Report of the Greenland Halibut (*Reinhardtius hippoglossoides*) Age Determination Workshop
The sketch below shows 5Zc and 5Zu are sub/subdivisions of the Subdivision 5Ze established by the Scientific Council for the purpose of recording and reporting catches from Canadian waters (5Zc) or USA waters (5Zu) (NAFO GC Doc. 86/2, 2nd revision, and GC Doc. 86/4, revised).
Foreword

In accordance with its mandate to disseminate information on fisheries research to the scientific community, the Scientific Council of NAFO publishes the Journal of Northwest Atlantic Fishery Science, which contains peer-reviewed primary papers and notes on original research, and NAFO Scientific Council Studies, which contains review papers of topical interest and importance. Each year since 1981, the Scientific Council has held at least one Special Session on a topic of particular interest, and many of the contributions to those sessions have been published in either of these NAFO publications.

There has been increasing concern regarding the interpretation of otolith structure, particularly for slow growing, long lived fishes. In general errors in age determination could lead to an under-estimation of the age of the fish and a consequent over-estimation in the annual yield that can be taken sustainably. Greenland halibut have long been known to be difficult to age and in 2004 NAFO’s Scientific Council recommended that an age determination workshop be convened to address this issue. To ensure the widest possible participation, the workshop was held in 2006 in St. John’s, Newfoundland and Labrador. A rigorous otolith exchange and cross-validation program was undertaken prior to the workshop and used to identify individual and systematic errors arising from the variety of methods used. Results from the application of age validation methods were presented and appropriate conclusions drawn. It was found that older and larger specimens were being under-aged, although it was not possible to identify the extent of this with the methods currently available.

This NAFO Scientific Council Studies No. 41 provides an accurate and illustrated account of the progress made during the meeting. It includes many excellent and annotated photographs of otoliths that will provide a valuable reference tool for all fish aging laboratories. The many powerpoint presentations are also included in the appendices and give a fantastic overview of the current problems and solutions in the aging of Greenland halibut.

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Anthony Thompson
General Editor,
Journal of Northwest Atlantic Fishery Science
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Report of the Greenland Halibut (*Reinhardtius hippoglossoides*) Age Determination Workshop

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**ABSTRACT**

The workshop was held in St. John’s, Newfoundland and Labrador, February 21–24, 2006. Prior to the workshop there was an exchange of otoliths and scales collected during the 2005 EU survey in SA3. During the workshop each lab presented information on ageing methods using scales, otolith whole and otolith section: no two labs used the same method. Research related to methods and age validation was also presented. Observations have been made in recent years that suggest Greenland halibut are longer lived and slower growing than previously thought. The otolith cross-section methods presented during the workshop indicated older ages at a given length compared to surface ages. For the Alaskan stock it was suggested the methods deviate beginning at approx. 60 cm or age 7 yr. For the stock in NAFO SA0 deviations in the age bias plot of whole versus section age estimates began at about age 15 (approx. 50 cm). For the Northeast Atlantic stock off the Norwegian coast ages derived from a revised whole otolith method began to deviate beginning at ages 4–5 (approx. 40 cm). Dark “featureless” translucent margins on large otoliths indicate an accumulation of compacted small annual zones. It became clear during the workshop that bias between age readers could not be solved by simply agreeing to common interpretation practices. Workshop participants provided several conclusions and recommendations.

**INTRODUCTION**

Concern with regards to the difficulty of age determination in Greenland halibut (*Reinhardtius hippoglossoides*) and the precision and accuracy of ages produced by current whole otolith and scale methods has persisted for many years. Some Institutes have stopped ageing Greenland halibut altogether while other labs feel that differences in ages may be small and could be resolved with some comparative exercises and quality control. In 2004 the NAFO Standing Committee on Fisheries Science recommended at Scientific Council that age-readers of Greenland halibut in Subarea 2 and Divisions 3KLMNO participate in a 2005 Workshop to reach agreement upon common age reading practices and eliminate biases in age interpretation (pg. 168 in NAFO 2004). This workshop was postponed to 2006 and expanded to include participants from labs outside the NAFO convention area (e.g. Northeast Atlantic and the Pacific) (see Appendix I for the workshop agenda).

The workshop was held in St. John’s, Newfoundland and Labrador, February 21–24, 2006. Prior to the workshop there was an exchange of otoliths and scales collected during the 2005 EU survey in SA3. Labs participating in the exchange were from the NAFO area; Canada, Greenland, Spain, Portugal and Russia. During the workshop each lab presented information on their ageing methods and research related to methods and age validation. There were opportunities to examine samples using various methods and to discuss the potential of new methods. Several conclusions and recommendations were made.

This report summarizes presentations and discussion heard during the workshop and provides conclusions and recommendations regarding the age determination of Greenland halibut to date. PDF copies of the slides from the power point presentations can be found in Appendices III to XVIII.
TERMS OF REFERENCE

The terms of reference proposed for this meeting are similar in many ways to the terms of reference identified for the ICES/NAFO meeting held almost 10 years earlier. For example in 1997 participants planned to inter-calibrate age reading and describe a protocol for handling Greenland halibut otoliths as well as develop diagrams and reference photographs to illustrate age reading criteria. These objectives were not entirely achieved and 10 years later we are hoping to try again to make some progress. However, since 1997, two Institutes that participated in the workshop at that time have chosen to stop ageing Greenland halibut because of concerns over accuracy and precision in the age estimates they were producing. Other labs are starting to question the traditional whole otolith method and we have some results from validation research that supports this concern. Thus, with these points in mind the following terms of reference were developed:

1) Review and evaluate various methodologies used by member states to determine age of Greenland halibut.
2) Present and discuss results of pre-workshop whole otolith exchange amongst member states.
3) Consider results of recent validation studies to determine if ageing work should continue for Greenland halibut and if so, develop guidelines.
4) Produce recommendations to establish a set of standard protocols and methodology for age determination of Greenland halibut to achieve consistency between participating member states and plan the next steps in this process.
5) Document workshop proceedings and methods, which will be reported to NAFO Scientific Council in June 2006, including conclusions on whether current ageing practices should continue to be used in assessments and guidelines on how to proceed.

OTOLITH STRUCTURE AND ORIENTATION

Participants reviewed a list of terms commonly used in age determination and developed the following definitions for use in discussing Greenland halibut ageing methods. Figure 1, 2 and 3 illustrate Greenland halibut otolith morphology.

![Fig. 1. Left and right Greenland halibut otoliths (from Esther Roman, this workshop). The reference bar on each photo measures 1000 μm.](image-url)
The following terms were reviewed by workshop participants and definitions agreed to after plenary discussion to clarify meaning in some cases.

**Accuracy**: the closeness of a measured or computed value to its true value.

**Age estimation, age determination**: terms preferred when assigning ages to a fish. Often synonymous with ageing, but the term ‘ageing’ refers to a time-related process.
**Age-group**: the cohort of fish that have a given age (ex. the 5-year-old age-group). Synonymous with age class.

**Annulus**: one of a series of concentric zones on a structure that may be interpreted in terms of age. The annulus is defined as either a continuous translucent zone (depending on lighting) that can be seen along the entire structure as a ridge or a groove in or on the structure and forms once per year. Usually the “winter” growth zone is considered the annulus, which marks the end of the year of growth. With transmitted light it will appear light with reflected light it will appear dark.

**Annual growth zone**: A growth zone that consists of a fast growing zone (opaque) and a slow growing zone (translucent).

**Bias**: Error that affects accuracy or the proximity of the age estimate to the true value. Differences are systematic (i.e. Over-ageing or under-ageing over a range of ages).

**Birth Date**: Based on an accepted standard, all Greenland halibut are assumed to have a birth date of January 1.

**Check**: A stress induced, often indistinct translucent zone. Not a “true” annulus, though it might appear as one forming in an opaque zone where you would not expect it. It may be confused with an annulus, especially if it is prominent. The check represents a slowing of growth for some reason and is often not as distinct as an annulus, usually merging with it or is discontinuous. Terms like false annulus should be avoided. Checks may also be referred to as a split or doubles depending on whether they merge with annuli or not.

**Circulus**: A concentric ridge formed on a scale by the periodic addition of material to the edge of the basal plate. Could vary between species and how the scale is formed. The plural term is circuli.

**Cohort**: A group of fish born during the same year (Jan 1–Dec 31). Synonymous with year class.

**Corroboration**: A measure of the consistency or repeatability of an age determination method. This does not necessarily mean that the ages are accurate. For example, if two different age readers agree on the number of zones present in a hard part or if two different age estimation structures are interpreted as having the same number of zones.

**Crystallized otolith**: An otolith displaying a different type of mineralization that doesn’t usually have discernable zonation that can be interpreted for age.

**Daily increment**: An increment formed within the otolith over a 24 hour period. Usually used when ageing larval fish.

**Distal Surface**: The surface of an otolith that faces away from the brain/center and that contains a growth pattern that is commonly used for age determination. In Greenland halibut it has a convex shape.

**Hyaline zone**: A zone that allows a greater quantity of light to pass through than an opaque zone. The preferred term is translucent zone.

**Edge type**: Opaque/translucent deposition occurring on the outer edge of the age structure representing the most recent growth at the time the fish was captured.

**False annulus**: Sometimes used synonymously with “check”, it refers to a zone of slow growth that is not counted as an annulus.

**Finger(s)**: Narrow growth projections along the outer edge of whole Greenland halibut otoliths, particularly on the posterior-dorsal margin of the left otolith and anterior margin of the right otolith. Generally more pronounced in larger otoliths.

**Focus**: The origin of growth in the scale bound by the first circulus.

**Marginal increment**: The region beyond the last annulus at the margin of the structure used for age estimation. Quantitatively, this increment is usually expressed in relative terms, that is, as a fraction or proportion of the last complete annual or daily increment.

**Nucleus**: Origin of growth in the otolith. For Greenland halibut it is often opaque, located centrally in the left and asymmetrically in the right.

**Opaque zone**: A zone that restricts the passage of light when compared with a translucent zone. Under transmitted light the opaque zone doesn’t allow the passage of light and appears dark; under reflected light it appears bright.
**Proximal Surface:** The surface of an otolith facing the brain/center containing the sulcus. In Greenland halibut it has a concave shape with a peri-sulcular tuberosity (dome).

**Peri-sulcular tuberosity:** Dome-like structure, centrally located on the proximal side of the left otolith in Greenland halibut.

**Precision:** The closeness of repeated measurements to the same quantity, measured by independent re-aging of same structure by the same or different readers.

**Reflected light:** Illumination from above.

**Sagittae:** The largest of the three pairs of otoliths found in the head of a fish. It is the one that is used most often in age determination.

**Sulcus acusticus:** Normally referred to as a sulcus. It is a longitudinal groove extending down the proximal (convex) surface of an otolith. On the left otolith of Greenland halibut, it is found anterior to, and partially ascending the anterior slope of the peri-sulcular tuberosity.

**Transition zone:** A region of relative change in an otolith growth pattern between two similar or dissimilar regions. In most cases, a transition zone is recognized due to a change in relative width or size in growth zones that may be abrupt or slow. For example, transition zones are formed during the transition between larval to juvenile forms or from immaturity to mature life cycles.

**Translucent zone:** A zone that allows the passage of light. Under transmitted light it appears bright; under reflected light it appears dark.

**Transmitted light:** Illumination from below.

**Validation:** The process of measuring the accuracy of an age estimation method.

**Verification:** The process of establishing that something is true. Individual age estimates can be verified if a validated age estimation method has been employed. Verification implies the testing of something, such as a hypothesis, that can be determined in absolute terms to be either true or false.

**Winter zone:** Translucent growth (annulus, not a check) that is normally deposited during the fall and winter when fish are growing relatively slowly.

**Year-class:** The cohort of fish that were born in a given year (Jan 1–Dec 31) (ex. the 1990 year class).

**Zone:** Region of similar structure or optical density (opaque or translucent). Synonymous with ring, band, or mark. The term zone is preferred.

**GREENLAND HALIBUT BIOLOGY AND LIFE HISTORY**

Discussion started with a description of the distribution and behavior for the Alaskan stocks. Young fish are found on the shelf with adults in deeper offshore waters. Females less than 60 cm are usually found to be immature while females greater than 70 cm are usually mature. Abundance of fish that would be a mixture of mature and immature, those between 60 and 70 cm, is unusually low and it was suggested that they may be missing them in surveys due to reduced coverage in the rough slope areas. Tag and recapture studies have been conducted for several years in Alaskan waters. There have been a number of recaptures to date, including two fish that had been at-large for 16 and 20 years. Recent results from data storage tags show off-bottom migrations in January of up to 200 m twice a day. It has been hypothesized that this may be pre-spawning or spawning behavior.

In the North Atlantic young fish are also found on the shelves with larger fish found at deeper depths. Greenland halibut is generally a cold water fish, found in Arctic waters (e.g. -0.3°C in portions of Baffin Bay) as well as warmer waters of the Atlantic (e.g. 7.0°C along portions of the Grand Bank and Flemish Cap). It was noted that the Spanish long-line survey in Subarea 3 went down to 3 000 m but did not find any Greenland halibut beyond 2 200 m (De Cárdenas et al. 1996). Results from archival data storage tags applied in the Northeast Atlantic off the coast of Norway showed a change in behavior, with vertical off bottom migrations of 100 to 200 m beginning in August and extending through December. This behavior pattern seems to be repeated annually as the data tag extended through to mid October of the following year with similar behavior beginning again in early August. This behavior seems to be similar to that observed in the Alaskan stock with the difference being the time of year that it occurs.

There was some discussion as to whether it is possible for the growth of this deep, cold water species to be consistent at 4–5 cm throughout its lifespan which is suggested by results obtained using current whole otolith production...
ageing methods. It was suggested that this growth rate may only be true for the younger fish. Use of the whole otolith method to age Yellowtail flounder (*Limanda ferruginea*) produced a similar linear growth pattern which was later proven to be inaccurate based on tag returns and age validation (Annex 1 in Walsh and Burnett 2002, Dwyer et al. 2003). Another comment was that if Greenland halibut are maturing at between 8–12 years (60–70 cm) then this species is likely one that is long-lived.

**AGEING METHODOLOGY**

During the first and second day in plenary, the workshop spent time learning about the methods each lab had developed to age Greenland halibut. Most labs used some variation of the whole otolith method, others had developed section methods and we had presentations on two scale methods.

**Whole Otolith Methods**

Five labs gave presentations on the whole otolith method: Northwest Atlantic Fisheries Centre (NAFC-Canada), Instituto Nacional de Investigação Agrária e das Pescas (INIAP/IPIMAR-Portugal), Instituto Español de Oceanografía (IEO-Spain), Greenland Institute of Natural Resources (GINR) and Institute of Marine Research (IMR-Norway). Most prefer to use the left otolith but some consider the right as well and IMR has developed an alternate method using the right otolith only.

A copy of each presentation is included as an Appendix.

**NAFC-Canada-Prepared by K. Dwyer, R. Burry and B. Greene (Appendix III):** At NAFC otoliths from NAFO SA2 and 3 are collected and stored dry in paper envelopes. They are immersed in 95% alcohol in a watchglass and read using a stereomicroscope at 10x magnification with reflected light. Higher magnification may be used closer to the edge on large otoliths. The preferred age reading zone is within the widest half of the longitudinal axis (although this does vary) on the distal or convex side. Grinding has been used to try to clarify annuli. Translucent bands (dark under reflected light) are counted as annuli. Bowering and Nedreaas (2001) say that 0-group fish caught in August range from 5–8 cm and those caught between Oct–Dec have a modal length of 8.5 cm–10.5 cm. Whole otoliths from fish 5–8 cm in length were found to be approximately 1.09 mm in diameter. Examples of these 0 group otoliths were shown alongside other otoliths from young (1 to 3 year old fish) to illustrate the importance of determining the first annulus.

