APPENDIX H

REPORT OF THE WORKSHOP ON ESTIMATING AGE IN PATAGONIAN TOOTHFISH

(Center for Quantitative Fisheries Ecology, Old Dominion University, Norfolk, Va., USA, 23 to 27 July 2001)

CONTENTS

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INTRODUCTION

1.1 The Workshop on Estimating Age in Patagonian Toothfish was held at the Center for Quantitative Fisheries Ecology (CQFE), Old Dominion University, Norfolk, Va., USA, from 23 to 27 July 2001. The workshop was chaired by Dr I. Everson (UK) and attended by 17 participants. The list of participants is given in Attachment 1. Local arrangements for the meeting had been made by Dr J. Ashford (USA).

1.2 Dr Cynthia Jones (CQFE) welcomed participants to the workshop. She noted that, unlike in other branches of ecology, it was possible to determine the age of individual fish over time scales of days to years. This ability had influenced the development of fisheries models such that accurate and precise age determinations were now normal requirements for population assessments. The importance and value of Patagonian toothfish (*Dissostichus eleginoides)* internationally placed a high priority on achieving consensus on the best methods for age determination and she looked forward to a successful meeting.

1.3 The requirement for the meeting had been foreshadowed during the 2000 meeting of the Working Group on Fish Stock Assessment (WG-FSA-2000) when it was noted that some differences were present in the growth parameters being used for assessments of *D. eleginoides*. Dr Everson had been invited to contact all those undertaking age determination of *D. eleginoides* in order to determine whether these differences were real or else due to methods of otolith preparation and reading. Through SC CIRC 00/21 he had contacted colleagues interested in this research and they had agreed to participate in an otolith exchange project. They had also agreed to come together at a workshop whose primary aim would be to seek conformity in estimating the age of *D. eleginoides*, if such a meeting could be arranged. Although there is growing interest in age determination of other species, in particular *D. mawsoni*, it had been agreed by those who had responded to SC CIRC 00/21 that the primary aim should be to concentrate on the one species *D. eleginoides*.

1.4 In summary form, the main objectives for the workshop had been to consider and advise WG-FSA on:

(i) otolith collection protocols;
(ii) otolith preparation protocols;
(iii) agreed definitions of otolith structures used for age determination;
(iv) quality control and quality assurance; and
(v) validation.

1.5 In recent years there has been coordination of work on fish otoliths through the European Fish Ageing Network (EFAN) that had resulted in a series of reports. These reports were accessed through the EFAN website (www.efan.no) to provide background guidance in setting up the workshop and this was gratefully acknowledged.

1.6 Drs Ashford and Everson had developed an agenda and work plan that had been circulated just prior to the meeting and these were discussed by the workshop. In addition to topics directly concerned with the use of otoliths for age determination, it was agreed that it would be appropriate to include time for discussion on information from otoliths which might be used to improve knowledge of toothfish ecology. With this modification the agenda was adopted (Attachment 2).

1.7 The report of the meeting was prepared by all participants and collated by Dr Everson.

BACKGROUND INFORMATION ON *D. ELEGINOIDES*

2.1 High-quality age and growth information is critical to the accurate assessment of *D. eleginoides* stocks in the Southern Ocean. The techniques of estimating age and growth patterns for *D. eleginoides* from otoliths are influenced by a number of factors, including sampling regimes, preparation techniques, reader experience and analytical approaches. Thus, the age determination methodologies are heavily dependent on the specific laboratory and principal investigator. The goal of this workshop was to bring together scientists and provide a forum for exchange of ideas and insight into various techniques and methodologies related to age determination of *D. eleginoides* from otoliths. In addition, this workshop was structured to demonstrate age estimation methodologies for individuals who are considering using these techniques in different institutes, and to encourage collaborative efforts between interested scientists.

2.2 Currently used assessment techniques of *D. eleginoides* stocks rely heavily on age and growth information. For example, in recent assessments length-frequency data from trawl surveys have been analysed using mixture analysis in order to generate estimates of recruitment to the population of *D. eleginoides*. Length-at-age relationships are used as a guide for setting the initial conditions necessary to identify the number of cohorts present, as well as their mean lengths. During the 2000 meeting of WG-FSA, von Bertalanffy growth parameters (L_8, k and t_0) for the mixture analysis and general yield model (GYM) were based on several sources of age and growth information from several laboratories. For example, growth parameters for stocks around South Georgia were based on values estimated by combining the lengths at age from two sources: otoliths collected in the UK survey around South Georgia in January and February 1991; and an age–length key from readings of scales taken from the commercial longline fishery during February to May 1991. Other estimates of growth parameters were available, though the values were sometimes quite different depending on the study. WG-FSA was very concerned by the variability and uncertainties within and between these sets of growth parameters, and stressed that work to refine and validate age determination methods was a high priority. Further, WG-FSA had encouraged introduction, progress, and testing of alternative age-structured models for future assessments of *D. eleginoides*. Thus it is critical that age estimation techniques be refined to improve the quality of these assessments.

2.3 It was noted that the preparation and reading of otoliths was only one part of a continuum in the process of providing information on the age of individuals for stock assessments. In the first instance it was essential to decide on the purpose for which age determination was required. This should be used to indicate the number of otoliths which would need to be read, as well as optimal sampling protocols. Information from previous work would provide an indication of the likely precision associated with a given sample size. This and other information should be used in a 'feedback' system in order to determine the most cost-effective sampling and analysis program consistent with the aims of the study.

RESULTS OF THE OTOLITH EXCHANGE PROJECT

3.1 The three main laboratories engaged in age determination *D. eleginoides* had participated in the study. These were: National Institute of Water and Atmospheric Research, Nelson, New Zealand (NIWA), local coordinator Mr P. Horn; Central Ageing Facility, Victoria, Australia (CAF), local coordinator Dr K. Krusic-Golub; and CQFE, local coordinator Dr Ashford.

3.2 It is the practice at each of these centres for otolith readers to be given no more information than the date and location of capture of individual fish. No information is given to the reader of the size of the fish.

3.3 Otoliths for the study had been sent initially to Dr Everson and he, in conjunction with Dr M. Belchier (UK) had arranged for the circulation of samples and collation of results. Samples had been received and read twice, independently and separated in time by one week, by Mr Horn, Drs Krusic-Golub, Ashford, S. Wischniowski (CQFE) and E. Larson (CQFE). The CAF and NIWA otolith preparations were brought to the workshop.

