

Northwest Atlantic



Fisheries Organization

Serial No. N5276

NAFO SCR Doc. 06/49

## SCIENTIFIC COUNCIL MEETING – JUNE 2002

### Report of the Greenland Halibut (*Reinhardtius hippoglossoides*) Age Determination Workshop

Editors

Margaret A. Treble

Fisheries and Oceans Canada, Freshwater Institute,  
501 University Cres., Winnipeg, Manitoba, Canada R3T 2N6

Karen S. Dwyer

Northwest Atlantic Fisheries Science Center, Fisheries and Oceans Canada,  
P.O. Box 5667, St. John's, Newfoundland and Labrador, Canada, A1C 5X1

### 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 were using 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 bias plot of whole versus section ages 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. Greenland halibut have a larger size at maturity (40 cm for males and 60 cm for females) which is typical of many long-lived species. 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.

### 1. 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.

### 1.1 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 they had 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 now 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:

- Review and evaluate various methodologies used by member states to determine age of Greenland halibut.
- Present and discuss results of pre-workshop whole otolith exchange amongst member states.
- Consider results of recent validation studies to determine if ageing work should continue for Greenland halibut and if so, develop guidelines.
- 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.
- 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.

### 1.2 Participants (see Appendix II)

Karen Dwyer	Northwest Atlantic Fisheries Center, St. John's (Co-convener)
Brian Greene	Northwest Atlantic Fisheries Center, St. John's
Randy Burry	Northwest Atlantic Fisheries Center, St. John's
Margaret Treble	Freshwater Institute, Winnipeg (Co-convener)
Rick Wastle	Freshwater Institute, Winnipeg
Rob Perry	Department of Environment and Conservation, Corner Brook
Brian Healey	Northwest Atlantic Fisheries Center, St. John's
Esther Román	Instituto Español de Oceanografía, Vigo
Ricardo Alpoim	Instituto Nacional de Investigação Agrária e das Pescas, Lisbon
Delsa Anderl	NOAA Fisheries Alaska Research Center, Seattle
Jake Gregg	U.S.G.S. Western Fisheries Research Center, Nordland
Ole Thomas Albert	Institute of Marine Research, Tromsø
Merete Kvalsund	Institute of Marine Research, Bergen
Lars Heilmann	Greenland Institute of Natural Resources, Nuuk
Kaj Sünksen	Greenland Institute of Natural Resources, Nuuk
John Casselman	Queen's University Department of Biology, Kingston
Shayne Maclellan	Pacific Biological Station, Nanaimo
Taras Igashov	PINRO, Murmansk*

\*T. Igashov participated in the otolith and scale exchange prior to the workshop and prepared a report on the methods used at the Knipovich Polar Research Institute of Marine Fisheries and Oceanography (PINRO) but was not able to participate during the workshop.

### 1.3 Standardization of Age Reading Terminology

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

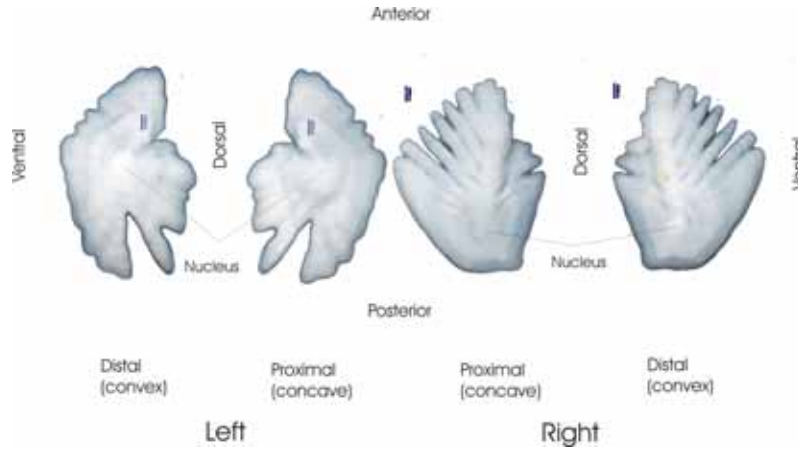


Fig. 1. Left and right Greenland halibut otoliths (from Esther Roman, this workshop). The reference bar on each photo measures 1000  $\mu\text{m}$ .

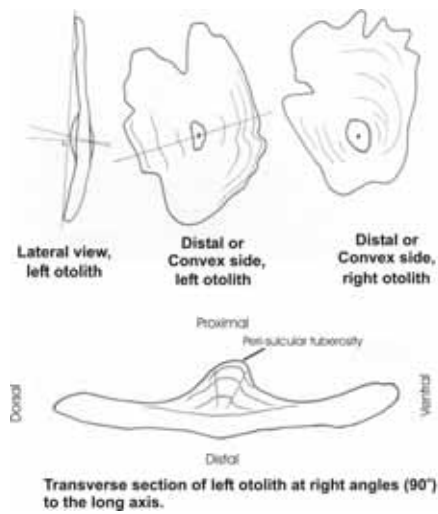


Fig. 2. Line drawings of the proximal side of Greenland halibut sagittae and a transverse section of the left sagittae (from Casselman and Slapkauskas this workshop).



Fig. 3. Lateral view the left sagittae clearly showing the concave shape and location of the peri-sulcular tuberosity on the proximal side (from Jacob Gregg this workshop).

### Glossary of Terms

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 (ie. 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-ageing 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.

## **2.0 Review of Ageing Methodology and Research at each Lab**

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.

### **2.1 Whole Otolith Methods**

Five labs gave presentations on the whole otolith method: Northwest Atlantic Fisheries Centre (NWAFC-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.

NWAFC-Canada-Prepared by K. Dwyer, R. Burry and B. Greene (Appendix III):

At the NWAFC 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. The alcohol is less dense than water and so debris tends to fall to the bottom allowing for more otoliths to be viewed before having to change the solution. 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. Bowring and Nedreas (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 5-8cm 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 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.

## 2.1 Otolith Section 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 Cornerbrook, Newfoundland and Labrador (DEC-Canada).

### FWI-Canada-Prepared by M. Treble and R. Wastle (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 max 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 they have been investigating section methods. 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 and so a single transverse cross-section was chosen instead. The 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 this 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 of 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. Increment 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.*, in press).

Three trials were conducted to test this new method. Trial 1 examined otoliths from 93 fish with mean length 40cm, Trial 2 examined 226 fish with mean length 75cm, and Trial 3 examined 75 fish with mean length 37cm. 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 for the cross-section and 4.15 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,  $M$ =0.18 and for the Atlantic,  $M$ =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.* in press). 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 tubercosity 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 S. MacLellan, Supervisor of the Fish Ageing Lab at 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. Annuli 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 NWAFC 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, 1200). 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]. So 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 Group2, 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 Friedman 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.

### 2.3 Scale Methods

There were two presentations on scale methods, one from the Knipovich Polar Research Institute of Marine Fisheries and Oceanography (PINRO-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 Krzykawsky (1975 and 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. They found the structure 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. 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.

### 2.4 Results of the Whole Otolith and Scale Exchange Held Prior to the Workshop

At the end of the first day K. Dwyer from NWAFC presented results of an exchange of age materials (otoliths and scales) amongst several of the labs that assess Greenland halibut ages in the Northwest Atlantic (Appendix XVI). In the previous exchange carried out in 1997 as part of the Icelandic Ageing workshop they found bias between readers and noted that percent agreement was low. A baked whole otolith method was recommended following discussions at the Iceland workshop. However, not many labs changed their methods. No progress has been made on improving precision or bias in age determinations since then. For the 2004/05 exchange 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.

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. Rus1 used the PINRO scale method described above and Can3 used the FWI scale method described above.

There were very few “consensus” ages. Ages estimated for most samples by readers 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  $\pm 1$  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.

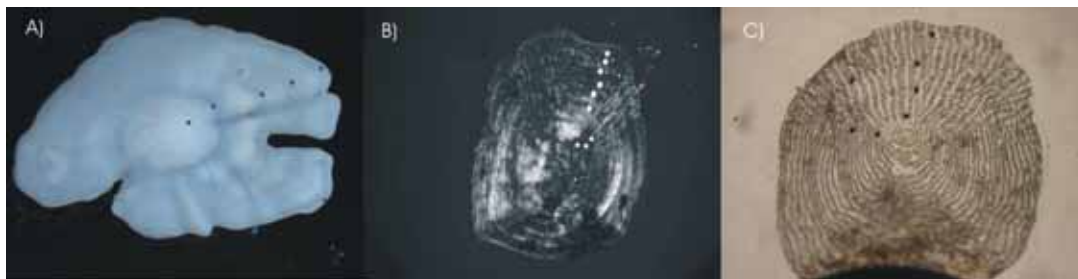


Fig. 4. Comparison of age determination structures from a 40 cm fish: A) whole otolith method, all readers age 5 years; B) Can3 scale method, age 9 years; C) Rus1 scale method, 4 years.

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 are used to using. These otoliths were non-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 about 60-70 cm (sexual maturity), growth is hypothesized to slow and therefore the whole otolith method would be expected to fail.

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 should be explored for this species.

## **2.5 Group Age Determination and General Discussion**

We ended the first day with the group ageing a set of 8 whole otolith images that were projected on the screen at the front of the room. 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.

## **2.6 Brief Discussion on 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 vertical 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 the young fish are also found on the shelves with larger fish found as you go to deeper depths. Greenland halibut are generally a cold water fish, found in the arctic waters (e.g.  $-0.3^{\circ}\text{C}$  in portions of Baffin Bay) as well as warmer waters of the Atlantic (e.g.  $7.0^{\circ}\text{C}$  along portions of the Grand Bank and Flemish Cap). It was noted that the Spanish long-line survey in Subarea 3 went down to 3000 m but did not find any Greenland halibut beyond 2200m (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 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. Another comment was that based on observations from other long-lived species, if Greenland halibut are maturing at between 8-12 years (60-70cm) then this species is likely one that is long-lived.

## **2.7 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 collect images and document age determination methods and criteria in reference manuals. 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.

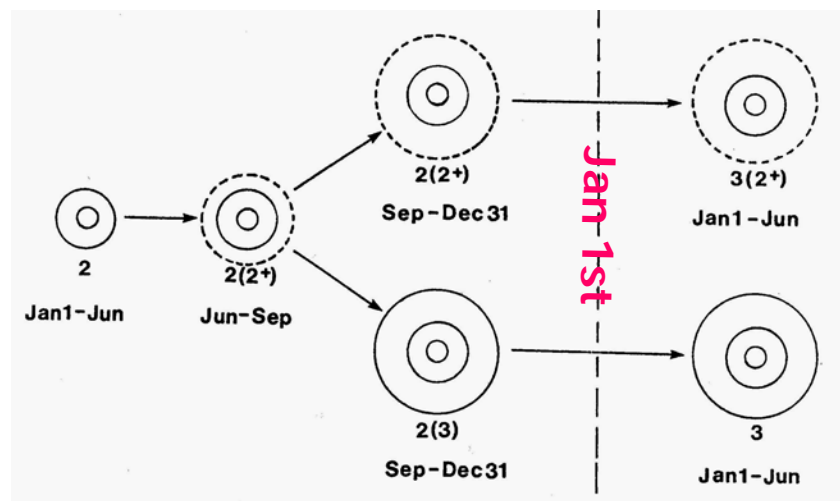


Fig. 5. Age designation system, international birthday of January 1<sup>st</sup>, used for marine fish at the Fisheries and Oceans Canada, Pacific Biological Station (from Shayne MacLellan).

## 2.8 Age Validation

Research into age validation for Greenland halibut has been conducted by FWI in collaboration with Dr. S. Campana from Bedford Institute of Oceanography, Fisheries and Oceans Canada, Dartmouth, Nova Scotia, Canada, Dr. C. Jones from the Center for Quantitative Fisheries Ecology at Old Dominion University, Norfolk Virginia, U.S.A. and J. Boje from the Danish Institute for Fisheries Research, Charlottenlund, Denmark (Appendix XVII and Treble et al. 2005). Two types of validation methods were applied, Oxytetracycline (OTC) and <sup>14</sup>C 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  $^{14}\text{C}$  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  $^{14}\text{C}$  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  $^{14}\text{C}$  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 ( $^{14}\text{C}$  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 that we used did not produce the results expected as it was difficult to determine annuli with confidence and ages tended to be lower than the  $^{14}\text{C}$  validation ages. Growth in Greenland halibut in the size range of 55 to 70 cm is in the order of 2-3 cm/yr.

## 2.9 Group Age Determination and Discussion

Day two ended and day three began with group ageing and discussion. K. Dwyer (NWAFC) 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  $2.0 (\pm 0.5)$  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  $^{14}\text{C}$  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.

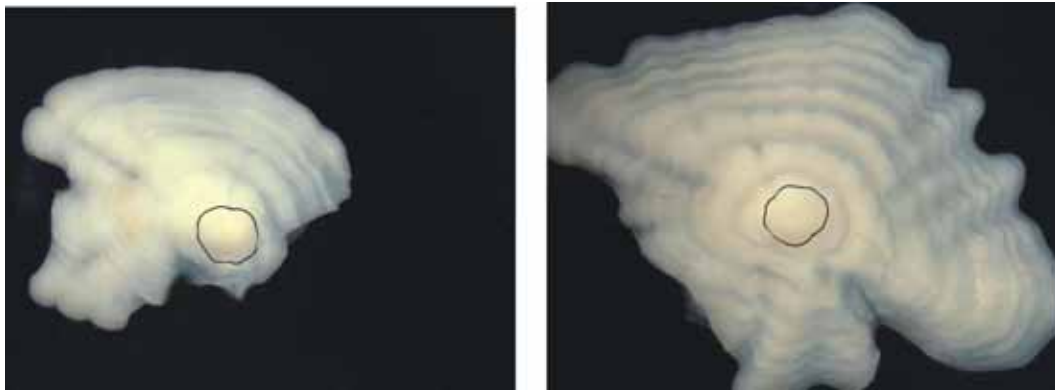


Fig. 6. Outline of 0+ (7 cm) otolith superimposed over images of larger otoliths illustrates how the first annulus may be mis-interpreted if a 0+ reference size has not been determined for a particular stock.

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.

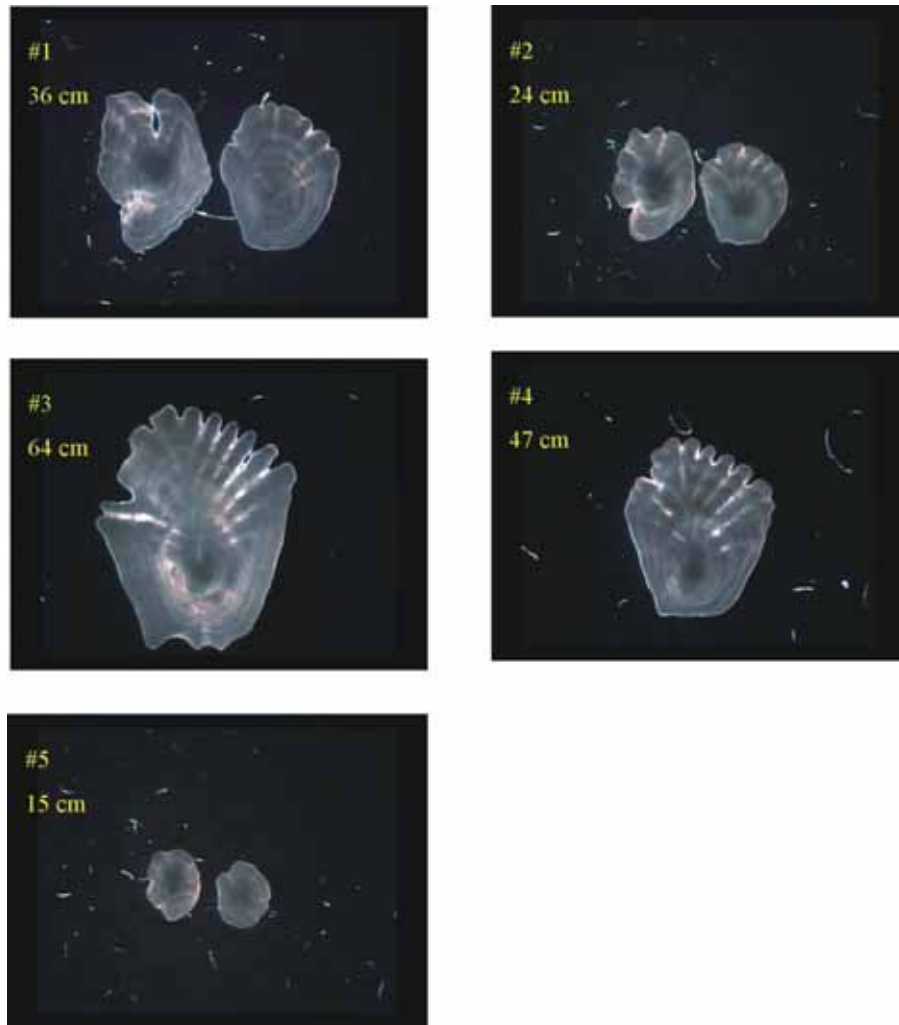


Fig. 7. Images of Greenland halibut otoliths prepared using the GINR whole otolith method.

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.



Fig. 8. Images of Greenland halibut otoliths prepared using the IMR refined method. Image #1 was from sample 2004805MarTN84004\_1\_15, #2 from sample \_1\_05 and #3 from sample \_1\_01.

## 2.10 Queen's University Assessment of Greenland Halibut Otoliths and Age Determination

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.

You can get the best insight into otolith growth 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  $325 \pm 20$  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 ( $-7.5 \pm 3.4$  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.

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).

A detailed objective study with quantitative measures could provide necessary insights into section orientation and zonation interpretation.

It is important to measure precision within readers and to get that minimized before trying to compare between readers. You need to have some level of validation for a method or when you start comparing between methods and readers you get into arguments as to who is right or wrong with no reference.

## **2.11 Group Age Determination and Discussion**

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).