**INIAP/IPIMAR-Portugal-Prepared by R. Alpoim (Appendix IV):** The INIAP/IPIMAR uses the method that was recommended after the 1996 Reykjavik workshop. Otoliths from NAFO SA3 are collected and stored dry in paper envelopes and then in preparation for ageing they are soaked in a 50:50 mixture of Glycerin and Thymol for 72 hours. They are then baked in an oven for 30 min. at 200°C. Prior to ageing the otoliths are placed in immersion oil for 24 hours. The otoliths are read using 10x magnification (sometimes higher to see the edge of larger otoliths) with reflected polarized light. Both the left and right otoliths are used and in most cases they are read on the convex side although they may switch between convex and concave when ageing the larger otoliths. Contrast is improved with this method but the otoliths become fragile and break very easily. Translucent bands (dark under reflected light) are counted as annuli.

Results from a study showing otolith age frequency and otolith growth were presented. Ages 5 to 8 were the most common in the Portuguese catches with 19 being the oldest observed age. Otolith growth increments began to decrease in fish greater than approximately 56 cm for both the left and right otolith. When this data is plotted against age class there is more of a difference between the two otoliths with otolith growth increments reduced to near 0 mm beyond age class 8 for the left (symmetrical) otolith while for the right (asymmetrical) otolith incremental growth begins to slow but does not reach an asymptote within the available age range (1–12 years).

**IEO-Spain-Prepared by E. Roman (Appendix V):** At the IEO otoliths are collected from NAFO SA3 and ICES Area II stored dry in paper envelopes but are soaked in a 10:90 mixture of Glycerin and Alcohol for 12–48 hours prior to examination. The wetting agent increases the resolution between translucent and opaque zones. Both otoliths are
placed in a black dish and viewed with a stereomicroscope using a fixed 12.5x magnification under reflected light, inclined at 45–60 degree angle to the surface of the otolith. The left otolith is usually found to be more suitable for age reading because it is generally more uniform in shape, has fewer fingers than the right and the nucleus is centered with possible reading axes all around it. The convex side is preferred for age reading although in some cases the edge of the concave surface is examined. Translucent bands (dark under reflected light) are counted as annuli.

Determining the first annulus is difficult and they also commented that age interpretation was especially difficult for fish over 10 years old. It is hard to interpret growth on the edge when growth zones become narrower. The maximum ages reached using this method were 21 years for females and 15 years for males.

GINR-Greenland-Prepared by K. Sünksen and L. Heilmann (Appendix VI): The GINR collects otoliths from NAFO SA1 and ICES XIBb2 and stores them dry in paper envelopes. Prior to ageing the small otoliths (<~ 45 cm) are placed in water and the large otoliths (>~ 45 cm) are placed in 50% ethanol. The convex side of both otoliths is viewed with a stereomicroscope with 8x–10x magnification under transmitted polarized light. However, the right otolith is chosen for the age determination and the left is used as support. Opaque bands (dark under transmitted light) are counted as annuli. Examples of the interpretation of annuli and checks were shown. A plot of the linear regression of length on age showed growth was fairly constant across ages 0–15 years at approx. 5 cm/yr.

IMR-Norway-Prepared by O. T. Albert (Appendix VII): At IMR otoliths are collected from ICES Areas I and II. A production ageing method using the left whole otolith has been used but recently they have identified problems with this method. For example the annual length increment increases from age 9 onwards when you would expect it to decrease and there is no increase in the standard deviation with age, possibly because fish length is used to assist with age determination. A refined method has been developed to address these problems. The right otolith was chosen as it has the longest growth axis along which to interpret annuli. It was noted that the right otolith continues to grow out around the margin and along the “fingers” while the left otolith grows more in thickness. The otoliths and surrounding tissue are collected and stored frozen in small vials. For age determination the fresh otolith is placed in water and a digital image taken. The image is digitally enhanced using Adobe Photoshop and the location of the first annulus determined based on average size of known age 1 otoliths. Age readers create individual layers on which they can indicate their interpretation of the structure. These layers can be turned on and off and allows for a visual comparison of interpretation within and between readers. The difference between a frozen otolith and a dry otolith, both viewed in water, was illustrated using photos and results from image analysis. It was noted that otoliths stored dry could be cleared by placing them in glycerin for 24 hours, although the result is not as good as using otoliths that had been frozen.

Data from a comparison of the production method and refined method were presented. The refined method results in a wider age distribution (1–27 years) than the production method (1–15 years). Growth rate is similar for the two methods up to age 5 after which it decreases for the refined method. Length modes of young (5–50 cm) fish were used to verify the early ages derived using the refined method. The age 1 and age 2 fish matched well with the first two modes of the length frequency however the third mode at approx. 30 cm was found to be comprised of a mixture of age 3 and 4 year olds with a few as old as age 5. Another verification of the method for the younger ages was the size of the third and fifth annuli measured in older fish (10–20 years) was found to be similar to the size of otoliths from fish aged 3 and 5 years as of Jan 1. The mean length increment per year for age 5+ fish using the production method was 4 cm and for the refined method it was approx. 2.4 cm which was similar to growth rates determined from tag-recapture data and an analysis of modal progression in spawning areas for years 1996–2003. Further development of the refined method was outlined with the intention that it be adopted for use in assessment work.

Otolith Sections Methods

Five labs contributed presentations on section methods: the Freshwater Institute (FWI-Canada), The Central Ageing Facility within the Department of Primary Industries (CAF-Australia), Alaska Fisheries Sciences Center within NOAA (AFSC-United States of America), the Pacific Biological Station (PBS-Canada) and the Provincial Department of Environment and Conservation lab in Corner Brook, Newfoundland and Labrador (DEC-Canada).

FWI-Canada-Prepared by M. Treble and R. Waste (Appendix VIII): At the FWI otoliths are collected from NAFO SA0. They found it difficult to interpret ages using the typical whole otolith method and the linear growth
model that resulted did not seem realistic. There was also evidence from oxytetracycline marked fish that annual growth on the edge of whole otoliths of fish 55 cm to 66 cm in size was difficult to determine if at all and these annuli would likely be missed using the whole method. Also the maximum whole otolith ages were below ages estimated by Carbon 14 validation (>20 years for fish >70 cm). For many species it has been shown that a cross-section of the otolith will reveal structure that was not visible on the surface and as a result more accurate and precise ages can be determined even for the largest fish so at FWI section methods have been investigated. Otoliths are collected and stored dry in paper envelopes. A transverse cross-section through the nucleus of the left otolith was chosen because this plane is approximately perpendicular to the sulcus (a standard sectioning practice for most species) and it passes through a thickened portion of the peri-sulcular tuberosity, present in the left otolith only, that shows good ring formation in cross-section. To prepare for age determination the otoliths are embedded in a transparent epoxy resin. The otolith core is marked to indicate where to cut the cross-section and a low speed saw with a diamond tipped blade is used to cut the otolith. Thin sections of 350 μm were found to be fragile. So instead a single transverse cross-section bisecting the nucleus was chosen. The two cut surfaces were polished by hand and then viewed under reflected light in water using a stereomicroscope with 30x–40x magnification. The structure along the thinner margins of the otolith was difficult to interpret and therefore annuli (translucent bands) were read in the area of the peri-sulcular tuberosity, usually on the ventral side of the sulcus.

Results of an age methods comparison trial were presented that showed whole ages under-estimated section ages beginning at 15–18 years and that the precision was better for the section method (CV 9%) compared to the whole method (12%). Maximum section ages from this trial were 25 years which compared fairly well to the ages estimated using the Carbon 14 validation method. However, some problems with the section method have been identified; 1) by taking a transverse section through the nucleus of the left otolith you are not capturing the region with the maximum growth because the peri-sulcular tuberosity grows away from the nucleus at an angle. However, if you section through the thickest portion of the otolith you will miss the nucleus and possibly the first annulus; and 2) the structure could change depending on the lights incident angle, suggesting that a stain treatment or thin section method may be preferable. Further refinement of the section methods may improve precision and increase the level of confidence in ages.

CAF-Australia-Prepared by C. Green and presented by M. Treble (Appendix IX): The CAF has developed expertise in assessing fish ageing structures for use in production ageing. They have experience with a wide range of both marine and freshwater fish, including long-lived species such as Orange Roughy (Hoplostethus atlanticus) whose otoliths have complex structure similar in some respects to Greenland halibut, although Greenland halibut have a peri-sulcular tuberosity on the left otolith which is absent in Orange Roughy otoliths. The CAF was contracted by FWI to assess preparation and ageing techniques for the age determination of Greenland halibut.

An initial 21 otoliths were examined whole in water using reflected light and then several section planes were tested using both left and right otoliths in order to determine the best preparation method and develop a protocol for otolith increment interpretation for age estimation. The preferred method was a transverse section through the nucleus of the left otolith. Two ageing planes were compared, one in the thickened peri-sulcular region and the other out along the thinner dorsal or ventral margin. A second batch of 168 otoliths from SA0, ranging in size from 8 to 90 cm with a primary mode at 50 cm and secondary modes at 15cm and 25 cm (using 5cm length classes) was prepared and aged according to this method.

Otolith mass was measured prior to embedding and sectioning to use as a diagnostic tool for assessing potential errors in age estimates. Assessing otolith mass is part of the standard age determination protocols used at the CAF. Otoliths will stop growing in diameter, particularly after maturity, but will usually continue to thicken and grow in weight. This rate of growth in otolith weight is generally constant and when plotted against age results in a linear or a two-stage linear relationship, with one rate prior to maturity and a second lesser rate after maturity. In long-lived species it has been observed that plots of otolith mass against estimated age can show an increasing slope at older ages (rather than a linear relationship) if the ages have been underestimated. Also, a large variation in the otolith mass-age relationship may indicate a lack of precision in the age estimates. A two stage growth relationship for otolith mass and age was described by Fenton et al. 1991 for Orange Roughy.

Increments were visible in whole otoliths in smaller fish however ageing increased in difficulty with otolith size. The otolith margin in larger fish was often relatively opaque due either to narrow increment formation or the curvature
of the margin, impeding increment clarity. The majority or increments visible on whole otoliths were clearer in the finger-like structures. However, inconsistency in morphology made defining a consistent ageing plane difficult between samples. For many species otolith growth in young fish is on the dorsal-ventral plane and as fish grow older growth is directed towards the proximal side, making surface ageing more difficult. If preparation constraints are a concern it would be feasible to estimate the age of fish from whole otoliths up to a certain age or size. The threshold would depend on the point in which otolith growth slows on the dorsal-ventral plane and continues on the proximal side.

The peri-sulcular tuberosity or “dome” appears to continue to grow as the otolith grows. This dome is only apparent on the left otolith. Relatively clear, consistent increments within and adjacent to the dome could be counted from the primordium to the edge within a cross-section along the transverse plane of the left otolith. However, clarity of the structure on the otolith margin in larger otoliths was reduced as increments were relatively close together. Incremental structure was also seen along a “ridge” formed immediately adjacent to the distal face. The ridge formed the longest growth axis and so there was a lot of incremental structure visible. There were many “checks” that made interpretation difficult.

Ages estimated from the “dome” area varied between 0+ and 31 years with the mode at 10 years. Ages estimated from the “ridge” area varied between 0+ and 34 years with the primary mode at 14 years and a secondary mode at 4 years. Greenland halibut have a decaying growth function for fish length and otolith mass which indicates that growth rate for the fish does slow and a similar relationship should be expected in the fish length-age relationship. This was not the case with the estimated “dome” ages although the relationship looks somewhat better for the “ridge” ages. Since the growth rate of the fish slows and the otolith weight continues to increase in mass (and presumably age) it could be expected that the otolith mass and age relationship would be almost linear or fit a two-stage linear growth function with the inflection point attributable to the time of maturation. The linearity of the ridge estimates was greater than the dome estimates while the relationship for the dome estimates showed an increasing slope with age and considerable variability in otolith mass at age.

The relationship between fish length and dome age estimates illustrates that there is virtually no cessation of growth for the older fish. There are a number of hypotheses that could explain this relationship: 1) it could be the true representation of Greenland halibut growth; 2) it could be due to a non-representative catch of the larger fish; 3) it could be due to under estimation of age for larger fish. However, the relationship between fish length and distal ridge age estimates did produce a relationship similar to a decaying growth function suggesting the latter is a possibility. Incremental structure in the cross-section is relatively well defined for small to medium sized fish but for larger fish it is difficult to decipher, especially close to the margin and so the periodicity of increment formation in this area remains unclear. Additional re-captures of OTC marked fish along with other validation techniques would be required to be confident that increments are formed annually and interpreted accurately.

AFSC-United States of America-Prepared by J. Gregg (Appendix X): At the AFSC otoliths are collected from the north Pacific, Bering Sea and Aleutian Island fisheries. They were looking for a method to improve the precision of age estimates that could be adapted to production ageing. They had low confidence in surface ageing, especially near the margin in older otoliths and chose to examine the peri-sulcular tuberosity on the left otolith more closely using a section and stain method. The otoliths were collected onboard the vessels and stored immediately in a Glycerin-Thymol solution. They were prepared for age determination by embedding in clear polyester resin. A single cut was made slightly oblique to the transverse plane and was adjusted for each otolith to insure that the saw blade bisected the nucleus, passed through a thick section of the peri-sulcular region and extended out the centre of a prominent dorsal finger. Surfaces of the cut otoliths were polished to remove saw marks. Polyester blocks that contained cut otoliths were submerged in a solution of 1% Aniline Blue in 1% acetic acid at a temperature of 20–23°C for 13 minutes. Otoliths were then rinsed with water and wiped clean to remove residual stain and acid. The polished surface was covered with mineral oil to eliminate surface glare and both halves were examined under a stereomicroscope at 12x to 50x magnification using reflected light. Blue stained translucent zones were counted as annuli. A more detailed description of the method can be found in (Gregg et al. 2006).

Three trials were conducted to test this new method. Trial 1 examined otoliths from 93 fish with mean length 40 cm, Trial 2 examined 226 fish with mean length 75 cm, and Trial 3 examined 75 fish with mean length 37 cm. Results
of a two reader ageing study were presented. Surface readings and stained cross-section reading were compared. Precision and symmetry were tested for both methods. A comparison of ages obtained from the two methods was also made.

