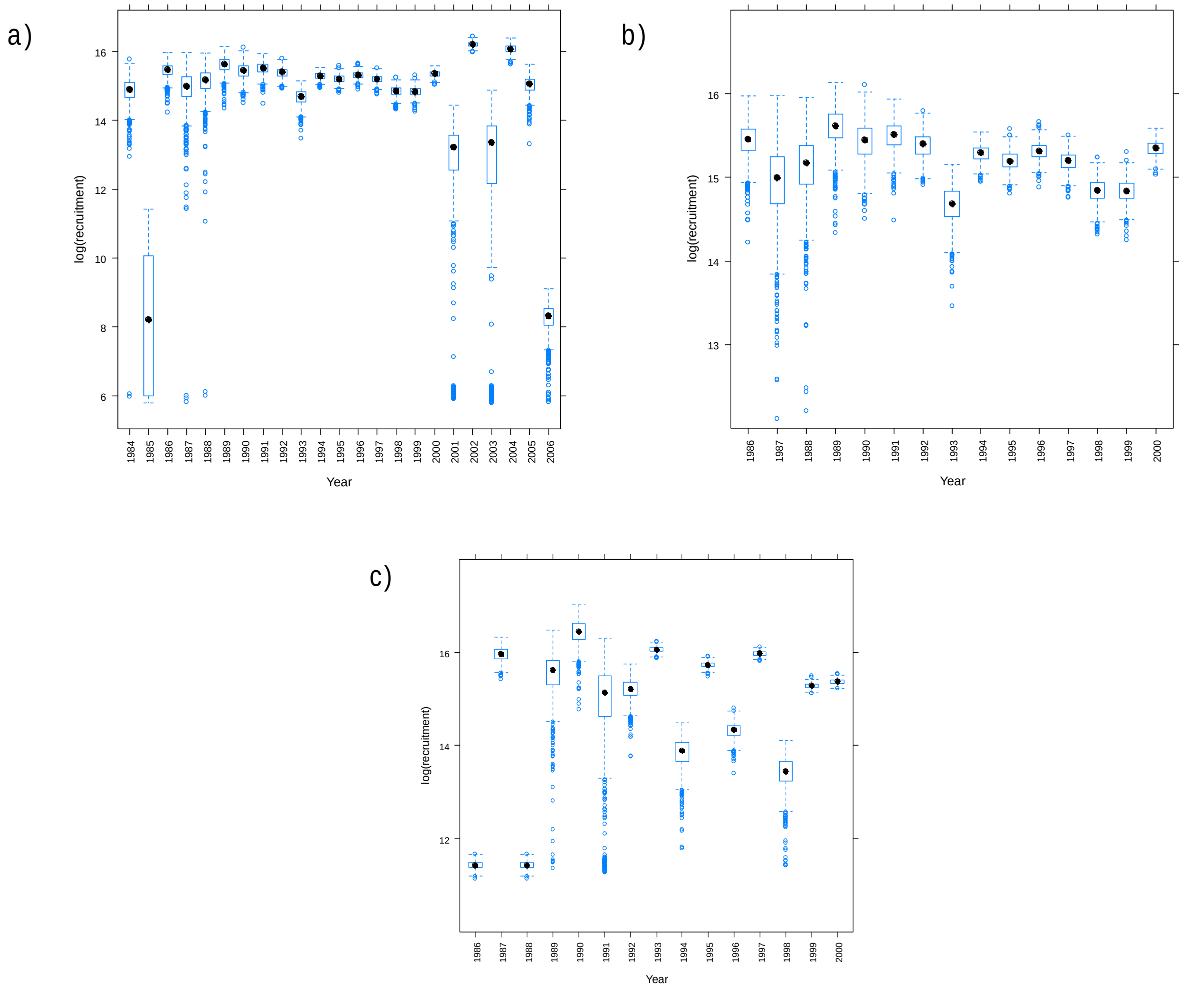

Figure 19. Recruitment series box plots based on 1000 MVN samples using the MPD estimates and the Hessian matrix for model *a2-2008-alkall-ir4* (a) maximum year range (b) middle year range (c) middle year range for model *a2-ess-2008*.

There was only a minor difference between CASAL model outputs when pooled versus unpooled ALKs were used to construct the survey abundance-at-age data (cf: model *a2-2008-alkall-iir4* versus model *a2-2008-alkallsg1-iir4* outputs in Table 4).

Figure 19 and Table 4 show that the variability in recruitment is reduced to values below 0.8 only when the ageing error matrix is based on a readability of a least 4, assuming the 'middle' (1986-2000) part of the recruitment series is used to determine CV_R (Table 4). Values of CV_R lower than approximately 0.6 have been observed, in general, by the author to not result in the depletion rule being the limiting factor in detemining the allowable catch.

The output from the integrated assessment of most interest in this study is the CV of the estimated historical recruitment series, CV_R, since this parameter strongly influences the effect of the depletion rule on the allowable catch. Compared to the assessment that did not incorporate catch-at-age or abundance-at-age data, the aged-based assessment dramatically lowered the CV_R, from around 1.5 to 1.8 down to approximately 0.3 to 0.4 if a readability score 5 was assumed or if for a score of 4 the most stable subset (1986-2000) of the full historical series (1984-2006) was used to estimate the CV. There was no reduction in CV_R if a readability score of 3 was assumed. The difference between a readability of 3 and 4 in ageing error is that zero ageing errors are relatively less prevalent (e.g. for age 8 the percentage of errors that were zero was estimated from previous work at 40% for score 3 and 48% for score 4, the corresponding ± 1 yr errors had prevalence of 46% and 45%, respectively) (see Appendix 5). A ± 1 year error may seem minor relative to the complete age range modelled of 1 to 35 yr, however most fish caught are in a more restricted age range. The highest age of fish in the main survey that have an upper selectivity greater than 0.2 was approximately 12 yrs while the corresponding values for the trawl and longline fisheries were 15 and 20 yrs respectively (Figure 15).

The results presented suggest that future ageing work would give a greater improvement to the integrated assessment if otoliths with readability score of at least 4 can be obtained in sufficient numbers to allow ALKs to be constructed only using ages obtained from these otoliths. When a large sample of otoliths available, given the results reported in this study it would be desirable to have the ability to screen out otoliths with lower readability (i.e. score of 2 or 3) before they were prepared for reading or at least in the early stage of preparation. For example, using a preliminary examination of unprepared otoliths with 'blind' or 'double-blind' comparisons of pre- and post-preparation readability scores could be used to investigate the accuracy of pre-preparation assessments of readability in terms of their consistency pre- and post-preparation, both within and between readers. This assumes that readability is not linked to growth rate or growth habit so that screening otoliths in the above way does not introduce bias in estimation of proportions-at-age in the population.

Benefits and adoption

This study has demonstrated the feasibility of collecting large size at age datasets for toothfish using high throughput otolith processing. It has also demonstrated the benefits of including age length keys into the integrated assessment for the HIMI toothfish fishery. These achievements have particular benefit for industry and management stakeholders in the HIMI fishery, as they can have increased confidence in the current assessment framework providing a robust and plausible assessment of stock status and dynamics, while integrating across a range of datasets. This is important during a period when management advice has recently moved from being developed based on the GYM modeling framework to the CASAL integrated assessment framework.

This work also has benefits for the Macquarie Island toothfish fishery, which is currently moving from a trawl only, tag-based assessment to a multi-gear integrated assessment. The outcomes of this project will assist stock assessment scientists in considering the value of age-based data in such a framework at an early stage of its development in the Macquarie Island fishery. Furthermore, the statistical methods developed for statistically representing reader error and effective sample sizes have application in any fishery using integrated assessments and/or age-length keys derived from multiple readers.

It is notable that the high level of industry and AFMA support was fundamental to the success of this project. This gives a strong indication of the commitment that industry and management stakeholders have to full adoption of the implication of this project for developing management advice and research strategies based on its outcomes.

Further development

Several aspects of this project would benefit from further development, but are outside the scope or timeframe of a tactical research fund project. These include:

Cost –benefit analysis of additional ageing

Processing and analyzing otoliths from under-represented and future fishing activity at HIMI will have benefits in terms of the precision and robustness of future HIMI stock assessments and management advice based on these assessments. Currently, stock assessments are preformed every year. Using the methodology described in this report, it is feasible to process a large number of otoliths, collected across all gear types in a season for inclusion in the assessment model with a lag of less than one year between the otoliths being collected and the resultant age-length data being available for inclusion in the assessment. However, this would incur costs in terms of the laboratory work required to do the analyses, as well as to a lesser extent the time spent by observers collecting them at sea. A formal cost-benefit analysis, *sensu* Francis (2006) could be implemented, using a simulation framework to represent the spatial and temporal structure of the HIMI fishery, observer sampling intensity, to determine the optimum investment in otolith processing to return best 'performance' of the HIMI assessment model as defined by stakeholders.

Use of ancillary data to improve ageing

Covariates of age such as otolith weight, which are available for nearly all fish sampled in this project, has been used in other studies to improve the precision of allocating individuals to age classes (Francis *et al.* 2005). With the dataset now available, exploring methods of using otolith weight, as well as other covariates such as sex has the potential to make development of age length keys more precise and cost-effective. It would also be desirable to develop a method to screen out otoliths with lower readability (i.e. score of 2 or 3) before they were prepared for reading. For example using a preliminary examination of unprepared otoliths with 'blind' or 'double-blind' comparisons of pre- and post-preparation readability scores could be used to investigate the accuracy of pre-preparation assessments of readability in terms of their consistency pre- and post-preparation both within and between readers. This assumes that readability is not linked to growth rate or growth habit so that screening otoliths in the above way does not introduce bias in estimation of proportions-at-age in the population.

Expansion of the reference collection

The analyses of reader error presented in Candy *et al.* (2009) would be improved through the inclusion and analysis of additional slides in the reference collection by multiple readers. In sampling extra otoliths a priority should be to increase the number in the poorly represented readability classes 2 and 5, and in the tails of the age distribution (i.e. above age 18 and below age 5). These could be achieved through selecting old and young fish from the existing otoliths samples, and/or selectively sampling and processing otoliths form individuasl that have a corresponding length in the upper and lower tails of the distribution.

Refinement of growth models for HIMI toothfish

Previous growth functions developed to describe growth of toothfish at HIMI (Candy *et al.* 2007) primarily derived from fish aged prior to the development of methods that target older fish such as trapping and long lines. The data set derived for this project includes many of the largest and oldest fish captured at HIMI, so including this data in a revised growth function has the potential improve of age structure from catch at length where direct ageing using otoliths is not possible.

International interlaboratory comparisons

While all the CCAMLR managed fisheries that include integrated assessments incorporate some form of age-length data in their input, the implications of reader error, as formulated for the first time by Candy *et al.* (2009) has not been explored in those assessments. A further development of this work would be to exchange reference collections with other laboratories and develop error matrices which can be incorporated into other toothfish assessments conducted by CCAMLR.

Planned outcomes

All planned outcomes have been achieved.

1. Refinement of integrated stock assessments for toothfish at HIMI through the inclusion of age-length data.

The results of this project, in particular the updated assessment, provide a firm basis for stock assessments scientists, fishers and resource managers to evaluate the costs and benefits of including season and gear specific age-length keys in the HIMI toothfish assessment in future, providing increased confidence in the current stock assessment approach.

2. Improved standards in ageing methodology and quality control for large scale ageing of toothfish.

During this project a robust methodology of the efficient throughput of large numbers of toothfish otoliths, while maintaining a high standard of readability and consitency. This methodology, coupled with the development of a reference collection has not only allowed over 2400 new age estimates to be incorporated in the HIMI integrated assessment, but also the reconciliation of large quantities of age estimates provided by the Central Ageing Facility for samples collected prior to 2003 for inclusion into the dataset used for the assessment.

3. Efficient collection of otoliths by observers in the HIMI fishery in future.

The subsampling framework, and the method used to estimate reader error and construct the age length keys used in this project has resulted in clear recommendations as to the the appropriate sampling method by oberves and for processing otoliths. Sampling every season, across all the sub-fisheries used in the assessment framework, including the survey and commercial fisheries should continue, to maximize options for including age-length data into the assessment in future. This sampling should concentrate on all length-classes in the catch at length samples for the fishery, hence the current 'length bin' sampling protocol, where observers collect otoliths from fish in all length classes measured, should continue, with increased emphasis on under-represented size classes at the extremes of the size distribution encountered in the fishery. This protocol ahas already been included in instrcutiosn provided to observers, and will ensure that the minimum amount of time is spent at sea collecting otoliths which will have the maximum usefulness in future assessments, as opposed to *ad hoc* protocols which may have been used in the past.