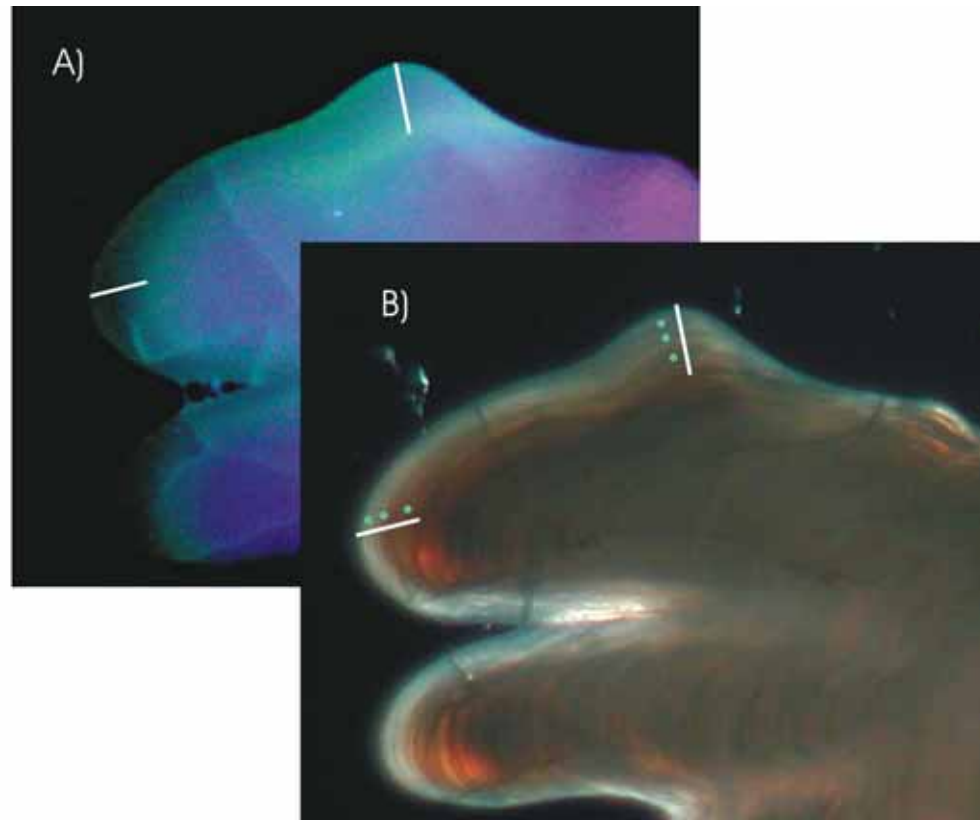


Fig. 9. A portion of the right otolith of a Greenland halibut re-captured in Cumberland Sound, Canada, 3 years and 10 months since marking with OTC. The first image shows the location of the OTC mark (pale green band) and the second image is of the same area taken using transmitted light. Green circles indicate the possible location of annuli beyond the mark.

Whole otoliths and their corresponding section images were selected from the samples we had looked at on the first day. Three of the four otolith samples that we 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.

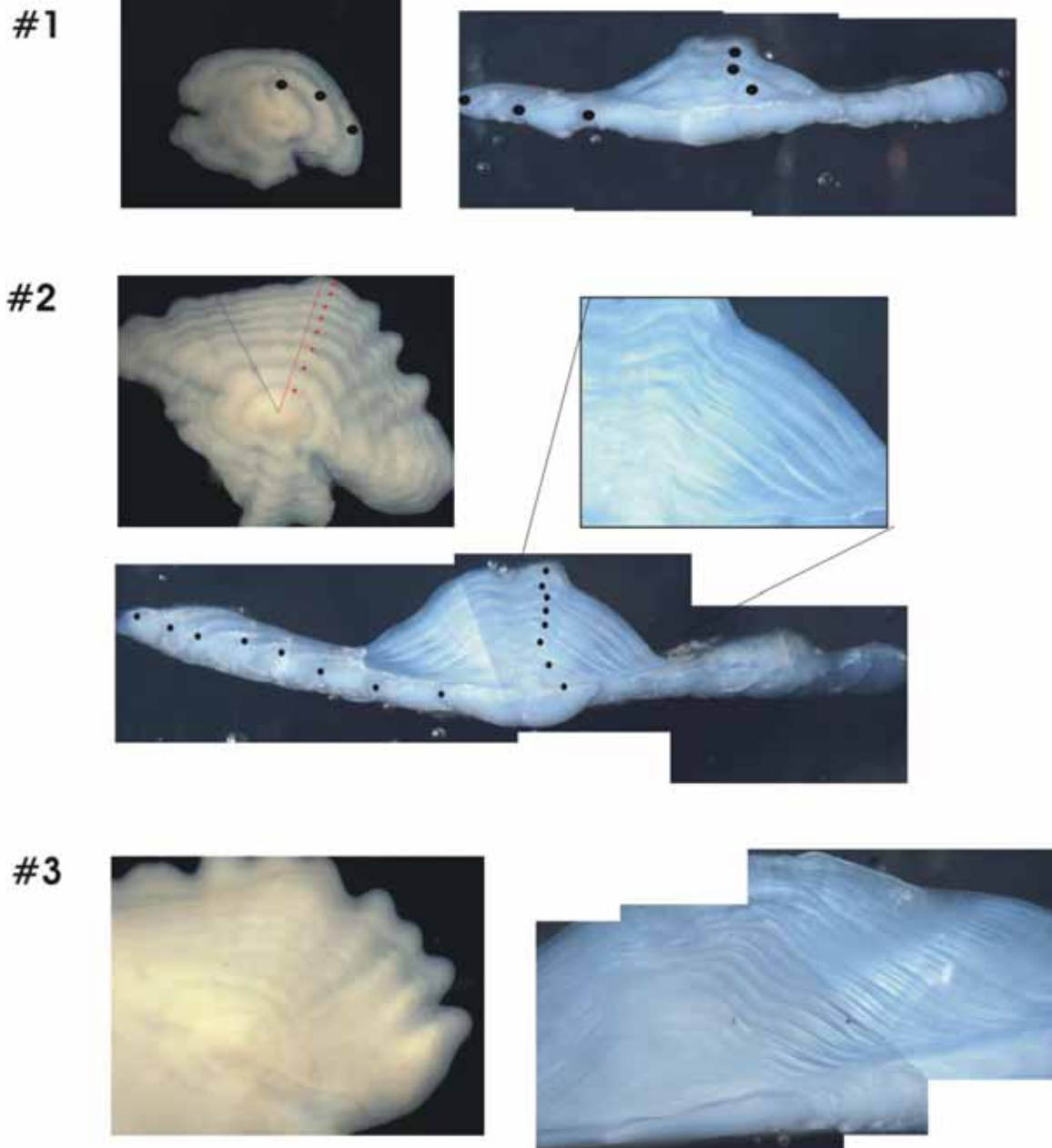


Fig. 10. Three samples from the set of otoliths that we had aged whole as a group the first day. Technicians at NWAFC sectioned them during the workshop: #1 - 30 cm female caught during the fall, surface age=3 years; #2 - 57 cm female caught during the fall, surface age=8 years; #3 - 101 cm female caught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

## 2.12 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 ( $C^{14}$ ) 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 (INIAP/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.

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 and Recommendations

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 morphometry 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.

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 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

- Bowering, W. and K. Nedreaas. 2001.
- Cooper, D.W., K.E. Pearson, and D.R. Gunderson. (submitted) Annual fecundity and an indirect estimate of natural mortality rate for *Reinhardtius hippoglossoides* (Greenland turbot or Greenland halibut). *Fishery Bulletin*.
- De Cárdenas, E., CASAS, J. M., ALPOIM, R., MURUA, H., 1996. Preliminary Results of the European Long-line Survey in the NAFO Regulatory Area. *NAFO SCR Doc.* 96/34, Ser. No N2709, 6p.
- Fenton, G.E., S.A. Short, and D.A. Ritz. 1991. Age determination of orange roughy, *Hoplostethus atlanticus* (Pisces: Trachichthyidae) using  $^{210}\text{Pb}$ :  $^{226}\text{Ra}$  disequilibria. *Marine Biology* 109: 197-202.
- Gregg, J., D. Anderl, and D. Kimura. (in press). Improving the precision of otolith-based age estimates for Greenland halibut, *Reinhardtius hippoglossoides*, found in Alaskan waters. *Fishery Bulletin*.

- Igashov. T. 2004. Results of comparative age reading of Greenland halibut *Reinhardtius hippoglossoides* (Walbaum) by scales and otoliths. NAFO SCR. Doc. 04/7, 3 pp.
- NAFO. 2004. Northwest Atlantic Fisheries Organization Scientific Council Reports 2004. Northwest Atlantic Fisheries Organization. Dartmouth, Nova Scotia, Canada, 298 pp.
- Treble et al. 2005. An assessment of age determination methods, with age validation of Greenland halibut from the Northwest Atlantic. NAFO SCR Doc. 05/43, 22 pp.

**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 21<sup>st</sup> (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 22<sup>nd</sup> (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 23<sup>rd</sup> (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 24<sup>th</sup> (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:

Karen Dwyer	Fisheries and Oceans Canada, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, Newfoundland and Labrador, Canada, A1C 5X1. Phone: +709-772-0573 - Fax: +709-772-4188 – E-mail: <a href="mailto:DwyerK@dfo-mpo.gc.ca">DwyerK@dfo-mpo.gc.ca</a>
Brian Greene	Fisheries and Oceans Canada, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, Newfoundland and Labrador, Canada, A1C 5X1. Phone: +709-772- - Fax: +709-772-4188 – E-mail: <a href="mailto:GreeneBr@dfo-mpo.gc.ca">GreeneBr@dfo-mpo.gc.ca</a>
Randy Burry	Fisheries and Oceans Canada, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, Newfoundland and Labrador, Canada, A1C 5X1. Phone: +709-772- - Fax: +709-772-4188 – E-mail: <a href="mailto:BurryR@dfo-mpo.gc.ca">BurryR@dfo-mpo.gc.ca</a>
Brian Healey	Fisheries and Oceans Canada, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, Newfoundland and Labrador, Canada, A1C 5X1. Phone: +709-772-8674 - Fax: +709-772-4188 – E-mail: <a href="mailto:HealeyBP@dfo-mpo.gc.ca">HealeyBP@dfo-mpo.gc.ca</a>
Shayne MacLellan	Fisheries and Oceans Canada, Pacific Biological Station , 3190 Hammond Bay Rd., Nanaimo, British Columbia, Canada, V9T 6N7. Ph: +250-756-7189 or 7179 – Fax: +250-756-7053 – E-mail: <a href="mailto:MacLellanSh@pac.dfo-mpo.gc.ca">MacLellanSh@pac.dfo-mpo.gc.ca</a>
John Casselman	Queen's University, Department of Biology, 2406 Biosciences Complex, 116 Barrie St., Kingston, Ontario, Canada, K7L 3N6. Ph: +613-533-6000 ext. 75371 – Fax: +613-533-6137 – E-mail: <a href="mailto:casselmj@biology.queensu.ca">casselmj@biology.queensu.ca</a>
Margaret Treble	Fisheries and Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, Canada, R3T 2N6. Phone: +204-984-0985 – Fax: +204-984-2403 – E-mail: <a href="mailto:TrebleM@dfo-mpo.gc.ca">TrebleM@dfo-mpo.gc.ca</a>
Rick Wastle	Fisheries and Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, Canada, R3T 2N6. Phone: +204-983-5143 – Fax: +204-984-2403 – E-mail: <a href="mailto:WastleR@dfo-mpo.gc.ca">WastleR@dfo-mpo.gc.ca</a>
Rob Perry	Senior Aquatics Species Biologist, Wildlife Division, Department of Environment and Conservation, 117 Riverside Drive, Corner Brook, NL A2H 7S1. Phone: +709-637-2023 – Fax: +709-637-2004 – E-mail: <a href="mailto:RobPerry@gov.nl.ca">RobPerry@gov.nl.ca</a>
Esther Román	Instituto Español de Oceanografía. Centro Oceanográfico de Vigo. C / Cabo Estay, s/n - Canido. 36200 - Vigo (Pontevedra). Spain Phone: +00.34.986.492111 - Fax: +00.34.986.498626 – E-mail: <a href="mailto:esther.roman@vi.ieo.es">esther.roman@vi.ieo.es</a>
Ricardo Alpoim	Instituto Nacional de Investigacao Agrária e das Pescas (INIAP/IPIMAR), Av. De Brasília, 1449-006 Lisbon, Portugal. Phone: +351 21 302 7000 – Fax: +351 21 301 5948 – E-mail: <a href="mailto:ralpoim@ipimar.pt">ralpoim@ipimar.pt</a>
Delsa Anderl	NOAA Fisheries Alaska Fisheries Science Center 7600 Sand Point Way NE, Seattle, WA 98115-0070 Phone: +206-526-4218 – E-mail: <a href="mailto:Delsa.Anderl@noaa.gov">Delsa.Anderl@noaa.gov</a>

- Jacob Gregg U.S.G.S., Western Fisheries Research Center, Marrowstone Marine Field Station, 616 Marrowstone Point Road, Nordland WA, 98358 USA. Phone: +360 385 1007 ext. 223 - Fax +360 385 7207 – E-mail: [jgregg@usgs.gov](mailto:jgregg@usgs.gov)
- Ole Thomas Albert Institute of Marine Research, Pb 6404, N-9294 Tromsø, Norway. Phone: +47 7760 9736 – Fax: +47 7760 9701 – E-mail: [oleta@imr.no](mailto:oleta@imr.no)
- Merete Kvalsund Institute of Marine Research, P.O. Box 1870 Nordnes, N-5817 Bergen, Norway. Phone: +47 55236992 – Fax: +47 55238687 E-mail: [Merete.Kvalsund@imr.no](mailto:Merete.Kvalsund@imr.no)
- Lars Heilmann Greenland Institute of Natural Resources, P.O. Box 570, DK-3900, Nuuk, Greenland. Ph: +299 36 1095 – Fax: +299 36 1212 – E-mail: [Lars@natur.gl](mailto:Lars@natur.gl)
- Kaj Sünksen Greenland Institute of Natural Resources, P.O. Box 570, DK-3900, Nuuk, Greenland. Ph: +299 36 12 43 – Fax: +299 36 1212 – E-mail: [Kaj@natur.gl](mailto:Kaj@natur.gl)



Participants (top to bottom): Brian Healey, Randy Burry, Ricardo Alpoim, Ole-Thomas Albert, John Casselman, Lars Heilmann, Kaj Sünksen, Merete Kvalsund, Rick Wastle, Delsa Anderl, Margaret Treble, Brian Greene, Shayne MacLellan, Esther Román and Karen Dwyer (missing Jacob Gregg and Rob Perry).

## 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

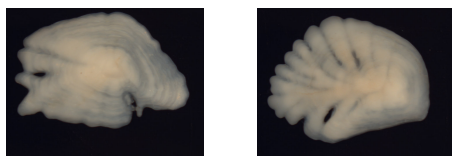
## Age Determination Methodology

- 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

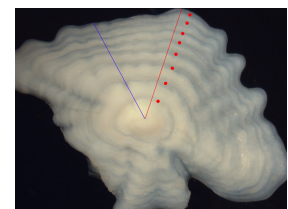
## Age Determination Methodology



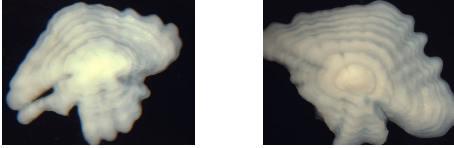
- Both left and right otoliths are used but the left otolith is preferred because nucleus is at centre with reading axes all around

## Age Determination Methodology

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

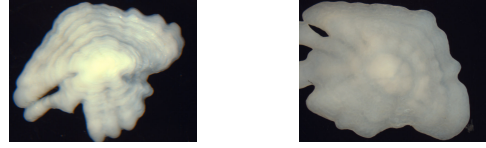


## Age Determination Methodology



- Commercial otolith versus RV otolith

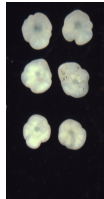
## Age Determination Methodology



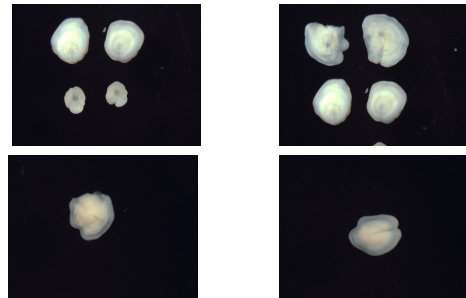
- Easily interpreted otolith versus a "bad" otolith
- Difficult species to age

## 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

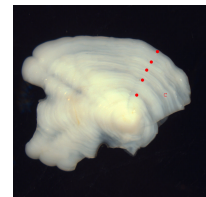


## Annuli

- 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. Alpoim method

### Whole otolith method from Portugal



Ricardo Alpoim  
Instituto Nacional de Investigação Agrária e das Pescas, INIAP/IPIMAR.  
Av. Brasília 1449-006 Lisboa, Portugal

2006

R. Alpoim

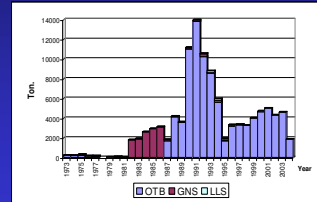
Whole otolith method from Portugal

1 / 11

### 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.



R. Alpoim

Whole otolith method from Portugal

2 / 11

### Methodology

The otoliths before burned were:

1994 - 1996 - cleaned with lixivina 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 280°C.  
1996 onwards - burned in a oven for 30 min at 200°C (Godinho and Alpoim).

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

R. Alpoim

Whole otolith method from Portugal

3 / 11

### Methodology

The otoliths were read:

at approximately 10x magnification (sometimes higher to see the edge of older otoliths),  
with reflected polarised light,  
both left and right otoliths are used,  
in most otoliths convex side up, but for the bigger ones changing between the convex and the concave side up is needed.

The otoliths after age reading are clean with alcohol and stored in envelopes.

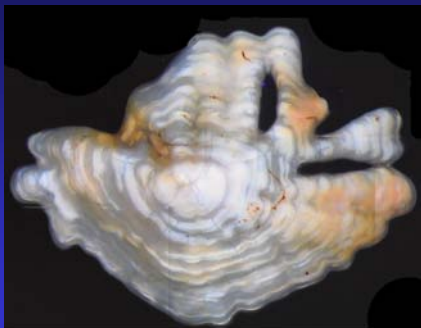
R. Alpoim

Whole otolith method from Portugal

4 / 11

### Results

Left otolith - symmetric



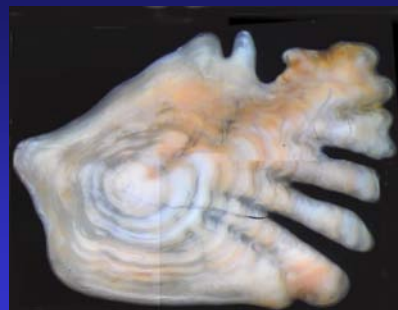
R. Alpoim

Whole otolith method from Portugal

5 / 11

### Results

Right otolith - asymmetric



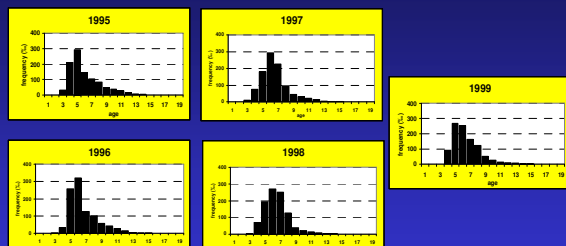
R. Alpoim

Whole otolith method from Portugal

6 / 11

## 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.

R. Alpoim

Whole otolith method from Portugal

7 / 11

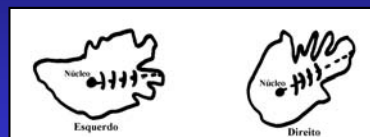
## Results

### Measures

#### OTOLITHS USED:

354 pairs from 1999

From the center of the nucleus to the end of each hyaline zone.