Stained cross-sections did increase precision of age estimates in older fish (Trial 2). The benefit of this method seems to increase with increasing age (size) of the fish being aged. In trials containing many small fish (Trials 1 and 3) precision did not increase. Additionally, trouble interpreting the second annulus, and resulting bias, had more effect in trials containing many small fish. Post-hoc correction of this bias was made in trial 1, and did result in higher precision for stained cross-sections. Bowker’s test of symmetry of age estimates (reader bias) was found to be significant in only two instances, the surface method in Trial 2 and the cross-section method in Trial 3. For Trial 2 lengths ranged from 57–98 cm and stained cross-section ages were older (mean 17.1 years) than surface ages (mean 12.4 years). For Trial 3 where lengths ranged from 12–63 cm there was no significant difference between age methods with mean age of 4.29 years for the cross-section and 4.15 years for the surface. This suggests there may be a size below which the cross-section method is not necessary. Differences in cross-section and surface ages plotted against age and length suggest that for this stock the limits may be approx. 60 cm or age 7 yr. Von Bertalanffy parameters were estimated for both methods with the \( L_\infty = 103.7 \) for the surface method and \( L_\infty = 86.2 \) for the cross-section method. This difference in estimated growth between the two methods would have an effect on mortality estimates. Current natural mortality rate used for the Bering Sea is \( M = 0.18 \) and that for the Atlantic stocks is \( M = 0.20 \). From this AFSC study the following natural mortality rates were estimated, surface method, \( M = 0.149 \) and the cross-section method, \( M = 0.115 \). Natural mortality has also been estimated using a GSI method, \( M = 0.112 \) (Cooper et al. 2007). The amount of time it takes to age an otolith using the stained cross-section method from making labels through sectioning, staining and age reading was estimated to be 15 minutes per otolith but with additional experience time spent at the microscope manipulating the cross-section could be reduced and overall time could be lowered significantly. Images of whole otoliths and their corresponding cross-section were shown for a range of fish sizes.

**AFSC-United States-Prepared by D. Anderl (Appendix XI):** A series of images were presented showing both the whole otolith and the corresponding cross-section prepared using the stained cross-section method described above. Images covered the full length spectrum from 17 cm to 98 cm and included age increment annotations so you could see how the structure had been interpreted. It was noted that there was some difficulty in locating the first annulus in the cross-sectioned otolith. There were also some interesting patterns in the peri-sulcular tuberocity with crossing over and merging of the bands.

**PBS-Canada-Prepared by S. MacLellan (Appendix XII):** A small set of otoliths from SA0 were sent to the Pacific Biological Station. They use a burnt otolith section method that involves breaking otoliths through the nucleus and then burning the cross-section using an alcohol lamp. Annuuli are burnt dark (brown) enhancing clarity. However, to control the process of burning, a baking method was used for these samples. The Greenland halibut otoliths were baked at 500°C for 5 minutes. They were then embedded in epoxy resin and thin sections cut using a slow-speed Buehler sectioning machine equipped with a diamond studded blade. Images of five otoliths were presented, each with a series of treatments; whole with reflected light, whole baked with reflected light, baked thin section with reflected light, baked thin section with transmitted light. The baked whole surfaces and baked thin sections using reflected light appeared to have the clearest patterns for interpretation. The area on sections near the sulcus appeared to contain the best (clearest and most consistent) axes of growth for age estimates. However, the sample size was small suggesting further work.

**DEC-Canada-Prepared by R. Perry (Appendix XIII):** The DEC examined two section methods using fish sampled by NAFC in SA3, the thin section method and the acetate peel method. Otoliths were embedded in a resin epoxy mixture. After testing various section planes using both the right and left otoliths a transverse section of the left otolith was chosen. A section, 400–500 μm thick, was cut from the otolith using two diamond tipped wafering blades separated by plastic spacers. Care was taken to ensure the nucleus was included. The section was mounted on a glass slide using the same epoxy/resin mix that was used to embed the otolith. The exposed surface was ground and polished using successively finer grades of sandpaper (600, 800, 1 200). To prepare the acetate replicate the polished surface was etched with a mild hydrochloric acid (2%) for 1–2 minutes. The amount of time the section must be exposed to the acid varies in accordance with species and fish growth rate. Within an increment the acid will etch into the otolith at
different rates depending on the calcium formation present. The next step is to use acetone and a small piece of acetate to make a topographic impression of the etched surface. The replicate is transparent allowing for easier interpretation and phase contrast further enhances the image. Sections and replicates were viewed at 40x to 100x magnification using transmitted light and were aged along a transect within the peri-sulcular tuberosity. There were difficulties defining the first annulus in the acetate replicates and this method under-aged the section method for the younger fish.

Inconsistencies in growth were noted; discontinuous bands and new growth centres. [Note: This pattern was also observed on the stained cross-section images presented by D. Anderl from AFSC, see above]. Standardization of methods and interpretation would be important with the section or acetate replicate methods.

The section and acetate replicate methods were tested by three age readers who performed three trial readings for each method. A paired t-test of the dataset overall showed no difference between the techniques (p = 0.899). An age bias plot suggested that there may be bias between the methods with the section method over-aging prior to age 20 and under-aging beyond age 20 compared to the acetate method. The paired t-test was re-run on the data in these two sub-groups and results were significant. For Group 1 (ages 1 to 20), p < 0.001 and the mean difference was 0.941. For Group 2 (ages 21 to 40), p < 0.001 and the mean difference was 3.430. Paired t-test results showed a significant difference for all three readers for Group 2, while for Group 1, Readers 1 and 3 had a significant difference and Reader 2 did not. A paired t-test comparison of results from the within reader trials showed no differences for Readers 2 and 3, while Reader 1 did have significant differences between Trials 1 and 3 (p = .005) and Trials 2 and 3 (p = .004) for the acetate peel method and between Trials 1 and 2 (p = .05) and Trials 2 and 3 (p = .018) for the section method.

A comparison among interpreters using the Freidman repeated measures analysis of variance on ranks test showed that for both methods Reader 1 and 3 differed from Reader 2. A paired t-test comparing coefficient of variation (CV) values between methods showed no significant difference in CV between methods either within reader or overall. Conclusions and observations from this study were: 1) 9 of the 50 otoliths sampled were interpreted to be older than 20 years using both methods; 2) Acetate replicates and sections gave the same level of precision; 3) Interpretations from acetate replicates on average were 3.5 years older than sections; 4) Increments of younger fish are difficult to see; 5) Incremental growth may be discontinuous.

Scale Methods

There were two presentations on scale methods, one from the Knipovich Polar Research Institute of Marine Fisheries and Oceanography (PINRO-Murmansk, Russia) and another from FWI.

**PINRO-Russia-Prepared by T. Igashov and presented by K. Dwyer (Appendix XIV):** The PINRO lab has been using a scale method to age fish captured in NAFO SA3. Their method has been based on early research comparing various ageing structures, including scales, otoliths, vertebrae and fin rays from Milinsky (1944) and Krzykawski (1976). The size of scales varies depending on where they are removed but studies done at PINRO show that scales taken from the dorsal area, above the lateral line, give similar ages to larger scales taken from the caudal area and both are similar to the ages derived from whole otoliths (Igashov 2004). Scales are removed from the dorsal area above the lateral line with care taken to clean the knife used to ensure only scales from other fish do not get mixed into the sample. Scales are dried flat in paper envelopes in a cool place (approx. 20°C). At lower temperatures the scales can decay, at higher temperatures they can stick together and become brittle. The scales are soaked in ammonium hydroxide to remove any mucous membrane that may be left on them. Scales that are not damaged and that have a uniform size are selected for age reading. Scales which are far larger or far smaller than scales from other fish of the same sex and length should not be selected. Once several scales have been selected they are pressed between two glass slides, and read with a microfiche using transmitted light. A combination of widely spaced and narrowly spaced (seen as a dark band on the scale) circuli, are considered an annulus.

**FWI-Canada-Prepared by M. Treble and R. Wastle (Appendix XV):** At the FWI scales were examined for their potential in age estimation. Scales were removed from the dorsal region and dried in paper envelopes. To begin the ageing procedure the scales from each fish were placed in water and viewed with a stereomicroscope at 20x to 30x magnification. The largest scale in good condition was chosen and cleaned if necessary. The structure was hard to interpret when the scale was viewed under regular transmitted light, but a pattern emerged when a circular polarizing filter was attached to the microscope. The scale was turned back and forth under the polarized transmitted light during
age reading to aid in annuli determination. A single pair of dark and light bands was considered an annulus and ages determined along the longest axis.

Initial results using this scale method looked promising. The method produced older ages than the whole and the cross-section method and they were within the range of ages estimated by the Carbon 14 validation for fish greater than 70 cm. Precision was also better with a CV of 6.0 compared to a CV of 9.1 for the cross-section and 12.4 for the surface method. However, there were no fish < 20 cm in the sample to help with the interpretation of the first few annuli. When samples were obtained, scale ages were found to over-estimate whole otolith ages which corresponded well to previously verified ages for this size range, suggesting that the scale ages were not accurate for fish <20 cm. Also, for a small set of samples, ages from larger scales found in the caudal area were found to be greater than ages from smaller scales found in the dorsal area of the same fish. Therefore, while the polarized transmitted light method looked promising initially there are some problems and without validation it is not possible to determine whether the structure of light and dark bands are in fact annuli.

QUALITY ASSURANCE AND QUALITY CONTROL (QA/QC)

Quality control and quality assurance are important aspects of the age determination process that are often overlooked. Tools that can be included in quality control protocols include bias and precision testing within and between age readers, establishing reference collections that are read routinely by lab age readers. It is useful to determine common practices amongst labs involved in age determination for the same species or stock and to collect images and document age determination methods and criteria in reference manuals (e.g. Yellowtail flounder (Walsh and Burnett 2002 and Dwyer 2005)). These manuals don’t have to be static but could be looked at as living documents that are updated or added to as new information or material is collected.

A group based on the west coast of North America, formed in 1982, meets every two years to share ideas and discuss age determination questions. It is called the Committee of Age Reading Experts (CARE) and is a working group of the Technical Subcommittee (TSC) of the Canada/US Groundfish Committee. Their mandate is to standardize and document age methodologies used for co-managed fisheries. They have developed methods manuals and organize exchanges for calibration and training. They participate in species of interest exchanges regularly in the years between meetings. Information on CARE’s history, species they age, ageing manual and other activities can be found on their web site: http://www.psmfc.org/care/.

The AFSC also has a very good web site (http://www.afsc.noaa.gov/refm/age/) that describes their age and growth program. They have posted a paper that describes quality control methods at their lab. They have also developed an interactive video demonstrating age reading methods for several species including the AFSC cross-section and stain method used for Greenland halibut.

Technology available today can allow for images to be shared amongst readers and even between labs to compare and allow for discussion of different age interpretations for the same sample which should make development and implementation of quality control easier than it may have been in the past. Prior to initiating an exchange it is important to establish a standard way of expressing age (i.e. January 1st birth date), age designation system and confidence index. For example the age designation system used by PBS is shown in Fig. 5. The dashed lines of growth in the illustration represent unfinished opaque growth. The number in front of brackets indicates age class (used for analysis) based on what is seen (inside brackets) taking into account the date caught and January 1st birth date. Pay close attention to the system in fall and spring, it allows correct age class to be interpreted despite the kind of zone on the otolith margin. It is the presence/amount of opaque/translucent growth and time of year that dictates the assignment of age class.

No matter what age designation system is chosen it doesn't matter what zones are counted to come up with the age as long as everyone has agreed on what an annual zone represents. That is, that an annual zone is made up of one opaque zone and one translucent zone. They should come up with the same answer.

Spreadsheets used at PBS to track age readings and monitor quality control were shared with the group and all labs were encouraged to establish quality assurance and quality control procedures in their labs.
AGE VALIDATION

Research into age validation for Greenland halibut has been conducted by FWI in collaboration with Dr. Steve Campana from Bedford Institute of Oceanography, Fisheries and Oceans Canada, Dartmouth, Nova Scotia, Canada, Dr. Cynthia Jones from the Center for Quantitative Fisheries Ecology at Old Dominion University, Norfolk Virginia, U.S.A. and Jesper Boje from the Danish Institute for Fisheries Research, Charlottenlund, Denmark (Appendix XVI and Treble et al. 2005, 2008). Two types of validation methods were applied, Oxytetracycline (OTC) and 14C radiocarbon assay of otolith cores from fish from locations within SA0, SA1 and SA2 born during the nuclear bomb testing in the 1960’s. The growth of tag-re-captured fish was also examined as an indirect way of verifying age and growth rates.

Three OTC marked fish have been recovered from a marking program conducted in Cumberland Sound from 1997–2000. Photos were taken of both whole otoliths, as well as left otolith sections (transverse plane) under ultraviolet (UV) light. On the whole otolith, material that has incorporated the OTC fluoresces light green under the UV light and becomes less visible as time-since-marking increases from 1 yr 11 months to 2 years 11 months and finally to 3 years 10 months. There was particularly little growth visible along the ventral edge which is a preferred area for surface reading due to its relative consistency (lack of “fingers”) between samples. The maximum growth areas seemed to be in the outer areas of the rostrum and the “fingers”. It was noted during discussion that OTC will be most readily incorporated into active growing areas of the otolith (e.g. the dome and some fingers) and this could give a clue as to where we should be looking to do the ageing. It was just possible to make out a mark visible on the edge of the left and right otoliths from the 66 cm fish that had been at large for 3 years 10 months. The whole age assigned to this fish was 18 years. However, on the surface of the left otolith it was not possible to distinguish corresponding annuli beyond the mark using either reflected or transmitted light. The mark could be clearly seen at the edge of the otolith cross-section for all three re-captured fish, although it was necessary to use higher magnification and reflected light to determine presumed annuli in numbers that corresponded to the number of years since marking. However, in some areas of the section it was not as distinct as in others, additional growth bands (that might be interpreted as annuli) could be observed under different focal lengths.

For the 14C radiocarbon validation a reference curve unique to Greenland halibut was developed using known age 1–3 year old fish born between 1955 and 1997. This curve was extended to the years prior to 1959 by using otolith cores from fish aged 10 years or older captured in the early 1960’s. Otoliths from adult fish sampled between 1967
and 1989 were chosen for validation against the reference curve. Whole ages were determined, sections containing the otolith core were prepared, digital images taken and enhanced using Adobe Photoshop. Material in the otolith core (first three years of growth) was extracted from each section, de-contaminated and submitted for 14C assay. Results were compared to the values in the reference chronology to determine the most plausible range of year-classes and a minimum age assigned to the validation samples. Minimum estimated ages for the 12 validation samples ranged from 12 years to 27 years with seven falling between 21–24 years. Section ages ranged from 12 to 20 years with five falling between 15 to 18 years. Left otolith whole ages estimated for a sub-set of these samples ranged from 14–20 years while the right whole ages were aged slightly older at 16 to 22 years. These fish ranged in lengths from 70 to 85 cm. Minimum core ages over-estimated the whole otolith age by 3 to 11 years and section ages by 1–15 years. The maximum observed 14C based age was 27 years while for the left whole otolith and section age it was 20 years and the max age for the right whole otolith was slightly greater at 22 years. A plot of length vs. age for the reference curve samples (young fish with ages based on the whole otolith method) and the validation samples (14C assay based minimum age values) suggests that a linear growth model for Greenland halibut is not appropriate and that Greenland halibut may in fact grow more slowly as they age.

Growth analysis from tag-recapture data from research conducted by the GINR with some additional data from the FWI Cumberland Sound project were analyzed using the GROTAG model developed by Francis (1988). A Gulland and Holt (1959) model was also tested to the full dataset as well as a sub-set of fish at-liberty for one year or more. Time at liberty varied from 0.08 to 7.17 years and length at re-capture ranged from 44 to 87 cm. Growth rates were estimated by the GROTAG model for 50 cm and 70 cm fish (these sizes fell well within the distribution of sizes in the data) at 2.86 cm and 3.01 cm, respectively. Both these rates were consistent with results from the Gulland and Holt regression of growth rate on average length. However, the GROTAG model estimates of bias ($m = -1.1$) and standard error for measurement bias ($s = 2.77$ cm) were large relative to the estimates of growth indicating that there is considerable uncertainty in our growth estimates using this model.