Conclusion

This project has successfully developed statistical methods for the sensitivity testing of the HIMI integrated assessment model to the inclusion of length-at-age estimates from over 5500 individual toothfish, in the form of gear and season specific age-length keys. Hence the primary objective of this project has been achieved.

This study has demonstrated that the inclusion of age-length data results in significant refinements to the estimates of several key parameters in the assessment. These include more precise estimates of the level and variability of recruitment of juvenile toothfish to the stock relative to when size at age is estimated using a von Bertalanffy growth function alone. This highlights the value of age at length data, drawn from a broad range of seasons and all gear types used in the fishery to date, in producing precise and plausible results under the assessment framework used for the HIMI toothfish fishery.

This project has also achieved its other objectives, relating to the development of robust and effienct otolith collection, processing and interpretation. High-quality, high-throughout ageing of toothfish otoliths is shown to be feasible, such that season and gear specific age-length keys could be routinely incorporated into the HIMI assessment with less than one year lag. Otoliths collection should continue across all gear types in the HIMI toothfish fishery, including long lines, trawls and survey trawls, sampling across all length classes present in the catch, as it is clear that each gear type samples a different part of the population.

This project has also provided a substantial dataset which can be used to enhance the assessment in future through the revision of the growth function, as well as providing the basis for more formal evaluation of the impact of ageing in assessment and management strategy evaluation.

A firm basis is therefore provided for stock assessments scientists, fishers and resource managers to evaluate the costs and benefits of including season and gear specific age-length keys in the HIMI toothfish assessment in future, providing increased confidence in the current stock assessment approach, as well as providing statistical tools that can be readily applied in other fisheries were age length keys and/or integrated assessments are used.

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Appendix 1. Intellectual Property

No intellectual property is identified as arising from this project.

The dataset generated from this project is housed in a secure database at the AAD. A metadata record describing the datasets and terms of use has been lodged with Australian Antarctic Data Centre.

Appendix 2. Staff

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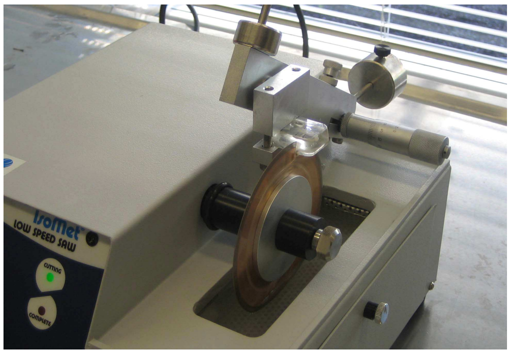
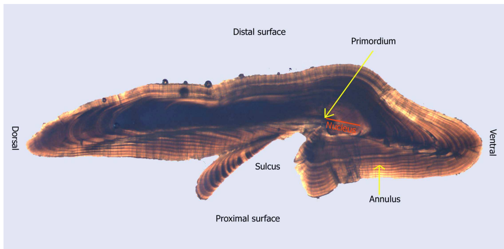
Appendix 3.

Otolith preparation and ageing of Patagonian toothfish, *Dissostichus eleginoides*, at the Australian Antarctic Division





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Department of the Environment, Water, Heritage and the Arts
Australian Antarctic Division

Fisheries Research and Development Corporation

Gabrielle Nowara, Jeremy Verdouw and Joe Hutchins

Cover photos:

Toothfish photograph: AFMA Observer program

Other photos: Gabrielle Nowara

Photos of otolith sections: Joe Hutchins & Jeremy Verdouw

Other photos: Gabrielle Nowara

Figure A3.1: Dirk Welsford

November 2008

Otolith preparation and ageing of Patagonian toothfish, *Dissostichus eleginoides,* at the Australian Antarctic Division.

Gabrielle Nowara, Jeremy Verdouw and Joe Hutchins

Department of Environment, Water Heritage and the Arts,
Australian Antarctic Division

November 2008

Introduction

Otoliths of Patagonian toothfish, *Dissostichus eleginoides*, are collected on board Australian fishing industry vessels at Heard and McDonald Islands by Australian Fisheries Management Authority (AFMA) observers. For a brief overview of the fishery at Heard Island and McDonald Islands (HIMI) and the sampling program see the document "*Data collection and the Fish database for Australia's sub-Antarctic fisheries*" (AAD 2007). The collection of otoliths is held at the AAD.

This manual is a guide to processing the otoliths for ageing to provide data to improve the stock assessments at HIMI. It was developed with the assistance of the Fisheries Research and Development Corporation as part of Tactical Research Fund Project TRF 2008/046, "Evaluating gear and season specific age-length keys to improve the precision of stock assessments for Patagonian toothfish at Heard and McDonald Islands."

The methods for weighing, embedding the otoliths in resin, marking and sectioning the blocks in a way which facilitates the best display of the rings are described; then the process of mounting the sections on slides. The second part describes the methods for viewing the slides under the microscope, capturing the images, and pointers for reading the rings.

Otolith preparation

Weighing

The fish otoliths are stored in paper envelopes in batches by the cruise number and sorted numerically by the fish serial number. Care is required when removing the otoliths from the envelopes as they are often stuck to the sides and can easily break when attempting to remove them. Each otolith is weighed separately on a highly accurate electronic balance.

In order to get an accurate weight, the otoliths need to be completely free of organic material or any other matter before they are weighed. If there is any residual material on the otolith, soak them in a container of milli-q water and leave to dry completely before continuing. When cleaning the otoliths, soak them in trays with numbered wells so that individual pairs of otoliths are kept separate and it is easy to keep track of the identification of the otoliths. When they are dry, return them to their envelopes.

The left and right otoliths should be identified (Figure A3.1) and each otolith weight recorded to 4 decimal places. This is done on the Mettler Toledo balance, which is located in the Science Laboratory on the ground floor of the Wild building. The balance is connected to a computer which enables the weights to be entered electronically into the database (Figure A3.2). Use a tared boat balance to hold the otolith being weighed.

Incomplete otoliths are not weighed; however broken otoliths where all the pieces are available are weighed, with this being noted in the database.

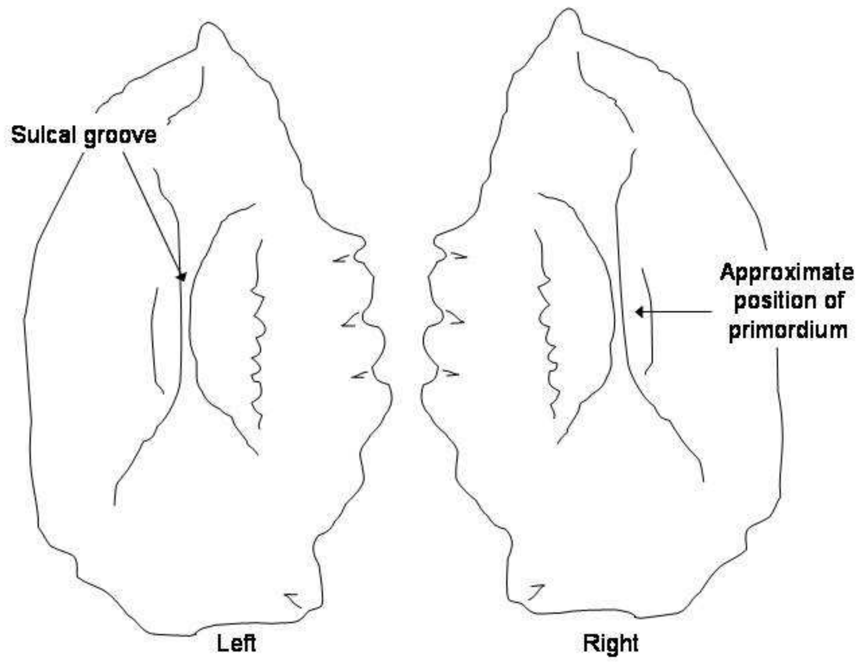

Figure A3.1. Identification of left and right otoliths of toothfish.

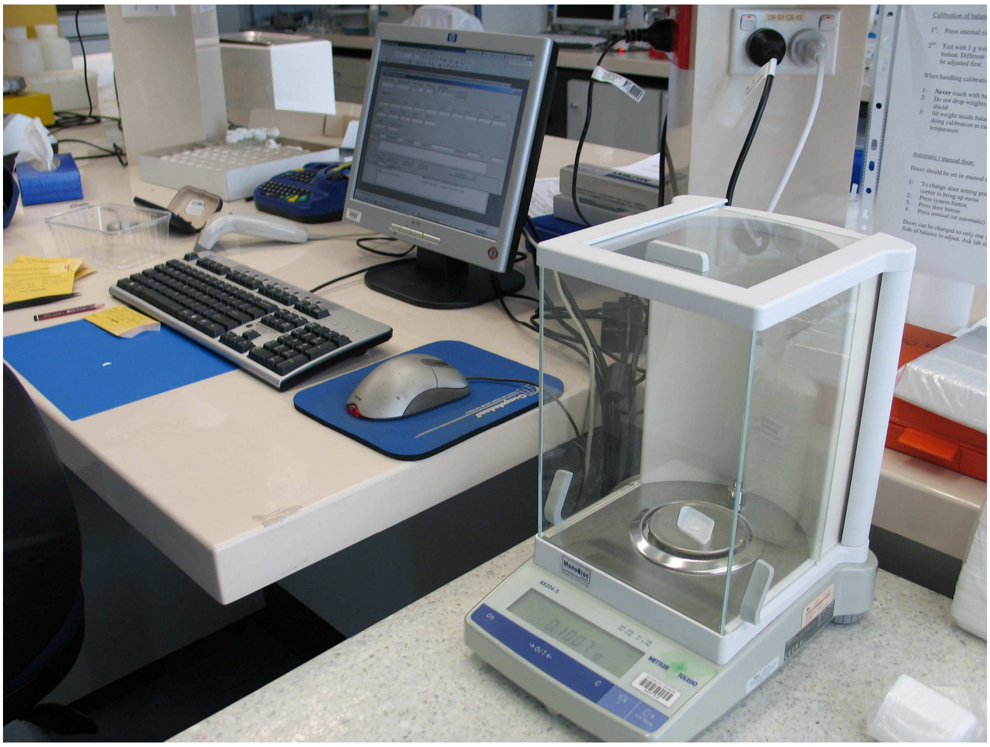

Figure A3.2. Electronic balance and computer setup for weighing otoliths.

Embedding, Sectioning and Mounting

Batch processing of otoliths (five at a time on a resin block) was tested, but it was decided that because Patagonian toothfish otoliths are difficult to read, better results are achieved when otoliths are processed individually. One otolith, selected at random, is embedded from each pair.

Individual otolith processing

Ensure that the otoliths have been weighed. Choose one of each pair of otoliths at random for sectioning. Individual otoliths are set in each well of an ice cube tray. Ice cube blocks are sectioned with either the Buehler Isomet Low Speed Saw or the Gemmasta high speed saw depending on the otolith size. All small and exceptionally large otoliths are to be sectioned on the Low speed saw to ensure a high quality section (these are the otoliths which are often the hardest to read) and all mid-sized otoliths can be sectioned using the high speed saw.

Setting the otoliths in resin

The ice cube trays are used as a mould.

1. Spray the ice cube trays sparingly with the Ease Release 2000 to facilitate the removal of the resin blocks when dry.
2. Prepare the resin (mix of 5:1 ratio of R180 to H180) and make a thin layer (approx 3mm) in each of the ice cube wells.
3. Allow to set for 24 hours.
4. Put one otolith in each well, aligning it so that the sulcal groove is up, and parallel to the long edge. Position it close to one of the short ends of the well (Figure A3.3).
5. Inset the label at the other end of the well. Labels can be printed in Excel (in 6pt font) with the *Fish serial number*.
6. Make up sufficient resin to cover the otoliths to about 3 mm.
7. Allow to cure for at least 24 hours before removing from the ice cube trays.
8. Full hardness of the resin is not reached for at least 3 to 4 days at room temperature (alternatively blocks can be placed in a 40°C oven for two days to speed up the hardening process).

Figure A3.3. Individual otoliths set in blocks in an ice cube tray.

Preparing for sectioning

Individual blocks are marked to prepare them for sectioning which ensures that the sections are cut precisely at the right angle to produce clear rings. There are three steps in this process.

1. Marking the primordium

Using a microscope with transmitted light, and the block on the stage, locate the primordium of the otolith and mark it with a dot using a permanent marker (e.g. Artline 725 superfine point permanent marker) (Figure A3.4).