3.4 Results from the independent age estimation were discussed. Whilst there was reasonably good agreement between readings on some otolith preparations, for others there was a significant disparity. The workshop noted that it is important to recall the differences in otolith preparation methods, discussed latter, that are in use at the different institutes. The otolith preparations were being read and interpreted by researchers closely familiar with their own laboratory's methods, but largely unfamiliar with those used elsewhere.

3.5 The results from the otolith exchange were used for two main purposes: firstly to indicate the level of precision present in independent estimates of age and secondly to highlight individual specimens that might be used to indicate both good and unclear examples of annuli. These topics were considered more fully under latter agenda items.

READING AGES FROM SAMPLES

4.1 The workshop received descriptions of the techniques in use at CQFE (from Dr Ashford), NIWA (from Mr Horn) and CAF (from Dr Krusic-Golub). In all of these it was emphasised that reading otoliths utilises skills in pattern recognition that had been developed over a significant period of time. Whilst for some fish, such as black drum (*Pogonias cromis*), examples of which were shown to participants, the pattern of otolith growth follows a regular pattern which can be understood with reasonable ease, this is not the case with *D. eleginoides* otoliths. Growth in whole *D. eleginoides* otoliths follows complex patterns that include many crenulations and spikes as shown in Figure 1. Arising from this, it is extremely difficult to prepare a section in one plane that displayed all annuli, in a clear manner but without artefacts. This means that the reader must have in mind the three-dimensional structure of the otolith in order to take account of annuli and be able to distinguish these from false checks.

4.2 Mr Horn described the otolith reading technique in use at NIWA for *D. eleginoides* otoliths collected from the southern New Zealand Exclusive Economic Zone and Subarea 88.1. He noted that some aspects of the interpretation may not apply to otoliths collected from other areas.

4.3 An example otolith preparation is shown in Figure 2. The number of complete translucent zones is counted. Zone counts are generally made on the ventral part of the section, either on the proximal surface adjacent to the sulcus or else along the dorso-ventral axis. However, all areas of the section are examined to find the area where the zonation pattern is clearest. Sometimes the count is started near to the sulcus, but finished in some other area of the proximal surface; counts in the two areas are linked by tracing a clear and continuous zone across the section.

4.4 The clarity of the zonation pattern varied considerably between otoliths. Examination of a number of otoliths in which the zonation was relatively clear indicated that many had an exceptionally dark fourth zone. Sometimes this darker zone occurred at the third or fifth zone. Measurements from the primordium to the longest axis of the first and third zones (on the ventral part of the section) were approximately 1.2 and 1.9 mm respectively. Interpretation of the first three to five growth zones was often complicated because of an abundance of what were considered to be false rings. However, the dark zone was also generally apparent in these otoliths and this band could be used as a boundary inside which the false rings could usually be subjectively, but logically, grouped into three (but sometimes two or four) multi-banded zones. The approximate measurements made on clear otoliths to the first and third zones were also used to help to indicate the likely position of these zones in otoliths with apparent multi-banding. Zones outside the dark growth zone were generally narrow and regular in width, but sometimes a region of transition was apparent outside the darkest zone where consecutive annuli became increasingly narrow before becoming regular in width. Also split zones were sometimes apparent in the area outside the dark zone. A zone was considered to be split if two opaque bands merged to form a single clear zone in any part of the section between the sulcus and ventral margin on the proximal side of the otolith.

4.5 Dr Krusic-Golub described the otolith reading technique in use at CAF for *D. eleginoides* otoliths. An example otolith preparation is shown in Figure 3. All sections of each row of otoliths are inspected and the section showing the clearest annuli is used for age estimation. This is generally, but not always, the section closest to the primordium. Estimation of age is made using the area of the otolith section in which annuli can be counted most clearly and consistently. Generally the sector from the primordium to the proximal edge of the section, on the ventral side of the sulcus is used. However for some preparations, increments formed on the dorsal side are at least as clear as those on the ventral side.

4.6 Under transmitted light, otolith sections are predominantly opaque especially near the nucleus. The first two to seven increments are generally broader and more opaque than the later increments. A transition period has been observed between the ages of 3 to 9. This transition period is recognised as a point of sudden change in increment width, however in some sections the transition from wide to narrow is gradual rather than sudden. Interpretation of the first three to five annuli is often difficult due to the presence of fine checks that are considered to be subannual. Generally these checks are irregularly spaced and are not continuous throughout the section. Zones after this period become far more regular in width and appearance and the annuli are easier to interpret.

4.7 Dr Ashford described the otolith reading technique in use at CQFE for *D. eleginoides*. An example otolith preparation is shown in Figure 4. The count path followed the large annuli along the dorsal axis, moving to the regular annuli along the proximal dorsal axis as the dorsal axis became compressed. Structures occurred at different scales in all regions: in the regular region, the narrowest annuli were considered annual as long as they persisted clearly either side of the count path. Marks or structures that did not persist far to either side of the count path or occurred irregularly at a lower scale were considered false checks. In the region of large annuli, it was more difficult to distinguish between annuli and checks: annuli are considered to be larger, have stronger contrast between opaque and translucent zones, and to persist either side of the count path notably into the compressed medial region. Checks tend to be confined to one region, particularly the proximo-ventral, or vary considerably in clarity between regions. Evidence of splitting was particularly clear in the distal dorsal area, a single translucent zone running along the distal side in contrast to a translucent zone with associated check running along the proximal side. In the nucleus, a discontinuity was observed running diagonally between the core and the dorsal protrusion. The edge of the nucleus was defined as the inner border of the first translucent zone, which was typically clearer than the succeeding translucent zones. As the hatch date of *D. eleginoides* is not known, the nucleus may not represent a full year's growth, so the outer edge of the nucleus was considered as time 0. The birthday of all fish was taken to be 1 July, so that the annulus was counted if the fish was taken after 1 July but not if taken before.

4.8 The workshop thanked Mr Horn, Dr Krusic-Golub and Dr Ashford for their presentations.

4.9 It was noted that otolith preparations had been examined under reflected light (CQFE and NIWA) and transmitted light (CAF). This difference was due to the current practice in the respective laboratories. The workshop agreed that such a difference would be very unlikely to introduce bias into the results. Since the appearance of the translucent and opaque zones of the otolith to the reader is strongly dependent on the form of illumination and to avoid confusion in the interpretation of results, the workshop agreed the definitions of the zones as set out in Table 1.

4.10 The occurrence of split zones or checks had been noted by the three primary readers. This characteristic is illustrated in Figure 5. Otoliths believed to contain split zones were examined and each reader described which zones they considered to be split and why. There was a general agreement on what constitutes a split zone. Any pattern of split zones was generally consistent between the dorsal and ventral sides of the section. It was concluded that the three readers interpreted split zones similarly.