In conclusion, the whole otolith method underestimates the true age of Greenland halibut. The section method applied in this case did not produce the results expected as it was difficult to determine annuli with confidence and ages tended to be lower than the 14C validation ages. Growth in Greenland halibut in the size range of 55 to 70 cm appears to be in the order of 2–3 cm/yr.

**COMPARISON OF AGEING METHODOLOGIES**

**Whole Otoliths and Scales – Pre-Meeting Exchange Exercise**

Bias between readers and low percent agreement was found in the previous exchange carried out in 1997 as part of the Icelandic Ageing workshop. A baked whole otolith method was recommended following discussions at this workshop. However, not many labs changed their methods and no progress has been made on improving precision or bias in age determinations since then.

An exchange of age materials (otoliths and scales) amongst several of the labs that assess Greenland halibut ages in the Northwest Atlantic was initiated in 2004/05 in preparation for this meeting. 100 pairs of otoliths and a selection of scales from the dorsal area, above the lateral line were collected from fish 15–57 cm in length captured during the EU survey of NAFO Div. 3M in July. Otoliths were placed in plastic vials with sea water which was replaced later with distilled water. Scales were removed and placed in paper envelopes. At the end of the first day of our workshop K. Dwyer from NAFC presented the results from this exchange (Appendix XVII).

Seven age readers from 5 labs aged the otoliths (Canada1, Canada2, Portugal1, Spain1, Greenland1, Russia1 and Russia2) and two age readers from two labs read the scales (Canada3 and Russia1). The otoliths were read whole, without manipulation, grinding or any type of preparation. Readers used stereomicroscopes and the otoliths were placed in water or alcohol prior to reading. Russia1 used the PINRO scale method described above and Canada3 used the FWI scale method described above.

There were very few “consensus” ages. Ages estimated by readers for most samples differed by at least a year. Coefficient of variation (CV) between readers was calculated for the otolith ages and varied between 3.5% and 17.8%.
Rus1 and Rus 2 had the lowest at 3.5% and the second lowest was 6.7% for Can1 and Gre1. Percent agreement was generally below 50% for all but the two Russian readers who had 85% agreement. Agreement increased in all readers for ±1 year, with values ranging from 61% to 99%. Seven of 21 paired t-test comparisons for the detection of bias in the otolith age determinations between readers were significant. Age bias plots were prepared to better assess the presence of bias between readers. Some form of bias was detected in almost all comparisons with the exception of Can1 and Spa1, Can1 and Por1, and Rus1 and Rus2. In general Can2 and Gre1 assigned older ages while Can1, Spa1, Rus1 and Rus2 assigned younger ages, compared to the other readers.

There were extreme differences between the ages assigned by the two scale readers with Can3 assigning much older ages than Rus1, both readers were using a method familiar to them although the methods differed between the labs. Can3 ages ranged from 5–15 with one aged as 21 while Rus1 scale ages ranged from age 2–8. The scale ages and otolith ages assigned by Rus1 were similar with slight bias to younger ages for scales at the oldest ages. Rus1 was the only reader who had experience reading both scales and otoliths and this is why we only had one within reader comparison between these two methods.

An example of each method applied to the otolith and scales from one of the fish sampled is shown in Figure 4.

There are factors which might have made age determination in this exchange more difficult such as the fact that many age readers were reading the otoliths without using preparation methods that they normally use. These otoliths were un-treated, and some readers commented that they found that the otoliths became too “clear” when soaked in water. Nonetheless the otoliths collected for this exchange were taken from fish whose length was less than 58 cm, before which many of the potential ageing problems take place. After approximately 60–70 cm (sexual maturity), growth is hypothesized to slow and therefore the whole otolith method would be expected to fail.

Fig. 4. Comparison of age determination structures from a 40 cm fish: A) whole otolith method, all readers age 5 years; B) Canada3 scale method, age 9 years; C) Russia1 scale method, 4 years.
The report from the Greenland Halibut Ageing Workshop held in Iceland (Anon., 1997) indicated that there was low precision between readers, and also bias detected. This is still the case 9 years later. Precision was low between most readers, and is low overall. This is a problem when it comes to assessments that use more than one country’s ageing input. However, there is also low precision between the two Canadian readers, indicating that more quality control is needed and if the whole otolith method is to continue, more comparative reading should be done to reduce these values.

In the absence of age validation of either whole otoliths or scales, the results from this exchange and others mean very little. Obviously, age validation studies must be further explored for this species.

**Whole Otolith and Section Methods – Examined During the Meeting**

The first day ended with the group ageing a set of 8 whole otolith images that were projected on a screen. There was general consensus for 2 of the 8 otoliths and it was noted that there was a need to work out the size of the first annulus to assist with interpreting its location.

During the workshop the IMR refined method was used to interpret the right otolith from the re-captured fish from Cumberland Sound that had been marked with OTC 3 years 10 months previously. The OTC mark was visible on the surface near the anterior edge. It was possible to identify potential annuli beyond the mark, corresponding to the 3+ years since marking, on the image of the whole otolith taken using transmitted light (Fig. 9). This had not been possible on the surface of the left otolith (Treble *et al.* 2005 and Appendix XVII).

![Fig. 9](image_url)

A portion of the right otolith of a Greenland halibut re-captured in Cumberland Sound, Canada, 3 years and 10 months since marking with OTC. Image A) shows the location of the OTC mark and image B) is of the same area taken using transmitted light. Circles indicate the possible location of annuli beyond the mark.
Whole otoliths and their corresponding section images were selected from the samples examined on the first day. Three of the four otolith samples viewed are shown below in Fig. 10. Annuli are clearly distinct on the surface of examples one and two and there was consensus on the ages for these fish, 3 years and 8 years, respectively. The sections for these two samples were also fairly clear and participants were able to identify annuli that matched the surface age. However, example three is more typical, even for fish that are smaller than the 101 cm female in this case. It is difficult to determine annuli on the surface and the edge is translucent, suggesting a build up of compact annuli. Annuli are not as distinct in this cross-section as in the first two examples, there is a lot of structure which makes interpretation challenging. A more refined section method or stain treatment may help to better define these annuli.

Fig. 10. Three samples from the set of otoliths that we had aged whole as a group the first day. Technicians at NAFC sectioned them during the workshop: #1 – 30 cm female caught during the fall, surface age = 3 years; #2 – 57 cm female caught during the fall, surface age = 8 years; #3 – 101 cm female caught during the fall, surface age = 12 to 20+ years.
Assessment by Queen’s University

A presentation on the assessment of Greenland halibut otoliths and their potential for use in age determination was prepared by Dr. J. Casselman and R. Slapkauskas (Appendix XVIII). Dr. Casselman is an experienced fisheries scientist who has specialized in age determination and growth research during his career at the Ontario Ministry of Natural Resources (OMNR). He is currently a Scientist Emeritus at OMNR and an adjunct professor at Queen’s University in Kingston, Ontario. Dr. Casselman was invited to participate in our workshop and in order to prepare he asked to have some samples sent to his lab at Queen’s University.

One of the first objectives was to examine the morphology and orientation of the otoliths. R. Slapkauskas made a series of hand drawings depicting various views of both otoliths (e.g. lateral, convex, transverse sections, etc.). They noticed immediately that the morphology of Greenland halibut otoliths is different from most other species. Greenland halibut otoliths are very thin and do not grow uniformly. The location of the nucleus differs between the left (centrally located) and the right (located near the posterior edge). The left otolith forms a thick peri-sulcular tuberosity or “dome” that grows at an angle away from the nucleus towards the posterior edge. This dome may begin to form after the first year but it is unclear what might cause it as metamorphosis is complete within the first 6 months and otoliths of other flatfish do not have this feature.

The best insight into otolith growth can be gained by examining the oldest samples. Ideally you want to maximize the material you have available for interpretation as our ability to resolve zonation improves with distance. So we should be ageing along a plane of maximum growth. In the OTC marked fish growth was active in the dome area of the left otolith and in some of the “fingers”. The dome was the most consistent area within the section. If you can track annuli throughout the structure your precision will improve and you will be more consistent.

However, because growth is not symmetrical, a transverse section at 90 degrees through the nucleus of the left otolith would not encompass the full extent of the dome. Also, in a cross-section you want the zonation to be at right angles to the section plane. You can test for this by focusing up and down through a polished thin section (recommend 325 ± 20 microns, 240 microns is too thin). If the zonation moves the section has not been taken at a right angle. To resolve these issues we might consider a transverse section of the dome with the blade angled obliquely (-7.5 ± 3.4 degrees) in order to ensure the section is 90 degrees to the zonation and that it includes both the nucleus as well as the most recent active growth layers. It is possible to set the blade at an angle using a protractor on certain models of sectioning machines. We should look for symmetry in the cross-section of the “dome” to assess whether we are at right angles to the plane of growth. A detailed objective study with quantitative measures could provide necessary insights into section orientation and zonation interpretation.

In the 16 otolith samples that were examined the females gave better images and zonation, the males were more difficult indicating that there may be more uniform growth in the females. Acetate replication (see DEC above for a description of this method) was tested but was found not to be any better than the section for the age range of the samples examined (i.e. 8–16 years, 38–85 cm).

Additional comments concerned the importance of achieving good precision within a method for an individual reader before comparing between readers. Also, there should be validation for a method before comparing between methods and readers or discussion as to which method is right or wrong can develop in the absence of independent reference criteria.

Discussion of Ageing Methodology

Day two ended and day three began with group ageing and discussion. K. Dwyer (NAFC) provided information that suggested the first annulus may be smaller than what is sometimes determined. According to Bowering and Nedreaas (2001) fish < 10 cm would be 0+. An outline of an otolith from a 0+ (7 cm) fish was superimposed on two images of larger otoliths taken at the same magnification (Fig. 6). The diameter of the first annulus on fish aged 0+(1) will vary between fast growing and slow growing fish and between stock area but it would be useful to determine the dimensions of this first annulus for each stock area to assist in age determination. For example IMR measured the size of the first annulus in their stock as 2.0 mm (± 0.5) and use this to help determine the first annulus in their revised whole otolith method. For fish aged 1 year and 9-11 mm in size, used in the development of the reference curve for the 14C validation research at FWI, otolith length varied between 1.82 and 2.51 mm and otolith width varied between 1.47 and 2.01 mm.
K. Sünksen presented a series of 5 otolith images from fish ranging in size from 15 cm to 64 cm (Fig. 7). There was consensus for #1 (5 years), #2 (2+ years) and #5 (1+ years) and estimated ages varied between 4 and 6 years for #4 and between 4 and 9 years for #3.

O. T. Albert and M. Kvalsund provided images from their archive that were taken as part of their refined whole otolith method and participants had a chance to use Adobe Photoshop software to apply age interpretations on individual layers (example shown in Fig. 8).

Participants spent some time discussing methods and examining materials provided by other labs at microscope stations set up at the back of the meeting room.

**SUMMARY**

A summary discussion on the third day was lead by S. MacLellan who is the supervisor of the Fish Ageing Lab at Fisheries and Oceans Canada’s Pacific Biological Station in Nanaimo, British Columbia. The presentation and discussion were organized into three sections: What do we know?; What don’t we know?; and Where do we go from here?.

**What do we know?** Three general methods were examined, each with various techniques, scales, otolith whole and otolith section, no two labs were using the same method.

Quality assurance and quality control measures were not routine for many labs. There was no consistent system within most agencies to assess precision in production ageing but some labs were beginning to implement testing procedures. There had been some exchanges between agencies to compare precision but there were variations or inconsistencies between readers and agencies for all methods due to criteria differences. Work on accuracy has been initiated with a bomb radiocarbon (C14) study and oxytetracycline mark and recapture study conducted by FWI. However, these studies looked at larger, older fish (55 to 85 cm) and not the entire age range.

Observations have been made in recent years that suggest Greenland halibut are longer lived and slower growing than previously thought. The otolith cross-section methods presented during the workshop indicated older ages at a given length. Data presented by AFSC showed the surface and section methods started to deviate at about age 7 (57–60 cm). FWI showed deviations in the bias plot of whole versus section ages began at about age 15 (approx. 50 cm, Treble et al. 2005). IMR has shown increased surface ages following a revision of their method and criteria with deviations beginning at ages 4–5 (approx. 40 cm). Dark “featureless” thick translucent margins on large otoliths indicate an accumulation of compacted small annual zones. Greenland halibut have a larger size at maturity which is typical of many long-lived species. Studies have shown size at maturity to be variable but in general females mature at approx. 60 cm
and males at approx. 40 cm. Growth rates for long-lived species are generally non-linear. Otolith mass data from the CAF study showed a decaying growth function, with fish reaching an asymptotic length as otolith weight continued to increase. This is typical for most teleost fish and indicates that Greenland halibut growth over time should also be non-linear. Greenland halibut inhabit a deep, cool/cold water environment which suggests they could be slow growing. Could fisheries be in trouble due to under-ageing inflating natural mortality? Inaccurate methods can give a false
impression that the stock is able to withstand higher mortality rates than is really the case. Increased fishing mortality can result in the loss of or significant decline in the older fish and this wouldn’t be detected with inaccurate methods.

**What don’t we know or what don’t we have?** We don’t have standardization within methods. In other words we don’t have standard criteria, terminology, counting axes, or age designation system. We don’t have precision or consistency measures within most labs or between labs for the same methods. We have not determined the accuracy of any methods for the entire age range. Data is not available to assess efficiencies of methods in terms of ageing rates, cost of materials and equipment for most techniques. The methods need to be well documented including training procedures and policies that include precision analysis and records of number of samples aged per day. Standardized exchange mechanisms should be established between agencies, particularly if they are contributing data to a single stock assessment.

**Where to go from here?** We should take steps to document our methods and techniques including specific criteria with both text and images to describe preparation, standard axis, interpretation of edge growth and description of plus groups. The CARE web site provides a description of key criteria that would be a good reference to start with. We should establish a standard age designation system and determine an objective confidence index or repeatability index for comparison within and between readers. A quality assurance and quality control system should be established to evaluate and measure precision. Procedures for exchanges should be developed including the frequency of exchanges, the sample size and how to standardize them. We should continue with the current exchange and have M. Kvalsund (IMR) read the right otoliths using their refined method followed by R. Alpoim (INIA/IPIMAR) who will bake them using their method. R. Wastle (FWI) will read the sections taken from the left otolith. The additional data should be included in the draft report of the exchange presented at this workshop. Validation work should continue in order to evaluate the entire age range. Can we agree on where to go from here and determine the next steps? In order to achieve
progress communication should be maintained with a commitment to continue our research and QA/QC procedures identified above with another meeting in a few years time to share results.

What are the implications for stock assessment? The impact of the workshop conclusions on current stock assessments was not discussed during the workshop. Following the workshop, Dr. John Casselman kindly provided his observations concerning Greenland halibut age interpretation and advice for the application of ages from current methods as well as research into more refined methods. His comments are attached in Appendix XIX.

**CONCLUSIONS**

The workshop concluded that:

A. Current production methods underage old fish but it is not known to what extent or at what size/age the under ageing begins.

B. Validation methods that have been applied; bomb radiocarbon dating and tagging and oxytetracycline marking for Greenland halibut, have been carried out for NAFO Div. 0B and 2G that indicate longevity of this species goes beyond that indicated by present techniques.

C. Biological methods that indicate longevity have been applied for the Barents Sea, analysis of otolith morphology and length measures, and show much greater age expectancy and this affects the fishable portion of the stock.