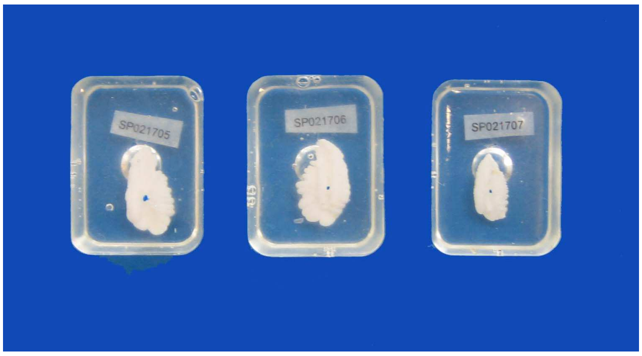

Figure A3.4. Otoliths in individual blocks with the primordium marked.

2. Marking the extension of the sulcus line

The aim of this part of the process is to mark the otolith block in line with the straight part of the sulcus which runs across the primordium. A line at right angles to this will mark the line of cutting of the block for the section.

On a piece of paper or cardboard, preferably not white, draw a straight line of approx 100 mm. Place the block with the otolith sulcus up on the paper, lining up the straight middle part of the sulcus along the line. The sulcus and the line should be aligned when viewed from directly above. Holding the block in place, mark both ends of the block with a fine permanent marker pen using the line drawn on the paper to accurately mark the extension of the line going through the straight part of the sulcus (Figure A3.5a).

3. Marking the cutting line

A line at right angles to the middle, straight part of the sulcus needs to be marked to obtain the correct angle for cutting the sections. This line becomes the guide for lining up the block in the saw for sectioning.

Prepare a piece of non-white paper or cardboard with 2 lines at right angles drawn on it in black pen. Draw some additional parallel lines on the vertical plane at 5 mm intervals. Place the otolith block so that the marks on the anterior and posterior ends of the block lie on the horizontal line and the primordium lies at the point where one of the vertical lines cross. Draw a line on the block, lining up with the one marked on the template, making sure that it is done when viewed directly from above. Mark a second line parallel to and about 1 cm away from the line going through the primordium on the side towards the centre of the block (Figure A3.5b). This line will assist with lining it up in the chuck on the saw (Figure A3.6). Note that these lines will not necessarily be parallel to the edge of the resin block. The important angle is the one relative to the sulcus of the otolith.

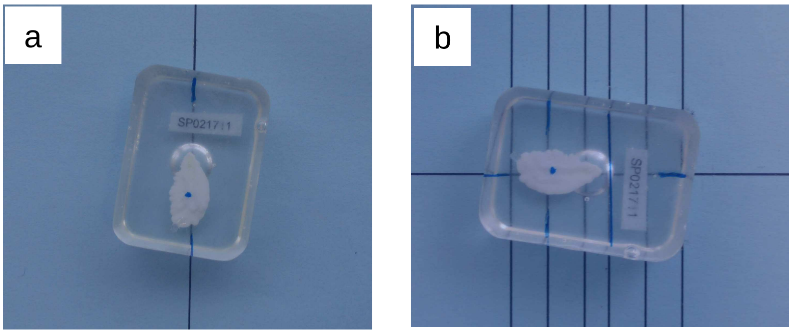

Figure A3.5. Marking the a) anterior and posterior ends (blue marks) and the b) cutting angle (blue lines) on the otolith block.

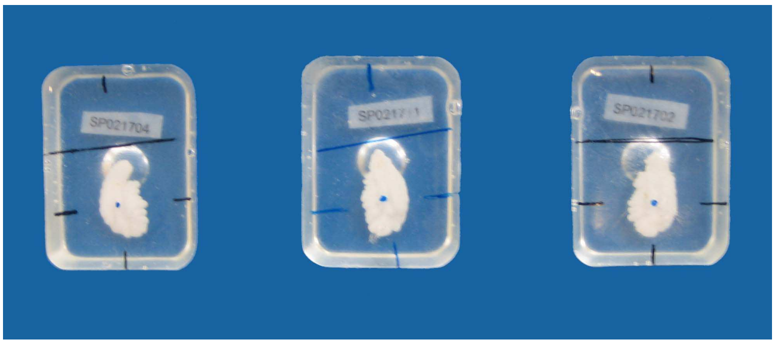

Figure A3.6. Individual otoliths in resin blocks marked with lines to guide the cutting angle in the saw.

Sectioning (Low speed Buehler saw)

1. Insert the block into the chuck, making sure that the line on the block will be parallel to the edge of the saw blade by aligning the second mark from the otolith with the edge of the chuck (Figure A3.5b). Attach the chuck to the saw.
2. Turn the microtome until the line going through the primordium of the otolith is aligned with the cutting edge of the blade.

3. The aim is to cut three sections of 350µm thickness (35 units on the microtome), with the second section enclosing the mark that was made on the primordium.
4. Move the microtome back 110 units* and cut (Figure A3.7). This marks the start of the sections.
5. Move the microtome forward 70 units to form the first slice of 350µm thickness. Number the cut (1) when it comes off the saw.
6. Rinse the section in milli-q water and lay out to dry on absorbent tissue.
7. Continue moving the microtome along 70 units and making slices until there are three sections.
8. Number each section (with a pencil) as it comes off the saw so that you can keep track of the sequence of sections (Figure A3.8).
9. Rinse each section and lay out on the tissue.
10. When dry, place all the sections on a tray or into a plastic bag along with the remaining block.

*Note: if intending to cut more than three sections, the microtome must be moved back the appropriate distance to ensure that the primordium is included in the central cut. Calculate this using the blade thickness of 0.35mm (35 units on the saw microtome) and the desired section thickness at 0.35mm.

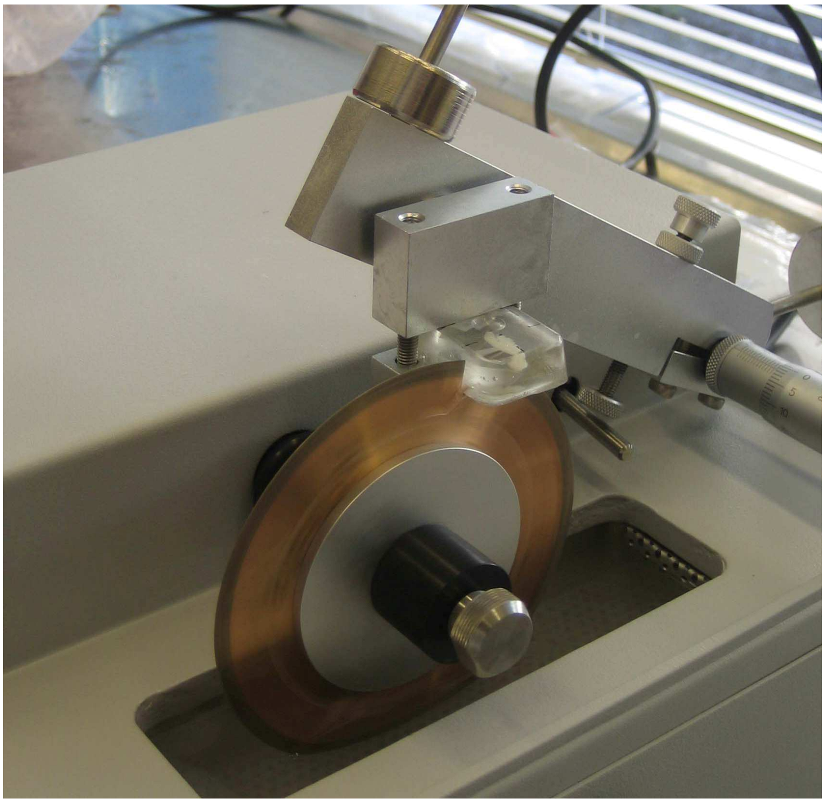

Figure A3.7. Otolith block positioned in the chuck and being sectioned on the Buehler saw.

Figure A3.8. Sectioned otoliths numbered in order of position coming off the saw.

Sectioning (High speed Gemmasta saw)

1. Insert the block into the chuck on the saw, making sure that the line on the block is parallel to the edge of the chuck (if needed, thin resin sections may be used as chocks to keep the block horizontal in the chuck).

2. Turn the microtome until the line going through the primordium of the otolith is aligned with the cutting edge of the blade.
3. The aim is to cut four sections of 350 μm thickness (3.5 units on the microtome), with the third section enclosing the mark that was made on the primordium.
4. Move the microtome back 18 units* and cut (Figure A3.8). This marks the start of the sections.
5. Move the microtome forward 6.5 units to form the first slice of 350μm thickness. Number the cut (1) when it comes off the saw.
6. Rinse the section in milli-q water and lay out to dry on absorbent tissue.
7. Continue moving the microtome along 6.5 units and making slices until there are four sections.
8. Number each section (with a pencil) as it comes off the saw so that you can keep track of the sequence of sections (Figure A3.10).
9. Rinse each section and lay out on the tissue.
10. When dry, place all the sections on a tray or into a plastic bag along with the remaining block.

*Note: if intending to cut more than four sections, the microtome must be moved back the appropriate distance to ensure that the primordium is included in the central cut. Calculate this using the blade cutting thickness of 0.3mm (3.0 units on the saw microtome) and the desired section thickness at 0.35mm.

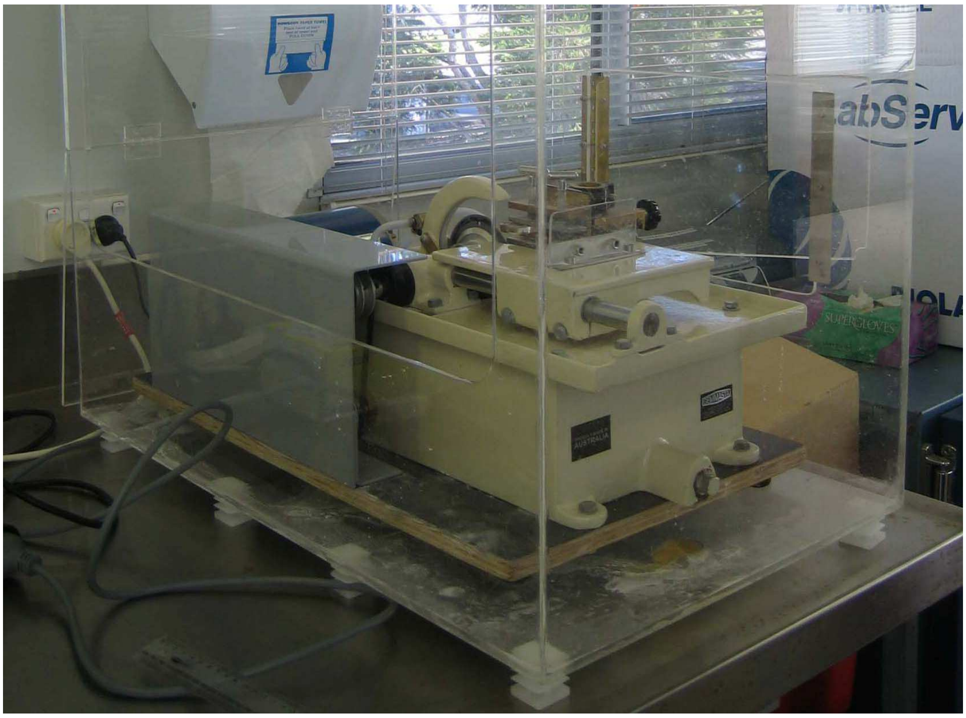

Figure A3.9. Gemmasta saw used for sectioning otoliths inside the perspex spray cabinet.

Mounting the sections on slides

1. The sections from the otolith of one fish are mounted onto one 76 x 26 mm slide with a 50 x 22 mm cover slip.
2. Write the last two digits of the Fish serial number on the resin below each otolith section with a pencil (Figure A3.8). If necessary, carefully cut the strip to a narrower width with a pair of scissors. This needs to be done slowly and carefully to ensure that the resin does not crack or the otolith sections fall out.
3. If using slides with a frosted end, write the *Fish serial number* on with a pencil or make up a printed label to put at the top of the slide in the resin.
4. To clean the sections before mounting, dip them for a few seconds firstly in Milli-Q water and then in Ethanol. Allow to dry.
5. Leave at least 5 mm at the top and bottom of each slide clear to prevent difficulty in accommodating the slide in a slide box.
6. Mix up some polyester clear casting resin (98% resin and 2% MEKP catalyst).