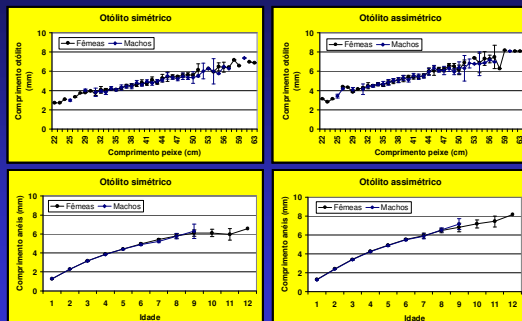
R. Alpoim

Whole otolith method from Portugal

8 / 11

## Results

### Measures



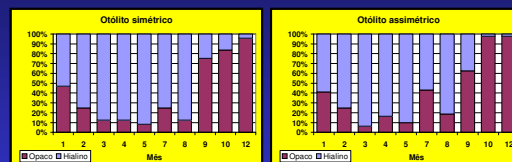
R. Alpoim

Whole otolith method from Portugal

9 / 11

## Results

### Hyaline vs Opaque (1999)



R. Alpoim

Whole otolith method from Portugal

10 / 11

## 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.

R. Alpoim

Whole otolith method from Portugal

11 / 11



Spanish Institute of Oceanography  
VIGO


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

## Age determination methodology of Greenland halibut

Whole otolith method from Spain

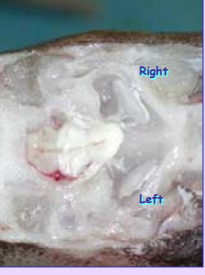
Esther Román

### 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.

### Collection and storage of age samples



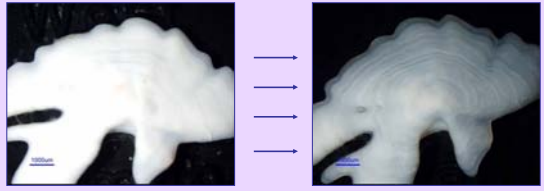
The sagitta otoliths are removed by dissection of the head of the fish and stored dry in paper envelopes.

The following information is recorder for each specimen sampled: length, sex, maturity, weight, vessel name, haul, etc.

### 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 45-60 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 (\*)

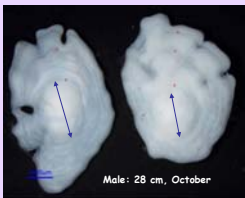
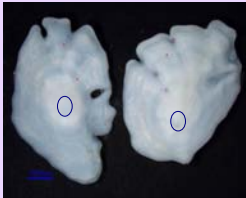





**Reading Procedure**

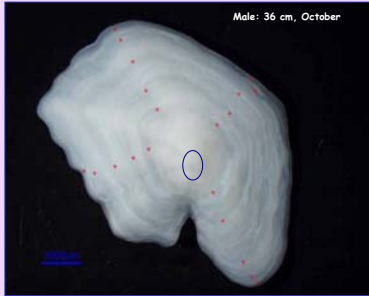
Although, the left sacculus otolith is more suitable for age reading because it is generally more uniform in shape and has less "fingers" than the right one, and the nucleus of the left otolith is situated in the center with possible reading axes all around.

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







**Reading Procedure**




Different axes or areas are used for ageing on the convex surface.




**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




**Reading Procedure**


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

Maximum ages reached using this method have been:


♀ :	Northwest - 21 years
	Northeast - 21 years
♂ :	
	Northwest - 15 years
	Northeast - 17 years



**Determination 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.

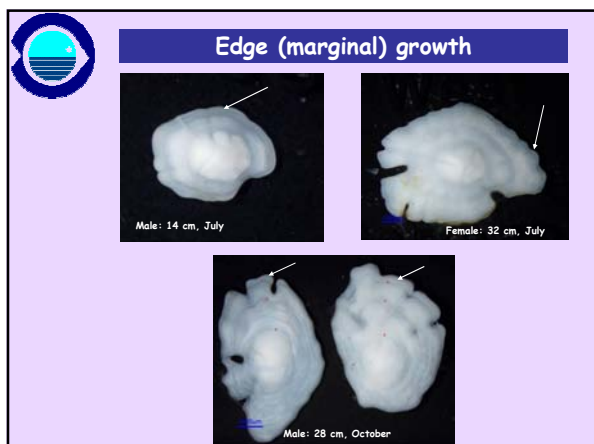


**Edge (marginal) growth**

The interpretation of the extent and type of growth at the otoliths 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.





### 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. Sunksen

Pingortitaleriffik  
Greenland Natural Institute  
Greenland Institute of  
Natural Resources

### Whole otolith method used in Greenland




Kaj Sunksen & Lars Heilmann

Pingortitaleriffik  
Greenland Natural Institute  
Greenland Institute of  
Natural Resources

### Examined areas


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



Pingortitaleriffik  
Greenland Natural Institute  
Greenland Institute of  
Natural Resources

### Procedure

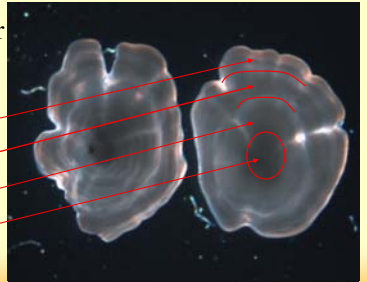
- No preparation
- Otolith examine solution:
  - Small (< ~ 45 cm): Water
  - Large (> ~ 45 cm): 50% Ethanol
- Right otolith, -left supports
- Stereomicroscope, 8-10x magnification
- Transmitted polarized light
- Up to 8 years with acceptable confidence.
  - Record: 25 years



Pingortitaleriffik  
Greenland Natural Institute  
Greenland Institute of  
Natural Resources

### Examples

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

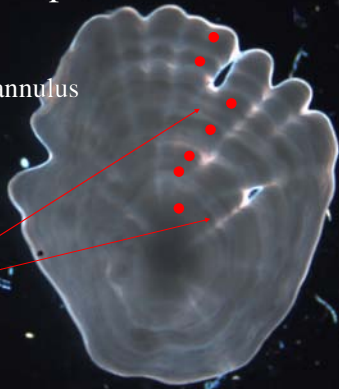


3rd summer  
2nd summer  
1st summer  
Nucleus

## Examples, II

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

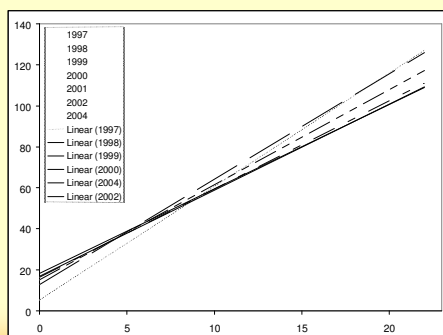
Dots: Summers  
Checks



Thanks !




## Age vs. length



## Appendix VII. O. T. Albert refined method

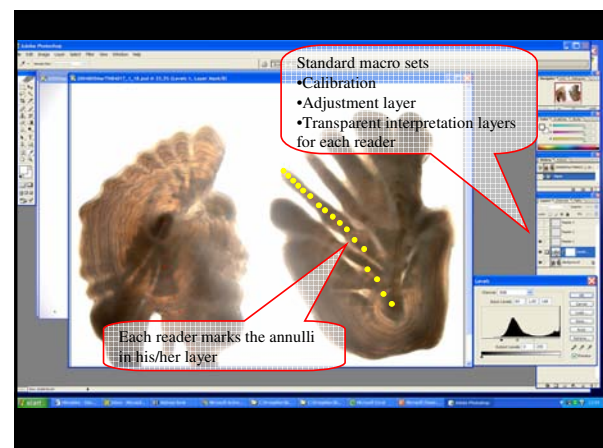
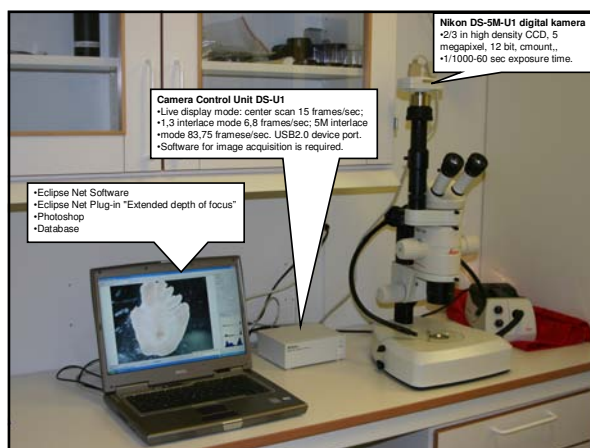
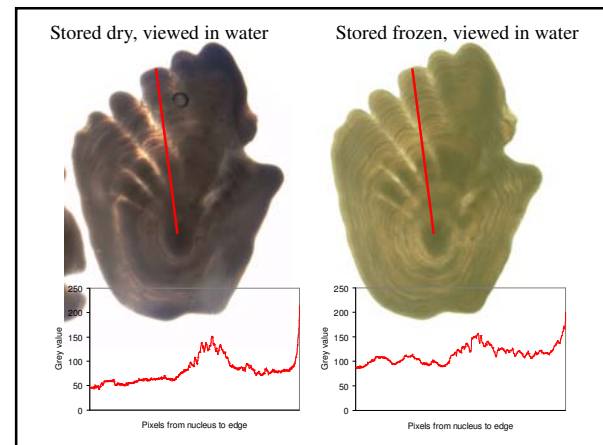
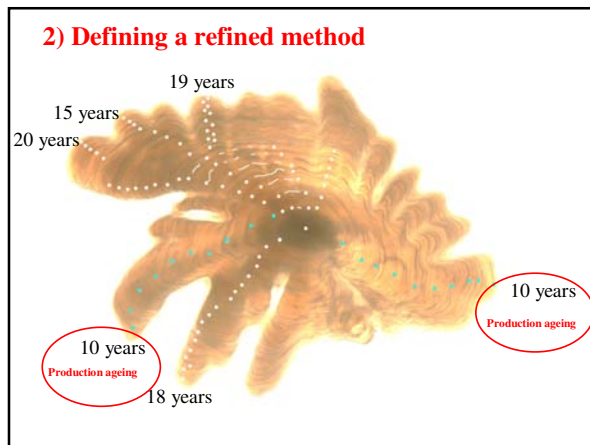
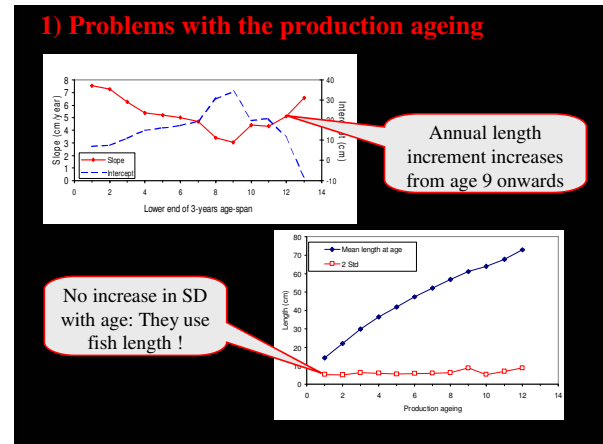
**A refined method for estimating age of Northeast Arctic Greenland halibut**

- 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



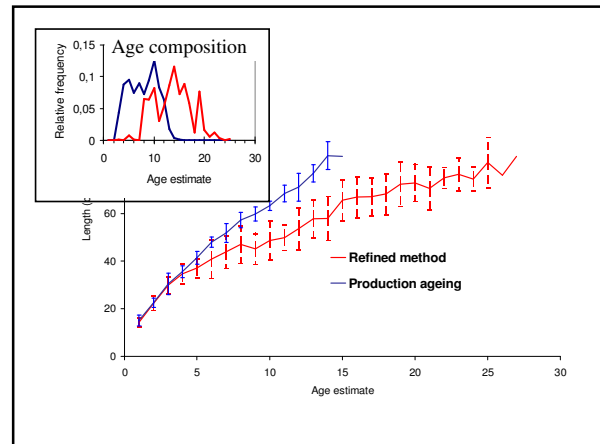
Ole Thomas Albert

Institute of Marine Research, Tromsø, Norway

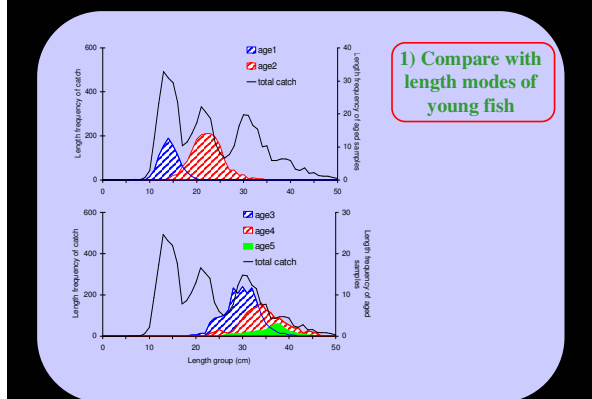


### Method: Production Refined

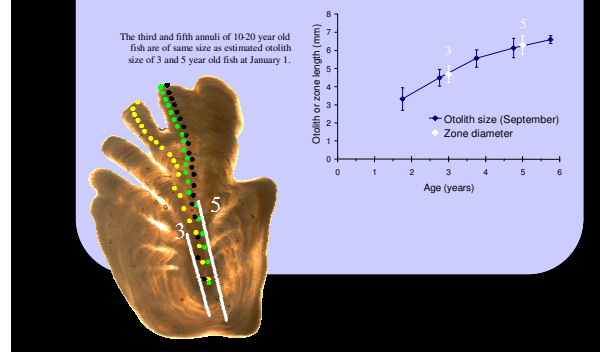
Storing	Dry	Frozen
Viewed in	Water	Water
Otolith studied	Left	Right
Visual aid	Microscope	Digital photos
Light	Transmitted	Transmitted
Interpretation	Count	Count/mark
Available info	Fish length	Length of first zone



### 3) Validating the refined method



### 2) Size of the first few annuli in older fish



### 3) Age-information in otolith morphometry

		Otolith measures					
		WEIGHT <sup>1/3</sup>		AREA <sup>1/2</sup>		LENGTH	
		Ref.	Prod.	Ref.	Prod.	Ref.	Prod.
		Meth.	ageing	Meth.	ageing	Meth.	ageing
Right otolith	Correlation	0,15	0,10	0,08	0,09	0,08	0,11
	p-value	0,06	0,20	0,43	0,36	0,38	0,25
	Significance	*	ns	ns	ns	ns	ns
Left otolith	Correlation	0,19	0,07	0,26	0,13	0,22	0,11
	p-value	0,04	0,45	0,02	0,24	0,06	0,32
	Significance	**	ns	**	ns	*	ns

### 4) Compared with growth estimates



#### •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

## Appendix VIII. FWI section method

### Otolith Section Methods Assessed by DFO Central and Arctic

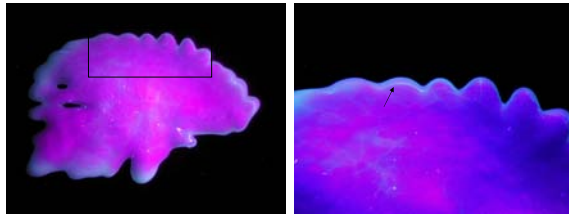
Prepared by  
Margaret Treble and Rick Wastle  
Winnipeg, MB

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

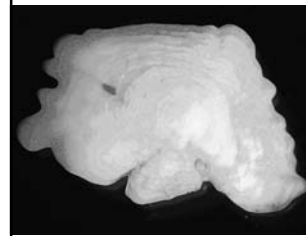
### Why Sections?

- 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.

Greenland Halibut OTC Recapture  
(60cm female, 3 Years, 10 Months after marking)



Left otolith – UV Light

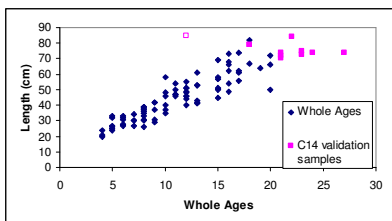


Left Otolith (Distal Surface)  
In Water, Reflected Light –  
Age 10+



Left Otolith thin section  
Reflected Light – Age 18+

63 cm Female (SA3) sent to Australia for methods testing



Whole otolith ages from n=80 samples examined in our age structure comparison. Age estimates from 10 Carbon 14 validation samples are also shown.

### 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 lowspeed saw with a diamond tipped blade is used to cut the section (e.g. Buehler Isomet™ saw).

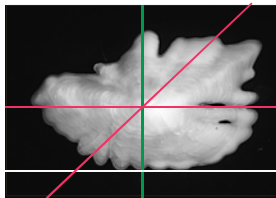
## Section Method Con't

- We found thin sections (350  $\mu\text{m}$ ) 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  $\mu\text{m}$  and 9  $\mu\text{m}$  lapping film and finished with 0.3  $\mu\text{m}$  dry film.
- The cross-section was then viewed under reflected light in water using a dissecting microscope with 30x-40x magnification.

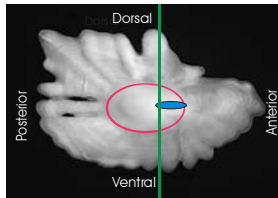
## Section Plane

- 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

### Greenland Halibut Left Otolith Section Planes



Distal View  
Section Planes Attempted  
Green – accepted  
Red – rejected

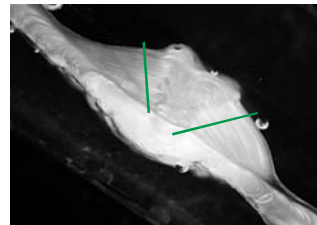
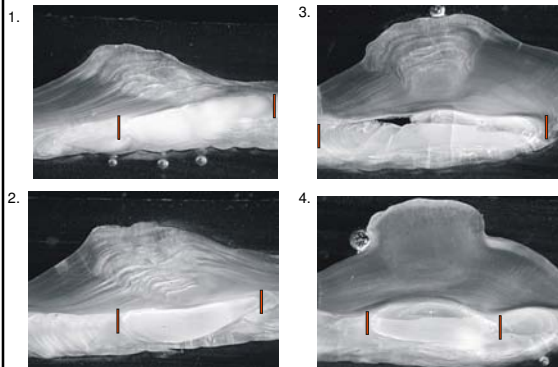


Proximal View  
Red Oval – Approx. Location of the Dome  
Blue Solid Oval – Approx. Location of the Sulcus

## Section Plane con't

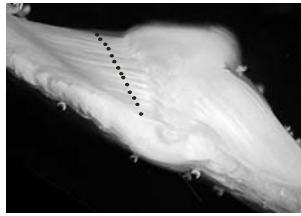
- Sections were also taken in the thickest portion of the otolith in order to hit the peak of the dome.
- But the dome thickens towards the posterior, away from the core
- The nucleus would be missed and the first year could be missed as well.