D. Precision and bias are still problematic due to a lack of standard application of methods and criteria.

E. The current scale method under-estimated the current otolith methods at the oldest ages.

F. Systematic studies of new methods and comparisons there of are needed to determine a reliable method for production ageing.

**RECOMMENDATIONS**

The workshop recommends that:

1) Each institute document their current method for production techniques and begin documenting new methods that are under development. This will be done partially in the NAFO report in a manual that outlines such things as: method preparation, choice of left/right otolith, lighting, axis read, etc. Year 1

2) Age validation studies should be conducted for each stock area; e.g. tagging and mark-recapture through injection of oxytetracycline or other internal marking methods. These should be initiated as soon as possible. Year 1 (Barents Sea, NAFO Div. 1A)

3) A comparison of methods be carried out within/among regions and that specifically, three methods be examined: Norwegian new whole otolith method, bisected otolith method and a thin section method and each method should be tested for efficiency in terms of production ageing. Year 1

4) An exchange should take place between labs that are active in production ageing within each stock area, which would include otoliths across all lengths, sexes, and seasons. Establish a set of rules to conduct the exchange such as assigning designated numbers to samples and age interpreters in order to reduce bias. Year 2

5) The end-users of ageing data need to understand the limitations of the current scale and otolith surface methods. We recommend an overlap of production ageing methods and any new methods, therefore the current production ageing methods in the NAFO area should continue until alternative methods are developed and agreed upon.

6) Within methods, quality assurance and quality control procedures should be developed, standardized and implemented. Year 1

7) A workshop should be held again within 2 years for each stock area and within 4 years to compare results from method development.
REFERENCES


Appendix 1. Agenda for the Greenland Halibut Ageing Workshop

February 21-24, 2006
The Fluvarium, St. John’s, NL

Terms of Reference (TOR):

1. Review and evaluate various methodologies used by member states to determine age of Greenland halibut.
2. Present and discuss results of pre-workshop whole otolith exchange amongst member states.
3. Consider results of recent validation studies to determine if ageing work should continue for Greenland halibut and if so, develop guidelines.
4. Produce recommendations to establish a set of standard protocols and methodology for age determination of Greenland halibut to achieve consistency between participating member states and plan the next steps in this process.
5. Document workshop proceedings and methods, which will be reported to NAFO Scientific Council in June 2006, including conclusions on whether current ageing practices should continue to be used in assessments and guidelines on how to proceed.

Agenda

Note: The agenda presented here was the original agenda. However, it was modified during the course of the workshop to accommodate late arrivals of some participants and to allowing for additional hands on time to view samples and have one on one and informal discussion amongst participants.

Tuesday, February 21st (0800–1700 h)

Co-conveners: Karen Dwyer and Margaret Treble

• Set up network; housekeeping items (Karen Dwyer)
• Outline TOR for the workshop. Brief review of TOR (1997) from last workshop and how this workshop differs. (Margaret Treble)
• Review glossary. Members to finalize glossary by end of workshop. (Karen and group)

Break: 10:30–10:45

• Background of Greenland halibut ageing by method and region.
  * Whole otoliths (5 minutes each)
    • “Whole otolith method in Canada” (Brian Greene)
    • “Whole otolith method from Portugal” (Ricardo Alpoim)
    • “Whole otolith method from Spain” (Esther Roman)
    • “Whole otolith method and results from Greenland” (Lars Heilmann)
    • “New whole otolith method from Norway” (Ole Thomas Albert)
  * Sectioned otoliths (15 minutes each)
    • “Otolith section methods assessed by DFO Central and Arctic” Canada (Margaret Treble)
    • “Improving the precision of otolith-based age estimates for Greenland halibut, Reinhardtius hippoglossoides, with preparation methods adapted to fragile sagittae” United States (Jake Gregg)

Lunch: 12:30–1:30

• Scales (5 minutes each)
  • “Russian age estimation method of Greenland halibut using scales” (Taras Igashov)
  • “Scale ageing using polarized transmitted light” Canada (Rick Wastle)
  • Other “Scale impressions of Greenland halibut using laminated plastic slides” Canada (Karen Dwyer)

Break: 3:00–3:15

• Results of whole otolith and scale exchange between regions including examples on-screen and ageing together. (Karen)

Wednesday, February 22nd (0900–1700 h)

• Section method (continued)
  • “Acetate replicate method using Greenland halibut otoliths” Canada (Rob Perry)
• Review of Greenland halibut biology (environment, physiology, behaviour, etc.) and how this affects age and growth. (Led by Ricardo, with contributions from others on specific stocks)

Break: 10:30–10:45
• Hands-on ageing of some new methods (whole, section and scale). Participants welcome to bring materials from their regions. Experts will be available to demonstrate their methods.

Lunch: 12:30–1:30
• Brief discussion on quality assurance and control (QA/QC) (accuracy, precision, age validation). (Overview – Shayne MacLellan)
• Presentation: “Age validation of Greenland halibut sectioned otoliths using bomb radiocarbon dating and oxytetracycline marking of tagged fish”. (Margaret)
• Presentation of report “Assessment of preparation and ageing techniques for the age determination of Greenland halibut” from the Central Ageing Facility in Australia (Margaret)

Evening: Dinner for participants (7:30, D’jango’s Restaurant)

Thursday, February 23rd (0900–1700 h)
• Discussion on whether the scale and whole otolith methods produce age data accurate enough for continued use in age-based assessments and how new methods have potential to improve accuracy and precision. (Led by Shayne MacLellan)

Break: 10:30–10:45
• Slideshow, ageing and group discussion of criteria used to interpret otolith sections. (Led by Shayne and Margaret)

Lunch: 12:30–1:30
• Participants read subsets of exchange otoliths ($n = 25$) that have been sectioned for comparison of methods.

Friday, February 24th (0900–1500 h)
• Discussion and assessment of age results from yesterday’s subsets of sectioned otoliths, including side by side images of whole otoliths, scales and sections. (Karen)

Break: 10:30–10:45
• Discussion and recommendations regarding methodologies reviewed during workshop. That is, can the membership decide to promote a preferred method for determining the age of Greenland halibut? (Margaret)

Lunch: 12:30–1:30
• Finalizing and acceptance of glossary of terms. (Margaret)
• Meeting summary and recommendations for next steps. Make recommendations to workshop membership and to agencies.
### Appendix II. Workshop Participants

**Greenland Halibut Ageing Workshop**

**Participants List**

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Appendix III

Age Determination Methodology at NAFC, Canada

Greenland halibut ageing workshop
St. John's NL
(February 21-24, 2006)

Age Determination Methodology

- Two age readers (one reads commercial, one reads RV otoliths)
- Little testing done between readers
- About 5000-6000 otoliths read per year
- Age data going back to 1960s for commercial data, and to 1950s for RV data

Age Determination Methodology

- Whole otolith method
- Both otoliths removed from fish head and stored dry in paper envelopes (often these break, but since dealing with so many otoliths this is considered best)

Age Determination Methodology

- Immersed in 95% alcohol in black watchglass
- Reflected light
- 10X magnification (closer to the edge on large otoliths may use higher magnification) using stereomicroscope

Age Determination Methodology

- Convex side is preferred
- Preferred axis is within the widest half of the longitudinal axis but variable
- Grinding sometimes done

Age Determination Methodology

- Both left and right otoliths are used but the left otolith is preferred because nucleus is at centre with reading axes all around
Appendix III

Age Determination Methodology

• Commercial otolith versus RV otolith

Age Determination Methodology

• Easily interpreted otolith versus a “bad” otolith
• Difficult species to age

Whole otoliths

• Nucleus and first annulus
• According to Bowering and Nedreaas (2001) the first mode of length frequency plots of G. halibut is at 5-8 cm
• From fish this size, otoliths corresponding to 0 group fish are approximately 1.09 mm

Photos of whole otoliths from young fish

Annuli

• True annuli – usually defined as a ring that can be followed all the way around the perimeter
• False annuli and checks – weak or incomplete

Checks

• Settling checks?
• Spawning checks
• Other
Appendix IV

Whole otolith method from Portugal

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Introduction

Portugal start a Greenland halibut direct fishery in 1982. From 1982 to 1986 with gillnetters inside the Canadian EEZ, from 1987 onwards with trawlers outside the 200 miles.

Portugal only started ageing Greenland halibut in 1994 using otoliths. In 2000 stopped to read otoliths in a routine manner due to the discrepancies with other countries and also because that the Portuguese commercial age/length keys were not used in the G. halibut assessment.

Methodology

The otoliths before burned were:
1994 - 1996 - cleaned with lixivia with a help of a little brush.
1996 onwards - soaked in glycerine-thymol (50:50) for approximately 72 hours.

After the otoliths were burned:
1994 - in a electric plate for a few seconds.
1995 - 1996 - burned in a oven for 10-30 min at 280ºC.
1996 onwards - burned in a oven for 30 min at 200ºC (Godinho and Alpoim).

Before age reading the otoliths were immersed in immersion oil for 24 hours.

Results

Left otolith - symmetric

Right otolith - asymmetric
Results
Length frequency Div. 3L, 1995-1999

Ages 5 to 8 are the most common in the Portuguese catches, but the older age observed were 19.

Appendix IV

Results

Measures

OTOLITHS USED:
354 pairs from 1999
From the center of the nucleus to the end of each hyaline zone.

Comments

The methodology used regularly in Portugal since 1996 is the one agreed after the Reykjavik workshop 1996.

The contrast is better with the treatment of the otolith, but the otoliths after burning became very fragile and break very easy.
Appendix V

Collection and storage of age samples

The specimens of Greenland halibut (Reinhardtius hippoglossoides) examined are from samples taken in the Commercial fishery, Experimental fishing and Research Surveys throughout the North Atlantic.

Preparation of structures for age determination

Whole otoliths are stored in small vials and soaked in glycerine-alcohol (10:90) prior to examination for approximately 12-48 hours. The wetting agent increases the resolution between the translucent and opaque zones.

Reading Procedure

Both otoliths of Greenland halibut are examined under binocular stereomicroscope with a reflected light illuminator inclined at 45–50 degrees at magnifications of 12.5x (we use a fixed magnification for to maintain a constant perception). Under reflected light, winter (hyaline) zones appear dark and summer (opaque) zones are white.

Reading Procedure

Both otoliths are placed in black plastic holder and then immersed in the wetting agent (glycerine-alcohol) for examination. Convex side is preferred (*)

[Images of Greenland halibut otoliths and collection and storage setups]
Appendix V

**Reading Procedure**

Although, the left sacculus otolith is more suitable for age reading because it is generally more uniform in shape and has less "fingers" than the right one, and the nucleus of the left otolith is situated in the center with possible reading axes all around.

In some cases, we use the concave surface to examine the edge.

![Male 26 cm, October](image1)

![Different axes or areas are used for ageing on the convex surface.](image2)

**Reading Procedure**

When all otoliths have been read once, the differences in age determination between readings of the same fish are discussed by viewing it on a video monitor.

![Discussion Stereomicroscope](image3)

**Reading Procedure**

Interpretation of age is sometimes difficult, especially for fish over 10 years old.

Maximum ages reached using this method have been:

- Female: Northwest - 21 years
- Male: Northwest - 15 years
- Northeast - 17 years

**Determinination of the first annulus**

The determination of the first annulus (the first distinct and complete dark zone formed outside the nucleus) is very difficult in many cases.

![Female 17 cm, July](image4)

**Edge (marginal) growth**

The interpretation of the extent and type of growth at the otolith’s edge is related to the time of year the fish was caught and the internationally accepted convention of a standard January 1st birthday.

Edge growth may be very difficult to interpret on older otoliths at any time of the year as the growth zones are so small.
Appendix V

Otolith Age Validation of Greenland Halibut

Otolith age validation studies on Greenland halibut have not been carried out in our laboratory.

However, our lab will begin research activities in order to facilitate more precision and ensure accuracy of age interpretation of Greenland halibut (Marginal increment analysis and length-frequency modes) in the current year.
Appendix VI

Whole otolith method used in Greenland

Examined areas

- 4 areas:
  - Eastgreenland, offshore
  - Westgreenland, offshore
  - Diskobay, inshore
  - Uummannaq, inshore
- ~ 500 otolith pairs aged from each area
- ¾ working year in lab

Examined areas

Procedure

- No preparation
- Otolith examine solution:
  - Small ( < ~ 45 cm): Water
  - Large ( > ~ 45 cm): 50% Ethanol
- Right otolith, left supports
- Stereomicroscope, 8-10x magnification
- Transmitted polarized light
- Up to 8 years with acceptable confidence.
  - Record: 25 years

Examples

- Nucleus and first annulus
- Fish 22 cm
- Caught summer
- Age: 2 year

Examples, II

- A check vs. an annulus
- Fish 45 cm
- Age: 6 year

Examples, II

Thanks !
A refined method for estimating age of Northeast Arctic Greenland halibut

1) Problems with age estimates from production ageing
2) Describing a refined method
3) Validating the refined method
4) Further development of the refined method

Ole Thomas Albert
Institute of Marine Research, Tromsø, Norway

1) Problems with the production ageing

- Annual length increment increases from age 9 onwards
- No increase in SD with age: They use fish length!

2) Defining a refined method

- 10 years
- 15 years
- 18 years
- 20 years
- 10 years

Stored dry, viewed in water
Stored frozen, viewed in water

- Nikon CO-MF41 digital camera
- Camera Control Unit CO-U1
- 1/1000-60 sec exposure time
- Software for image acquisition is required

- Eclipse Net Software
- Eclipse Net Plug-in "Extended depth of focus"
- Photoshop
- Database

Appendix VII

- Adjustment layer
- Transparent interpretation layers for each reader
- Each reader marks the annuli in his/her layer
- Standard macro sets
- Calibration
- Adjustment layer
- Transparent interpretation layers for each reader
### Appendix VII

#### 3) Validating the refined method

1) Compare with length modes of young fish

2) Size of the first few annuli in older fish

   - The third and fifth annuli of 10-20 year old fish are of the same size as estimated otolith size of 3 and 5 year old fish at January 1.

3) Age-information in otolith morphometry

<table>
<thead>
<tr>
<th>Otolith measures</th>
<th>WEIGHT</th>
<th>AREA</th>
<th>LENGTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation</td>
<td>0.15</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>p-value</td>
<td>0.10</td>
<td>0.20</td>
<td>0.08</td>
</tr>
<tr>
<td>Significance</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

| Correlation      | 0.19    | 0.04  | 0.26   | 0.22  | 0.22  | 0.24   | 0.22  | 0.22  | 0.24   |
| p-value          | 0.07    | 0.45  | 0.13   | 0.11  | 0.11  | 0.32   | 0.11  | 0.11  | 0.32   |
| Significance     | ns      | ns    | ns     | ns    | ns    | ns     | ns    | ns    | ns     |

#### 4) Compared with growth estimates

- Data source and method:
  - Production ageing (Linear regression of ages 5+)
  - Refined ageing (Linear regression of ages 5+)
  - All tag recaptures (mainly <3 years at large)
  - Recaptures after 8+ years at large
  - GROTAG predicted values (all tag recaptures)

- Mean length increment per year (cm)
Appendix VII

4) Further development of the refined method

- Implement the method in assessment work
- Archiving and database of images and annotations
- Analyses of annotations
- Automatic diagnostics report (of interpreter and interpretations)
- Open web-access for comparisons and training
- Images of fresh otoliths at sea

Thank You

Ole Thomas Albert
Institute of Marine Research, Tromsø, Norway
## Why Sections?