7. Using a plastic disposable pipette, place a small amount (approx. 2mm in depth) of resin onto a slide covering an area approximately the size of the cover slip.
8. Arrange the sections on top of the resin and then flip them. Push the sections to the bottom of the resin making sure that there are no air bubbles trapped beneath them. Use forceps to drag any bubbles away from the sections that may interfere when viewing them. Use the same process to add the label to the resin. (Figure A3.10).
9. Gently lower a cover slip over the sections starting from one side and making sure that no air bubbles are trapped beneath the cover slip.
10. Allow to cure for 24 hours.

Figure A3.10. Sections from single otoliths mounted on slides.

Estimating age from mounted sections

Once the otolith sections have been mounted on slides they are ready to be examined under the microscope to count the number of rings for ageing. The best section for each otolith is located and images are taken and saved for further examination.

Microscope and camera setup

The microscope used for the reading of otoliths is a Leica MZ95 with a Leica DFC320 camera connected to a computer running the Leica application suite software (Figure A3.11).

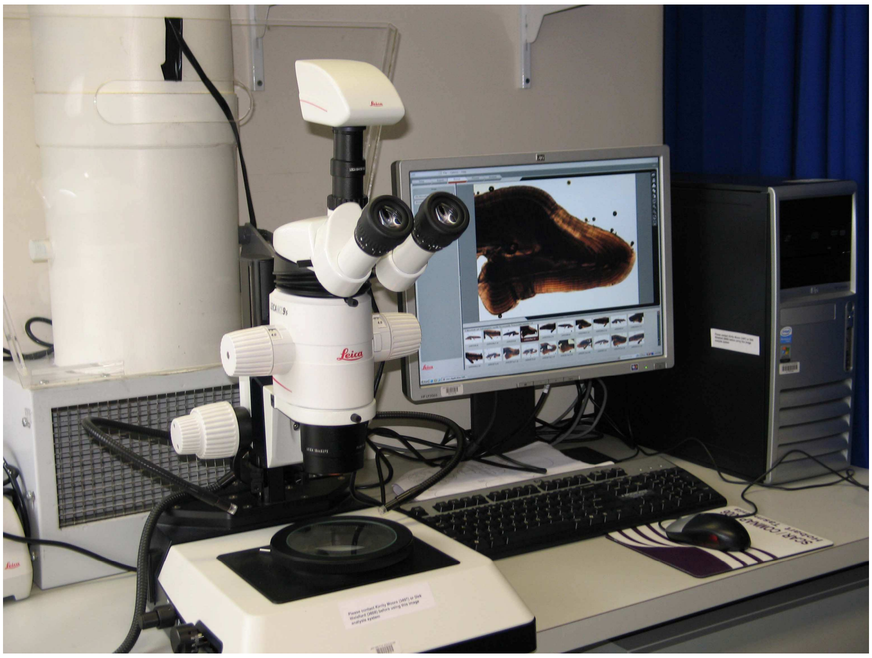

Figure A3.11. Microscope and imaging software on the computer.

Capturing images of otolith sections

With the microscope set at 20x magnification search through the mounted sections on a slide to find the clearest image which includes the primordium of the otolith. Capture an image of the selected otolith section, using the highest magnification (usually between 16× and 25×) which contains the entire otolith in the field of view. Save the image using the appropriate calibration configuration and give it the name of the fish serial number. Increase the magnification to 32× or 40× and capture separate images of the dorsal and ventral sides of each otolith section. Save these as the fish serial number, followed by 'rc1 or rc2' for ring count one and ring count two. All images should be saved in a relevant capture folder based on voyage.

Estimating age from captured images

Age estimates are made by counting opaque rings or increments in the otolith structure which have been shown to correspond to annual increases in growth. Estimates of otolith age are entered into the Fish database.

Pointers for reading rings

Ring counts are generally made down the ventral side of an otolith section closest to the sulcus, however the dorsal side may also be referred to if the ventral side is unclear (Fig 12). Identify the path of a ring around as much of the lobe as possible before deciding that it is an annulus, as there are often sub-bands and false checks. If a clear path for reading is not visible for counting in one area, follow a ring around to another part of the otolith to continue the count. The shape of the otolith is a useful rough guide to the age of the otolith. See section 4.3.5 for examples of fish of various ages.

Identification of first increment

The first and most important step is to identify the 1st increment. In toothfish this may be difficult to determine, as there is a large dark region at the centre of the otolith. The first ring is most easily located by looking for the first translucent zone outside of the dark core, and looking for the adjacent dark (opaque) band which forms the first ring. For sections which incorporate the primordium, the sulcus acusticus usually penetrates to the edge of the 1st increment (Kalish and Timmiss, 2000). Where measurements are available, it can be expected that the radius from the primordium to the inside of the first opaque zone will be between 0.6 and 0.9mm (ventral side, Figure A3.12).

Split opaque zones

Sometimes it will be noticeable that two bands appear very close together and it is unclear whether these represent one or two opaque zones. The workshop on ageing toothfish (SC-CAMLR 2001) recommended that where the split zones are within the first eight years of life that they be considered a split annulus, but if they occurred after eight years it should be counted as two annuli. This concept can be best understood by comparing the annuli in the outer area of a section from a large (older) fish, and comparing the form of the annuli to those closer to the core of the otolith.

Definitions

Some definitions from a workshop on the estimating of age in Patagonian toothfish (SC-CAMLR 2001) are useful and are reproduced here. The features are illustrated in Figure A3.12.

*Annulus***:** working from the nucleus, this comprises one opaque and the next adjacent translucent zone. Thus:

Year 1: that part of the otolith from the nucleus extending out to the outer edge of the first translucent zone; and

Year 2: that part of the otolith that extends from the inner edge of the first opaque zone after the nucleus to the outer edge of the second translucent zone.

Checks: translucent growth zones, denoting a slowing of growth that forms within the opaque zone; do not form annually but reflect various environmental or physiological changes.

Distal surface: the external surface of the whole otolith, opposite the sulcus.

*Nucleus***:** includes the primordium and extends outwards to the inside edge of the first translucent zone.

*Primordium***:** The point from which all growth in the otolith originates, formed when fish are still embryonic.

Proximal surface: the internal surface/sulcus-side of the whole otolith.

Plus growth: opaque zone forming on the edge of the otolith; not counted in age class designation.

Sulcus: the groove on the proximal surface through which the auditory nerve passes.

Transition zone: a region of change in the form (e.g. width or contrast) of the increments. The change can be abrupt or gradual. Transition changes are often formed in otoliths during significant habitat or lifestyle changes, such as movement from a pelagic to demersal habitat or the onset of first sexual maturity.

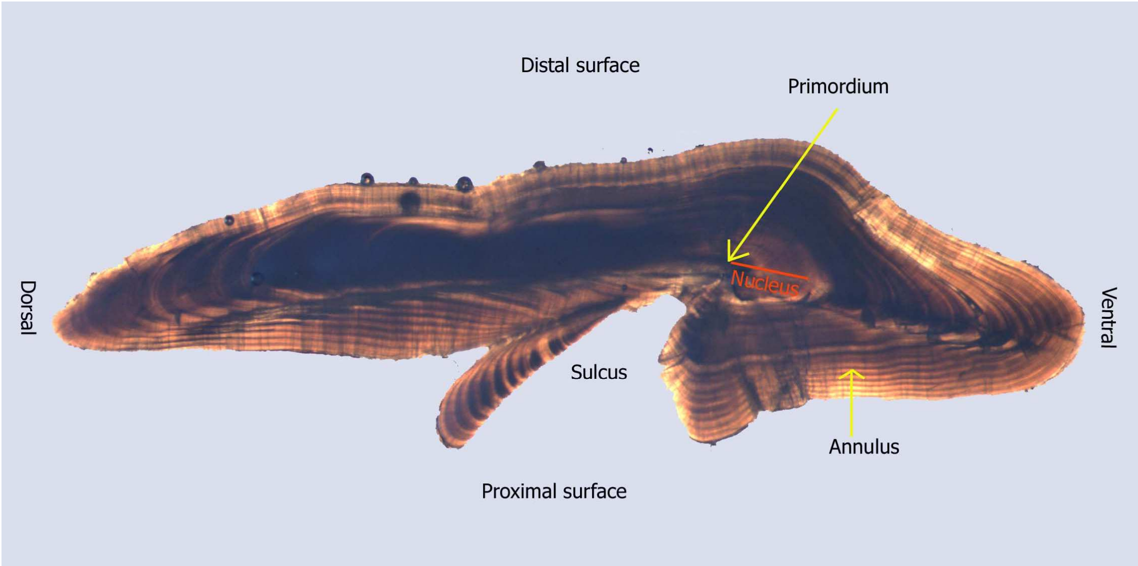

Figure A3.12. Definitions of otolith features.

Example otoliths

Examples of otoliths of various ages are provided to show the change in shape with age (Figures A3.13-A3.19).

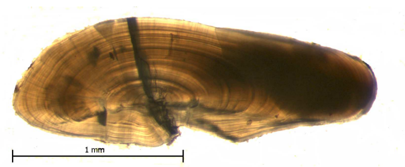

Figure A3.13. An otolith of a 0+ toothfish.

Figure A3.14. An otolith of a 1 year old toothfish.

Figure A3.15. An otolith of a 2 year old toothfish.

Figure A3.16. An otolith of a 5 year old toothfish.

Figure A3.17. An otolith of a 10 year old toothfish.

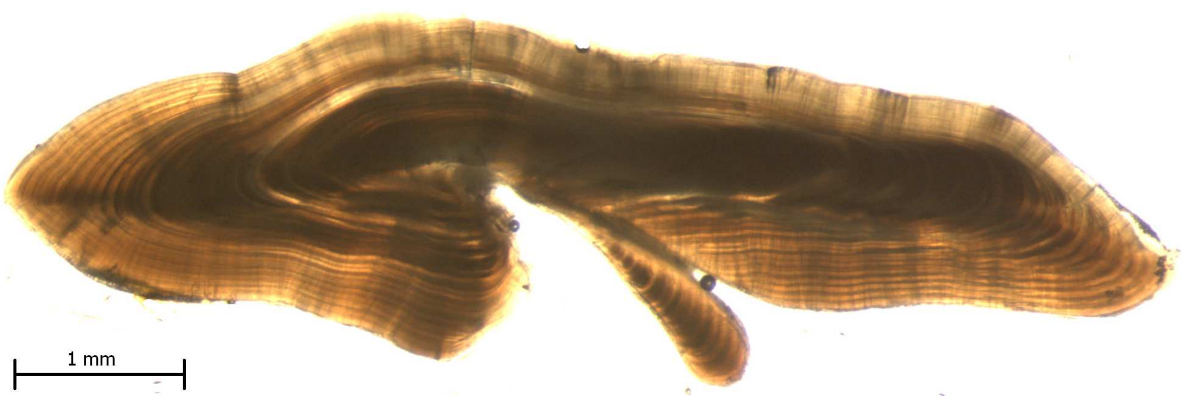

Figure A3.18. An otolith of a 15 year old toothfish.

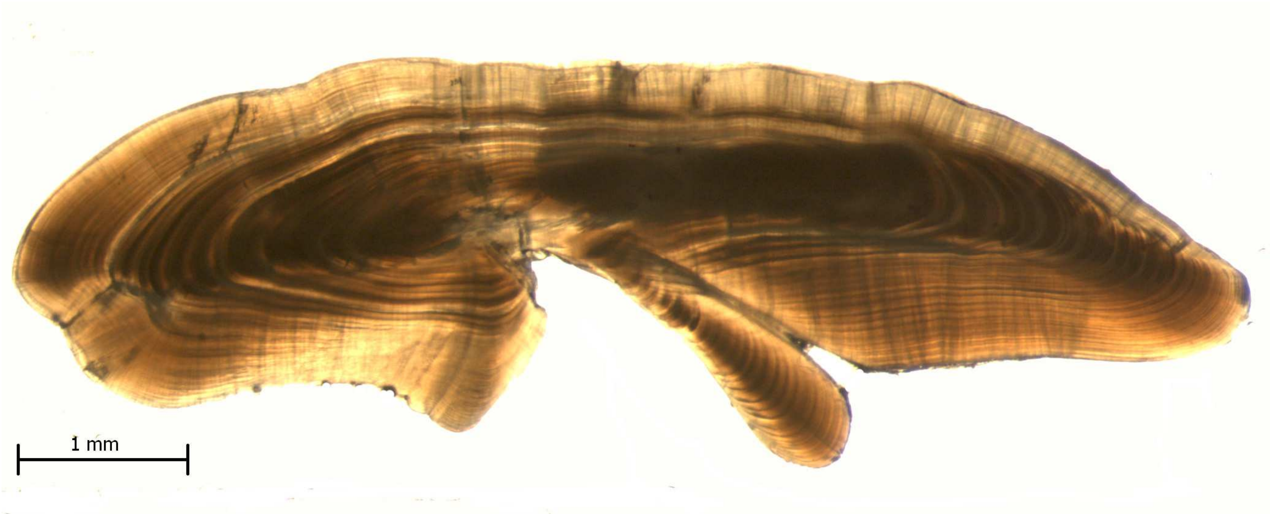

Figure A3.19. An otolith of a 20 year old toothfish.