4.11 It was acknowledged that, on occasions, it will be difficult to determine whether an area of predominantly translucent material constitutes a single split annulus or two distinct annuli. In such a situation it was resolved that if the problem area occurred in the first eight years of life, then it should be considered a split annulus, and if it occurred after eight years, then two annuli should be assumed. This criterion is based on two themes, i.e. the relatively high abundance of split zones in the early years of growth, and a desire to age conservatively (from a resource management point of view).

4.12 Arising from the presentations and the subsequent discussion it was apparent that there were minor differences in the definitions being used for nucleus and annuli. Arising from plenary discussion a series of definitions were agreed; these are listed below and shown diagrammatically in Figures 6 and 7 and on actual preparations in Figures 2 to 5.

Primordium: The point from which all growth in the otolith originates.

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

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.

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 the 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.

4.13 The Workshop agreed that 1 July was the most appropriate birthday to be used for *D. eleginoides*. This date was chosen because it:

- conforms with the best knowledge of the timing of spawning (Kock and Kellermann, 1991); and
- is also congruent with the best available knowledge of the time of formation of the translucent zone (Horn, 1999, 2001).

4.14 An illustration of the model of otolith growth adopted by the workshop is presented in Figure 7a. Because formation of the translucent zone coincides with spawning, the use of a 1 July birth date allows for correct year-class assignment (e.g. fish spawned in 1998 are always assigned to the 1998–1999 year class).

4.15 As a comparison to the otolith growth model adopted for *D. eleginoides*, a model is illustrated for a hypothetical fish which spawns or hatches in September and with annulus formation in May. This is shown in Figure 7b. In this example, the use of a 1 January birth date allows for correct year-class assignment (e.g. fish spawned in 1998 are always assigned to the 1998 year class). However, the use of a 1 September birth date, while being the correct biological birth date, causes the incorrect year-class assignment (i.e. fish harvested from January through August and belonging to the 1998 year class are mistakenly assigned to the 1999 year class).

OTOLITH SAMPLE PREPARATION

5.1 Mr Horn described the technique in use at NIWA to prepare otoliths for reading. The sequence of activities in use is as follows:

- clean dry otoliths are marked transversely through the primordia with a pencil;
- the otoliths are baked whole in an oven at 275°C for about 12 minutes, until amber in colour;
- the otoliths are embedded in rows in epoxy resin, and sectioned along the pencil lines (NB: all preparation and use of epoxy resin must be conducted in a fume cupboard by a technician using protective gloves);
- the sectioned surfaces are coated with paraffin oil prior to examination; and
- sections are viewed using reflected light under a binocular microscope at a magnification of x40.

5.2 Dr Krusic-Golub described the technique in use at CAF to prepare otoliths for reading. The sequence of activities is as follows:

- clean dry sagittal otoliths are embedded in rows of five in blocks of clear casting polyester resin ensuring that the primordium of each of the otoliths is in line. (NB: a well ventilated room and the use of organic gas masks is recommended);
- a minimum of four transverse sections (approximately 300–400 μm thick) are cut from the centres of the otoliths using a modified Gemmasta™ lapidary saw fitted with a 0.25 mm wide diamond impregnated blade;
- the sections are cleaned in water, rinsed with alcohol and dried;
- sections are mounted on microscope slides under glass cover slips with further polyester resin; and
- sections are viewed under transmitted light at x25 and x40 magnification.

Generally the otoliths are not baked in this process although this can be undertaken if so desired.

5.3 Dr Ashford described the technique in use at CQFE to prepare otoliths for reading. The sequence of activities is as follows:

- one of each pair of otoliths is selected randomly and baked at 400°C for approximately three minutes;

- otoliths are ground by holding the anterior side against the grinding wheel of a Hillquist Thin Section Machine until an internal mark is revealed which has been found to lie consistently just anterior to the nucleus;

- the ground face is then mounted on a glass slide using Krazy-Glu, left to dry, and ground from the posterior side to form a thick transverse section incorporating the nucleus and avoiding crenellations;

- the section is finally polished using Mark V Laboratory 3M aluminium oxide polishing paper, covered with Flo-Texx; and

- sections are viewed using reflected light under a binocular microscope at a magnification of x25.

5.4 The workshop concluded that the methods for preparation and reading of otoliths in use at CAF, CQFE and NIWA gave essentially similar estimates of age. Accordingly the workshop recommended to WG-FSA that these methods are the best currently available for the estimation of age in *D. eleginoides*.

5.5 It was noted that although these protocols provide satisfactory estimates of age, they are not necessarily the only ones that might be appropriate. Whilst favouring the current protocols the workshop accepted that new or revised protocols might be equally effective.

SAMPLING AND EXPERIMENTAL DESIGN

Assessment of Precision

6.1 Preliminary analyses of the data obtained from the otolith exchange project were undertaken using the 'Age-comparisons' spreadsheet (Eltink, in Eltink et al., 2000) available from the EFAN website. Only data from those experienced readers who routinely analyse *D. eleginoides* otoliths were included in the analysis. A total of 149 otoliths were analysed. Some technical difficulties were encountered whilst using the 'Age-comparisons' spreadsheet as it had been designed for age reading comparisons for fish less than 15 years old. Since it was not possible to rectify the problem during the course of the workshop, a small proportion of the results, 15%, were not included in the analysis. In spite of this difficulty, the spreadsheet enabled a quick and easy analysis of the precision of otolith age estimates of *D. eleginoides*.

6.2 Overall there was reasonable agreement in age estimations between all three readers. The close agreement in overall CV (Table 2) obtained from the three sets of otoliths strongly suggests that the method of sample preparation does not affect the precision of estimated age. There is little evidence that the variability of age estimates increases when readers are presented with material prepared by methods with which they are unfamiliar. Although there is no evidence that preparation method has an effect on precision, an analysis of bias plots (Figure 8) for each reader shows that the age estimates made by one reader (reader 3) were consistently lower than those of the other two readers. This trend is in broad agreement with the results of a previous otolith exchange exercise undertaken between readers 2 and 3. It is suggested that differences in the interpretation of the first few annuli are probably the main cause of these differences.

6.3 The results obtained from the preliminary otolith exchange project have highlighted the value of continued otolith exchanges between those laboratories which routinely use otoliths to estimate the age of *D. eleginoides*. The workshop recommended that exchanges should occur annually and should include any new laboratories that wish to start reading *D. eleginoides* otoliths.