- We found the whole otolith difficult to interpret.
- Linear growth derived from whole ages did not seem realistic.
- We saw very little growth on the edge of an otolith from an OTC marked fish.
- Our whole otolith ages were below the ages estimated by the Carbon 14 validation (>20 yrs for fish >70 cm).
- For most species it had been shown that whole otoliths under-estimate true age and sections have been determined to be the preferred method.

## Sectioning Method

- A thin layer of transparent epoxy resin is placed in the bottom of a mould, once it is tacky the otolith is placed in the mould and covered with another layer of resin.
- Once the block is cured it is removed from the mould, the otolith core is located and a mark placed on the block to indicate where to take the section.
- A low speed saw with a diamond tipped blade is used to cut the section (e.g. Buehler Isomet™ saw).
Section Method Con’t

• We found thin sections (350 µm) from otoliths embedded in epoxy resin to be fragile and chose instead to use a single transverse cut.
• The surface was polished by hand using wet 30 µm and 9 µm lapping film and finished with 0.3 µm dry film.
• The cross-section was then viewed under reflected light in water using a dissecting microscope with 30x-40x magnification.

Section Plane

• left otoliths have a well developed dome on the proximal surface which show better ring formation in cross-section than the right otolith.
• three sectioning planes were attempted on the left otolith but the transverse plane had a number of advantages
  – the structure was more distinct and easier to interpret
  – it is roughly perpendicular to the path of the sulcus (a standard sectioning practice for many species)
  – it is roughly a cross section through the dome
  – it can be standardized by creating the section line perpendicular to the almost straight ventral edge of the otolith

Section Plane Con’t

• Sections were also taken in the thickest portion of the otolith in order to hit the peak of the dome.
• But the dome thickens towards the posterior, away from the core
• The nucleus would be missed and the first year could be missed as well.

Left Otolith – Series of Four Thin Sections

• Annuli were usually read on the left slope of the central “dome”.
• We found the structure out along the thinner margins of the otolith hard to interpret.
• Green lines indicate our preferred ageing zone
• But we did observe that the structure changed sometimes when the angle of the reflected light was changed.
Appendix VIII

Possible Adjustments to Section Method

- Treating the otolith in some way either with a stain, with heat (baking or burning), or by applying an acid bath and acetate peel could enhance the annuli and minimize or eliminate the variability related to light angle.
- We have tested these methods on only a few samples so can’t yet recommend one over another.

Better results when we combined the two stains

50% Neutral Red to 50% Toluidine Blue (59 cm Female, Baffin Bay) 66% Neutral Red to 33% Toluidine Blue (58 cm Female, Cumberland Sound)

Thin Section Method

- The Central Ageing Facility in Australia was contracted to assess preparation and ageing techniques for Greenland halibut.
- They use a clear polyester casting resin that is harder than the epoxy resin that we used for our thin section trials which seems to solve the breaking problems we had.
- A lapidary saw with a diamond tipped blade (e.g. Gemmasta™) was used to cut a series of four thin sections (350 µm) from otoliths embedded in sequence in the casting resin.
- Sections were mounted on a glass slide and viewed using a dissecting microscope with transmitted light (up to 40x magnification).
Summary

- Precision of the section method was better than the whole method and ages were within the range of the C14 validation samples.
- However, interpretation of the structure in Greenland halibut otolith sections was not as easy as we had hoped.
- There are several variations to the section method that may improve precision further and increase our level of confidence in the estimated ages.
Assessment of preparation and ageing techniques for the age determination of Greenland halibut

Prepared by
Corey Green
Central Ageing Facility
Department of Primary Industries
Queenscliff, Victoria, Australia

Presented by Margaret Treble, DFO, Central and Arctic

Greenland Halibut Age Determination Workshop
February 21-24, 2006
Fluvarium, St. John’s, NL

Introduction

- The Central Ageing Facility in Australia was contracted to assess preparation and ageing techniques for Greenland halibut.
- They initially examined 21 otoliths, viewing them in water using reflected light.
- They did trials with various section planes on both the left and right otoliths

Methods

- Otolith mass was used as a diagnostic tool for assessing potential errors in age estimates.
- In long-lived species, plots of otolith mass against estimated age show an increasing slope at older ages if the ages have been underestimated.
- Also, large variation in the relationship may indicate a lack of precision in the samples.

Results

- Increments were visible in whole otoliths in smaller fish however ageing increased in difficulty with otolith size.
- The otolith margin in larger fish was often relatively opaque due either to narrow increment formation or the curvature of the margin, impeding increment clarity.
- The majority of increments visible on whole otoliths were clearest in the finger-like structures.
- However, inconsistency in morphology made defining a consistent ageing plane difficult between samples.
- For many species otolith growth in young fish is on the dorsal-ventral plane and as fish grow older growth is directed towards the proximal side, making surface ageing more difficult.
- If preparation constraints are a concern it would be feasible to estimate the age of fish from whole otoliths up to a certain age or size.
- The threshold would depend on the point in which otolith growth slows on the dorsal-ventral plane and continues on the proximal side.

Appendix IX

Found that the transverse section of the left otolith had the clearest discernable structure.
- The dome appears to continually grow as the otolith grows
- This dome is only apparent on left otoliths
- Viewed transversely, increments within and adjacent to the dome were relatively clear, consistent in formation and could be readily counted from the primordium to the edge
- However, the clarity of the structure on the otolith margin in larger otoliths was reduced as increments were relatively close together.
- Incremental structure was also discernable along a ridge formed immediately adjacent to the distal face.
- The ridge formed the longest growth axis and so there was a lot of incremental structure visible. There were many “checks” visible that presented difficulties in interpretation.
Appendix IX

- Dome age relationship is relatively linear, not typical, despite a decaying growth model for the otolith mass and fish length.
- May be due to reduced sample sizes in the largest size classes or under-age estimation of the larger fish.
- However, the relationship between length and ages from the distal ridge produces a relationship similar to a decaying growth function.
- Incremental structure is relatively well defined for small to medium sized fish but for larger fish it is difficult to decipher, especially close to the margin.
- Additional re-captures of OTC marked fish along with other validation techniques would be required to be confident that increments are formed annually and interpreted accurately.
Improving the precision of otolith-based age estimates for Greenland halibut, with preparation methods adapted to fragile sagittae

Jake Gregg^1, Delsa Anderl^2, and Dan Kimura^2

1-USGS, Western Fisheries Research Center, Marrowstone Marine Field Station
2-NOAA, Alaska Fisheries Science Center, Age and Growth

Objectives

• Improve the precision of Greenland halibut age estimates
• Adapt methods to production ageing
• Validate new method

Pilot Work

• Trial and error with methods from literature
• Low confidence in surface ageing, especially near the margin in older otoliths
• Wanted to examine large peri-sulcular tuberosity on left otolith
• Sectioning and staining

Preparation

• Embedding in polyester resin
• Cutting and polishing
• Staining with Aniline Blue in 1% Acetic Acid
  - (Richter and McDermott 1990)
Appendix X

Methods

- Two reader ageing study
- Aged surfaces and stained cross-sections
- Examined discrepancies together and assigned consensus ages
- Tested precision, symmetry, and compared consensus ages

Summary of samples used in three Greenland halibut ageing trials

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample Size</th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>Collection Year, Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>93</td>
<td>93</td>
<td>12.84</td>
<td>40</td>
<td>1998.85</td>
</tr>
<tr>
<td>Trial 2</td>
<td>226</td>
<td>226</td>
<td>57.98</td>
<td>75</td>
<td>1994.41</td>
</tr>
<tr>
<td>Trial 3</td>
<td>75</td>
<td>75</td>
<td>12.83</td>
<td>37</td>
<td>1994.85</td>
</tr>
</tbody>
</table>

Results

Trial 1

<table>
<thead>
<tr>
<th>Method</th>
<th>CV</th>
<th>(\chi^2)</th>
<th>df</th>
<th>p &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>11.33</td>
<td>30.4</td>
<td>22</td>
<td>0.1091</td>
</tr>
<tr>
<td>Cross-section</td>
<td>19.68</td>
<td>74.0</td>
<td>28</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Trial 2

<table>
<thead>
<tr>
<th>Method</th>
<th>CV</th>
<th>(\chi^2)</th>
<th>df</th>
<th>p &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>16.31</td>
<td>94.2</td>
<td>71</td>
<td>0.0342</td>
</tr>
<tr>
<td>Cross-section</td>
<td>9.46</td>
<td>76.9</td>
<td>68</td>
<td>0.2159</td>
</tr>
</tbody>
</table>

Trial 3

<table>
<thead>
<tr>
<th>Method</th>
<th>CV</th>
<th>(\chi^2)</th>
<th>df</th>
<th>p &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>8.11</td>
<td>19.5</td>
<td>11</td>
<td>0.0532</td>
</tr>
<tr>
<td>Cross-section</td>
<td>9.96</td>
<td>32.3</td>
<td>12</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

Trial 2: Comparison of age frequencies for estimates made from surfaces and stained cross sections.

- Surface mean = 12.4 years
- Stained cross-section mean = 17.1 years

Bowker's test of symmetry
Appendix X

Trial 3: Comparison of age frequencies for estimates made from surfaces and stained cross sections.

<table>
<thead>
<tr>
<th>Age estimate (yr)</th>
<th>Surface</th>
<th>Stained cross section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Graph" /></td>
<td><img src="image2.png" alt="Graph" /></td>
</tr>
<tr>
<td>2</td>
<td><img src="image3.png" alt="Graph" /></td>
<td><img src="image4.png" alt="Graph" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="image5.png" alt="Graph" /></td>
<td><img src="image6.png" alt="Graph" /></td>
</tr>
<tr>
<td>4</td>
<td><img src="image7.png" alt="Graph" /></td>
<td><img src="image8.png" alt="Graph" /></td>
</tr>
<tr>
<td>5</td>
<td><img src="image9.png" alt="Graph" /></td>
<td><img src="image10.png" alt="Graph" /></td>
</tr>
<tr>
<td>6</td>
<td><img src="image11.png" alt="Graph" /></td>
<td><img src="image12.png" alt="Graph" /></td>
</tr>
<tr>
<td>7</td>
<td><img src="image13.png" alt="Graph" /></td>
<td><img src="image14.png" alt="Graph" /></td>
</tr>
<tr>
<td>8</td>
<td><img src="image15.png" alt="Graph" /></td>
<td><img src="image16.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

When to cross-section age?

Frequency (cross-section - surface)

Comparison plot of length-at-age for surface and cross-section age estimates, with Von Bertalanffy growth curves

Surface: $L_{\infty} = 103.7$, $K = 0.104$, $t_0 = -0.333$

Cross-section: $L_{\infty} = 86.2$, $K = 0.125$, $t_0 = -0.233$

Mortality Implications

- Current natural mortality parameters used
  - Bering Sea: $M = 0.18$
  - Atlantic: $M = 0.20$
- From this study (Hoenig 1983)
  - Surface: $M = 0.149$
  - Cross-section: $M = 0.115$
- From GSI method (Cooper et al.)
  - $M = 0.12$

Current Research

- Radiometric age validation
  - Analyzing two samples to determine feasibility
  - Base line activity of Pb-210 and Ra-226
  - Determining how many otolith cores are needed
- Treble et al.

Efficiency

- Time per otolith: 15 min
  - 0:49, Making labels
  - 1:00, Otolith preparation, drying, etc.
  - 2:15, Embedding
  - 1:38, Marking
  - 1:50, Cutting
  - 6:27, Polishing
  - 1:04, Staining
- Lowered ageing time significantly
Bowker’s test of symmetry

\[ \chi^2 = \sum_{i=1}^{m} \sum_{j=i+1}^{m} \frac{(n_{ij} - n_{ji})^2}{(n_{ij} + n_{ji})} \]

Where: \( n_{ij} \) is the number of specimens aged \( i \) by the reader and \( j \) by the tester

\( m \) is the maximum age
Appendix X

arc 065
TL = 12 cm
Age est = 1, 1

arc 049
TL = 19 cm
Age est = 2, 2

vst 096
TL = 66 cm
Age est = 9, 20

Arc 058
TL = 24 cm
Age est = 3, 3

Arc 109
TL = 30 cm
Age est = 3, 4
Appendix XI

Surface and Section Images from Greenland Turbot Covering a Wide Range of Sizes

By
Delsa Anderl from NOAA, Alaska Fisheries Science Center

http://www.afsc.noaa.gov/refm/age/interactive.htm
http://www.psmfc.org/care

ARC048L
s=2; xs=2
170 mm unsexed

ARC049L
s=2; xs=2
190 mm unsexed
Appendix XI
Appendix XI
Appendix XI
Appendix XI
Appendix XI
Appendix XI

VEST022L
S=14; xs=28
860 mm female

VEST065L
S=22; xs=28
960 mm female
Appendix XI
Appendix XI
Appendix XII

Results of the Examination of Greenland Halibut Otoliths
By
Shayne E. MacLellan, Fish Ageing Lab
Pacific Biological Station, Fisheries & Oceans Canada

Fish #1 (L&R) Reflected

Fish #1 (L) Un/Baked Reflected

Fish #1 (L) Baked Reflected

Fish #1 (L) Baked Thin XS Reflected

Fish #1 (L) Baked Thin Xs Transmitted

Fish #1 (R) Thin XS Reflected
Appendix XII
Appendix XII
Appendix XII

Fish # 5 (L&R) Reflected

Fish # 5 (R) Baked Reflected

Fish # 6 (L&R) Reflected
Age Interpretation for Halibut Otoliths
Thin Sectioning vs Acetate Replication

by
Rob Perry
Senior Aquatics Species Biologist, Wildlife Division, Dept. of Environment and Conservation, Corner Brook, NL

Otolith Preparations

Step 1
- Halibut otoliths were embedded in a resin epoxy mixture
  - Upon drying the mixture is very hard, making the otolith easy to mount and cut
  - It reduces chipping of the otolith
  - Allows for thin sectioning
  - The resin also makes an exceptional mounting medium

Step 2
- Sectioning and Polishing
  - A transverse section is cut from the otolith (400-500um) using two diamond tipped wafering blades, separated by plastic spacers. Careful attention is taken to ensure the nucleus of the otolith is included.
  - Otolith section is mounted on a glass slide using the same epoxy/resin mixture used to embed
  - The exposed surface is ground and polished using successively finer and finer grades of sandpaper (600, 800, 1200)

Step 3
- Acid etching
  - The polished surface is etched using a mild hydrochloric acid (2%)
  - The amount of time the otolith must be exposed to the acid will vary in accordance with fish growth rate and species
  - Within an increment, based on the calcium formation present the acid will etch into the surface at differing rates

Step 4
- Replication of Otolith Section
  - Using acetone and a small piece of acetate a topographic impression is made of the etched surface
  - The replicate is transparent allowing for easier interpretation
  - Phase contrast will further enhance the image

Section Images

Acetate
13.5 years

Section
14.0 years

Acetate
Mean 31 years

Section
Mean 26 years
Appendix XIII

30 years

Replicate images

Section
Mean age 15
Acetate
Mean age 14

20 years

Interpretations

Trial 1: 50
Trial 2: 50
Trial 3: 50

Trial 1: 50
Trial 2: 50
Trial 3: 50

Trial 1: 50
Trial 2: 50
Trial 3: 50

Trial 1: 50
Trial 2: 50
Trial 3: 50

Trial 1: 50
Trial 2: 50
Trial 3: 50

Trial 1: 50
Trial 2: 50
Trial 3: 50

Age Bias Plot Comparing all Sectioned and Acetate Ages

Paired t-test results
No overall difference between techniques
N= 434 pairs, t = 0.128, P = 0.899.