Readability Index

In order to assist with assessing the quality of otolith sections and the accuracy of the reading, a readability index is assigned when reading each section. The index is given in the following table:

Readability Index	Description
1	Unreadable
2	Poor
3	Fair
4	Good
5	Very good

An example of each of the 5 stages with a description of the main characteristics of each stage is presented here as a guide. The categories are somewhat subjective but once some familiarity is developed, it becomes easier to judge what category to choose.

Readability index 1

Sections where the rings are extremely unclear or discontinuous and/or the section does not go through the primordium, where the count is not possible or would be highly unreliable, should be marked unreadable (Figure A3.20).

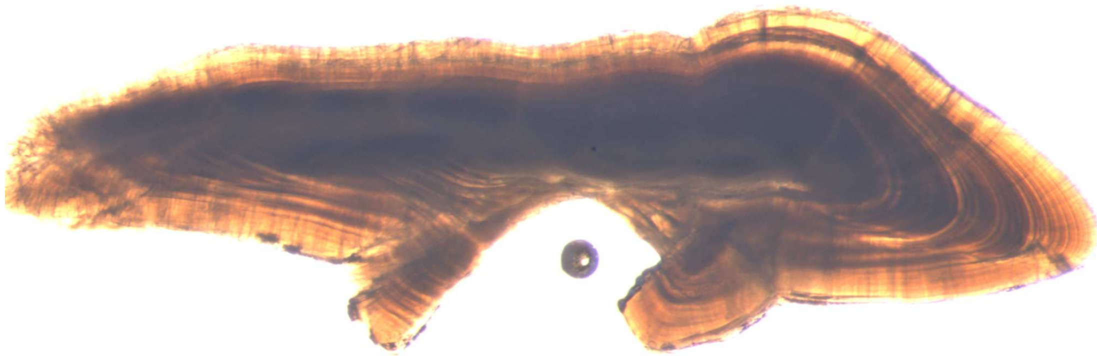

Figure A3.20. An otolith with a readability index of 1.

Readability index 2

The section is through the primordium but the rings are unclear and not continuous for very long sections, or there are large areas where rings are not distinguishable (often in the centre), leaving the count with a high degree of uncertainty (Figure A3.21).

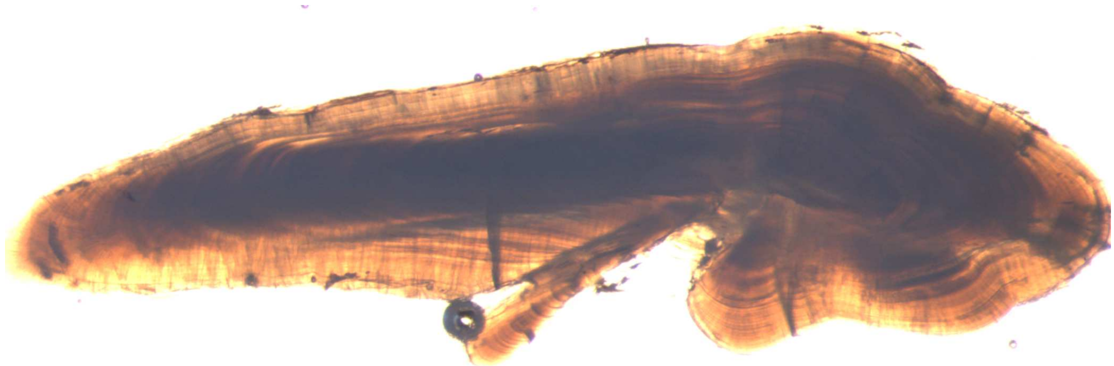

Figure A3.21. An otolith with a readability index of 2.

Readability index 3

Rings are visible around most of the section and fairly distinguishable, but some uncertainty still exists in differentiation and interpretation of rings (Figure A3.22).

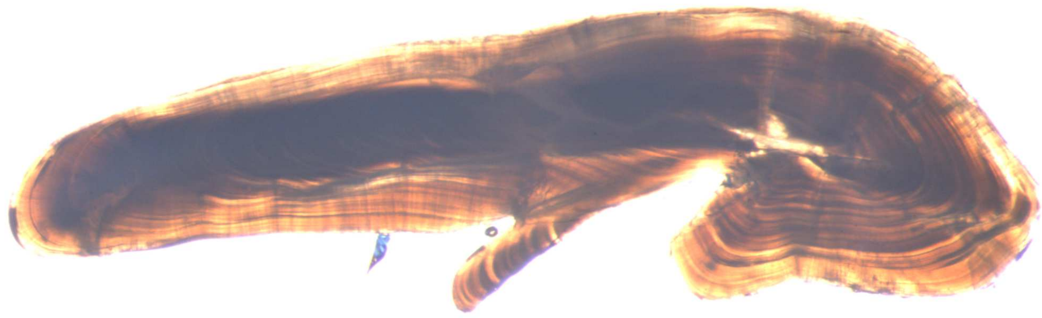

Figure A32. An otolith with a readability index of 3.

Readability index 4

Rings are clear over almost all of the otolith section, but there is perhaps one area that has some ambiguity e.g. towards the outer edge in this example (Figure A3.23).

Figure A3.23. An otolith with a readability index of 4.

Readability index 5

Rings are clearly visible around the proximal half of the otolith enabling an accurate count of the rings and confidence in repeatability of the count (Figure A3.24).

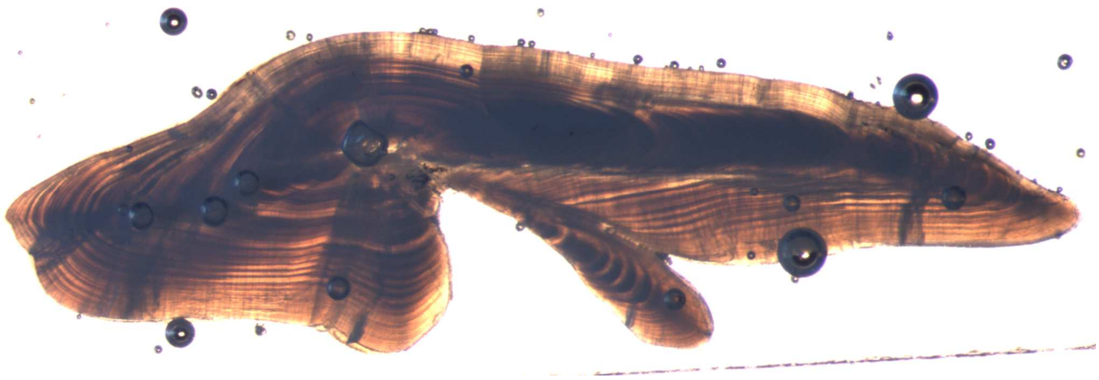

Figure A3.24. An otolith with a readability index of 5.

Reference collection

A selection of otoliths was put together as a reference collection. This collection of 200+ otoliths covers the range of lengths, ages and readability from otoliths collected from the fishery at Heard Island and McDonald Islands. The three types of fishing methods used in the fishery are represented; trawling, long lining and potting. The reference selection is designed to enable comparisons between readers and also to be read at intervals during a reading process to identify any drifting of readers.

Acknowledgements

Thanks to Dirk Welsford for useful suggestions on the content. The single otolith cutting technique was refined from a technique first learned by the primary author at Western Australian Marine Research Laboratories and this is gratefully acknowledged.

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Appendix 4 Otolith sub-sampling strategy

John McKinlay

Introduction

The otolith collection used for this project comprises over 21,000 toothfish otoliths collected up to 2007 from commercial and research fishing in the Heard Island and McDonald Island (HIMI) region. At the commencement of the project, more than 3,200 otoliths from fish captured between 1997 and 2003 had been processed to provide age estimates, leaving a non-aged collection of around 18,000 fish. Since preparing and ageing otoliths is an expensive process, the project budget did not allow for all remaining non-aged fish in the collection to be processed and a sub-sampling strategy was necessary.

Ideally, an optimised sub-sampling strategy would be one that provided the greatest improvement in the accuracy of the HIMI stock assessment for a fixed cost. This could be achieved through simulating catches from an operating model of the fishery and applying different sub-sampling strategies to catches (for which actual ages are known from the operating model) (e.g. Francis 2006). These strategies could then be assessed by finding the best sampling intensity by length-class for improving the accuracy of the TAC determined by applying the usual HIMI integrated assessment to the simulated data.

An approach such as that described above involves considerable overhead, both in terms of programming effort and assessment processing time, and was considered beyond the scope of the current study. Instead, we proposed for this study a resampling approach that utilises information from those fish already aged in order to determine optimal sample numbers from the non-aged collection. The rationale for this approach is as follows. With over 3,200 specimens already aged, useful information is known about the length-at-age relationship. Growth in toothfish tends to slow with increasing age, such that large length-classes typically contain a wider distribution of ages when compared with small length-classes. For example, the smallest length-class currently used the HIMI assessment (200-249mm) contains just 1-2 age-classes, while the largest (1200-1249 mm) may incorporate more than 10 age-classes (Welsford and Nowara 2007). The resampling approach we implement uses this age-length information to determine the sample sizes that would be necessary, per length-class, to detect an acceptable proportion (pre-specified by the user) of the ages present in any length-class.

Methods and Results

A function was developed in the software environment R (R Development Core Team 2008) to facilitate sub-sampling of the non-aged otolith collection. The function takes as primary input an extract of fish length-frequency information from the AAD fisheries database (FRESH FISH). Input data includes information about all aged and non-aged fish in the collection. Several categorical variables (fishery, year, 'randomness' status, readability of otolith) can be used to subset both input (aged) and output (non-aged) data, and to stratify data displays and the sampling scheme for selecting non-aged fish for aging. For exploratory purposes, the software tabulates (in numbers and proportions) and plots (by boxplot) age-length distributions for the aged collection (see Annex A for examples). The resampling procedure itself was determined to have several requirements:

1. Selection for aging should be stratified to occur within 50 mm length classes, as this reflects the resolution used in length frequency data incorporated into the current assessment model (Candy and Constable 2008).
2. Selection of non-aged specimens for aging should be random within length-classes.
3. Length-at-age information available from the population of fish already aged should be utilised to ensure optimal sample-size selection for each length-class.
4. Sample selection should accommodate stratification of the data according to fishery and season.

The resampling procedure to determine sample-sizes to be aged per length-class was constructed to operate in the following way. Under suitable stratification of season and/or fishery, the length distribution of aged fish is resampled with replacement from within a length-class to determine sample-sizes that – *post hoc* – would have been required to adequately determine age distributions. Here, use of the term ‘adequately’ is taken to mean that the procedure satisfies a number of preset conditions specified by the user, to be discussed shortly. The procedure allows the following type of question to be answered: ‘What sample sizes would I need, per length-class, to ensure I sample 80% of the ages in each length-class to a minimum of 5 fish in the outer ages (where ‘outer ages’ corresponding to 80% coverage would be defined as the 10th and 90th percentile of the age distribution)?’. In this way, existing aged data are used to ensure larger length-classes containing many age cohorts are adequately sampled to ensure a pre-specified coverage of the majority of age classes likely to be present.

The approach detailed above is predicated on an assumption that, within a length-class, randomly sampled aged fish in the collection provide a reasonable approximation of the age-distribution by length (i.e. sufficient sample size, and sufficient coverage of fishery/season). This assumption will not always be met, either at a broad scale (poor coverage for a fishery or season) or fine scale (poor coverage for an individual length-class within a combination of fishery and season).

When sufficient coverage of aged data is not available at a broad level, the software allows data to be conveniently aggregated over fishery and season. However, developing code to automatically determine levels of aggregation was not feasible given the timeframe and scope of this work, and such decisions must be determined heuristically by examining age-length frequency tables (a standard output of the software). For the purposes of the current analysis, aged data disaggregated by fishery and/or year were too sparse to adequately represent age distributions within length-classes, and were therefore aggregated. This necessity to pool data was not altogether unanticipated, since one of the goals of the project was to improve the coverage of aged data within the HIMI stock assessment (i.e. aging was to occur predominantly within seasons and fisheries not previously aged). However, any subsequent aging work would be expected to benefit from appropriate stratification of the data.