6.4 The workshop suggested the following scheme for a future routine otolith exchange program:

- Each participating laboratory should select pairs of otoliths from 40 fish (80 otoliths total).
- One otolith of each pair should be prepared and read using the routine methods of the 'originating' laboratory.
- In order to assess the effects of differences in preparation methods between laboratories the remaining otoliths of each pair should be divided between the other two laboratories, designated 'receiving laboratories', (20 otoliths each) for preparation and reading.
- The preparations to be archived and the results collated into a single annual report by the receiving laboratory.
- Organisation for such an exchange and the eventual central archiving of samples could be undertaken through the CCAMLR Otolith Network.
- The same archived otolith preparations should be made available for all new laboratories wishing to read *D. eleginoides* otoliths thus providing a source of reference material for all methods of otolith preparation.

Reference Otolith Sets and Validation Tests

6.5 The three main laboratories estimating age in *D. eleginoides* already use reference otolith sets in internal protocols as standards to prevent drift in the reader's estimates of age over time. Dr Ashford indicated that CQFE had data documenting this type of error in age estimation of *D. eleginoides* during training of a reader.

6.6 The workshop participants considered that the use of sample collections with standard ages were essential in preventing drift, and should be recommended. It was suggested that the proposed central archive of otoliths from the CCAMLR Otolith Network (see paragraph 6.4) might be used as a CCAMLR-wide standard set which could be passed around laboratories. The quality control (QC) methodology could then be used to see if significant biases occurred between estimated and standard ages.

6.7 Although standardised reference sets would allow the quality of age data to be monitored and corrections made when bias is found, the relationship between true and estimated age would be left unknown. The workshop considered that validation tests of the standard ageing methodology were of the highest priority.

6.8 Marginal increment analysis would allow the timing of zone formation in the otoliths to be ascertained. Although important, this would not however allow direct estimations of accuracy. These would be best achieved by tag–recapture studies with otoliths chemically marked by bomb-carbon analysis or by rearing experiments. These would allow a quantitative treatment comparing true ages and ages estimated by reading otoliths in an ANOVA design. However, the null hypothesis would be that there was no significant difference, and to test if this was true, a high statistical power would be needed. As a result, the group agreed that it was necessary to estimate the sample size needed for the correct level of power, using estimates of precision in repeat readings. It was observed that enough data now existed on precision for this to be possible.

6.9 Dr Krusic-Golub reported on a collaborative study with Mr R. Williams (Australian Antarctic Division). Sagittal otoliths collected from tagged and recaptured *D. eleginoides* were examined to determine if Strontium Chloride marks can be detected and secondly, the relationship between annulus formation and the period at liberty.

6.10 A strong mark had been detected on 66 of the 68 otoliths examined. This high rate of detection indicates that the technique is an effective method for marking *D. eleginoides* otoliths and provides a tool by which validation may be undertaken. For each year at liberty positive growth occurred and a single annulus was formed. Results from this preliminary study support the view that each annulus in the otolith, as defined by the current criteria, represents a year's growth.

Quality Control and Quality Assurance

6.11 Dr Ashford made a presentation showing that for *D. eleginoides* repeat readings within and between readers can be treated in a statistically rigorous manner. Thus, variance of repeat age estimates from the 1:1 relationship does not increase with age after the first three or four years. As a result, the residuals are normally distributed, usually show reasonably homogeneous variances between readings, and show no trends, fulfilling the assumptions of ANOVA. Using a design blocked on individual fish (Ashford, 2001), bias between readings and between readers can therefore be estimated using the difference between the estimated general mean and estimated treatment mean ($y_{..}$-$y_{i.}$), and reader variability can be estimated by the variance of the residuals. This allows data to be corrected for bias, and monitored for levels of variability to assure quality control. Corrections can then be made for any biases from true age in estimated ages that subsequently become evident through validation studies.

6.12 He also pointed out that estimates of reader variability using CV usually did not correct for bias beforehand, and were inflated as a result when bias was present. The distribution of residuals also meant that CV decreased with age, confounding comparisons between samples with different ages.

6.13 The workshop agreed that the method of estimating precision and variability in readings allowed a more sophisticated treatment of age data, and provided a rigorous framework for quality control of data.

6.14 In further discussion, the representatives of the three main laboratories estimating age in *D. eleginoides* agreed that they should exchange otoliths on a regular basis, and use the QC methodology to ensure that their readings were in agreement. Each laboratory would provide a sample of otoliths, processing and reading one randomly chosen otolith from each pair. Half of the remaining otoliths would be sent to each of the other laboratories to process and read.

Sampling for Age Data

6.15 Dr Ashford presented some results from a field trial of a sampling methodology designed with members of WG-FSA (Ashford et al., 1998; Ashford, 2001). The methodology used a multi-stage sampling design: essentially, a line is divided into 10 sections and two of these sections are randomly chosen. All fish caught on these sections are then sampled. The method allowed different observer tasks required to be integrated into a single random sampling design. Information from the trial indicated that most variability occurred within each section, but significant variability occurred at broader scales which needed to be accounted for. The trial also indicated that observers could sample fewer lines, thereby improving efficiency and freeing up time that could be allocated to other tasks.

6.16 The workshop agreed that this appeared a reasonable solution to the problem of obtaining representative samples from the *D. eleginoides* catch, and a subgroup was formed to consider the methodology further. The subgroup consisted of four participants who had acted as observers, with experience of a wide variety of longliner designs (Mr J. Selling, Germany), Mr P. Brickle (UK), Dr Belchier and Dr Ashford), and several others with experience in designing protocols for obtaining age data through observer programs, or for fisheries surveys (Dr C. Jones (USA), Mr Horn, Dr A. Arkhipkin (UK)).

6.17 Dr Jones pointed out that the CCAMLR *Scientific Observers Manual* did not include sampling for age data among the observer's highest priority tasks, even though this had been recommended by WG-FSA. He further observed that although the use of a randomised sampling design was recommended in the manual, none was provided to observers. The subgroup agreed that it was important that both these omissions should be corrected.

6.18 Dr Jones suggested that an important facet of sampling was the purpose for which the sampling was undertaken. The questions to be addressed should be defined beforehand. The subgroup then considered the methodology of Ashford et al. (1998). The members with observer experience agreed that the design was realistic, and easy to implement. The workshop agreed that, for obtaining population length data, this represented a considerable improvement over present ad hoc methods and should be incorporated in the *Scientific Observers Manual*.

6.19 For sampling for an age–length key, as it would be impossible to sample all fish within each line section, the workshop agreed subsamples should be taken instead. Various ways of achieving this were considered; eventually it was agreed that the first five fish of every selected line section should be subsampled for age. While recognising other methods would provide more statistical rigour, it was felt that this provided a practical solution in the interim until a methodology combining practicality and rigour could be developed. Meanwhile, sampling the beginning of the line section was a great improvement on the ad hoc method presently used.