### Left Otolith – Series of Four Thin Sections

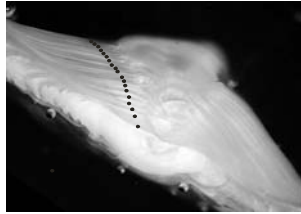


- Annuli were usually read on the left slope of the central "dome".
- We found the structure out along the thinner margins of the otolith hard to interpret.
- Green lines indicate our preferred ageing zone
- But we did observe that the structure changed sometimes when the angle of the reflected light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





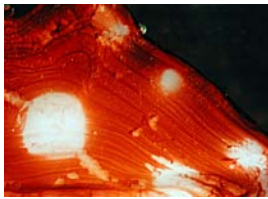
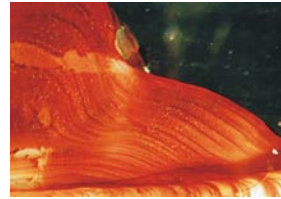
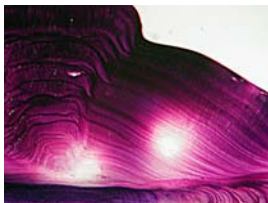
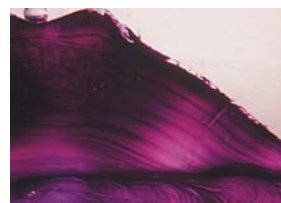
- 66 cm age=14 years



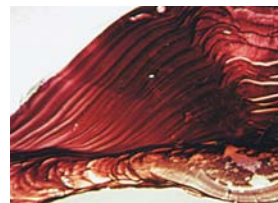
Same fish reflected light angle changed and interpretation of structure changed to age=19 years

### Possible Adjustments to Section Method

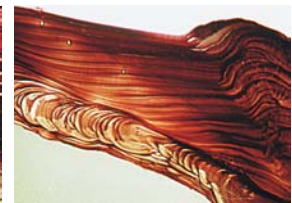
- Treating the otolith in some way either with a stain, with heat (baking or burning), or by applying an acid bath and acetate peel could enhance the annuli and minimize or eliminate the variability related to light angle.
- We have tested these methods on only a few samples so can't yet recommend one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



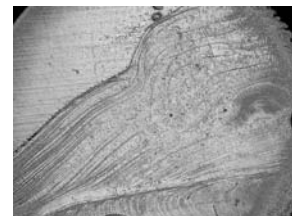
Better results when we combined the two stains



50% Neutral Red to 50% Toluidine Blue (59 cm Female, Baffin Bay)



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



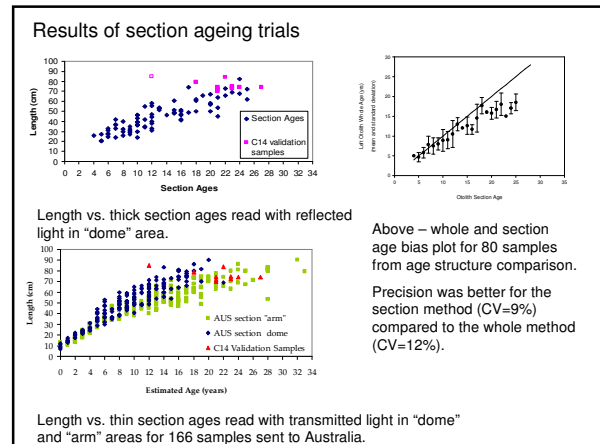
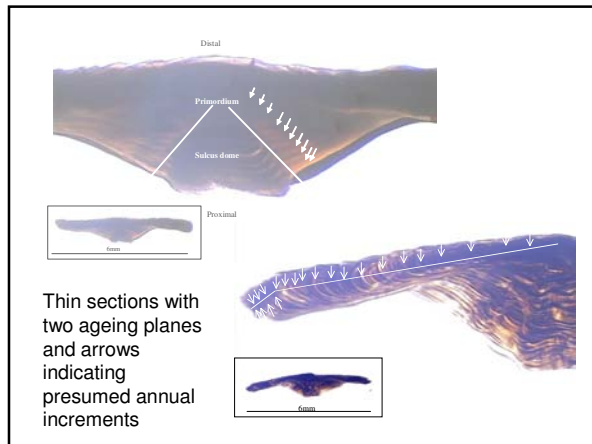
Acetate peel method – through core



Break and burn method – through thickest portion of otolith

### 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  $\mu\text{m}$ ) 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

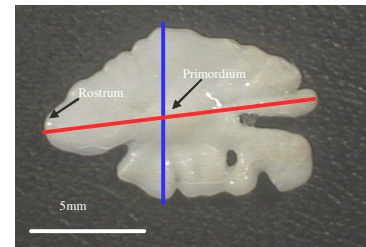
- The Central Ageing Facility in Australia was contracted to assess preparation and ageing techniques for Greenland halibut.
- They initially examined 21 otoliths, viewing them in water using reflected light.
- They did trials with various section planes on both the left and right otoliths

## 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.

## Methods

- Thin section methods were described previously

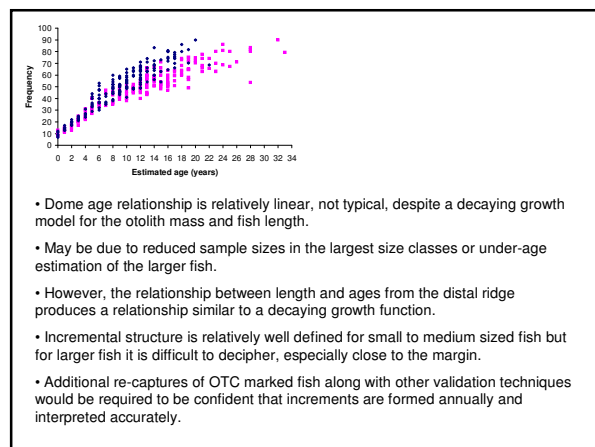
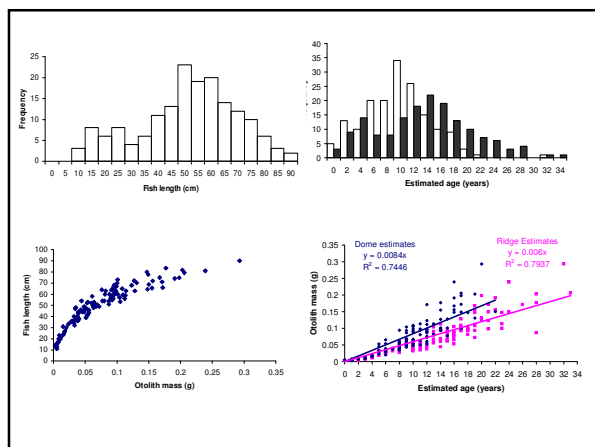
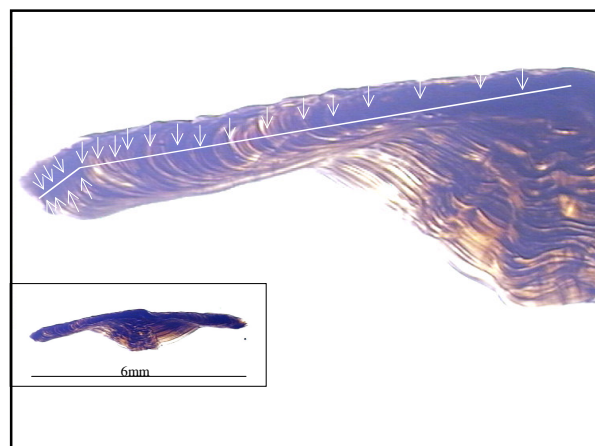
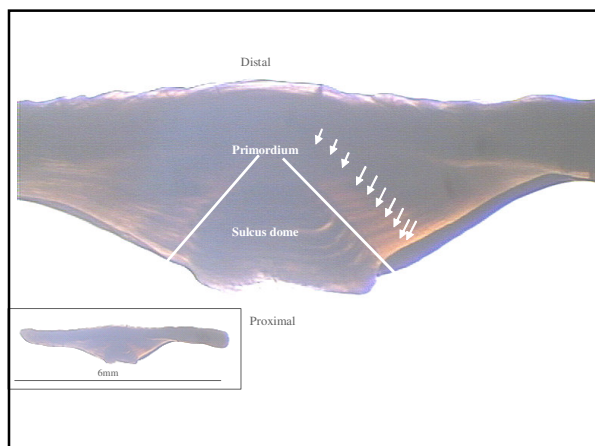



## Results

- 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.

## Results

- 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.








Improving the precision of otolith-based age estimates for Greenland halibut, with preparation methods adapted to fragile sagittae

Jake Gregg<sup>1</sup>, Delsa Anderl<sup>2</sup>, and Dan Kimura<sup>2</sup>

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

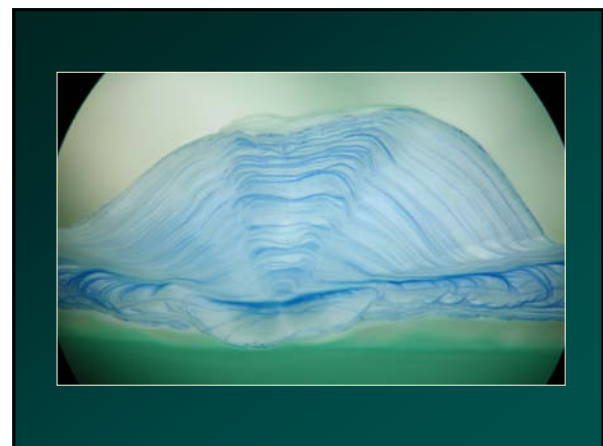
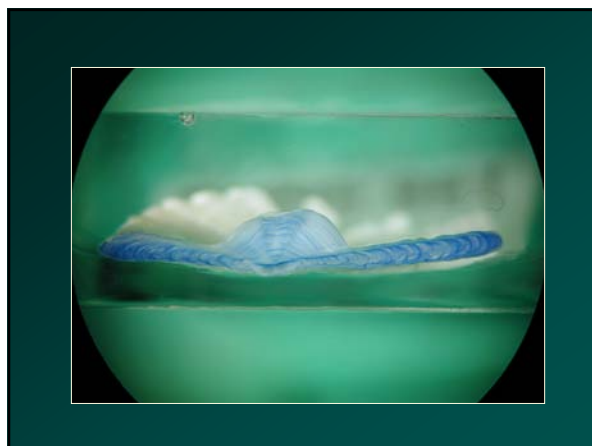

## Pilot Work

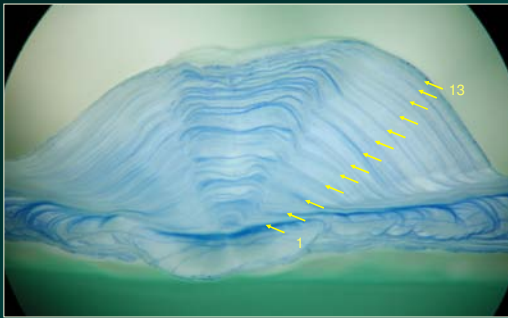
- Trial and error with methods from literature
- Low confidence in surface ageing, especially near the margin in older otoliths
- Wanted to examine large peri-sulcular tuberosity on left otolith
- Sectioning and staining



## Preparation

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





## 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

	<i>n</i>	Fish Length (cm)			Collection year, location
		range	mean	median	
Trial 1	93	12-84	40	31	1998, BS
Trial 2	226	57-98	75	72	1994, AI
Trial 3	75	12-63	37	39	1994, BS

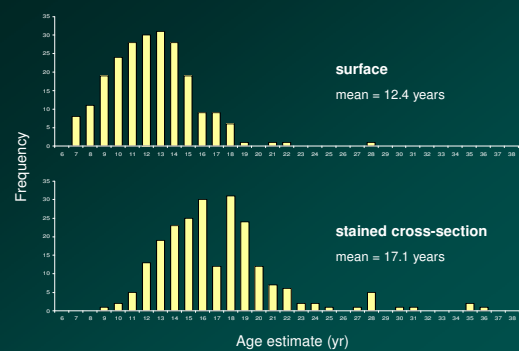
## Results

	method	CV	Bowker's test of symmetry		
			$\chi^2$	df	p <
Trial 1	surface	<b>11.33</b>	30.4	22	<b>0.1091</b>
	cross-section	<b>19.68</b>	74.0	28	<b>0.0001</b>
Trial 2	surface	<b>16.31</b>	94.2	71	<b>0.0342</b>
	cross-section	<b>9.46</b>	76.9	68	<b>0.2159</b>
Trial 3	surface	<b>8.11</b>	19.5	11	<b>0.0532</b>
	cross-section	<b>9.96</b>	32.3	12	<b>0.0012</b>

## Results

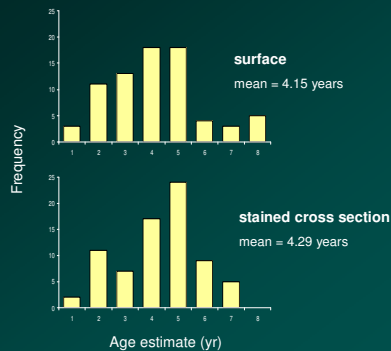
	method	CV	Bowker's test of symmetry		
			$\chi^2$	df	p <
Trial 1	surface	<b>11.33</b>	30.4	22	<b>0.1091</b>
	cross-section	<del>19.68</del> <b>7.68</b>	<del>74.0</del> <b>27.3</b>	<del>28</del> <b>23</b>	<del>0.0001</del> <b>0.2440</b>
Trial 2	surface	<b>16.31</b>	94.2	71	<b>0.0342</b>
	cross-section	<b>9.46</b>	76.9	68	<b>0.2159</b>
Trial 3	surface	<b>8.11</b>	19.5	11	<b>0.0532</b>
	cross-section	<b>9.96</b>	32.3	12	<b>0.0012</b>

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

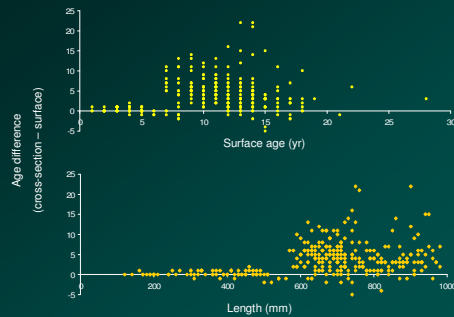




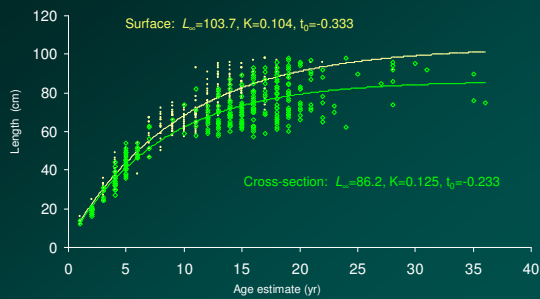
Trial 3: Comparison of age frequencies for estimates made from surfaces and stained cross sections.



## When to cross-section age?



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



## Mortality Implications

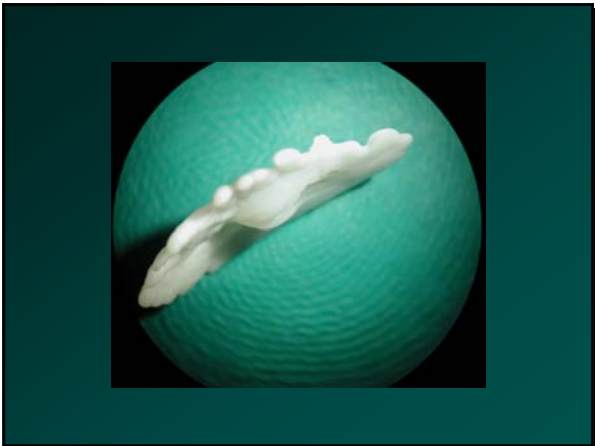
- Current natural mortality parameters used
  - Bering Sea:  $M = 0.18$
  - Atlantic:  $M = 0.20$
- From this study (Hoenig 1983)
  - Surface:  $M = 0.149$
  - Cross-section:  $M = 0.115$
- From GSI method (Cooper *et al.*)
  - $M = 0.12$

## 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 prep: rinsing, drying, etc.
  - 2:15, Embedding
  - 1:38, Marking
  - 1:50, Cutting
  - 6:27, Polishing
  - 1:04, Staining
- Lowered ageing time significantly