To accommodate poor coverage at a fine-scale (i.e. within a length-class), it was necessary to develop criteria based on minimum sample sizes, and some rules about how sampling should proceed when such events occur. These and several other adjustable rules developed for the routine are defined as follows:

1. *min.aged*: the minimum number of aged fish in a length-class before it is deemed representative of the age structure for that length-class. (default: *min.aged*=50). If there are fewer aged fish than this number, then rule 2 below is applied.

2. *mrs*: the minimum random sample of non-aged fish to take from a length-class when there are insufficient aged fish for determining an age distribution within a length-class (default: *mrs*=20).

3. *min.naged*: the minimum number of non-aged fish present in a length-class below which no random sampling to achieve a specified coverage is undertaken. In other words, the resampling routine aims to achieve an optimal sub-sample of non-aged fish in length-classes where there are *too many* non-aged specimens to age them all, but for some minimum number it makes no sense to sub-sample, we simply age them all (default: *min.naged*=20).

4. *age.coverage*: the middle proportion of the age distribution that is required to be captured by the determined sample size (default: *age.coverage*=0.8).

5. *excl.p.age*: since the tails of the age distribution for a single length-class can contain very few individuals, when resampling to achieve a specified level of age coverage it was necessary to exclude the tail for purposes of assessing *age.coverage*. This was achieved by truncating the age distribution to ensure that relative frequencies in the outer-most ages exceeded a minimum threshold value (default: *excl.p.age*=0.02). Note that samples with low probability ages excluded for the purpose of determining *age.coverage* (by virtue of the argument *excl.p.age*) can be selected in the resampling routine, albeit with low probability of selection.

6. *min.out*: resampling within a length-class proceeds until a minimum number of samples are achieved in the outer-most age-classes determined by *age.coverage* (default: *min.out*=2).

By way of a hypothetical example to illustrate these rules, consider estimating the sample size that would be required from the non-aged collection to ensure 60% coverage of ages for a single length-class (800-850 mm) (Figure A4.1). We begin with 111 aged fish within the length-class (Figure A4.1a). From this distribution of ages, two age-classes are excluded for the purposes of determining age coverage in the resampled data since their relative proportion is less than 0.02. Resampling (with replacement) of this distribution proceeds until the resulting distribution is shown to have achieved sufficient age coverage (60% in this example) to a depth of at least two samples in the outer-most age-classes (Figure A4.1b). The sum of samples in b) provides the sample size (in this case 43) that would be required to achieve 60% coverage of the age-distribution (truncated to exclude those ages with relative frequencies less than 0.02) to at depth of at least 2 samples in the outer-most age-classes.

Under this framework, the expected values of the relative frequencies of ages in the resampled distribution will converge toward those of the parent distribution as the number of resamples increases. The resampled distribution is similar in nature to a single bootstrap sample (*sensu* Efron and Tibshirani 1993), except that in this case the resulting sample size is not restricted to be equal that of the parent distribution. Optimisation by bootstrapping the entire process would comprise a natural extension of the work, however this was not possible in light of the grid search across all plausible parameter values (below) and time constraints for finalising sampling.

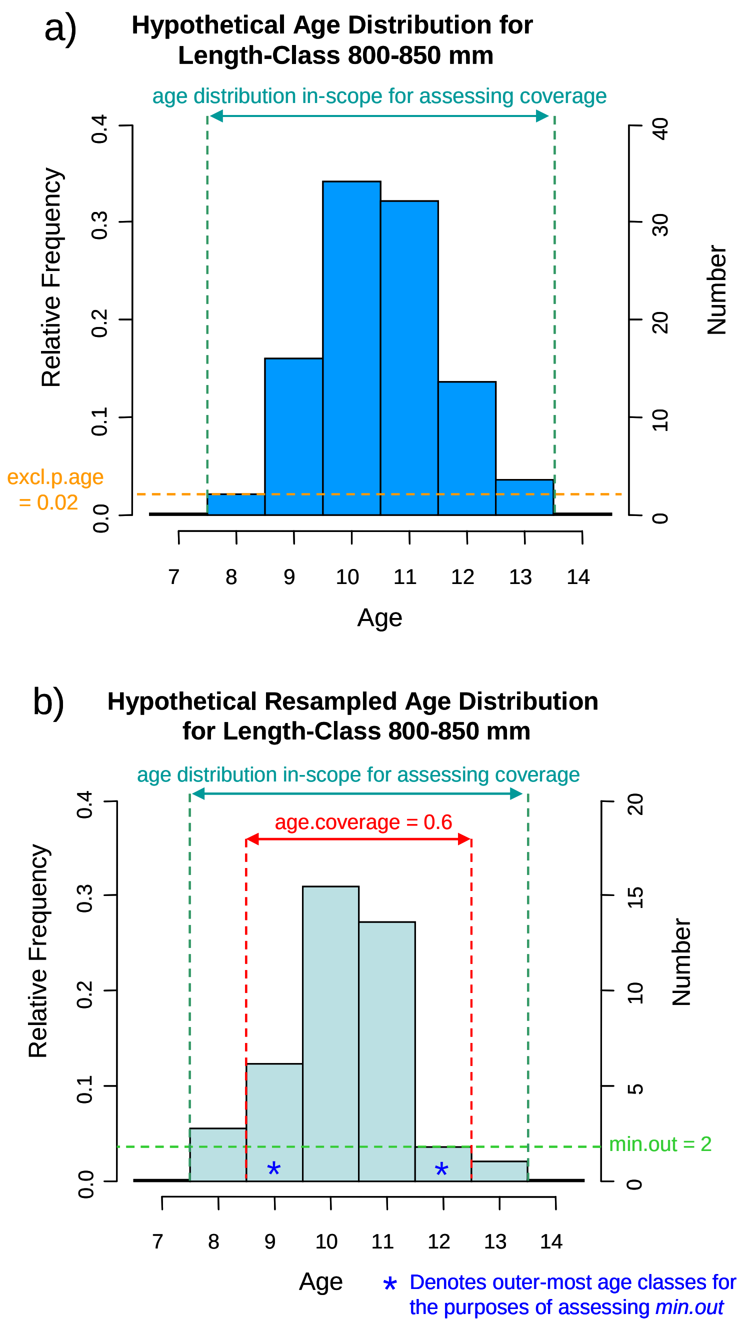

Figure A4.1. Plots of hypothetical data for an individual length-class, showing diagrammatically how function parameters are used to ensure adequate representation of the age distribution during resampling. Panel a) shows the age distribution from a single length-class determined from the collection of aged otoliths. Panel b) shows the distribution that might arise from the resampling process, with 60% coverage of the truncated distribution achieved when both outer-most age classes have at least 2 samples drawn (*min.out* = 2). See text for further detail.

The rules defined above show default values for the most important function arguments determining sample size estimation. However, to determine the optimum values for each parameter a grid-search over a large number of possible values was undertaken (Table A4.1). Taking all combinations of these values generated a set of 192 scenarios, each of which were sequentially applied to each year-by-fishery subset of the non-aged data. Stratification of the optimisation by year and fishery was necessary since the amount of aged data present varied between fisheries and years.

Table A4.1. Parameter values used in grid search, all combinations of which provide 192 scenarios.

Parameter Name	Description	Values
age.coverage	Desired age coverage (as proportion)	0.9, 0.8, 0.7, 0.6
min.out	Min. samples in outer-most ages	2, 1
min.aged	Min. aged fish for a length-class before deemed representative of age distribution	30, 50
mrs	Min. random sample taken in the event resampling not undertaken	20, 30
excl.p.age	Excluded ages for purposes of determining age coverage (based on low relative proportions)	0.001, 0.01, 0.02

All scenarios were applied to subsets of the non-aged data with sufficient coverage to warrant subsampling (Table A4.2). For those length-classes not able to be sampled because *min.aged* and/or *min.naged* rules were broken, a minimum random sample of up to *mrs* was taken (if available). Although length-class bin size is an adjustable parameter of the resampling routine, only 50 mm was used since this is the length-class size currently used within the integrated assessment of the HIMI fishery (Candy and Constable 2008).

Fishery 5 (Long line Ground C) and 6 (Long line Ground D) were not subjected to the resampling method for determining minimum sample sizes since there were typically less than 20 fish per 50 mm length-class across all length-classes; in these instances all non-aged fish were selected for aging.

Table A4.2. Fishery and season combinations with large otolith collections necessitating subsampling, and the sampling parameters used.

Fishery	Season	Parameter					
		age.cov	***min.out***	***min.aged***	***mrs***	***min.naged***	***excl.p.age***
1 Trawl Survey	2007	90	1	30	20	20	0.01
2 Trawl Ground B	2003	60	1	30	10	20	0.02
	2004	60	1	30	10	20	0.02
	2005	60	1	30	10	20	0.02
	2006	60	1	30	10	20	0.02
	2007	60	1	30	10	20	0.02
3 Trawl Ground C	2006	60	1	30	10	20	0.02

References

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Welsford, D.C. and Nowara, G.B. 2007 Summary of holdings of Patagonian toothfish (*Dissostichus eleginoides*) otoliths and size-at-age estimates from Heard and McDonald Islands (Division 58.5.2). *CCAMLR Document WG-FSA-07/45.*

Annex A – Example tabular and graphical output

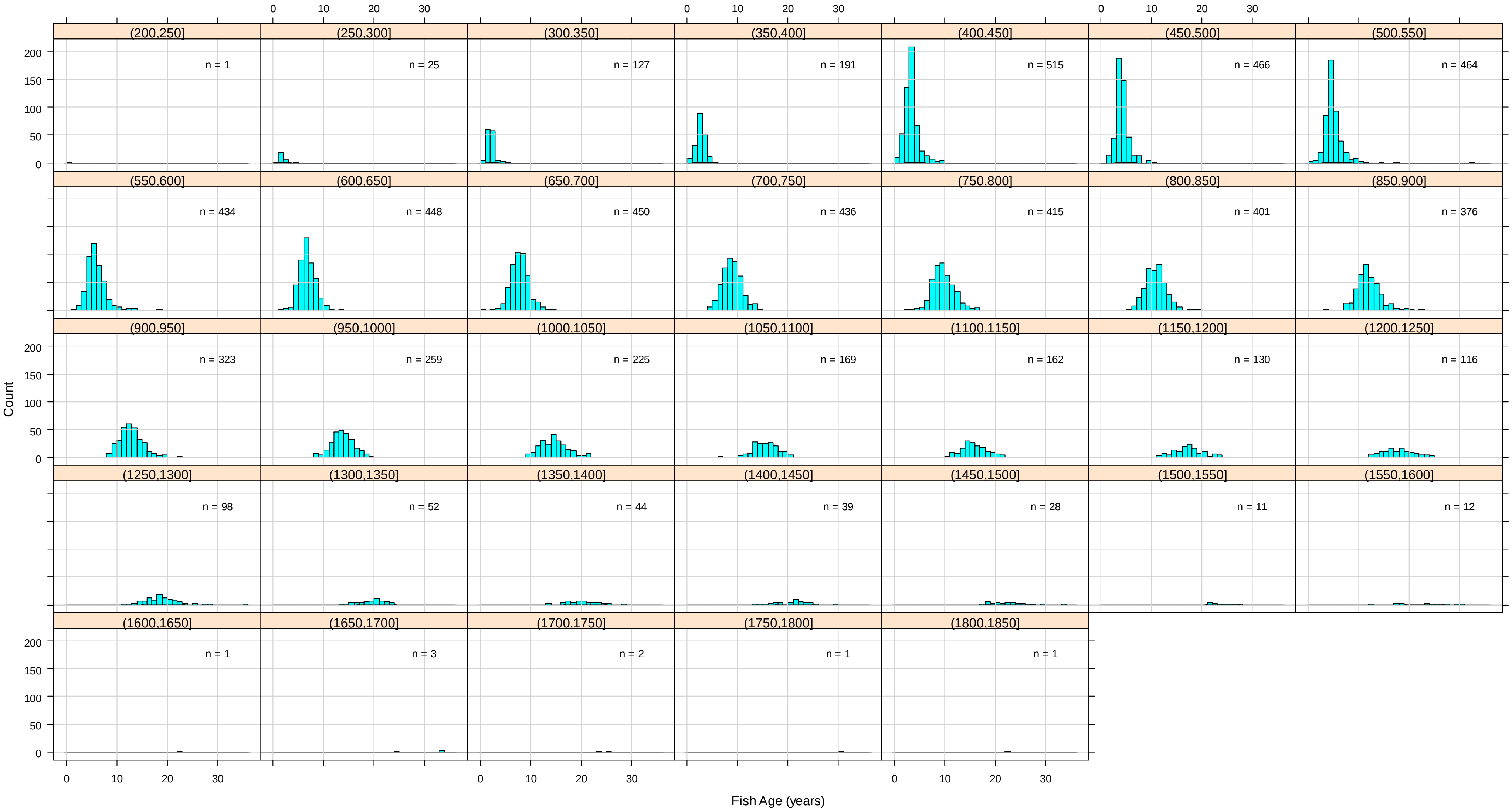
Table A4.3. Number of aged fish by season, summed across all fisheries, at the commencement of this project. These constituted the input (aged) data used for determining sample-sizes by length-class to be selected from the non-aged collection.