6.20 For obtaining age data for von Bertalanffy growth function estimates, the design should be stratified by 5 cm total length increments: thus, observers should use the methodology of Ashford et al. (1998), sampling for each 5 cm stratum until that cell is filled. This was considered a practical solution, although the workshop recognised that, because of the numbers-at-length, cells for increments between 80–100 cm would be filled quickly, while those for large and small fish would be filled more slowly. Thus the sampling frames for different cells would be somewhat different.

6.21 The workshop also discussed the numbers of samples requested by CCAMLR from each observer. It was felt that enough information was now available on precision levels in age estimations to calculate the sample numbers necessary for each purpose defined. The group asked Dr Ashford to undertake these calculations and present a report at the next meeting of WG-FSA.

OTOLITH STUDIES LINKED TO OTHER ASPECTS OF SOUTHERN OCEAN ECOLOGY

7.1 During discussions on future work, the workshop was given three small presentations on oceanography, some aspects of which may prove useful in the elucidation of *D. eleginoides* distribution and migration.

7.2 Dr Cynthia Jones (CQFE) told the workshop of her work on the trace elemental components that are incorporated into fish otoliths from the water column. CQFE uses a technique called laser ablation Inductively Coupled Plasma Mass Spectrometry (ICPMS) to measure the concentrations of trace elements from a small sample taken from the otolith. Trace element accumulation into fish otoliths varies among samples collected at different areas and reflects the characteristics of different waters. The concentrations of trace elements such as strontium and dO^{18} and dO^{16} isotope ratios have a relationship with salinity and temperature respectively. This technique is useful for looking at the spatial distribution of fish. It may also have implications in the study of fish movements and migration by investigating the trace elements in samples taken from earlier growth rings and the outer growth rings of otoliths.

7.3 Dr E. Hofmann (USA) talked to the workshop on Southern Ocean oceanography and how the structure of the environment affects ecosystems. She gave examples of where large and small-scale variability in the environment results in changes of the nature of biological interactions. She presented examples of new conceptual models that affect the nature of ecosystems. These included the Circumpolar Wave, a meteorological phenomenon, that has an effect on the extent of the sea-ice with a 4~5 year periodicity. Other examples included the interannual variations in the extent of the sea-ice, the distribution of upper Circumpolar Deep Water and the southern boundary of the Antarctic Circumpolar Current. The latter seems to have most effect on ecosystems in its boundary currents and a number of species are affected by this, examples given included krill and *Pleuragramma* spp. Dr Hofmann also presented a model for the oceanography of Drake Passage Scotia Sea area.

7.4 Dr Arkhipkin spoke of a project proposal to study the demography and migrations of the *D. eleginoides* in the Southwest Atlantic. He presented fishery data on *D. eleginoides* around the Falkland/Malvinas Islands. Dr Arkhipkin also described the distribution of juvenile *D. eleginoides* in the trawl fishery on the shelf and in the longline fishery in waters greater than 600 m. He described three areas, one in the north (50°S), one in the southeast (54°S) and one of lesser importance to the east where the fishery is concentrated. It is unclear as to whether these concentrations represent a single stock or several stocks originating from different regions in the Southwest Atlantic. Dr Arkhipkin presented a scheme of currents around the Falkland/Malvinas Islands and a hypothesis of the ontogenetic migrations of the *D. eleginoides* from the slope waters to the three major areas off the shelf in deeper waters associated with these currents. The aims of the project will include genetic screening of mitochondrial and microsatellite DNA, ICPMS for trace elemental analysis and parasitological studies to identify stocks and trace migrations of *D. eleginoides*.

FUTURE WORK ON *D. ELEGINOIDES* OTOLITHS AND ADVICE TO WG-FSA

Advice to WG-FSA

8.1 (i) the workshop agreed that although age determination of *D. eleginoides* was difficult, it could be achieved using otolith sections (paragraph 4.1);

(ii) key features to be taken into account in reading otoliths are set out in paragraphs 4.9 to 4.15;

(iii) three otolith preparation protocols were discussed, all of which were considered suitable for age determination of *D. eleginoides* (paragraphs 5.1 to 5.5);

(iv) the workshop recommended that a routine program to exchange otoliths for age determination between laboratories be established (paragraphs 6.4. and 6.14.);

(v) the workshop recommended that all protocols for age determination be subject to quality assurance and quality control as described in paragraphs 6.4, 6.5 to 6.8 and 6.14;

(vi) the workshop recommended that reference sets of otoliths be prepared in order to monitor the precision of experienced and new readers (paragraph 6.6); and

(vii) the workshop recommended that the CCAMLR *Scientific Observers Manual* should be revised to incorporate the randomised sampling methodology of Ashford et al. (1998), and reflect the priorities laid down by WG-FSA (paragraphs 6.17 to 6.21).

Future Work

8.2 The workshop agreed that further research is needed on the following topics:

(i) determine more precisely the time interval between the formation of the primordium and the formation of the distal edge of the first translucent zone or the edge of the nucleus (paragraph 4.13);

(ii) validation of the timing of annulus deposition through Marginal Increment Analysis (MIA) (paragraph 4.13);

(iii) develop other validation methods specifically to estimate accuracy (paragraph 6.7); and

(iv) follow modal progression of length density of pre-recruits from a single area with otolith ground-truthing, with the aim of better defining their growth (paragraph 6.7).

Coordination of Otolith Research

8.3 The workshop had provided a valuable opportunity for participants to discuss their work and to develop new ideas and collaborations. It was agreed that there would be considerable merit if this activity could continue and agreed to form its own CCAMLR Otolith Network (CON) to which all participants along with anyone interested in studies on otoliths of Southern Ocean fish could join. Initially CON would meet by correspondence through email although meetings might be arranged in the margins of symposia or CCAMLR meetings.

CLOSE OF THE MEETING

9.1 The Convener noted that the workshop could not have taken place without a great deal of hard work by many individuals. He thanked Drs Ashford and Krusic-Golub and Mr Horn for providing samples and leading the way with the otolith exchange exercise. He thanked all the participants at the workshop for their hard work throughout the meeting. Support for the workshop had come from CQFE and also from the US AMLR Program and this was gratefully acknowledged. Finally he thanked all the CQFE team for keeping the meeting running smoothly and efficiently. The CQFE team in turn thanked the Convener for his considerable efforts in initiating and chairing the workshop.