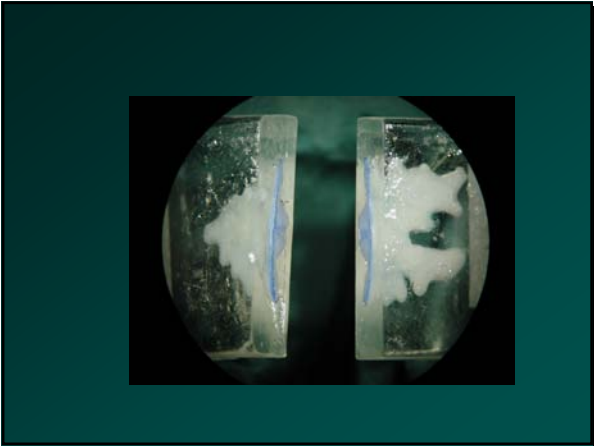
# Bowker's test of symmetry

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

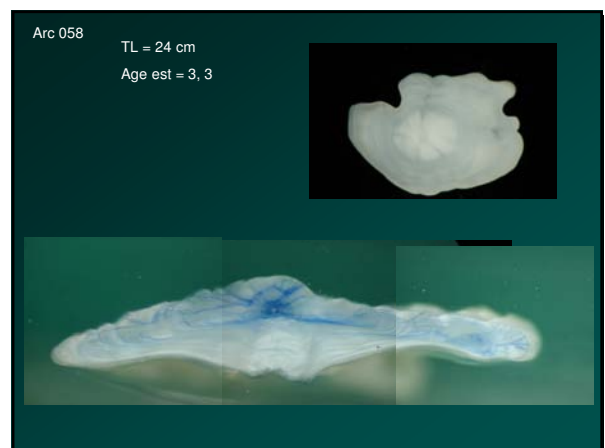
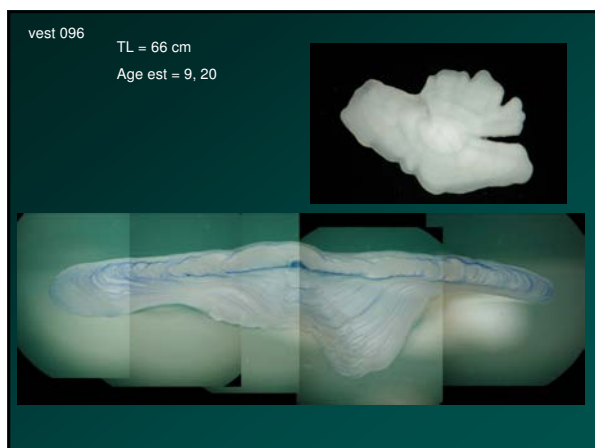
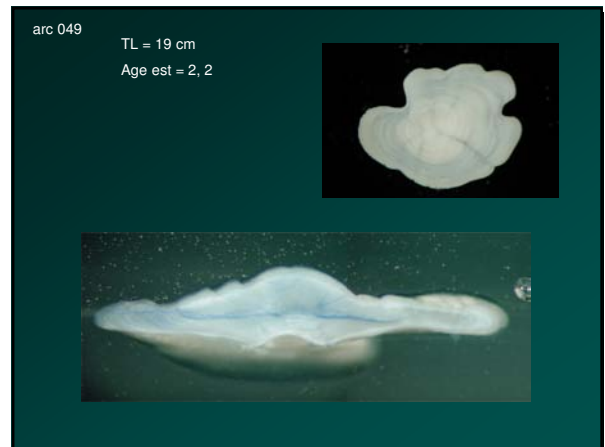
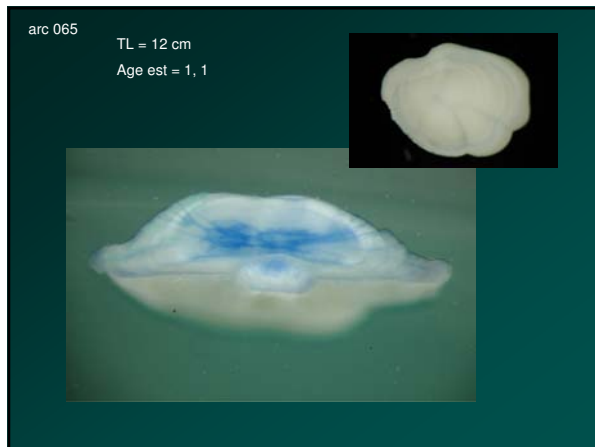
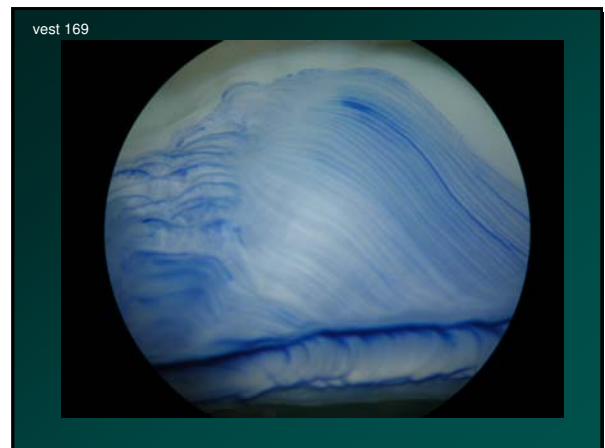
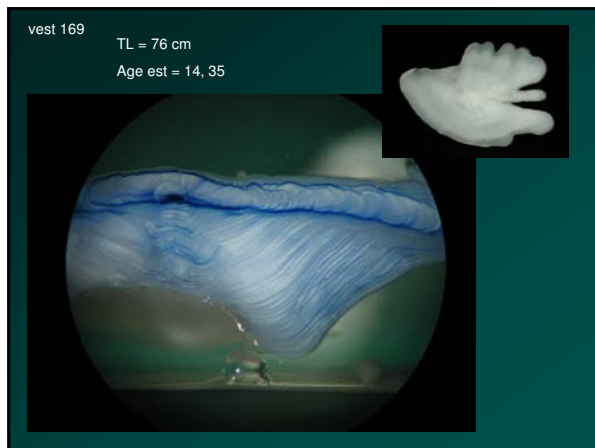
Where:  $n_{ij}$  is the number of specimens aged  $i$  by the reader and  $j$  by the tester  
 $m$  is the maximum age



	method	CV	Between reader % agreement			Bowker's test of symmetry		
			+/- 0 yr	+/- 1 yr	+/- 2 yr	$\chi^2$	df	p <
Trial 1	surface	11.33	44.1	81.7	90.3	30.4	22	0.1091
	cross-section	19.68	20.4	66.7	81.7	74.0	28	0.0001
	corrected	7.68	53.8	81.7	93.5	27.3	23	0.2440
Trial 2	surface	16.31	16.8	42.0	58.4	94.2	71	0.0342
	cross-section	9.46	16.3	44.3	68.1	76.9	68	0.2159
Trial 3	surface	8.11	65.3	89.3	93.3	19.5	11	0.0532
	cross-section	9.96	49.3	90.7	98.7	32.3	12	0.0012



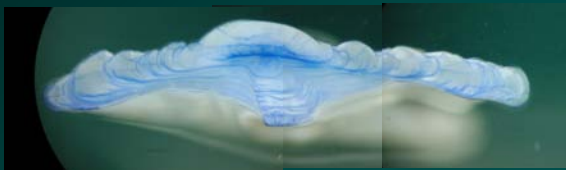
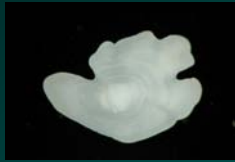




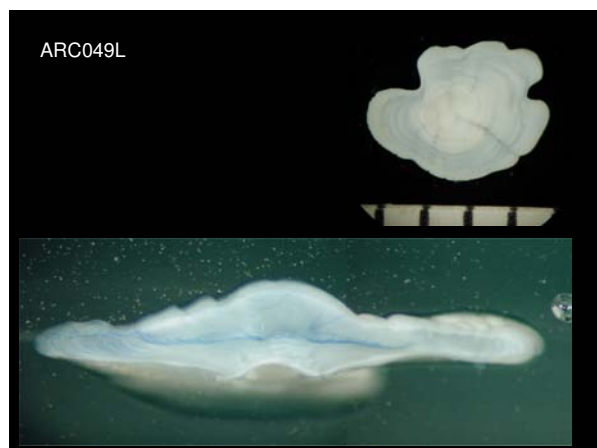
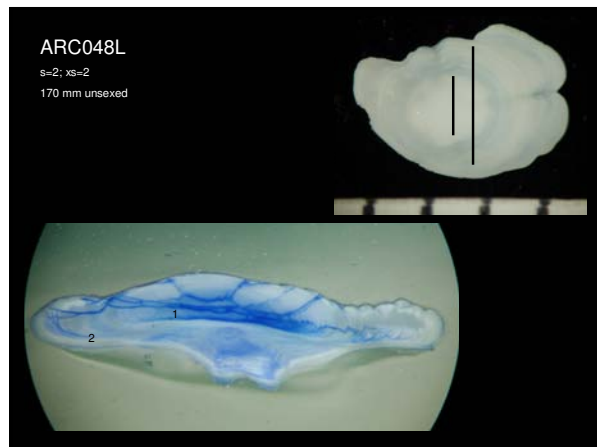
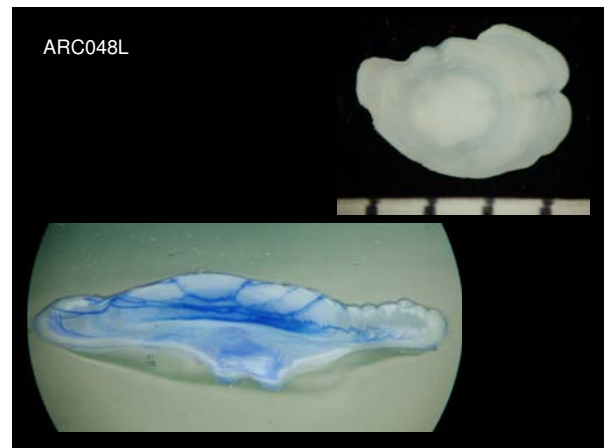
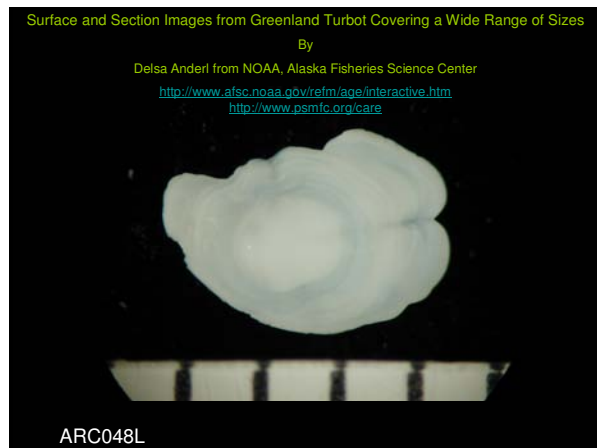
Arc 109

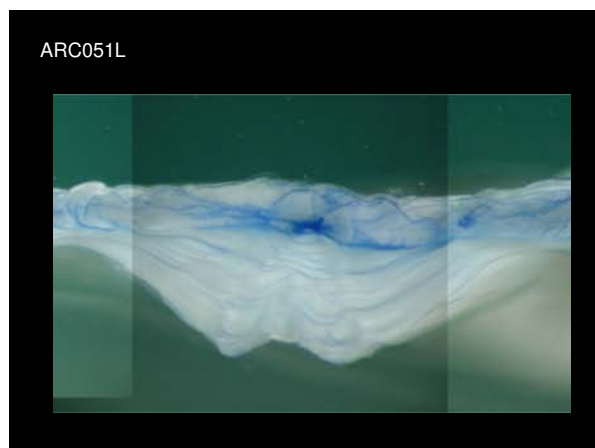
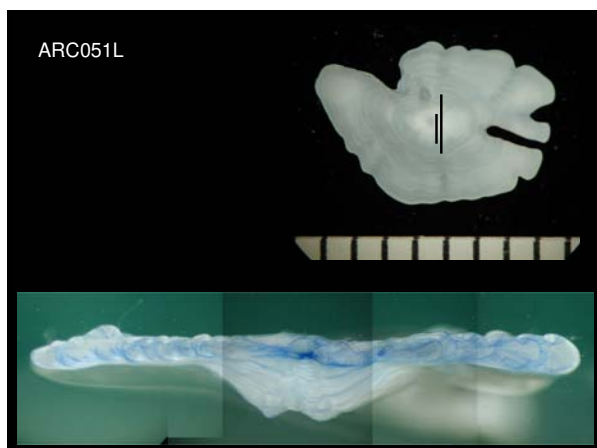
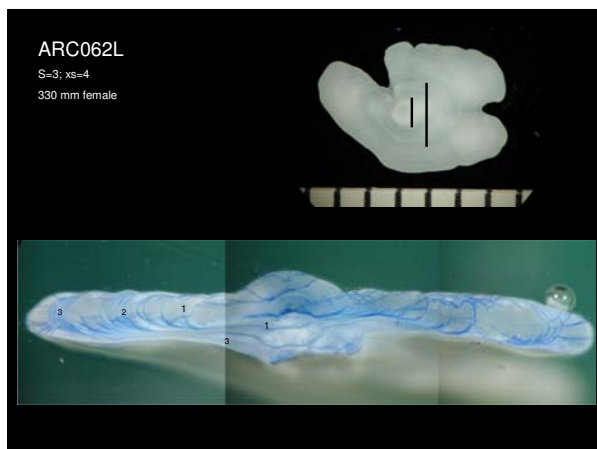
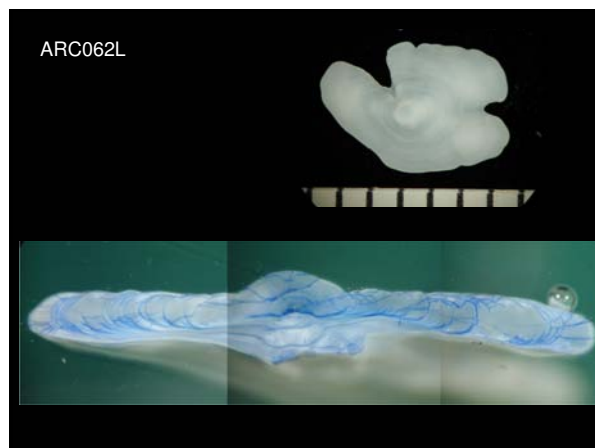
TL = 30 cm

Age est = 3, 4



## Appendix XI. D. Anderl



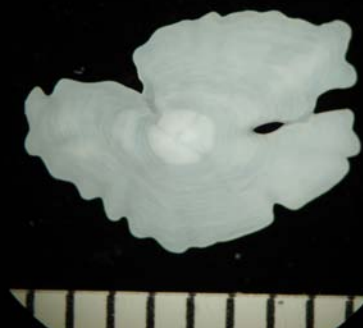


ARC051L

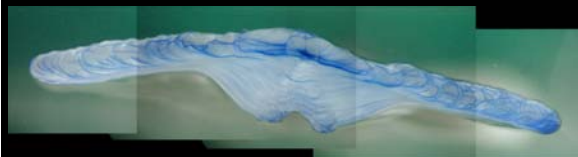
S=3; xs=4  
360 mm female



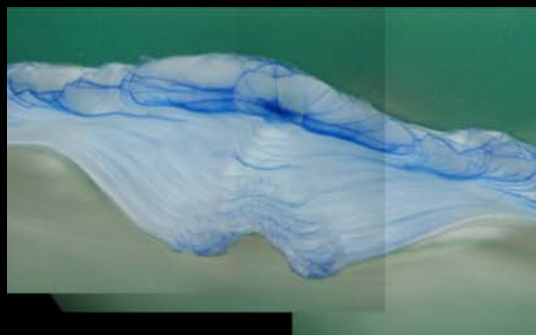
ARC082L



ARC082L

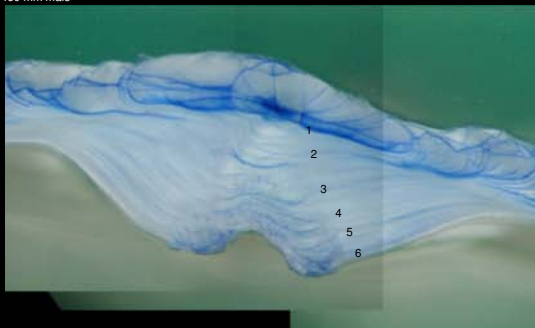


ARC082L



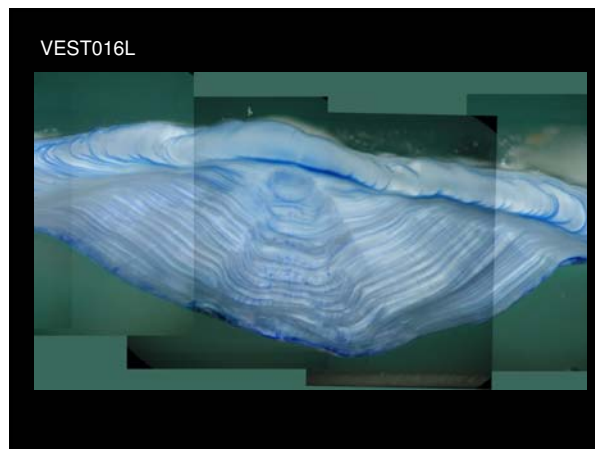
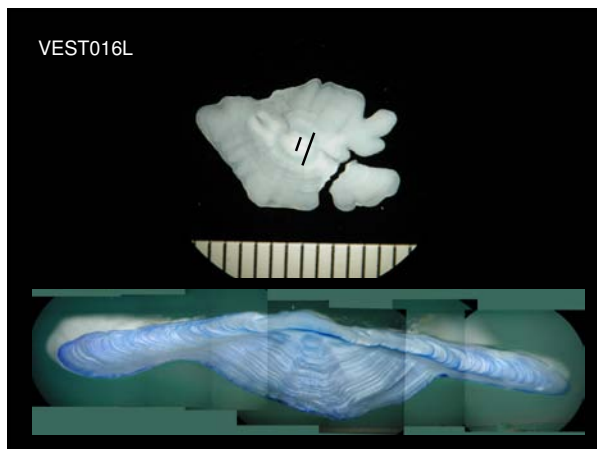
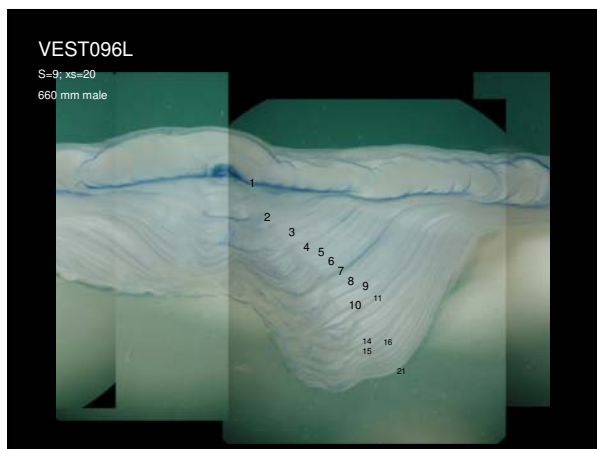
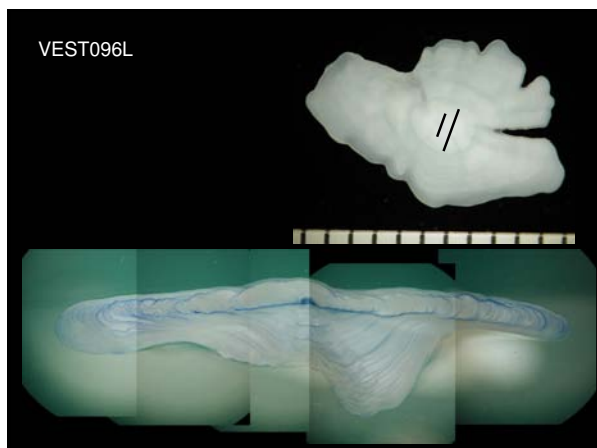
ARC082L