	CASAL Year										
Length Bin (mm)	1990	1992	1993	1997	1998	1999	2000	2001	2002	2003	Sum
100-149	.	.	.	.	.	.	.	.	.	.	.
150-199	.	.	.	.	.	.	.	.	.	.	.
200-249	.	.	.	.	.	.	.	.	.	1	1
250-299	2	3	.	.	.	.	2	2	.	9	18
300-349	19	17	2	.	.	2	12	9	.	23	84
350-399	13	59	4	.	.	.	22	8	1	1	108
400-449	47	40	21	.	5	24	23	42	20	2	224
450-499	51	27	25	.	1	44	46	36	43	.	273
500-549	42	.	11	.	.	48	56	46	44	.	247
550-599	4	.	8	.	.	45	66	43	51	.	217
600-649	.	.	3	.	.	54	58	40	49	40	244
650-699	.	1	2	.	.	48	55	39	49	47	241
700-749	.	.	.	.	.	52	51	48	47	49	247
750-799	.	.	.	.	4	45	51	42	44	43	229
800-849	.	.	.	.	1	41	57	39	44	43	225
850-899	.	.	.	.	4	34	51	32	34	45	200
900-949	.	.	.	.	3	13	21	38	26	46	147
950-999	.	.	.	.	3	6	22	24	10	41	106
1000-1049	.	.	.	18	23	6	7	14	15	2	85
1050-1099	.	.	.	9	21	7	11	7	6	.	61
1100-1149	.	.	.	6	25	9	8	9	7	.	64
1150-1199	.	.	.	4	21	7	8	3	3	.	46
1200-1249	.	.	.	4	17	4	8	5	1	.	39
1250-1299	.	.	.	4	11	1	9	3	4	.	32
1300-1349	.	.	.	3	4	4	1	3	2	.	17
1350-1399	.	.	.	1	6	1	2	3	1	.	14
1400-1449	.	.	.	1	5	2	.	1	1	.	10
1450-1499	.	.	.	2	1	2	2	.	.	.	7
1500-1549	.	.	.	.	1	.	1	.	.	.	2
1550-1599	.	.	.	.	2	.	1	.	.	.	3
1600-1649	.	.	.	.	.	.	.	.	.	.	.
1650-1699	.	.	.	.	.	.	.	.	.	.	.
1700-1749	.	.	.	.	1	.	.	.	.	.	1
1750-1799	.	.	.	.	.	.	.	.	.	.	.
Sum	178	147	76	52	159	499	651	536	502	392	3192

Table A4.4. Number of aged fish by season, summed across all fisheries, at the completion of this project. These are comprised of all aged fish used for determining age-length keys.

	CASAL Year														
Length Bin (mm)	1990	1992	1993	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Sum
100-149	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1
150-199	2	3	.	.	.	.	2	4	2	11	.	1	.	.	25
200-249	19	19	2	.	.	2	12	15	18	24	.	1	6	9	127
250-299	14	59	4	.	.	.	21	17	17	1	8	.	20	30	191
300-349	47	40	21	.	5	24	23	46	31	7	9	10	57	195	515
350-399	52	27	25	.	1	45	48	43	49	9	6	13	67	81	466
400-449	42	1	11	.	.	49	56	50	44	9	9	9	93	91	464
450-499	4	1	8	.	2	45	68	47	51	10	8	10	85	95	434
500-549	.	.	3	.	3	60	61	46	49	55	10	9	82	70	448
550-599	.	1	2	.	3	54	60	50	50	56	10	10	80	74	450
600-649	.	.	.	.	3	58	52	49	49	59	10	10	90	56	436
650-699	.	.	.	.	6	46	53	47	46	52	9	10	72	74	415
700-749	.	.	.	.	2	44	59	46	48	54	18	9	71	50	401
750-799	.	.	.	.	8	34	53	43	38	54	8	17	72	49	376
800-849	.	.	.	.	20	13	22	48	32	54	10	6	71	47	323
850-899	.	.	.	.	15	6	24	44	16	48	9	4	63	30	259
900-949	.	.	.	18	34	10	13	43	22	13	2	2	37	31	225
950-999	.	.	.	9	25	12	19	31	15	7	3	1	21	26	169
1000-1049	.	.	.	6	32	11	20	34	13	8	3	3	21	11	162
1050-1099	.	.	.	4	26	10	17	23	12	2	3	1	22	10	130
1100-1149	.	.	.	5	17	6	15	33	6	7	2	3	17	5	116
1150-1199	.	.	.	4	14	6	19	23	9	.	1	1	18	3	98
1200-1249	.	.	.	3	4	5	6	19	7	1	.	1	5	1	52
1250-1299	.	.	.	1	7	1	7	12	3	1	.	.	11	1	44
1300-1349	.	.	.	1	5	4	4	7	4	.	.	.	11	3	39
1350-1399	.	.	.	2	1	6	6	5	1	.	.	.	6	1	28
1400-1449	.	.	.	1	1	2	1	2	4	.	.	.	.	.	11
1450-1499	.	.	.	.	5	1	1	1	.	1	.	1	1	1	12
1500-1549	.	.	.	.	.	.	.	.	.	.	.	.	1	.	1
1550-1599	.	.	.	.	.	1	.	1	.	.	.	.	1	.	3
1600-1649	.	.	.	.	.	1	.	1	.	.	.	.	1	.	3
1650-1699	.	.	.	.	1	.	.	.	1	.	.	.	.	.	2
1700-1749	.	.	.	1	.	.	.	.	.	.	.	.	.	.	1
1750-1799	.	.	.	.	.	.	.	.	.	.	.	.	1	.	1
Sum	180	151	76	55	240	555	742	829	637	544	138	132	1102	1044	6425

Figure A4.2. Histograms showing the distribution of ages per 50 mm length-class, summed across all fisheries, at the completion of this project. These are comprised of all aged fish used for determining age-length keys.



Appendix 5 Ageing error matrices

Table A5.1 Ageing error matrix E for otoliths with readability 5.

1	0.749	0.247	0.004	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.208	0.741	0.047	0.005	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.004	0.206	0.733	0.056	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.004	0.204	0.724	0.065	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.005	0.202	0.716	0.076	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.005	0.198	0.707	0.087	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.005	0.195	0.699	0.099	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.006	0.190	0.690	0.111	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.006	0.186	0.681	0.123	0.004	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.006	0.181	0.672	0.136	0.005	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.007	0.176	0.663	0.149	0.006	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.007	0.172	0.653	0.162	0.007	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.007	0.167	0.644	0.174	0.008	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.008	0.163	0.634	0.186	0.009	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.008	0.159	0.624	0.198	0.010	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.009	0.156	0.615	0.209	0.012	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.009	0.153	0.605	0.220	0.013	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.010	0.150	0.595	0.230	0.015	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.010	0.148	0.585	0.240	0.017	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.011	0.146	0.574	0.249	0.019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.012	0.145	0.564	0.257	0.021	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.013	0.144	0.554	0.265	0.024	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.014	0.144	0.544	0.271	0.026	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.015	0.145	0.533	0.277	0.029	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.017	0.146	0.523	0.282	0.032	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.018	0.148	0.513	0.286	0.035	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.020	0.150	0.502	0.289	0.039	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.022	0.153	0.492	0.291	0.042	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.025	0.156	0.481	0.292	0.046	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.027	0.160	0.471	0.292	0.050	0.0	0.0	0.0
31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.030	0.165	0.461	0.290	0.053	0.0	0.0
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.034	0.171	0.450	0.287	0.057	0.0
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.038	0.177	0.440	0.283	0.061
34	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.108	0.184	0.430	0.278
35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.117	0.463	0.420

Table A5.2 Ageing error matrix E for otoliths with readability 4.

1	0.554	0.410	0.030	0.005	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.340	0.544	0.077	0.033	0.005	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.028	0.333	0.534	0.090	0.008	0.006	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.005	0.029	0.325	0.523	0.104	0.009	0.002	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.001	0.006	0.031	0.316	0.513	0.119	0.012	0.002	0.0	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.001	0.001	0.006	0.032	0.306	0.503	0.134	0.014	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.001	0.001	0.006	0.033	0.295	0.492	0.150	0.017	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.001	0.001	0.007	0.034	0.284	0.482	0.166	0.020	0.004	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.001	0.001	0.007	0.035	0.273	0.471	0.181	0.023	0.005	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.001	0.001	0.008	0.036	0.261	0.461	0.196	0.027	0.006	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	0.0	0.0	0.0	0.0	0.0	0.001	0.002	0.008	0.037	0.250	0.451	0.211	0.031	0.007	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.002	0.002	0.008	0.038	0.238	0.440	0.224	0.036	0.008	0.002	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.002	0.002	0.009	0.039	0.228	0.430	0.237	0.040	0.009	0.002	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.002	0.002	0.009	0.040	0.217	0.420	0.248	0.045	0.011	0.003	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.002	0.003	0.010	0.041	0.208	0.410	0.258	0.050	0.012	0.003	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.003	0.003	0.010	0.042	0.199	0.400	0.267	0.056	0.014	0.004	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.003	0.003	0.011	0.043	0.190	0.390	0.274	0.061	0.015	0.005	0.004	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.003	0.004	0.011	0.044	0.183	0.380	0.281	0.067	0.017	0.006	0.005	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.003	0.004	0.012	0.045	0.176	0.370	0.285	0.073	0.019	0.007	0.006	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.004	0.005	0.012	0.046	0.169	0.361	0.288	0.079	0.021	0.008	0.007	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.004	0.006	0.013	0.048	0.164	0.351	0.290	0.085	0.023	0.010	0.008	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.005	0.006	0.014	0.049	0.158	0.342	0.290	0.090	0.025	0.012	0.009	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.005	0.007	0.014	0.051	0.154	0.333	0.290	0.096	0.027	0.013	0.010	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.006	0.008	0.015	0.053	0.150	0.323	0.287	0.102	0.029	0.016	0.011	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.007	0.009	0.016	0.055	0.147	0.314	0.284	0.107	0.031	0.018	0.013	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.007	0.010	0.017	0.058	0.144	0.305	0.279	0.112	0.032	0.020	0.014	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.008	0.012	0.018	0.061	0.142	0.297	0.273	0.117	0.034	0.023	0.016	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.009	0.014	0.019	0.064	0.140	0.288	0.266	0.121	0.036	0.026	0.018	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.010	0.016	0.020	0.067	0.138	0.280	0.258	0.125	0.037	0.029	0.020	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.012	0.018	0.021	0.071	0.137	0.271	0.249	0.128	0.038	0.032	0.021
31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.036	0.020	0.023	0.075	0.137	0.263	0.240	0.131	0.039	0.036
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.040	0.063	0.024	0.079	0.136	0.255	0.229	0.133	0.040
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.044	0.070	0.066	0.084	0.136	0.247	0.218	0.134
34	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.048	0.077	0.068	0.224	0.137	0.240	0.206
35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.052	0.085	0.070	0.230	0.331	0.232

Table A5.3 Ageing error matrix E for otoliths with readability 3.