9.2 The Convener wished all participants a safe journey home and closed the workshop.

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Eltink, A.T.G.W., A.W. Newton, C. Morgado, M.T.G. Santamaria and J. Modin. 2000. Guidelines and tools for age reading comparisons. Version 1. (First sheet of age comparison.xls.) *EFAN Report* 3-2000: 75 pp.

Horn, P.L. 1999. Age and growth of Patagonian toothfish (*Dissostichus eleginoides*) and Antarctic toothfish (*D. mawsoni*) in waters from the New Zealand Exclusive Economic Zone to CCAMLR Subarea 88.1. Document *WG-FSA-99/43*. CCAMLR, Hobart, Australia.

Horn, P.L. 2001. Age and growth of Patagonian toothfish (*Dissostichus eleginoides*) and Antarctic toothfish (*D. mawsoni*) in waters from the New Zealand sub-Antarctic to the Ross Sea, Antarctica. *Fisheries Research:* in press.

Kock, K.-H. and A. Kellermann. 1991. Reproduction in Antarctic notothenioid fish: a review. *Ant. Sci.,* 3 (2): 125–150.

Table. 1: Descriptions of translucent and opaque zones as seen in otoliths when viewed under reflected and transmitted light

Definition	Light Source	
	Reflected Light	Transmitted Light
Translucent Zone – Zone that allows greater passage of light than opaque zone. Has been referred to by some authors as the hyaline zone.	Appears as darker bands on otolith surface when light is reflected.	Appears as lighter bands when light is transmitted.
Opaque Zones – Zone where passage of light is restricted.	Appears as lighter bands on otolith surface when light is reflected.	Appears as darker bands when light is transmitted.

Table 2: Coefficient of variation (CV) in total age estimations from otoliths prepared at different institutions.

Otolith Preparation (institution)	CV all Readers (%)
CQFE	14
MAFRI	19
NIWA	16

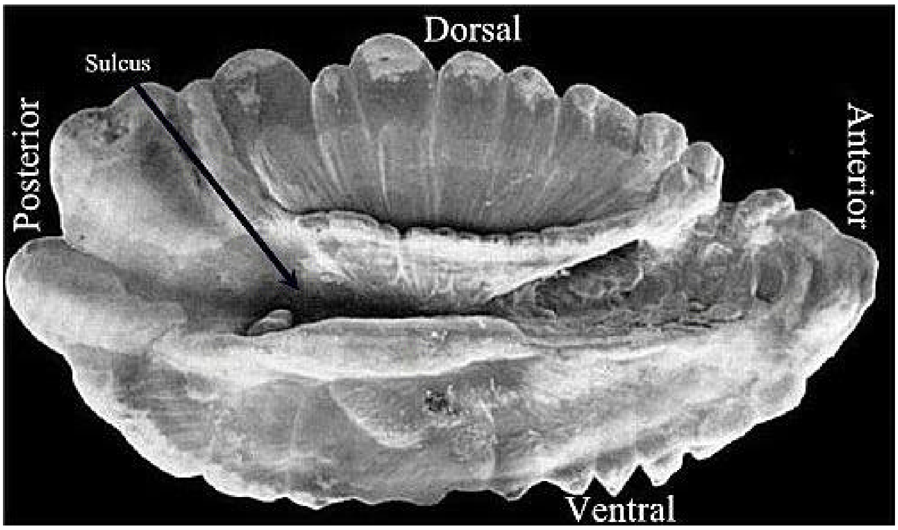


Figure 1: Proximal surface view of whole *Dissostichus eleginoides* otolith. Otolith SEM image © Australian Antarctic Division.

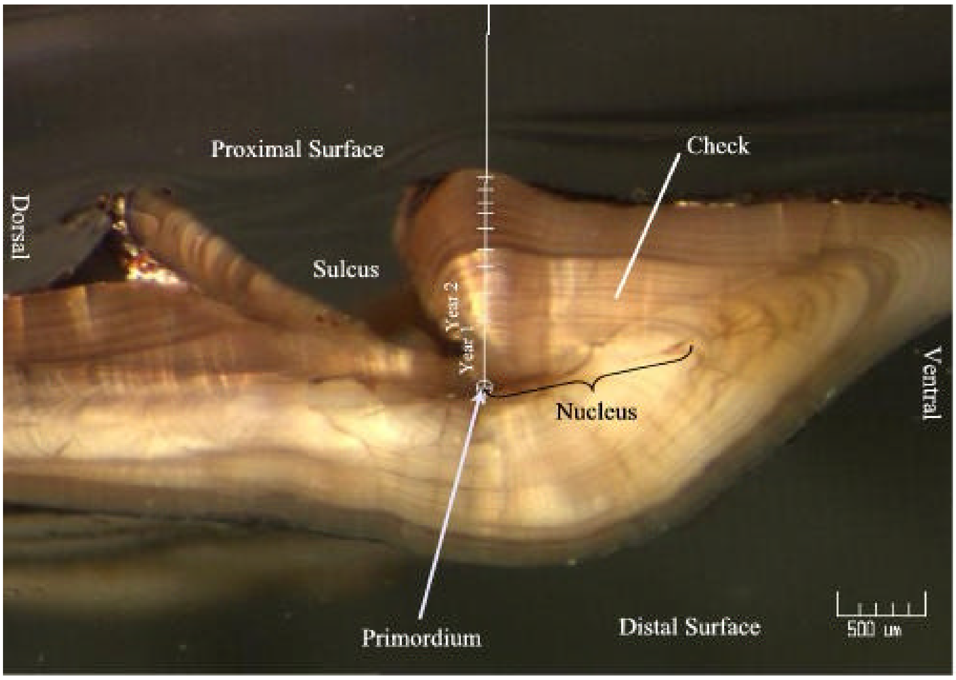


Figure 2: Features associated with sectioned *Dissostichus eleginoides* otolith prepared according to NIWA methodology and viewed under reflected light.