S=5; xs=6  
460 mm male

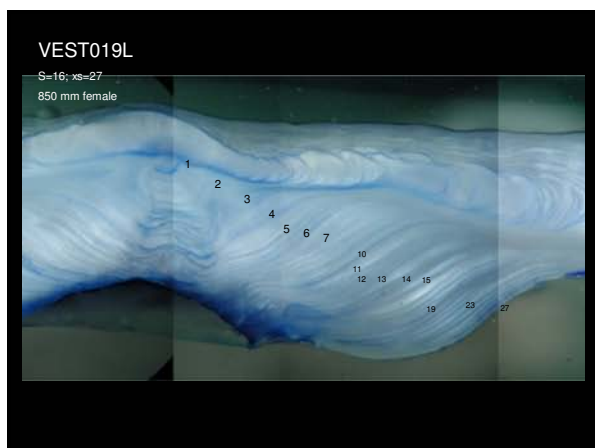
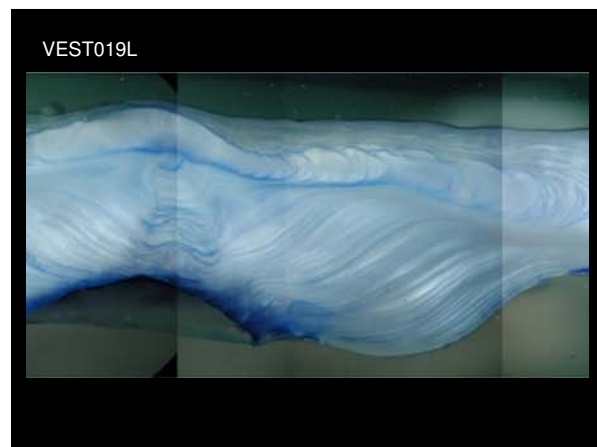
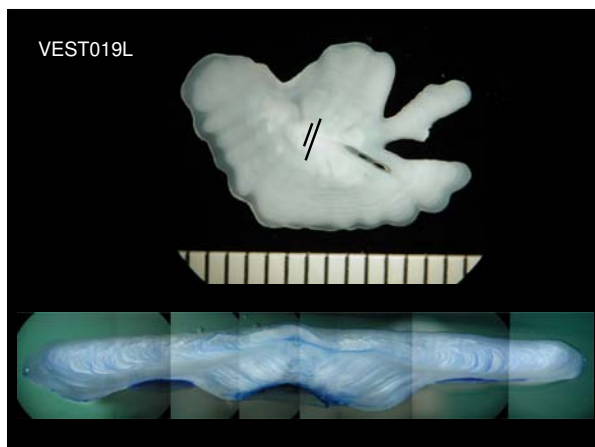
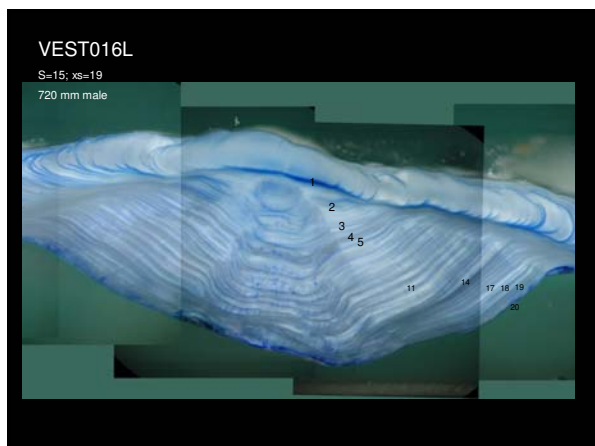


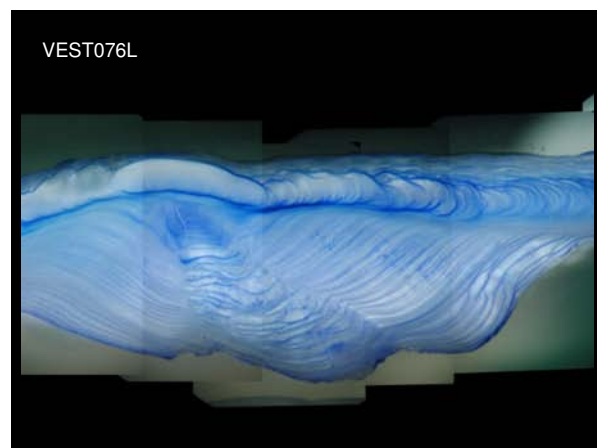
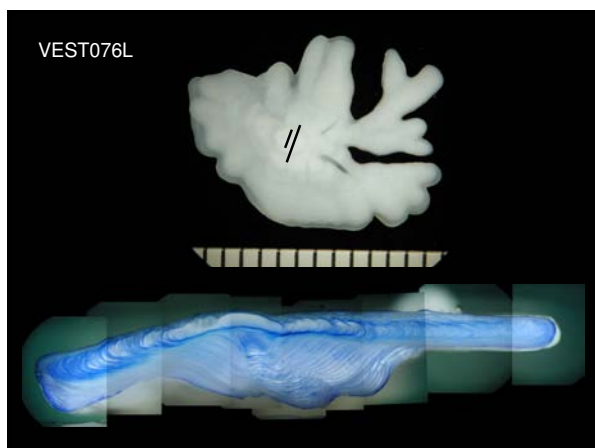
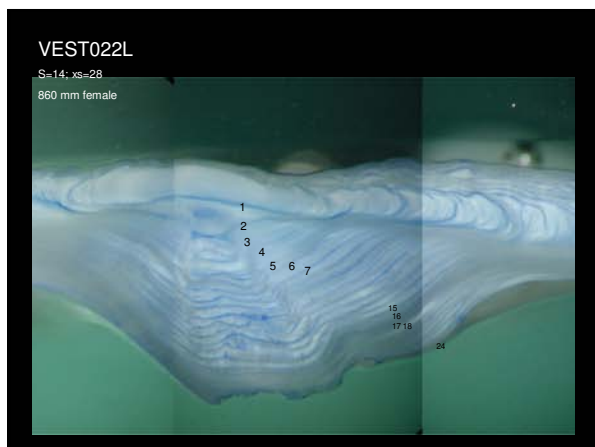
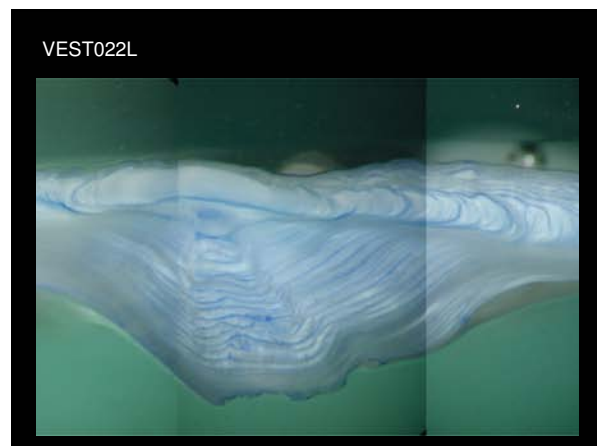
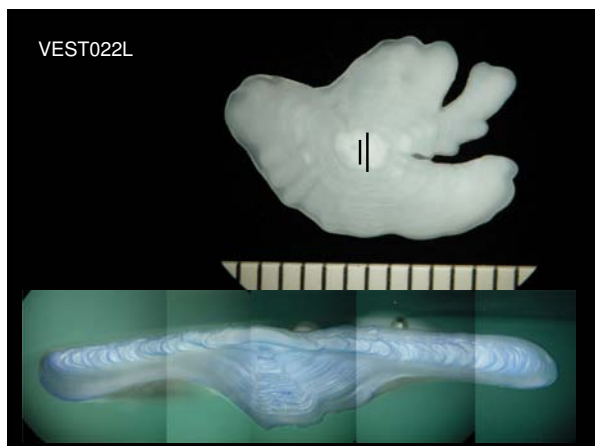
VEST096L



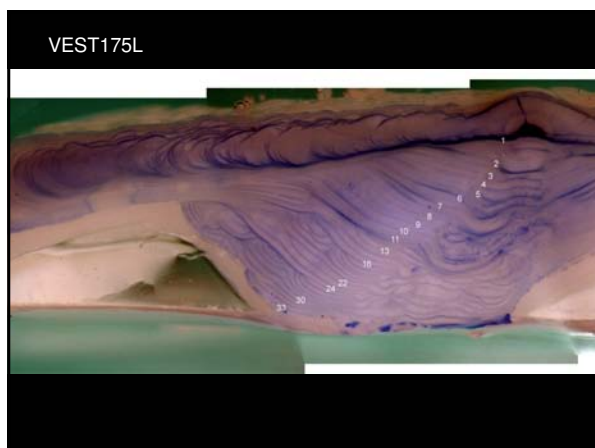
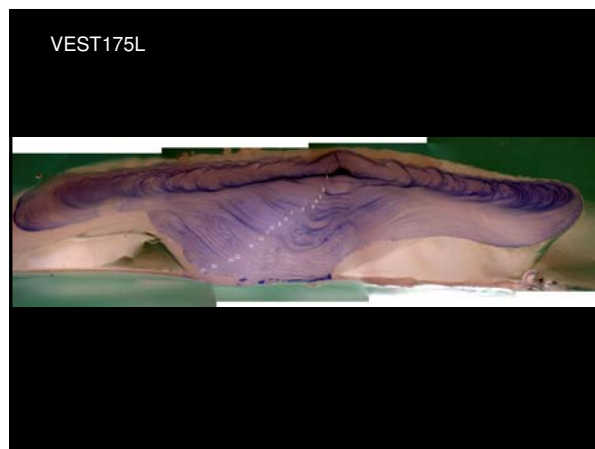
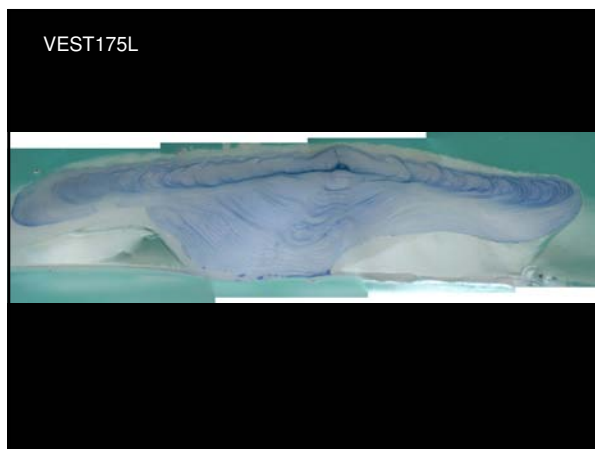
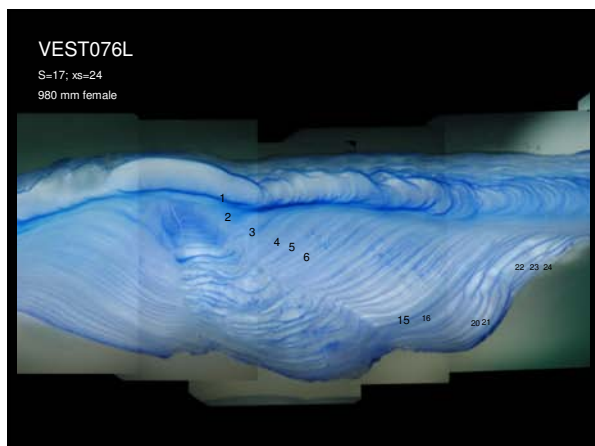




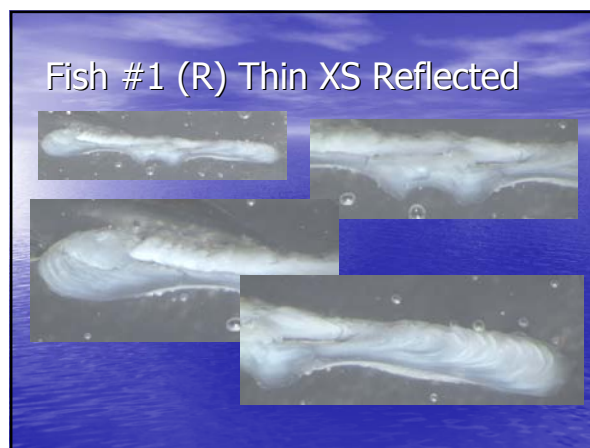
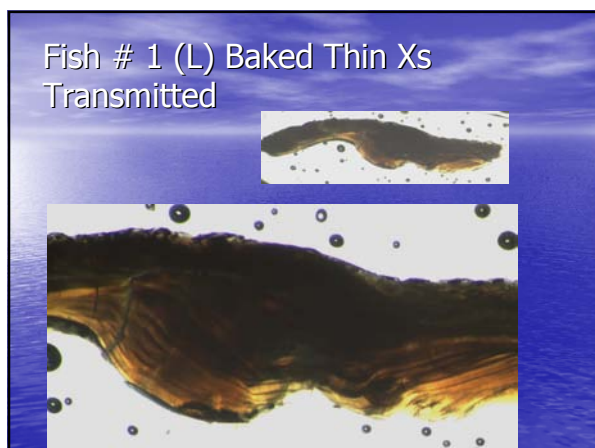
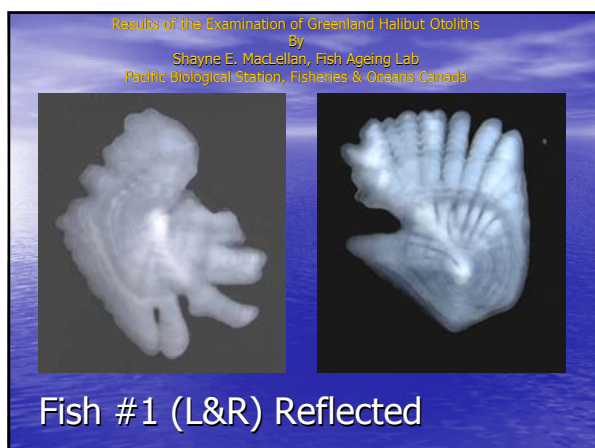