1	0.468	0.453	0.065	0.012	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.371	0.457	0.084	0.071	0.014	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.060	0.361	0.447	0.098	0.016	0.015	0.002	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.013	0.062	0.349	0.437	0.112	0.020	0.004	0.002	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.002	0.014	0.064	0.335	0.426	0.126	0.024	0.005	0.001	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.002	0.002	0.014	0.066	0.321	0.416	0.141	0.029	0.006	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.002	0.002	0.015	0.068	0.306	0.406	0.156	0.034	0.008	0.001	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.003	0.003	0.016	0.069	0.291	0.396	0.170	0.040	0.009	0.002	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.003	0.003	0.017	0.070	0.276	0.386	0.184	0.046	0.011	0.002	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.003	0.003	0.017	0.071	0.261	0.376	0.196	0.053	0.013	0.002	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	0.0	0.0	0.0	0.0	0.0	0.003	0.004	0.018	0.072	0.247	0.367	0.208	0.060	0.015	0.003	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0	0.0	0.0	0.004	0.004	0.019	0.072	0.232	0.357	0.219	0.068	0.018	0.004	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.004	0.005	0.019	0.073	0.219	0.348	0.228	0.076	0.020	0.005	0.004	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.004	0.005	0.020	0.073	0.206	0.338	0.235	0.084	0.023	0.006	0.005	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.005	0.006	0.021	0.074	0.194	0.329	0.241	0.092	0.026	0.007	0.006	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.005	0.006	0.021	0.074	0.183	0.320	0.246	0.100	0.029	0.008	0.007	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.006	0.007	0.022	0.075	0.173	0.311	0.249	0.108	0.032	0.010	0.008	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.006	0.008	0.023	0.076	0.163	0.302	0.250	0.116	0.035	0.012	0.010	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.007	0.009	0.023	0.076	0.154	0.293	0.251	0.124	0.038	0.014	0.011	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.007	0.010	0.024	0.077	0.146	0.285	0.249	0.131	0.041	0.016	0.013	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.008	0.011	0.025	0.078	0.139	0.276	0.247	0.139	0.044	0.019	0.015	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.009	0.012	0.025	0.079	0.133	0.268	0.243	0.146	0.047	0.022	0.016	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.010	0.013	0.026	0.081	0.127	0.260	0.238	0.152	0.049	0.025	0.018	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.011	0.015	0.027	0.083	0.122	0.252	0.233	0.158	0.052	0.028	0.021	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.012	0.016	0.028	0.084	0.117	0.244	0.226	0.163	0.054	0.032	0.023	0.0	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.013	0.018	0.029	0.087	0.113	0.237	0.218	0.168	0.057	0.035	0.025	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.014	0.020	0.030	0.089	0.109	0.229	0.210	0.172	0.059	0.040	0.027	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.016	0.023	0.032	0.092	0.106	0.222	0.202	0.175	0.060	0.044	0.030	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.017	0.026	0.033	0.095	0.103	0.215	0.192	0.177	0.061	0.048	0.032	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.019	0.029	0.034	0.098	0.101	0.208	0.183	0.179	0.062	0.053	0.035
31	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.058	0.033	0.036	0.102	0.098	0.201	0.173	0.179	0.063	0.057
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.063	0.098	0.038	0.106	0.097	0.195	0.162	0.179	0.063
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.067	0.107	0.102	0.111	0.095	0.188	0.152	0.177
34	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.072	0.117	0.103	0.290	0.094	0.182	0.142
35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.077	0.127	0.104	0.292	0.224	0.176

Appendix 6 Number fish aged by fishery and year, median readability score, length frequency sample size, and effective sample size for catch-at-age proportions.

Table A6.1 Number of toothfish aged and used in the revised HIMI assessment by fishery and year. F1=Trawl survey; F2= Trawl, Ground B; F3= Trawl, Ground C, F5= Longline Ground C, F6 = long line Ground D, F10 = Pot.

CASAL Fishery	Year										
	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Sum
f1	0	2	20	1	1	13	0	0	120	548	705
f2	73	495	630	682	526	284	138	132	217	144	3321
f3	53	3	36	5	63	38	0	0	195	0	393
f5	0	0	0	0	0	80	0	0	96	204	380
f6	0	0	0	0	0	100	0	0	222	148	470
f10	0	0	0	0	0	0	0	0	176	0	176
Sum	126	500	686	688	590	515	138	132	1026	1044	5445

Table A6.2 Median readability score for toothfish aged and used in the revised HIMI assessment by fishery and year. CASAL fisheries as described in Table A6.1.

CASAL	Year									
Fishery	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
f1	-	-	2.5	2	4	2	-	-	4	3
f2	3	3	3	3	2	3	4	4	3	3
f3	3	3	3	3	3	3	-	-	3	-
f5	-	-	-	-	-	2	-	-	3	4
f6	-	-	-	-	-	3	-	-	3	4
f10	-	-	-	-	-	-	-	-	3	-

Table A6.3 Total length frequency samples for fishery and year combinations for which number aged > 50. CASAL fisheries as described in Table A6.1.

CASAL	Year										
Fishery	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Sum
f1	-	-	-	-	-	-	-	-	2081	2050	4131
f2	8307	13930	19095	22561	14036	17420	16706	11570	11539	12967	148131
f3	2100	-	-	-	4191	-	-	-	3230	-	9521
f5	-	-	-	-	-	1696	-	-	5487	3996	11179
f6	-	-	-	-	-	2498	-	-	3514	1556	7568
f10	-	-	-	-	-	-	-	-	5888	-	5888
Sum	**10407**	**13930**	**19095**	**22561**	**18227**	**21614**	**16706**	**11570**	**29658**	**18519**	**182287**

Table A6.4 Total effective sample size for catch-at-length proportions for commercial fishery and year combinations for which number aged > 50. CASAL fisheries as described in Table A6.1.

CASAL	Year										
Fishery	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Sum
f1	-	-	-	-	-	-	-	-	1559	801	2360
f2	3238	4286	4436	8119	5410	6852	3210	2693	7538	5127	50909
f3	1291	-	-	-	2848	-	-	-	1889	-	6028
f5	-	-	-	-	-	868	-	-	6577	5388	12833
f6	-	-	-	-	-	3178	-	-	4865	2349	10392
f10	-	-	-	-	-	-	-	-	4278	-	4278
Sum	**6527**	**6285**	**6436**	**10120**	**10260**	**12901**	**5214**	**4698**	**27153**	**14871**	**104465**

Table A6.5 Total effective sample size for catch-at-age proportions for commercial fishery and year combinations for which number aged > 50. CASAL fisheries as described in Table A6.1.

CASAL	Year										
Fishery	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Sum
f2	66	1417	2018	1572	913	569	623	317	1021	429	8945
f3	89	-	-	-	43	-	-	-	581	-	713
f5	-	-	-	-	-	90	-	-	108	267	465
f6	-	-	-	-	-	90	-	-	256	165	511
f10	-	-	-	-	-	-	-	-	415	-	415
Sum	**2153**	**3416**	**4018**	**3573**	**2958**	**2752**	**2627**	**2322**	**4387**	**2868**	**31074**

Table A6.6 Sample fraction (%) of length frequency samples that were aged for commercial fishery and year combinations for which number aged > 50. CASAL fisheries as described in Table A6.1.

CASAL	Year									
Fishery	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
f1	-	-	-	-	-	-	-	-	5.77	26.73
f2	0.79	10.17	10.56	6.96	6.5	3.26	3.72	2.73	8.84	3.3
f3	4.23	-	-	-	1.02	-	-	-	17.98	-
f5	-	-	-	-	-	5.3	-	-	1.96	6.68
f6	-	-	-	-	-	3.6	-	-	7.28	10.6
f10	-	-	-	-	-	-	-	-	7.04	

Appendix 7 Approximating the age distribution in the population

If the marginal distribution of length is given by, $f_L(l)$ where

$$f_L(l) = \int_{a=0}^{\infty} f_A(a)\, f_{A,L}(a|l)\, da$$

then

$$f_A(a) = \sum_{j=1}^{s} \left\{ \int_{l=K_{j-1}}^{K_j} f_L(l)\, f_{A,L}(a|l)\, dl \right\}. \qquad \text{(A1)}$$

Equation (A1) can be re-expressed as

$$f_A(a) = \sum_{j=1}^{s} \left\{ \int_{F_L(l)=F_L(K_{j-1})}^{F_L(K_j)} f_{A,L}(a|l)\, dF_L(l) \right\}$$

where $F_L(l)$ is the cumulative density function corresponding to probability density function $f_L(l)$. Using the mid-point rule to carry out the integration gives

$$f_A(a) = \lim_{\Delta l \to 0} \sum_{j=1}^{s} \sum_{h=1}^{H_j} \left\{ F_L\left(K_{j-1} + h\Delta l\right) - F_L\left(K_{j-1} + (h-1)\Delta l\right) \right\} f_{A,L}\left(a \middle| l = K_{j-1} + \left(h - \tfrac{1}{2}\right)\Delta l\right) \qquad \text{(A2)}$$

where $H_j = \left(K_j - K_{j-1}\right)/\Delta l$.

If the approximation

$$F_L\left(K_{j-1} + h\Delta l\right) - F_L\left(K_{j-1} + (h-1)\Delta l\right) \cong \left\{ F_L\left(K_j\right) - F_L\left(K_{j-1}\right) \right\} \Delta l, \qquad \text{(A3)}$$

($h = 1 \ldots H_j$), is substituted in (A2) then

$$f_A(a) \cong f_A^*(a) = \sum_{j=1}^{s} \left\{ F_L\left(K_j\right) - F_L\left(K_{j-1}\right) \right\} \left\{ \int_{l=K_{j-1}}^{K_j} f_{A,L}(a|l)\, dl \right\}$$

so it follows that

$$f_A^*(a) = \sum_{j=1}^{s} P_L\left(l \in B_j\right) \left\{ \int_{l=K_{j-1}}^{K_j} f_{A,L}(a|l)\, dl \right\}. \qquad \text{(A4)}$$

Note that fewer (i.e. wider) length bins are required to adequately approximate $F_L(l)$ using equation (A3) compared to that required to approximate with the same accuracy the term $\int_{l=K_{j-1}}^{K_j} f_{A,L}(a|l)\, dl$ for all *j*. In addition, no approximation of this last integral is required by equation (A4).

Appendix 8 Calculation of the coefficient of variation (CV) of abundance-at-age data

If the estimated (i.e. 'observation' in CASAL) proportion of the survey catch of age a for a particular year is given by, p_a, from the application of ALK see equation (1) of Candy (2009), and the corresponding estimate of the population size vulnerable to the survey is given by $\tilde{N}$ then the estimated abundance (i.e. 'observation' in CASAL) of age a fish is given by $N_a = \tilde{N} p_a$.

If the expected value of p_a and $\tilde{N}$ are given by μ_{p_a} and $\mu_{\tilde{N}}$, respectively and given N_a can be expressed (exactly) by a second-order Taylor series expansion about $\theta = \left(\mu_{p_a}, \mu_{\tilde{N}}\right)$ as

$$N_a = \mu_{p_a}\mu_{\tilde{N}} + \mu_{\tilde{N}}\left(p_a - \mu_{p_a}\right) + \mu_{p_a}\left(\tilde{N} - \mu_{\tilde{N}}\right) + \left(p_a - \mu_{p_a}\right)\left(\tilde{N} - \mu_{\tilde{N}}\right)$$

then

$$E\left(N_a\right) = \mu_{p_a}\mu_{\tilde{N}} \text{ and}$$

$$Var\left(N_a\right) \cong \mu_{\tilde{N}}^2 Var\left(p_a\right) + \mu_{p_a}^2 Var\left(\tilde{N}\right) + Var\left(p_a\right) Var\left(\tilde{N}\right)$$

assuming $Cov\left(p_a, \tilde{N}\right) \equiv 0$.

The variance of p_a is given by $Var\left(p_a\right) = \mu_a\left(1 - \mu_a\right)/n'$ where n' is the effective sample size obtained using the method described by Candy (2009) for accounting for between-haul heterogeneity in commercial catch-at-length proportions, ALK sampling error, and random ageing error. The estimate of p_a was obtained using the standard non-parametric ALK method [equation (1) of Candy (2009)].

The only difference in calculation of n' for the survey data, compared to that for the commercial catch-at-length proportions (Candy, 2009), is that the effective sample size for the proportion-at-length data was calculated separately for each stratum (i.e. accounting for between-shot within stratum heterogeneity) and then these values were accumulated across strata to give an overall ESS for survey catch-at-length proportions. In addition, the catch-at-length proportions were obtained as stratum-area weighted estimates. The variance of $\tilde{N}$ was obtained using the standard stratified random sampling estimate (Cochran, 1977). Finally, the estimates for μ_{p_a} and $\mu_{\tilde{N}}$ of p_a and $\tilde{N}$, respectively, were substituted into the above variance formulae and the resulting estimate of the variance of N_a was expressed as a coefficient of variation for input to CASAL.

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