Arbitrary sub-groups based on acetate ages
Group 1
Age 1 to 20
Group 2
Age 21 to 40

Results paired t-tests
Group 1
Pairs = 338
t = -4.379, P < 0.001
Mean difference = -0.941

Group 2
Pairs = 96
t = 7.104, P < 0.001
Mean difference = 3.430
Table 1: Paired t-test Results for Individual Interpreters Age Sub-Groups
(Comparison between techniques)

<table>
<thead>
<tr>
<th>Group</th>
<th>Interpreter</th>
<th>Pairs</th>
<th>Mean Difference</th>
<th>t</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(1-20)</td>
<td>Don</td>
<td>117</td>
<td>-1.166</td>
<td>-4.067</td>
<td>P = 0.001</td>
</tr>
<tr>
<td></td>
<td>Nathan</td>
<td>114</td>
<td>-0.456</td>
<td>-1.131</td>
<td>P = 0.252</td>
</tr>
<tr>
<td></td>
<td>Rob</td>
<td>107</td>
<td>-1.056</td>
<td>-1.056</td>
<td>P = 0.008</td>
</tr>
<tr>
<td>Age(21-40)</td>
<td>Don</td>
<td>10</td>
<td>3.30</td>
<td>1.68</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Nathan</td>
<td>31</td>
<td>4.22</td>
<td>4.61</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Rob</td>
<td>35</td>
<td>2.05</td>
<td>1.064</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Paired t-test Results for among Individual Interpreters trials

No differences among trials for Nathan and Rob (either technique)

Acetate
- Trial 1 vs 3
  Mean = 0.7551, P = 0.005

Thin sections:
- Trial 1 vs 2
  Mean = 0.1622, P = 0.018

Conclusions
- 9 of the 50 otoliths sampled were interpreted to be older than 20 years using both techniques.
- Acetate replicates and sections gave the same level of precision.
- Interpreters from acetate replicates on average were 3.5 years older than sections.
- Increments on younger fish are difficult to see.
- Incremental growth may be discontinuous.
Appendix XIV

Russian Age Estimation Method of Greenland Halibut using Scales

by
Taras Igashov, Oleg Smirnov, Marina Vaganova and Aleksey Amelkin

Presented to the Age Determination Workshop by Karen Dwyer

Russian method

- No less than 50 scales removed from dorsal area to ensure a good sample of suitable scales.
- Ensure the knife is cleaned after sampling each fish.
- Scales are stored in paper envelopes and laid out to dry in a cool place. If the scales dry too quickly they will curl or crack and exfoliate.

Figure 1. Spot on the body surface to pick out the scales for Greenland halibut age reading using PINRO method.

Russian method

- Soaked in 4% ammonium hydroxide to clean the slime.
- Selection of scales suitable for age reading: 1) When selecting scales do not choose damaged scales or scales from the lateral line; 2) The sample of selected scales should be of uniform size and should be similar in size to other scales sampled from fish of the same sex and length.
- Scales are then placed between two glass microscope slides under pressure.

Figure 2. Examples of Greenland halibut scale preparations to determine age. Scale between two glass slides.

Russian method

- Examples of some scales with annuli indicated.

Exchange Sample #17
Exchange Sample #48

Russian method

- It is noted that the same fish has scales of varying sizes (see photo) but also varying "ages" (Igashov, 2004)

Exchange sample #23.
Appendix XV

Scale Age Determination Using Polarized Transmitted Light

Prepared by
Rick Wastle and Margaret Treble
Winnipeg, MB

Greenland Halibut Age Determination Workshop
February 21-24, 2006
Fluvarium, St. John’s, NL

Introduction

• We found the structure in scales hard to interpret and hadn’t considered using them until we happened to see a scale viewed under polarized transmitted light.

Method

• Place scales in water and view with a dissecting microscope at 20x-30x magnification
• Choose the largest scale that is in good condition, clean if necessary
• Circular polarizing filters are attached to the microscope and transmitted light is used.
• The scale is turned back and forth during the age reading to aid in annuli determination.
• A single pair of dark and light bands is considered an annulus.
• Aged along the longest axis.

Greenland Halibut Scales (Three Different Fish Sizes)
-same magnification

#41576 - Female; 20 cm
Aged 5 years

#40756 - Male; 36 cm
Aged 10-11 years

#40735 - Female; 61 cm
Aged 20-21 years

Comments

• Initial results looked promising
• This scale age method produced older ages than the whole otolith method and section method
• Ages were within the range of ages suggested by the Carbon 14 validation (>20 years for fish >70 cm)
• Precision was better (CV of 6.0%) compared to the otolith methods (12.4% for whole and 9.1% for sections)
Appendix XV

Comments Con’t

- No fish in our initial sample below 20 cm
- We were uncertain about the interpretation of the first few annuli
- Obtained 14 young fish < 20 cm from shrimp survey, scale ages tended to over-estimate whole otolith ages that had previously been verified based on the Peterson length frequency method.
- Examined scales from 12 larger fish 36 to 66 cm taken from both the caudal and dorsal areas of the body and found that the larger scales from the tail produced older ages.

Summary

- The polarized transmitted light scale method looked promising.
- Problems arose when we examined scales for very young fish and scales of different size from the same fish.
- Without validation, it is not possible to determine whether the structure of light and dark bands that we see using this method are in fact annuli.
Age Validation and Growth Analysis of Greenland Halibut from the Northwest Atlantic

by
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Greenland Halibut Age Determination Workshop
February 21-24, 2006
Fluvarium, St. John’s, NL

Introduction
• Age determination for Greenland halibut has primarily been conducted using whole otolith methods.
• Age verification has been conducted on the youngest ages using whole otoliths and the Peterson length frequency method.
• Concern with the accuracy and precision of the current age determination method prompted us to examine age validation.

Methods
• Validation in OTC marked fish
  - 3 fish re-captured after being marked during tagging project in Cumberland Sound, Baffin Island 1997-2000.
  - Photos taken of both whole otoliths, left otolith was embedded in epoxy and sectioned.
  - Sections viewed under ultraviolet light.

• Carbon-14 Validation
  - Reference curve - unique to Greenland halibut developed using known age 1-3 year old fish born between 1955 and 1997.
  - Extended to years prior to 1959 using otolith cores from fish aged 10 or older captured in the early 1960’s.
  - Material in otolith core (first three years of growth) was extracted from each section, de-contaminated and submitted for ¹⁴C assay.
  - Results compared to the reference chronology to determine the most plausible range of year-classes and a minimum age assigned to the validation samples.
Appendix XVI

Methods Con’t

- Growth Analysis
  - Data from Greenland Institute of Natural Resources tagging program.
  - GROTAG model (Francis 1988)
  - Gulland and Holt (1959) model applied to full dataset and a subset of fish at-large one year or more.

Results

- Growth in OTC marked fish
  – <1-1.5cm/year

Table 1. Data for three fish marked with oxytetracycline during the Cumberland Sound tagging project. All three recaptures were tagged in 1999 at 65.97° N and -66.68° W and were recaptured 2 to 4 years later in the same general area.

<table>
<thead>
<tr>
<th>No.</th>
<th>Date Tagged</th>
<th>Length at Tagging (mm)</th>
<th>Date Recaptured</th>
<th>Length at Recapture (cm)</th>
<th>Wgt. (g)</th>
<th>Sex</th>
<th>Time Since Tagging</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>April 20, 1999</td>
<td>650</td>
<td>March 15, 2001</td>
<td>65</td>
<td>1550</td>
<td>M</td>
<td>1yr, 10+ months</td>
</tr>
<tr>
<td>2</td>
<td>April 15, 1999</td>
<td>635</td>
<td>April 4-7, 2002</td>
<td>64</td>
<td>2340</td>
<td>F</td>
<td>2 yrs, 11+ months</td>
</tr>
<tr>
<td>3</td>
<td>April 20, 1999</td>
<td>690</td>
<td>March 4, 2003</td>
<td>66</td>
<td>2730</td>
<td>F</td>
<td>3 yrs, 10+ months</td>
</tr>
</tbody>
</table>
Figure 4. Section C from previous figure is shown with images taken using both reflected and ultraviolet light under increased magnification to highlight the location of the OTC mark in relation to annuli.

Growth Analysis using Tag Recapture Length Data

- Time at liberty varied from 0.08 to 7.17 yrs.
- Length at re-capture ranged from 44 cm to 87 cm.
- Growth rate estimated by GROTAG model:
  - 55cm fish = 2.86
  - 70cm fish = 3.01
- Both these rates were consistent with the Gulland and Holt regression of growth rate on average length.

\[ y = 0.0938x - 3.9106 \]
\[ R^2 = 0.0803 \]

Figure 5. Growth rate (cm/yr) and average length (length at tagging - length at recapture)/2 for fish at large longer than 0.9 years. The GROTAG model estimates are also shown.

Figure 6. Plot of \( ^{14}C \) values for Greenland halibut with line fitted using a lowess regression. The reference chronology characteristic of the Northwest Atlantic (Campana et al. 2002) is also shown.

Table 3. Results of \( ^{13}C \) assays for mature Greenland halibut otoliths selected for validation.

<table>
<thead>
<tr>
<th>Year Sampled</th>
<th>NAFO Div.</th>
<th>Length</th>
<th>Sex</th>
<th>Whole Age-Left</th>
<th>Whole Age-Right</th>
<th>Section Age-Done</th>
<th>Min Age-( ^{13}C )</th>
<th>Min Age-( ^{14}C )</th>
<th>( ^{13}C )</th>
<th>( ^{14}C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>BB</td>
<td>78</td>
<td>16</td>
<td>16</td>
<td>17</td>
<td>1996</td>
<td>21</td>
<td>-3.5</td>
<td>-20.7</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>BB</td>
<td>74</td>
<td>17</td>
<td>18</td>
<td>15</td>
<td>1996</td>
<td>21</td>
<td>-5.6</td>
<td>-21.9</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>BB</td>
<td>72</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>1996</td>
<td>23</td>
<td>-5.4</td>
<td>-17.4</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>BB</td>
<td>72</td>
<td>16</td>
<td>16</td>
<td>12</td>
<td>1996</td>
<td>21</td>
<td>-3.0</td>
<td>-29.2</td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>2G + c</td>
<td>84</td>
<td>2</td>
<td></td>
<td></td>
<td>19</td>
<td>1996</td>
<td>23</td>
<td>-2.8</td>
<td>-17.3</td>
</tr>
<tr>
<td>1996</td>
<td>2G + c</td>
<td>74</td>
<td>2</td>
<td></td>
<td></td>
<td>18</td>
<td>1996</td>
<td>23</td>
<td>-1.2</td>
<td>-19.1</td>
</tr>
<tr>
<td>1994</td>
<td>2G + c</td>
<td>75</td>
<td>2</td>
<td></td>
<td></td>
<td>15</td>
<td>1993</td>
<td>23</td>
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<td>-11.8</td>
</tr>
<tr>
<td>1994</td>
<td>2G + c</td>
<td>76</td>
<td>2</td>
<td></td>
<td></td>
<td>17</td>
<td>1994</td>
<td>23</td>
<td>-6.0</td>
<td>-26.8</td>
</tr>
<tr>
<td>1973</td>
<td>IC</td>
<td>58</td>
<td>15</td>
<td></td>
<td></td>
<td>55</td>
<td>1970</td>
<td>12</td>
<td>-2.7</td>
<td>-18</td>
</tr>
</tbody>
</table>

* c indicates otolith core samples of mature fish originally analyzed in the development of the reference curve.

Ages ranged from 12 to 27 with most falling between 21-24.
Figure 8. Age bias plots of minimum core age from $^{14}$C and a) otolith section age; b) left otolith whole age.

- Minimum core ages over-estimated the whole otolith age by 3-11 yrs and section ages by 1-15 yrs.
- Maximum observed $^{14}$C age was 27 and for whole and section ages it was 20.

Figure 9. Length and minimum age for samples used for reference curve (red) and validation (green). The reference sample ages are based on the whole otolith method while the ages for the validation samples are minimum ages based on the $^{14}$C assay (Table 3). One of the validation samples was an 85 cm female aged at 12 years (open circle), however, this is likely an under-estimate.

Conclusions

- The whole otolith method underestimates the true age of Greenland halibut.
- The section method did not produce the results we expected. It was difficult to determine annuli with confidence and ages tended to be lower than the $^{14}$C age.
- Growth for Greenland halibut in the size range of 55 cm to 70 cm is 2-3 cm/yr.

Acknowledgements

John Babaluk at Fisheries and Oceans Canada, Winnipeg, encouraged us to initiate an age validation project for Greenland halibut and provided advice for the use of the chemical marker oxytetracycline. Ray Bowering and Randy Burry at Fisheries and Oceans Canada, St. John’s and Ole Jørgensen and Claus Simonsen at the Greenland Institute of Natural Resources provided the historic samples necessary to carry out $^{14}$C validation. We thank Linda Marks, Jill Moore, and Warren Joyce for their expert technical assistance. Radiocarbon assays were carried out by NOSAMS under NSF Cooperative Agreement OCE-9807266, and by Beta Analytic Inc. Funding was provided by the Nunavut Wildlife Management Board and Fisheries and Oceans Canada-Nunavut Implementation Fund to MAT with additional funding provided by NSF OPP-9985884 to SEC and CMJ.

Thank You!
Greenland Halibut Ageing Exchange 2005-2006
Prepared by
Karen Dwyer

1997 Exchange

- Last exchange carried out in 1997 (Anon., 1997)
- Percent agreement was low between readers
- Bias also present
- Baked whole otoliths were recommended as the method of best resolution

2005-2006 Exchange

- 100 pairs of otoliths/scales collected from fish ranging in length from 15-57 cm in NAFO Div. 3M (EU annual survey – July 2005) (Thanks Ricardo!)
- Otoliths stored in vials with water
- Scales (removed from dorsal area) placed in envelopes

2005-2006 Exchange

- Significant differences in paired t-tests between most readers (with the exception of the two Russian age readers)

2005-2006 Exchange

- Age bias plots more useful
  - Bias detected between almost all readers with the exception of Can1 and Spa1; Can1 and Por1 and Rus1 and Rus2
Appendix XVII

2005-2006 Exchange

- Age bias plots more useful
  - Can2 and Gre1 aged fish older than other readers
  - Can1, Spa1 and Rus1 and Rus2 aged fish younger than other readers

2005-2006 Exchange

- Age bias plots more useful
  - Rus1 and Rus2 - no bias

20045-2006 Exchange Scales

- Age bias plots more useful
  - Rus1 scales and Rus1 otoliths no bias (slight underageing of scales)

2005-2006 Exchange Scales

- Age bias plots more useful
  - Rus1 scales and Can3 scales - bias
  - Extreme underageing of Rus1 scale ages compared to Can3 scale ages
  - Can3 scale ages were older than all other ages across the entire age range

2005-2006 Exchange Scales

- Large difference in scale methods (top from PINRO, bottom from FWI)
- Exchange Sample #39, 41 cm fish aged 5 years (PINRO top) versus 11 years (FWI bottom)
- Scales from different parts of the fish may be different ages

Comparison of structures

Exchange sample #40 - 40 cm in length

Whole otolith - all aged 5 years old

Can3 scale method - 9 years old

Rus1 scale method - 4 years old
Appendix XVII

Exchange sample #67 - 27 cm in length
Most aged either 3 or 4 years old

Can3 scale age - 8 years old

Rus1 scale age - 3 years old
Appendix XVIII

Preparation and Interpretation of Greenland Halibut Otoliths: Preliminary Observations and Considerations

John Casselman and Rob Slapkauskas
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casselmj@biology.queensu.ca
February 2006

To be considered

- Examination: morphology and orientation
- Preparation: embedding
- Thin sectioning: orientation and surface structure
- Grinding and Polishing: to enhance optical zonation
- Viewing: to enhance optical zonation
- Interpretation: in relation to region and zonation

GREENLAND HALIBUT OTOLITHS
General morphology to be considered

Lateral view, left otolith
Convex side, left otolith
Convex side, right otolith

Fig. 2: a) Image of whole otolith showing sections used in 14C study (C=Core) and b) image of section showing age reading zones (left “arm”, “short” and right “arm”).