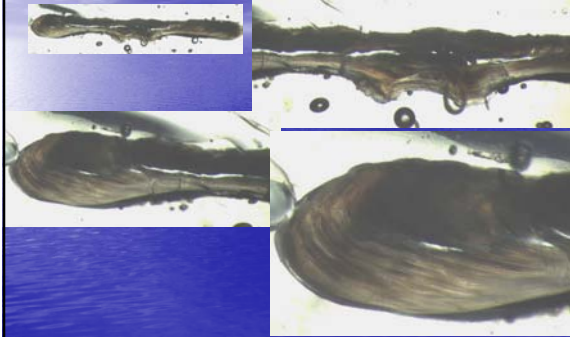




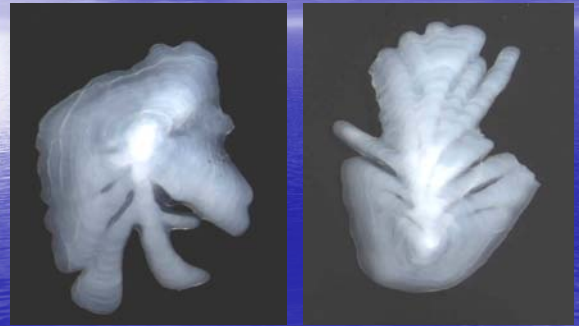
## Appendix XII. S. MacLelland methods



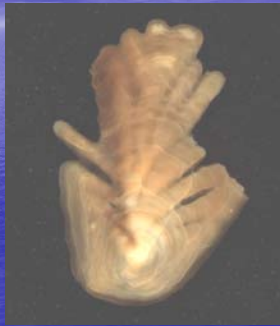
Fish #1 (R) Thin XS Transmitted



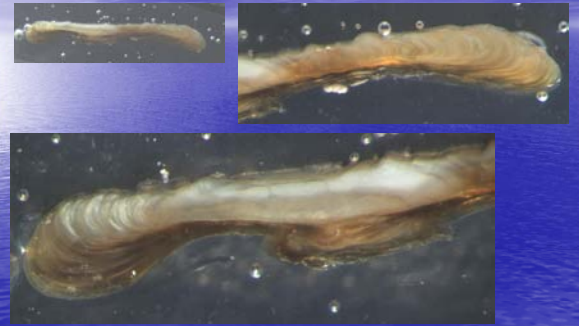
Fish # 2 (L&R) Reflected



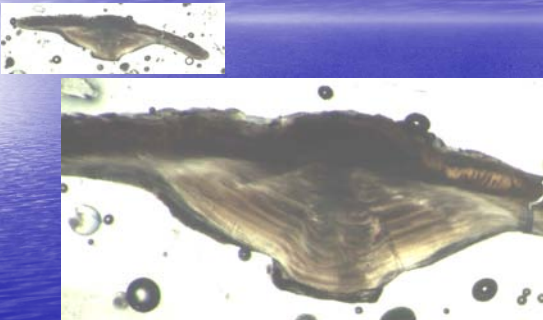
Fish #2 (R) Baked Reflected



Fish #2 (R) Baked Thin Xs Reflected



Fish #2 (L) Thin XS Transmitted

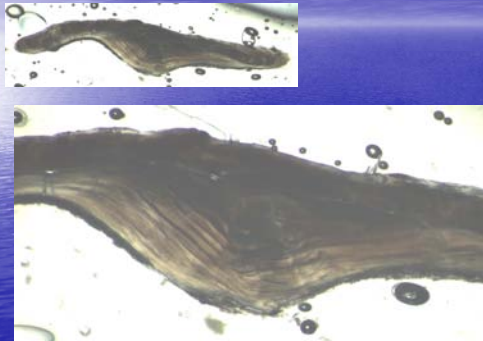


Fish # 2 (L) Thin XS Reflected

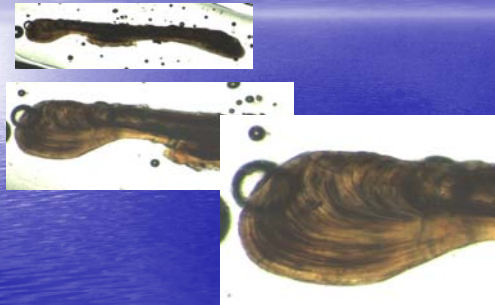




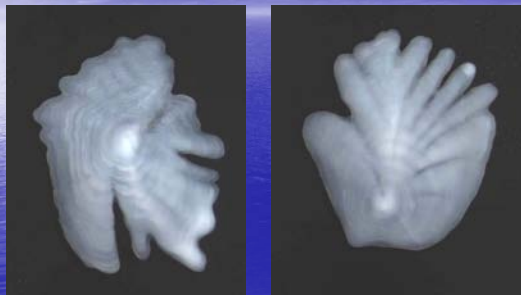
Fish # 2 (R) Thin XS Transmitted



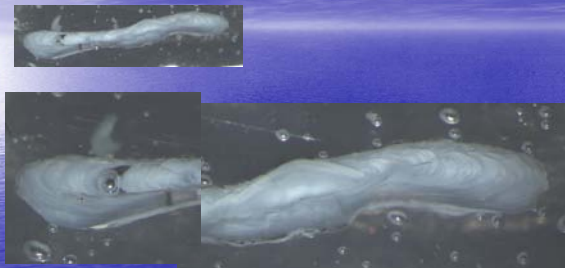
Fish #2 (L) Baked XS Transmitted



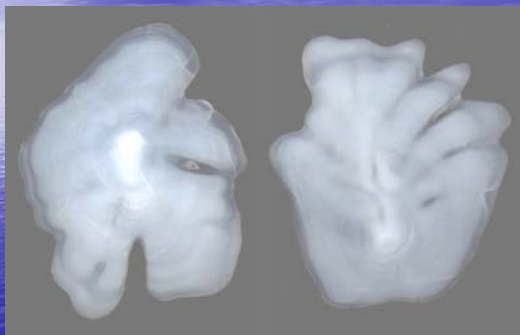
Fish # 3 (L&R) Reflected



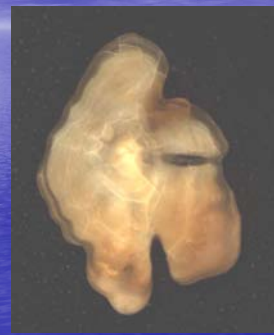
Fish # 3 Thin XS Reflected



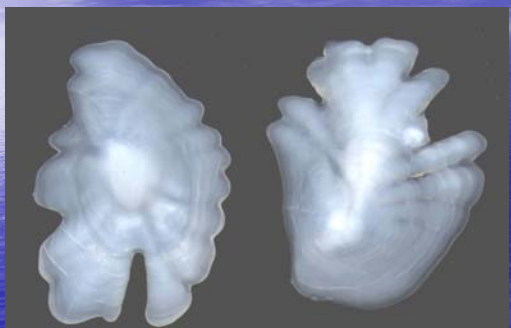
Fish # 4 (L&R) Reflected



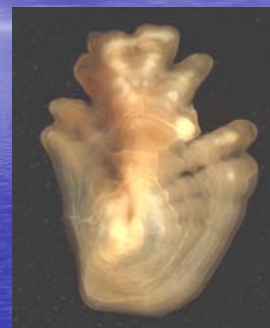
Fish # 4 (L) Baked Reflected



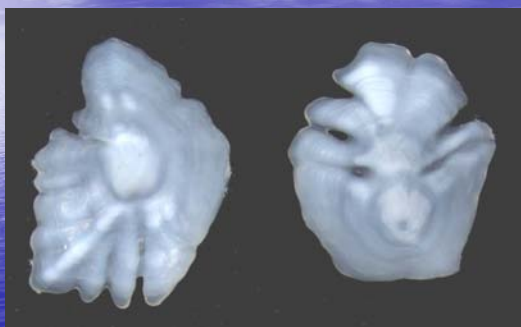
Fish # 5 (L&R) Reflected



Fish # 5 (R) Baked Reflected

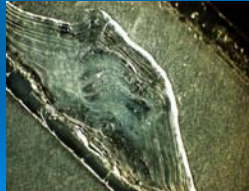
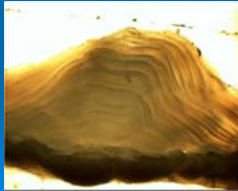


Fish # 6 (L&R) Reflected



## Appendix XIII. R. Perry Method

### Age interpretation for Halibut Otoliths Thin Sectioning vs Acetate Replication



### 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

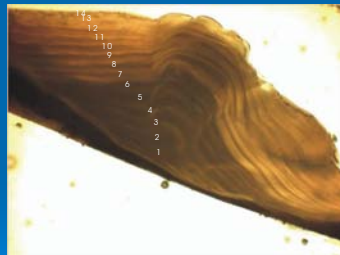
- Using acetone and a small piece of acetate a topographic impression is made of the etched surface
- The replicate is transparent allowing for easier interpretation
- Phase contrast will further enhance the image



### Section Images

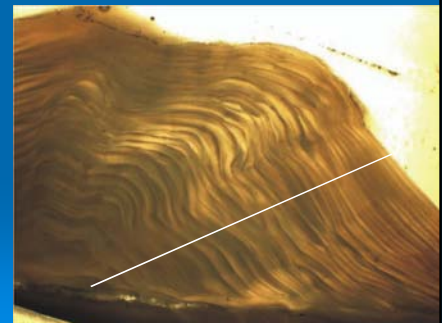
Acetate  
13.5 years

Section  
14.0 years

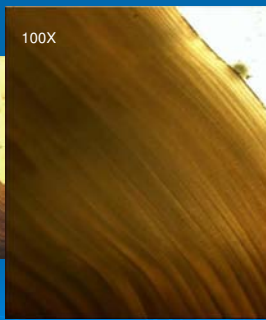
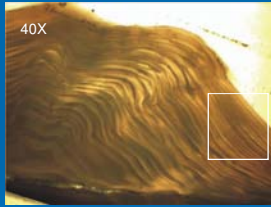


Acetate  
Mean 31years

Section  
Mean 26 years



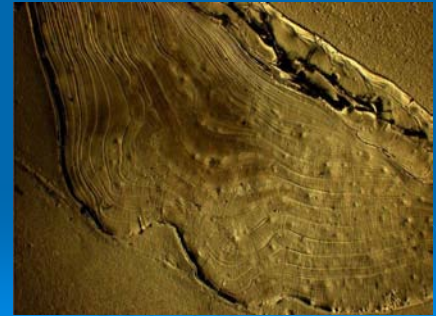
30 years



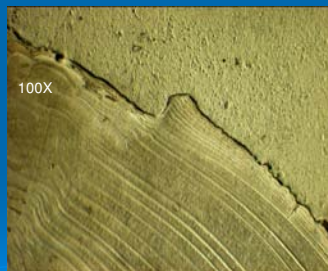
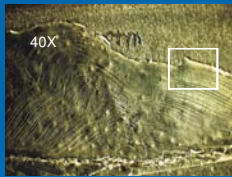
Replicate images

Section  
Mean age 15

Acetate  
Mean age 14



20 years



Interpretations



Acetate Replication (N=50) Trial 1: 50 Trial 2: 50 Trial 3: 50 → 150 interpretations

Thin Sections (N=50) Trial 1: 50 Trial 2: 50 Trial 3: 50 → 150 interpretations



Acetate Replication (N=50) Trial 1: 50 Trial 2: 50 Trial 3: 50 → 150 interpretations

Thin Sections (N=50) Trial 1: 50 Trial 2: 50 Trial 3: 50 → 150 interpretations



Acetate Replication (N=50) Trial 1: 50 Trial 2: 50 Trial 3: 50 → 150 interpretations

Thin Sections (N=50) Trial 1: 50 Trial 2: 50 Trial 3: 50 → 150 interpretations

Age Bias Plot Comparing all Sectioned and Acetate Ages

Paired t-test results

No overall difference between techniques  
N= 434 pairs,  $t = 0.128$ ,  
 $P = 0.899$ .

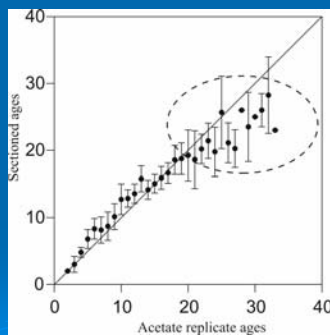
Arbitrary sub-groups based on acetate ages

Group 1

Age 1 to 20

Group 2

Age 21 to 40



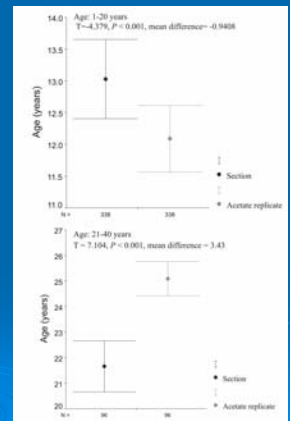
Results paired t-tests

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)**

Group	Interpreter	Pairs	Mean Difference	t	Significance
Age (4-20)	Don	117	-1.307	-4.067	$P < 0.001$
	Nathan	114	-0.456	-1.151	$P = 0.252$
	Rob	107	-1.056	-1.056	$P = 0.009$
Age (21-40)	Don	30	3.30	3.668	$P < 0.001$
	Nathan	31	4.22	4.610	$P < 0.001$
	Rob	35	2.85	3.964	$P < 0.001$

**Paired t-test Results for among Individual Interpreters trials**

**Dons trials had significant Differences**

**Acetate**

Trial 1 vs 3

Mean = -0.7551,  $P = 0.005$

Trial 2 vs 3

Mean = -1.2041,  $P = 0.004$

**Section**

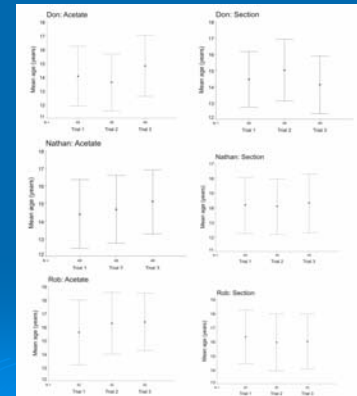
Trial 1 vs 2

Mean = -0.5714,  $P = 0.05$

Trial 2 vs 3

Mean = 0.8980,  $P = 0.018$

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



**Friedman Repeated Measures Analysis of Variance on Ranks (Comparison among interpreters)**

**Acetate replicates**

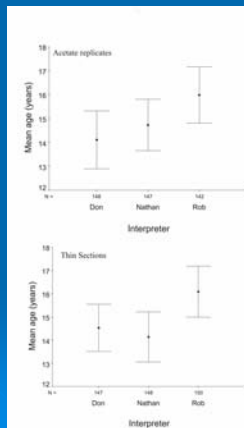
$\chi^2=17.067$ ,  $P < 0.001$

Don and Nathan differed from Rob

**Thin sections**

$\chi^2=42.451$ ,  $P < 0.001$

Don and Nathan differed from Rob



**Results: Paired t-test comparing coefficient of variation values (Comparison between techniques)**

Reader	Acetate CV	Section CV	t	Significance
Rob	0.154	0.128	1.089	$P=0.282$
Don	0.103	0.090	0.968	$P=0.338$
Nat	0.100	0.092	0.383	$P=0.704$
Overall	0.200	0.191	0.466	$P = 0.643$

## 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
- Interpretations 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. PINRO Scale Method

### 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.

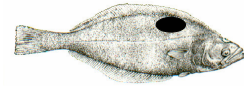


Figure 1. Spot on the body surface to pick out the scales for Greenland halibut age reading using PINRO method.

### 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.

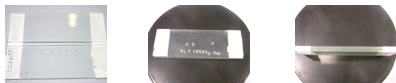
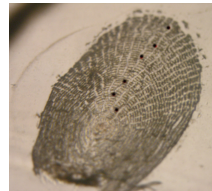


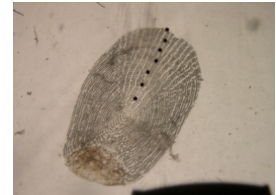
Figure 2. Examples of Greenland halibut scale preparations to determine age. Scale between two glass slides.

### Russian method

- Examples of some scales with annuli indicated.



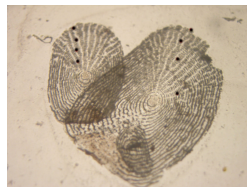
Exchange Sample #17



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.

## 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.



Transmitted Light



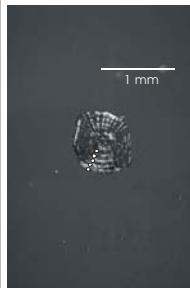
Polarized-Transmitted Light

(Male – 42 cm)

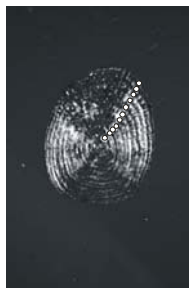
## Method

- 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.

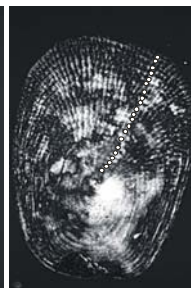
## Greenland Halibut Scales (Three Different Fish Sizes) -same magnification



#41576 - Female; 20 cm  
Aged 5 years



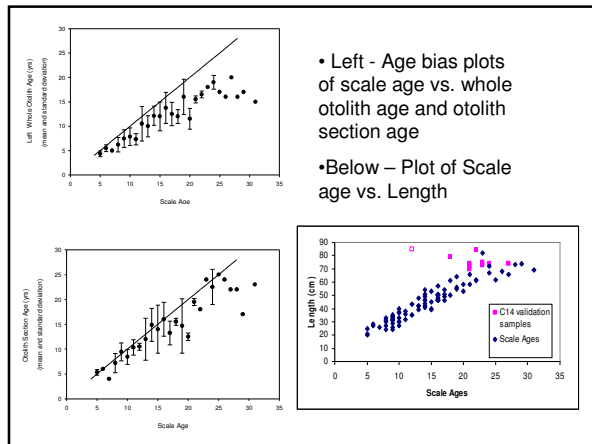
#40756 - Male; 36 cm  
Aged 10-11 years



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

## Comments

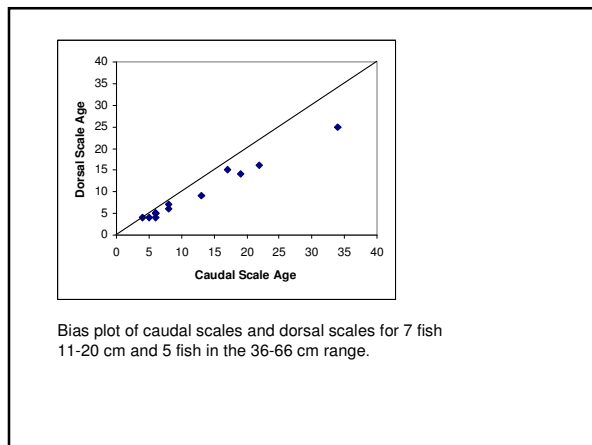
- Initial results looked promising
- This scale age method produced older ages than the whole otolith method and section method
- Ages were within the range of ages suggested by the Carbon 14 validation (>20 years for fish >70 cm)
- Precision was better (CV of 6.0%) compared to the otolith methods (12.4%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



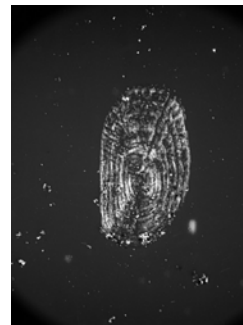
- Left - Age bias plots of scale age vs. whole otolith age and otolith section age
- Below - Plot of Scale age vs. Length

## Comments Con't

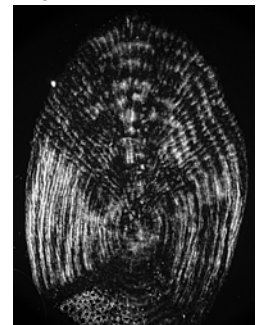
- No fish in our initial sample below 20 cm
- We were uncertain about the interpretation of the first few annuli
- Obtained 14 young fish < 20 cm from shrimp survey, scale ages tended to over-estimate whole otolith ages that had previously been verified based on the Peterson length frequency method.
- 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.



Greenland Halibut Dorsal and Caudal Peduncle Scales  
-36 cm fish, same magnification



Dorsal Scale  
-aged 8



Caudal Peduncle Scale  
-aged 12

## 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.

## 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

### 1997 Exchange

- Questionable how many recommendations were implemented
- No progress made on precision or bias since then

### 2005-2006 Exchange

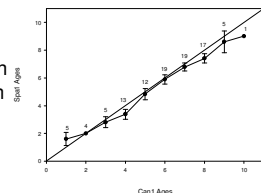
- 100 pairs of otoliths/scales collected from fish ranging in length from 15-57 cm in NAFO Div. 3M (EU annual survey – July 2005) (Thanks Ricardo!)
- Otoliths stored in vials with water
- Scales (removed from dorsal area) placed in envelopes

### 2005-2006 Exchange

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

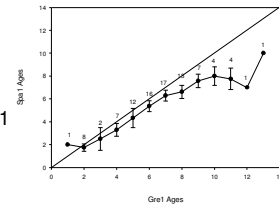
### 2005-2006 Exchange

- 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



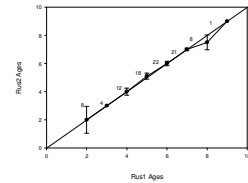
## 2005-2006 Exchange

- 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



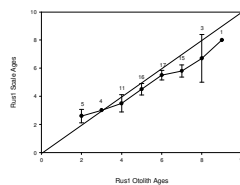
## 2005-2006 Exchange

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



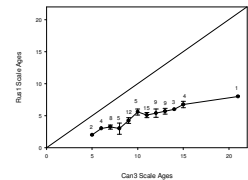
## 20045-2006 Exchange Scales

- 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



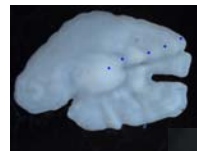
## 2005-2006 Exchange Scales

- 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



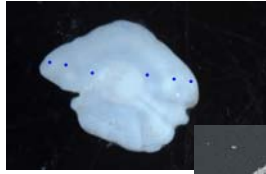
Whole otolith – all aged 5 years old

Can3 scale method- 9 years old



Rus1 scale method- 4 years old

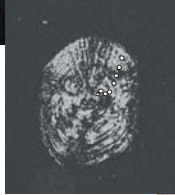




Exchange sample #67 - 27 cm in length

Most aged either 3 or 4 years old

Can3 scale age –  
8 years old



Rus1 scale age –  
3 years old



## Age Validation and Growth Analysis of Greenland Halibut from the Northwest Atlantic

by

Margaret A. Treble<sup>1</sup>, Steven E. Campana<sup>2</sup>, Rick J. Wastle<sup>1</sup>,  
Cynthia M. Jones<sup>3</sup> and Jesper Boje<sup>4</sup>

<sup>1</sup>Fisheries and Oceans Canada, Freshwater Institute, 501 University Cres.,  
Winnipeg, Manitoba, Canada R3T 2N6

<sup>2</sup>Fisheries and Oceans, Bedford Institute of Oceanography, 1 Challenger Drive,  
Dartmouth, Nova Scotia, Canada B2Y 4A2

<sup>3</sup>Center for Quantitative Fisheries Ecology, Old Dominion University, Norfolk, Virginia, U.S.A.

<sup>4</sup>Danish Institute for Fisheries Research, Charlottenlund Slot, 2920 Charlottenlund, Denmark

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.

## Introduction Con't.

- Validation
  - Oxytetracycline (OTC)
  - Nuclear bomb testing and <sup>14</sup>C: radiocarbon assay of otolith cores
- Growth of tag-recaptured fish

## 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.

## Methods Con't

- 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.
  - Validation- otoliths selected from fish sampled between 1967 and 1989.
  - Whole ages determined, thin sections prepared, digital images taken and enhanced in Adobe Photoshop.

## Methods Con't

- Validation Con't
  - Material in otolith core (first three years of growth) was extracted from each section, de-contaminated and submitted for <sup>14</sup>C 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.