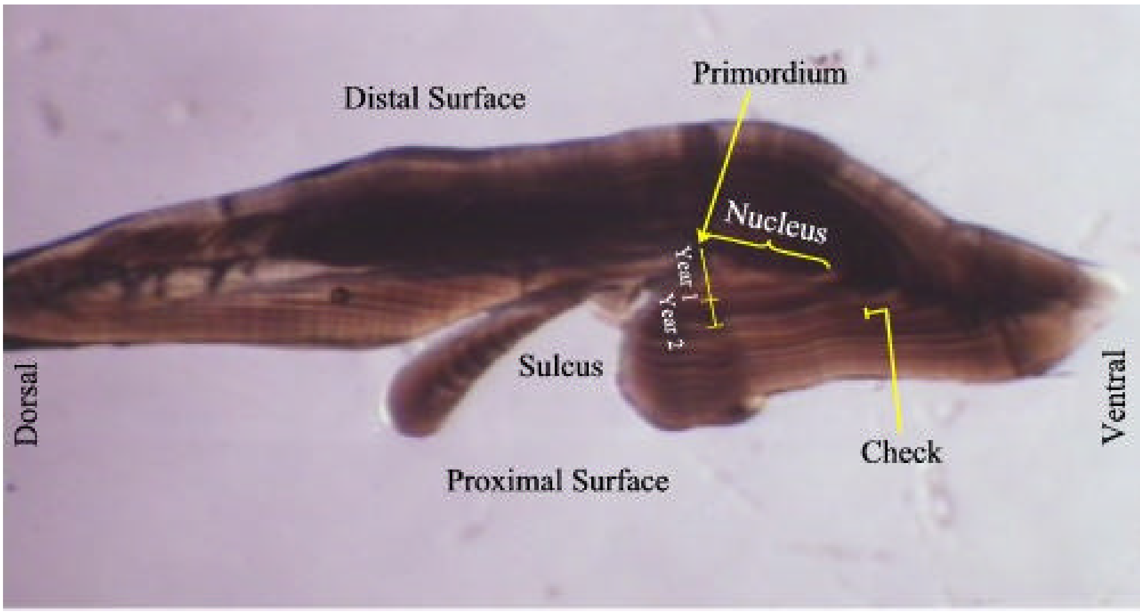


Figure 3: Features associated with sectioned *Dissostichus eleginoides* otolith prepared according to CAF methodology and viewed under transmitted light.

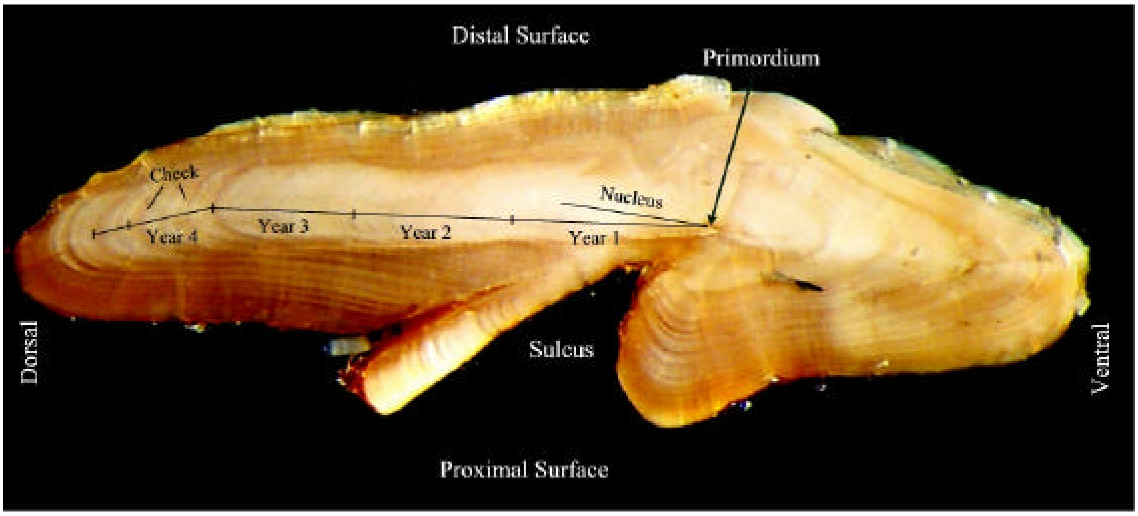


Figure 4: Features associated with sectioned *Dissostichus eleginoides* otolith prepared according to CQFE methodology and viewed under reflected light.

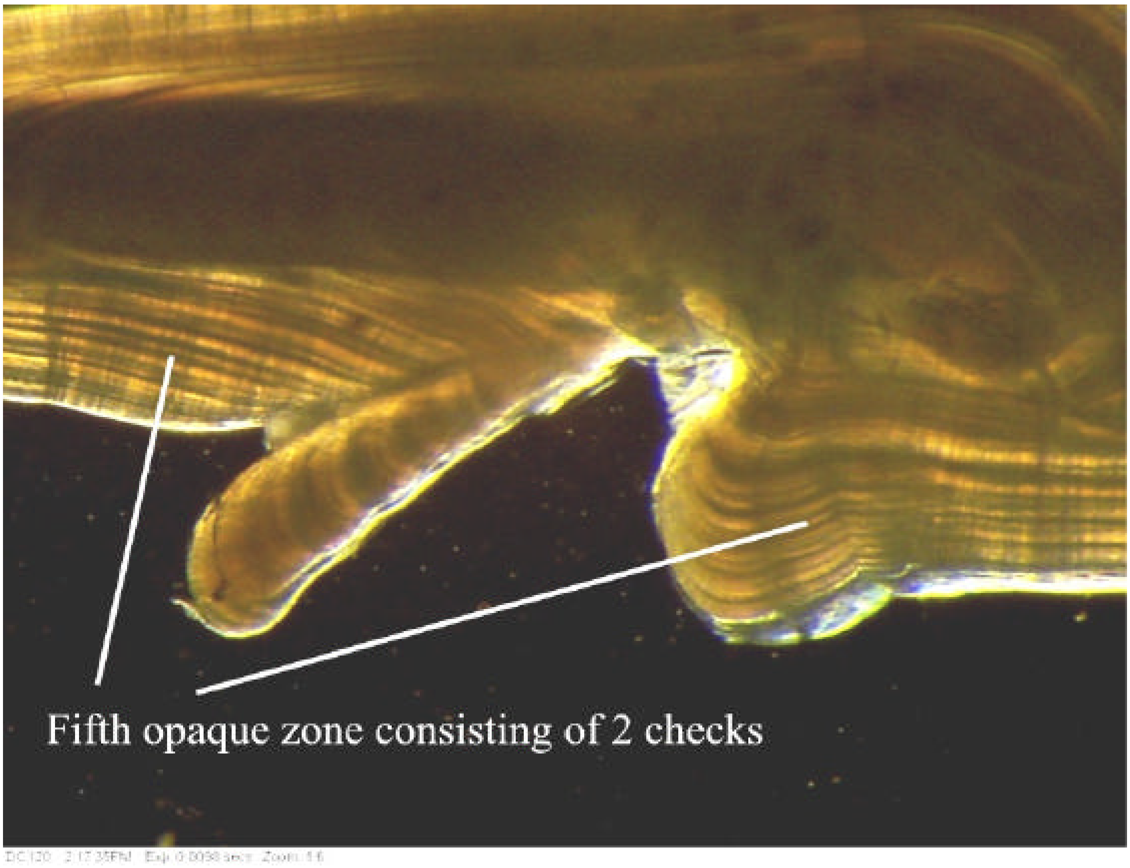


Figure 5: Checks associated with sectioned *Dissostichus eleginoides* otolith prepared according to CAF methodology and viewed under transmitted light.