Fig. 1: A) Top view of the edge of the otolith section. B) Cross-section of the otolith section. C) Cross-section of the otolith section showing lamellar structure. D) Cross-section of the otolith section showing lamellar structure.

Sectioning

Concave or Proximal Side
Appendix XVIII

**GREENLAND HALIBUT OTOLITHS**
Transverse section at right angles (90°) to the long axis - (numbers indicate otolith sample)

1. 3
2. 2
3. 21
4. 4b
5. 6
6. 5

**OTOLITHS OF GREENLAND HALIBUT – 9**
Transverse section indicating oblique angle (84°)

**OTOLITHS OF GREENLAND HALIBUT – 11**
Transverse section indicating oblique angle (82°)

**OTOLITHS OF GREENLAND HALIBUT – 8**
Transverse section indicating oblique angle (80°)
Appendix XVIII

Some observations

- Sectioning plane: transverse, generally at right angles to the long axis
- Through the nucleus: located from the convex side
- Through the dome: located on the concave side
- Adjusting for an oblique angle: transverse section is probably not best satisfied when applied at right angles to the long axis but should be more precisely located through the nucleus and the maximum radius of the dome – 7.5 ± 3.4 degrees off the right angle to the long axis; a slightly oblique angle

- Sectioned thickness: highly polished thin transverse slightly oblique angled sections (325 ± 20 microns), less preparation required if liquid immersion is used
- Zonation: zonation should not shift or move appreciably when focusing up and down; provides evidence about the orientation of the zonation in the section, best if at right angles to the section
- Slopes of the dome: provide best regions for interpretation, particularly if they are convex; dome and side slopes of dome provide evidence about growth radii

A detailed objective study with quantitative measures could provide necessary insights
Appendix XVIII

Discussion of Image Preparation and Interpretation Techniques

Images from workshop presentation
“Age interpretation for Halibut Otoliths Thin Sectioning vs Acetate Replication”
Rob Perry

Section Images

Acetate
13.5 years

Section
14.0 years

Acetate
Mean 31 years

Section
Mean 26 years

Replicate images

Section
Mean age 15

Acetate
Mean age 14
Appendix XVIII

Age Bias Plot Comparing all Sectioned and Acetate Ages

Paired t-test results
No overall difference between techniques
N = 434 pairs, t = 0.128, P = 0.899.

Arbitrary sub-groups based on acetate ages

Group 1
Age 1 to 20

Group 2
Age 21 to 40
Some comments concerning age interpretation of calcified structures of Greenland halibut provided by Dr. John M. Casselman following the meeting, March 2006.

For many species, scale and whole otolith interpretations as currently practiced significantly underestimate age of slow-growing and old fish. Many northern stocks of Greenland halibut seem to have some old individuals, and the species appears to be generally slow-growing. Under these circumstances, scales would not provide an accurate age and, indeed, if any other species are indication, scales may provide only half the true age for older fish. Also, scales, and to some extent whole otoliths, annually produce multiple checks and zones prior to maturity that can be misinterpreted as annuli. Under these conditions, age might be slightly overestimated. But for most species, once maturity is reached, the underestimation increases quite significantly, to the point where unless interpretation procedures are validated, they could result in considerable age underestimation bias and error.

If scale and whole otoliths are being used with unrefined interpretation procedures and are not validated, the resulting ages should not be used for stock management. Underestimations of age could provide erroneous impressions about the levels of exploitation that a stock can sustain. And quite importantly, fishing up of old individuals could occur and not even be detected.

If the fish are quite young (immature), these structures could provide accurate age. But unless validated, uncertainty exists. The collection of calcified structures should continue even if age assessment as currently practiced is discontinued. And new preparation and interpretation procedures should be developed as soon as possible. I am convinced, after examining some structures and participating in the workshop, that accurate age assessment of this species is possible.

Whole otolith procedures can provide valid age assessments. However, they usually require considerable refinement. Some new approaches were suggested at the workshop. Scales also might provide accurate age interpretations but would require more refined procedures than those usually used.

More marking studies should be initiated. The real advantage of labeling is that it provides considerable insights about structure growth. A good comparative study would also be helpful to examine various interpretation procedures and should include partly known-age samples (tag-recapture with labeling). “Blind” replication should also be conducted as part of the comparison.

Several otolith methods were discussed at the workshop that are more refined and seem feasible. These procedures would be quite different from those currently practiced. Generally, the calcified structures of this species are very difficult to interpret, probably because the fish live in deep water, grow at low temperature, and are quite slow-growing. There is no doubt that new interpretation procedures are required, but these should be carefully reviewed and tested before they are implemented.

Several techniques seem to have potential. 1) Use the right otolith with a new whole-otolith technique with clearing and incorporating image enhancement. As suggested in the workshop, this would include some procedure to retain translucency (freezing in otolithic fluid was suggested) or to clear the otolith. 2) Use the left otolith and a lateral grind and polishing technique on the convex side, concentrating on the anterior (?), using a grinding procedure applies a tilting pressure. A similar grinding and tilting procedure has been successfully used on old and slow-growing Arctic salmonids. 3) This same left otolith could then be cross-sectioned at an oblique angle through the nodule, or dome. This method requires some additional research, and the orientation of the sectioning technique might also consider a longitudinal, rather than a transverse, section through the nodule. Regardless of the method, the aim would be to section the nodule so that most of the optical zonation would be at right angles to the appositional growth.

These three methods could be used on the two otoliths of the same fish. The grinding and polishing of the left otolith does not interfere with the recommended cross-sectioning method, because the nodule develops on the concave side opposite to the convex surface that would be ground and polished.

I believe that otolith age interpretation procedures can be developed that will provide accurate age and reliable growth data for Greenland halibut. But the procedure must be more refined in this species than in most others. Otoliths of this species are very difficult to interpret, indeed even to prepare for interpretation. I believe that sectioning offers the greatest potential, but this remains to be seen through a proper comparative study.
In 1995, NAFO and ICES sponsored a successful symposium on the ecological role of marine mammals. This follow-up symposium will present new findings on the synthesises of information over ecosystem components, on biological and physical aspects of the environment, and on new research approaches to understanding the role of marine mammals.

Four sessions are planned:

- Biological and environmental factors affecting life history traits
- Foraging strategies and energetic requirements
- Theoretical considerations on apex predators and multispecies models
- Marine mammal - fisheries interactions

Contributed oral and poster presentations are welcome. Abstracts should be submitted by 1 May 2008. Final papers should be submitted by 30 November 2008 and will follow a peer-review process for publication in the Journal of Northwest Atlantic Fishery Science. Participants who are not giving presentations must register by 1 September 2008.
Early Stages of Fishes in the Western North Atlantic Ocean

by Michael P. Fahay

A hardcover two volume set containing over 1500 pages $120 CDN

This comprehensive scientific publication is the only up-to-date textbook providing detailed descriptions and accurate drawings of the early life-history stages of the fishes from the Northwest Atlantic Ocean north of 35°N and west of 40°W. The region covers the world’s most famous fishing grounds and includes the Davis Strait, southern Greenland, Flemish Cap, Georges Bank, northern Sargasso Sea and Middle Atlantic Bight to Cape Hatteras. This beautifully produced and published work includes:

- A checklist of 1075 fish species occurring in the study area
- Descriptions of egg, larval and juvenile stages of 760 species from 196 families
- Synopses of habitats from estuarine to abyssal
- Updated ranges and many species’ range extensions, often based on early stages
- Identification facilitated by numerous descriptive tables
- Morphological characters of developmental stages summarized and tabulated for 28 orders of teleosts, 15 suborders of Perciformes, 26 families of Percoidei and several other major groups
- Appendices with tabulations of meristic characters, museum reference material sources and collection data for original material
- Some 3000 drawings of eggs, larvae and juveniles and 2000 references
Review of Michael Fahay's 2007 monograph
"Early Stages of Fishes in the Western North Atlantic Ocean"

THE HISTORY

In the 1880s, naturalists such as Goode and Bean in the NW Atlantic, and Dannevig, Hjort, Schmidt, McIntosh and Prince in European waters, began to unravel the mysteries of the early development of fishes. There was ongoing conjecture surrounding the survival processes operating during the planktonic phase that generate the enormous and unpredictable variability in year-class strength. In 1914 Johan Hjort introduced the concept of the 'critical phase' in the early life history of fishes. Interest has inevitably surrounded the commercial species and it was as long ago as 1882 that fishermen joined the debate. The Scottish marine biologist William McIntosh was commissioned to study the effects of bottom trawling on the livelihoods of line fishermen. He incubated the reproductive products of captive fish and discovered that the young stages of fish, such as cod, haddock, whiting and lemon sole, were planktonic and hence not threatened by bottom trawling. This enraged the Scottish line fishermen who demonstrated outside his home and burned his effigy! Their actions heralded the first major confrontation between the fishing industry and scientists. For over a century, popular interest and scientific debate have embraced this fascinating world of the ichthyoplankton. In more recent times, fish quotas, growth and recruitment overfishing, species recovery programmes and global warming, have made the study of fish ontogeny even more relevant to our needs.

Survival of the early life history stages of fish and the subsequent strength of each year-class is fundamental knowledge in the management of commercial fish species. As a consequence, understanding the mechanisms which generate annual variability has attracted a considerable body of scientific investigation. Underpinning the biological and physiological research, is a comprehensive knowledge of their ontogeny. Accumulation of such knowledge has progressed steadily on both sides of the Atlantic to the point where the development of most of the commercially important species, plus many others, is now fairly well described. In 1976, F. S. Russell produced the first comprehensive work on the early life history of fishes. Interest has inevitably surrounded the specific study area. In this context there are many species, each species ensuring that this work has relevance to researchers outside the specific study area. In this context there are many species, particularly deeper water Maurolicus, Argentina, Argyropelecus, Maurolicus, Myctophids, Macourids, and many others, which occur on both sides of the North Atlantic.

Michael Fahay's professional interest in the early life histories of fishes started in 1965. He published an illustrated atlas of fish eggs and larvae in 1983, and recently spent three years expanding and updating this classic work. Michael retired in June 2006 and is currently co-authoring a book on temperate estuarine fishes.

THE REVIEW

Michael Fahay's current contribution represents a further major step forward in synthesising all our current knowledge of fish ontogeny in the western North Atlantic. It is a superbly presented monograph in two large volumes but it still comes as a surprise that the 760 species from 196 families described only represents about 70% of the adult species known to occur in the area. Since his original monograph, the study area has been extended and now covers the area from the Northern Sargasso Sea to the Davis Strait and east to 40° W. The work represents many hours of painstaking research and meticulous attention to illustrative detail. Michael generously acknowledges the help, in various ways, of his colleagues and in particular the skills of the larval fish illustrators whose descriptions of various stages he has been able to use. Those acknowledgements in no way detract from the huge contribution of the author in bringing together all of this information in such a readily accessible and usable form. I found that the summary information, on the essential characteristics of each order of fishes, including meristic characters, presented in tables, to be particularly valuable. The glossary of terms used is extensive and well complemented by clear explanatory figures. The individual species descriptions are a pleasure to read with relevant, useful and consistently clearly presented information. As he rightly points out, the proper identification and description of larval fishes is dependant on ontogenetic series rather than characteristics of individual specimens. It is particularly valuable to be given the full distribution range of each species ensuring that this work has relevance to researchers outside the specific study area. In this context there are many species, particularly deeper water Alepocephalus, Argentinus, Argyropelecus, Maurolicus, Myctophids, Macourids, and many others, which occur on both sides of the North Atlantic.

These two excellent volumes are a ‘must’ on the book shelves of all professional marine biologists on both sides of the North Atlantic. They will also prove to be of great interest to all keen amateurs who have the good fortune to be able to dip into the fascinating world of plankton and in particular ichthyoplankton.

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The Journal provides an international forum for the primary publication of original research papers on fisheries science in the Northwest Atlantic, with emphasis on environmental, biological, ecological and fishery aspects of the living marine resources and ecosystems. (Scientific publications during ICNAF times during 1949–79 are available at the Secretariat).

Vol. 6, No. 1, 2 – Miscellaneous papers, (17), June and December 1985, 179 pp.
Vol. 9 – Miscellaneous papers, (13), September and December 1989, 159 pp.
Vol. 27 – Symposium papers (22) (1 Note), Pandalid Shrimp Fisheries – Science and Management at the Millennium, December 2000, 289 pp.
NAFO Scientific Council Studies

This publication includes papers of topical interest and importance to the current and future activities of the Scientific Council.

No. 3 – Miscellaneous papers, (8), April 1982, 82 pp.
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NAFO Scientific Council Reports

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NAFO Statistical Bulletin

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Inventory of Sampling Data

This publication replaced ICNAF Inventory of Sampling Data 1967–1978 which was completed in 1986.

Inventory of Sampling Data 1979–1984, Published April 1989, 250 pp.

NAFO Index of Meeting Documents

This publication contains lists of all documents along with a subject and author index of the NAFO Scientific Council documents issued during 5-year periods.

1979–84 – Index of Meeting Documents, Published March 1985, 146 pp.
Information for Preparing Manuscripts for NAFO Scientific Publications

Journal of Northwest Atlantic Fishery Science

The Journal is for the primary publication of original practical and theoretical research that is unpublished and is not being submitted for publication elsewhere. While it is intended to be regional in scope, papers of general applicability and methodology may be considered. Space is also provided for notes, letters to the editor and notices. Each paper is assigned to an Associate Editor of the Journal's Editorial Board, and is normally reviewed by two referees regarding suitability as a primary publication.

NAFO Scientific Council Studies

The Studies publishes papers which are of topical interest and importance to the current and future activities of the Scientific Council, but which do not meet the high standards or general applicability required by the Journal. Such papers have usually been presented as research documents at Scientific Council meetings and nominated for publication by the Standing Committee on Publications. Studies papers are not peer reviewed.

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The paper should be in English. The sequence should be: Title, Abstract, Text, References, Tables and Figures.

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The paper should start with the title, followed by the name(s), address(es) and emails of the author(s) including professional affiliation, and any related footnotes.

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An informative concise abstract should be provided along with key words listed alphabetically.

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Materials and Methods should describe in sufficient detail the materials and methods used, so as to enable other scientists to evaluate or replicate the work.

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All Tables and Figures must be cited in the text. Tables and Figures must be numbered consecutively and correspond with the order of presentation in the text. Figure captions should be included as a separate page. Each table and figure should have a complete concise descriptive caption. Figures should always be submitted in black and white. Colour plots and photographs are acceptable only if colour is essential to the content. Preferably, all figures should be submitted as separate files in .eps or .ps format. Photographs, maps and contour plots can also be submitted in high quality .jpg format.

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