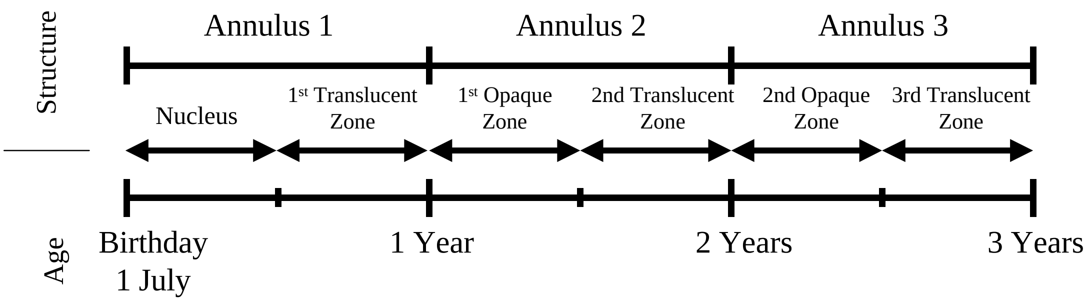
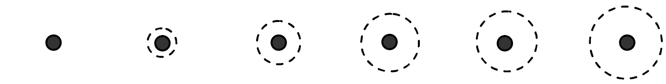
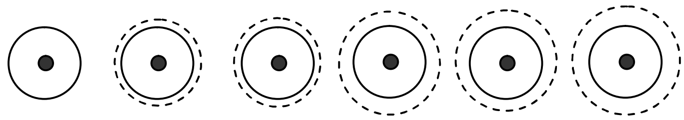


Figure 6: Defined timeline of otolith age and growth structures for *Dissostichus eleginoides*.

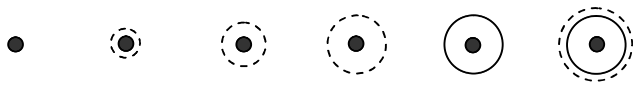


	Jul. 1998	Sept. 1998	Nov. 1998	Jan. 1999	Mar. 1999	May 1999
True Age	0	0.167	0.333	0.5	0.667	0.833
Assigned Age	0	0(0+)	0(0+)	0(0+)	0(0+)	0(0+)
True Year-Class	98-99	98-99	98-99	98-99	98-99	98-99
Assigned Year-Class	98-99	98-99	98-99	98-99	98-99	98-99

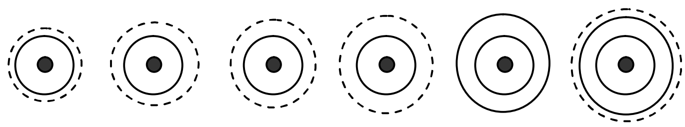


	Jul. 1999	Sept. 1999	Nov. 1999	Jan. 2000	Mar. 2000	May 2000
True Age	1	1.167	1.333	1.5	1.667	1.833
Assigned Age	1(1)	1(1+)	1(1+)	1(1+)	1(1+)	1(1+)
True Year-Class	98-99	98-99	98-99	98-99	98-99	98-99
Assigned Year-Class	98-99	98-99	98-99	98-99	98-99	98-99

Figure 7a: Model of otolith growth and annulus formation for *Dissostichus eleginoides*. Solid circles represent annuli and dashed circles represent plus growth.



	Sept. 1998	Nov. 1998	Jan. 1999	Mar. 1999	May 1999	Jul. 1999
True Age	0	0.167	0.333	0.5	0.667	0.833
Assigned Age	0	0(0+)	1(0+)	1(0+)	1(1)	1(1+)
True Year-Class	1998	1998	1998	1998	1998	1998
Assigned Year-Class	1998	1998	1998	1998	1998	1998



	Sept. 1999	Nov. 1999	Jan. 2000	Mar. 2000	May 2000	Jul. 2000
True Age	1	1.167	1.333	1.5	1.667	1.833
Assigned Age	1(1+)	1(1+)	2(1+)	2(1+)	2(2)	2(2+)
True Year-Class	1998	1998	1998	1998	1998	1998
Assigned Year-Class	1998	1998	1998	1998	1998	1998

Figure 7b: Model showing otolith growth and annulus formation for a fish which spawns in September and with annulus formation in May. Solid circles represent annuli and dashed circles represent plus growth. (a) Use of a 1 January birth date allows for correct year-class assignment. The age-class designation, or age, is written first followed by the actual number of annuli visible listed within brackets (e.g. 1(1+)). The presence of a '+' after the number in the brackets indicates new growth, or 'plus growth' visible on the structure's margin. Using this method, a fish sacrificed in January before annulus formation with one visible annuli would be assigned the same age, 2(1), as a fish with two visible annuli sacrificed in August after annulus formation, 2(2). (b) Use of a 1 September birth date, while the correct biological birth date, causes the incorrect year-class assignment.

ATTACHMENT 1

LIST OF PARTICIPANTS

Workshop on Estimating Age in Patagonian Toothfish
(Center for Quantitative Fisheries Ecology, Old Dominion University,
Norfolk, Va., USA, 23 to 27 July 2001)

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ATTACHMENT 2

AGENDA

Workshop on Estimating Age in Patagonian Toothfish
(Center for Quantitative Fisheries Ecology, Old Dominion University,
Norfolk, Va., USA, 23 to 27 July 2001)

1. Introduction and Welcome

2. Adoption of Agenda and Arrangements for the Meeting

3. Aims of the Project

4. Results of Otolith Exchange

5. Estimation Methodology
   - 5.1 NIWA
   - 5.2 CAF
   - 5.3 CQFE

6. Definitions of Nucleus and Annuli

7. Reading Ages from Samples

8. Sample Preparation

9. Sampling and Experimental Design

10. Reference Otolith Sets

11. Methods Report
    - 11.1 Otolith Preparation
    - 11.2 Otolith Reading

12. Further Work
    - 12.1 Validation
    - 12.2 Otolith Studies Linked to Other Aspects of Southern Ocean Ecology

13. Adoption of Report

14. Any Other Business

15. Close of Meeting.