## 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.

No.	Date Tagged	Length at Tagging (mm)	Date Recaptured	Length at Recapture (cm)	Rd. Wgt. (g)	Sex	Time Since Tagging
1	April 20, 1999	550	March 15, 2001	55	1550	M	1yr, 10+ months
2	April 15, 1999	635	April 4-7, 2002	64	2340	F	2 yrs, 11+ months
3	April 20, 1999	600	March 4, 2003	66	2730	F	3 yrs, 10+ months

Figure 1. Otoliths of OTC marked Greenland halibut shown under both ultraviolet and reflected light. The material that has incorporated the OTC shows up as a light green color and becomes less visible as time since marking increases: a) 1 year 11 months; b) 2 years 11 months; c) 3 years 10 months.

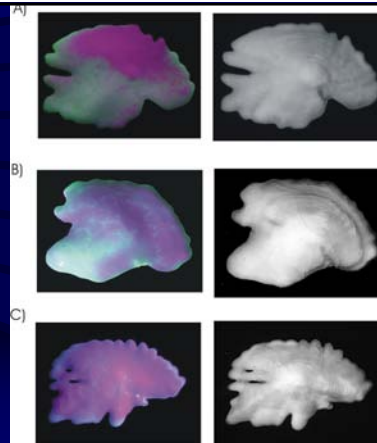


Figure 2. The OTC mark is visible at the edge in certain areas of the distal (A) and proximal (B) sides of the otolith sample taken from a Greenland halibut we recovered 3 years 10 months after tagging. Reflected light views of the same area are shown as well as a transmitted light view of the edge in A). The sectioning plane is also indicated.

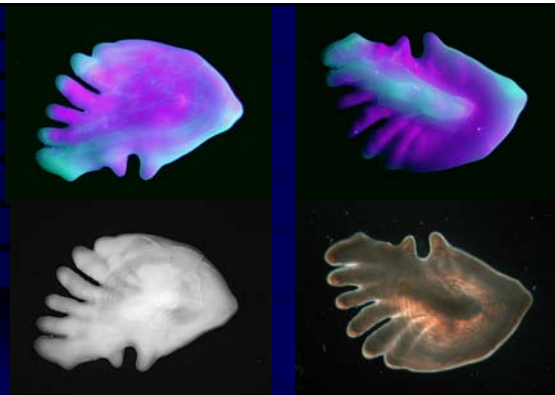
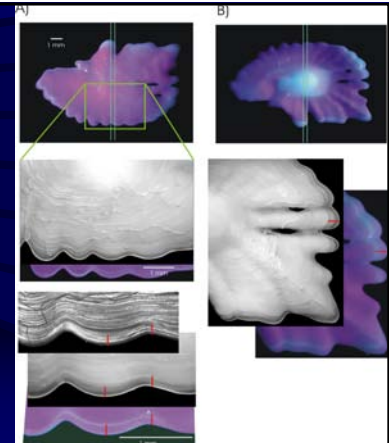
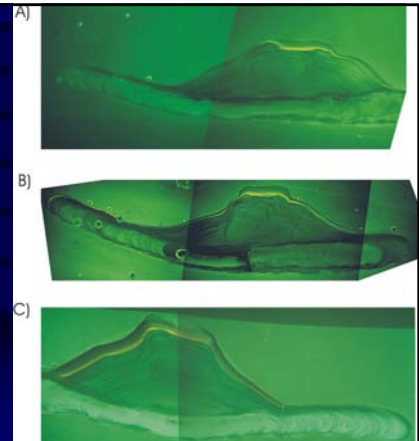


Fig. 1 Continued – UV, reflected and transmitted light images of the right otolith from the 3rd recaptured fish, 3 years 10 months at large.

Figure 3. Photos showing the entire otolith section under UV light for three Greenland halibut marked with OTC and recovered after: A) one year and 11 months; B) 2 years 11 months; and C) 3 years 10 months. The OTC mark shows up as a yellow band near the edge of the section.





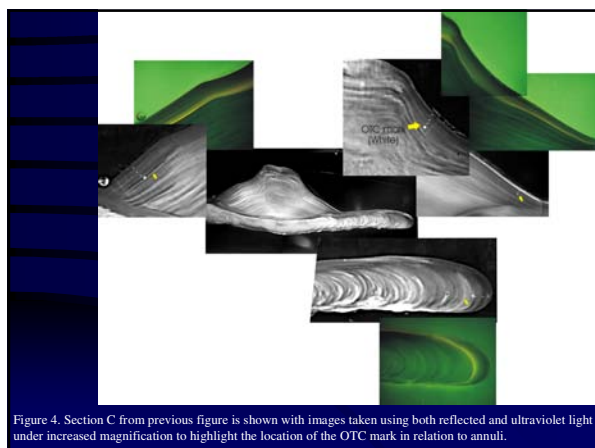


Figure 4. Section C from previous figure is shown with images taken using both reflected and ultraviolet light under increased magnification to highlight the location of the OTC mark in relation to annuli.

## 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.

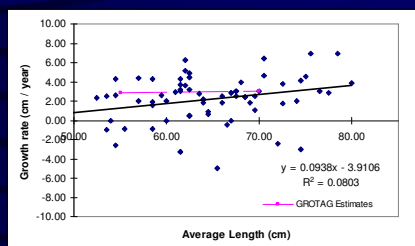


Figure 5. Growth rate (cm/yr) and average length ((length at tagging – length at recapture)/2) for fish at large longer than 0.9 years. The GROTAG model estimates are also shown.

## Carbon-14 Validation

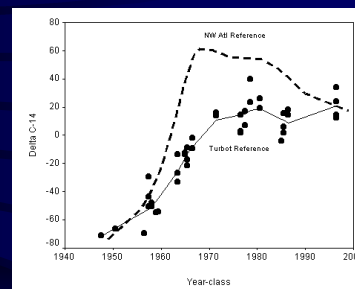
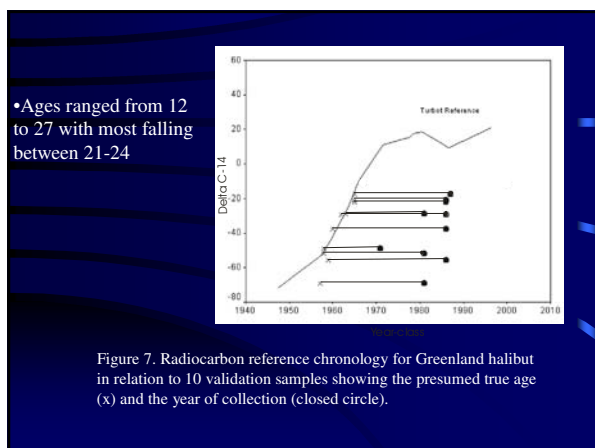


Figure 6. Plot of  $\Delta^{14}\text{C}$  values for Greenland halibut with line fitted using a lowess regression. The reference chronology characteristic of the Northwest Atlantic (Campana et al. 2002) is also shown.



•Ages ranged from 12 to 27 with most falling between 21-24

Figure 7. Radiocarbon reference chronology for Greenland halibut in relation to 10 validation samples showing the presumed true age (x) and the year of collection (closed circle).

Table 3. Results of  $^{14}\text{C}$  assays for mature Greenland halibut otoliths selected for validation.

Year Sampled	NAFO Div. *	Length	Sex	Whole Age-Left	Whole Age-Right	Section Age-Dome	Min. Year Class - $^{14}\text{C}$	Min. Age - $^{14}\text{C}$ Based	$\delta^{13}\text{C}$	$\Delta^{14}\text{C}$
1986	0B	70		16	16	17	1966	21	-3.5	-20.7
1986	0B	74		17	18	15	1966	21	-5.6	-21.9
1986	0B	72		20	20	20	1964	23	-3.4	-37.6
1986	0B	72		14	16	12	1966	21	-3	-29.2
1987	2G c	84	2	-	-	19	1966	22	-2.38	-17.4
1981	2G c	74	2	-	-	18	1958	24	-1.2	-69.1
1981	2G c	75	2	-	-	15	1959	23	-2.13	-51.8
1981	2G c	79	2	-	-	17	1964	18	-1	-28.8
1986	1C	74	2	16	22	12	1960	27	-2.51	-55.8
1971	1C	85		-	19	-	1960	12	-2.3	-49

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

- Minimum core ages over-estimated the whole otolith age by 3-11 yrs and section ages by 1-15 yrs.

- Maximum observed  $^{14}\text{C}$  age was 27 and for whole and section ages it was 20.

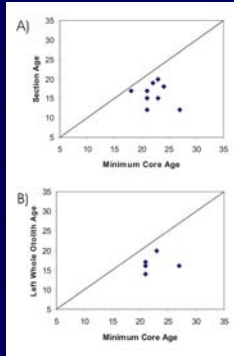


Figure 8. Age bias plots of minimum core age from  $^{14}\text{C}$  and a) otolith section age; b) left otolith whole age.

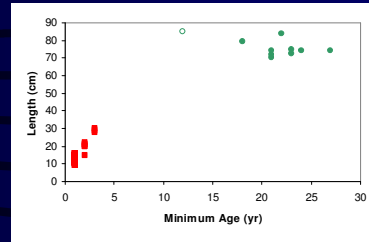


Figure 9. Length and minimum age for samples used for reference curve (red) and validation (green). The reference sample ages are based on the whole otolith method while the ages for the validation samples are minimum ages based on the  $^{14}\text{C}$  assay (Table 3). One of the validation samples 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  $^{14}\text{C}$  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  $^{14}\text{C}$  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!**

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

John Casselman and Rob Slapkauskas

Department of Biology, Biosciences Complex  
Queen's University, Kingston, Ontario K7L 3N6  
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

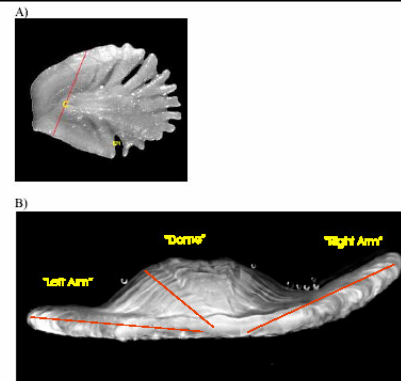
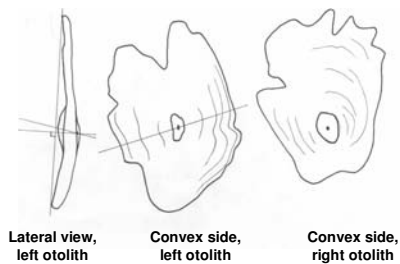


Fig. 2. a) Image of whole otolith showing section plane used in  $^{14}\text{C}$  study (C=Core) and b) image of section showing age reading zones (left "arm", "dome" and right "arm").

Treble et al. 2005

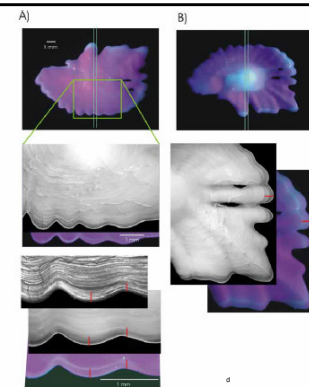
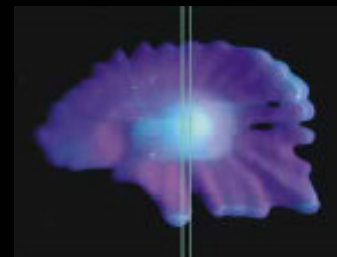


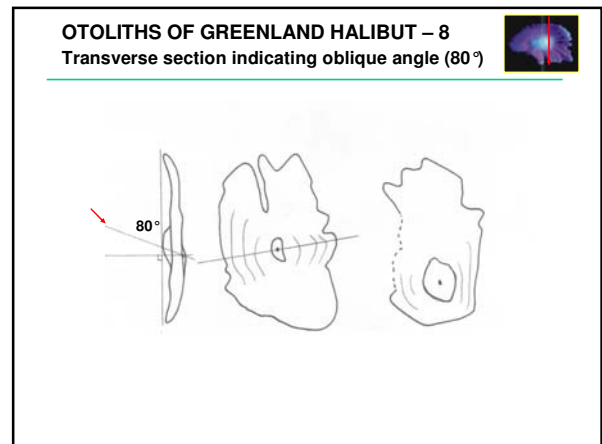
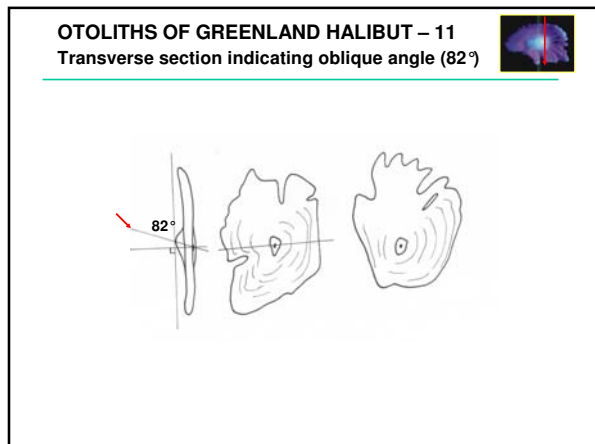
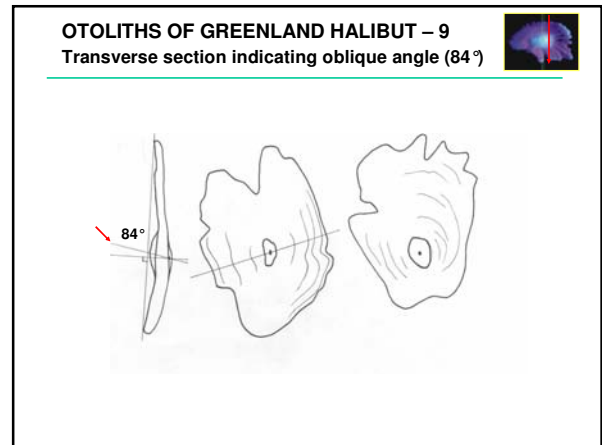
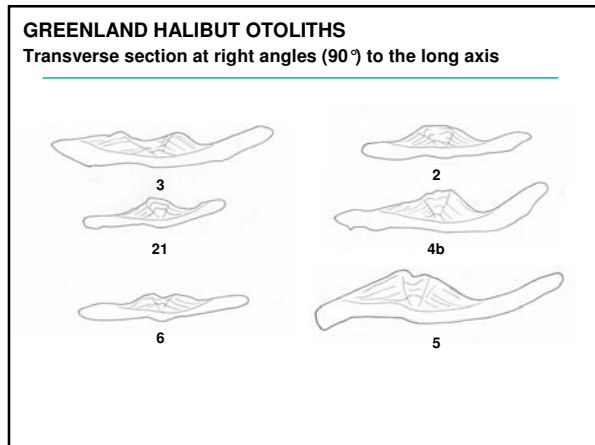
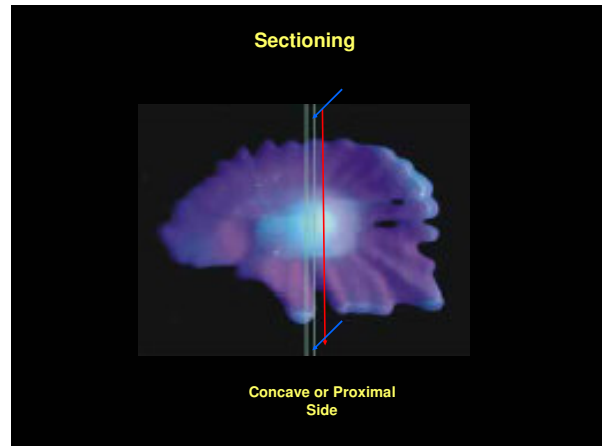
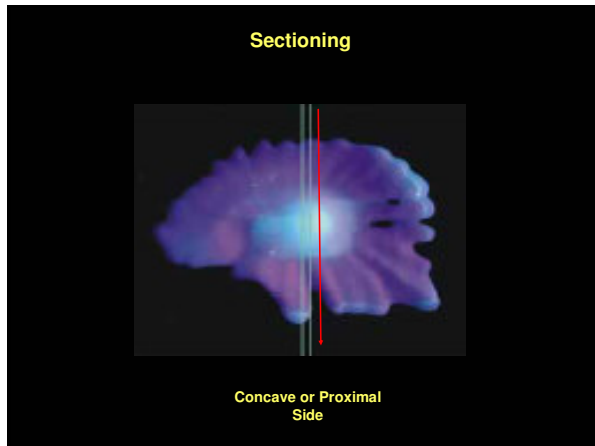
Fig. 4. The OTC mark is visible at the edge in certain areas of the ) distal (A) and proximal (B) ) sides of the otolith sample taken from a Greenland halibut we recovered 3 years (C) months after tagging. Reflected light views of the same area are shown as well as a transmitted light view of the edge in A). The sectioning plane is also indicated.

Treble et al. 2005

### Sectioning



Concave or Proximal  
Side



## GREENLAND HALIBUT OTOLITHS

Transverse section at oblique angle (82.5°) to the long axis

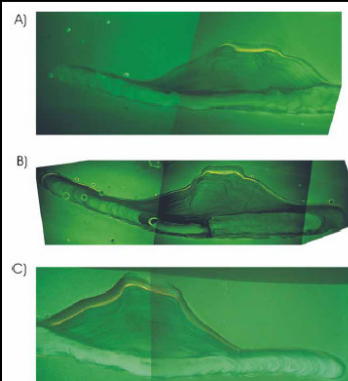
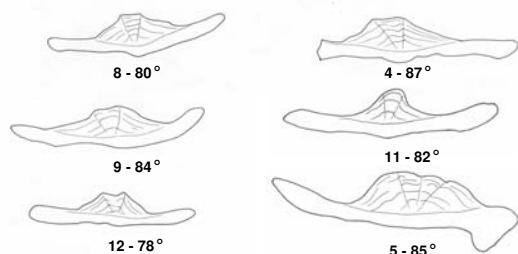


Fig. 5. Photos showing the entire otolith section under UV light for three Greenland halibut marked with OTC and recovered after: A) one year and 11 months; B) 2 years 11 months; and C) 3 years 10 months. The OTC mark shows up as a yellow band near the edge of the section.

Treble et al. 2005

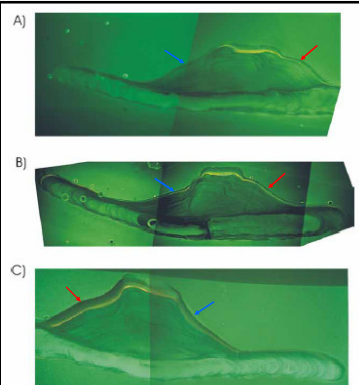
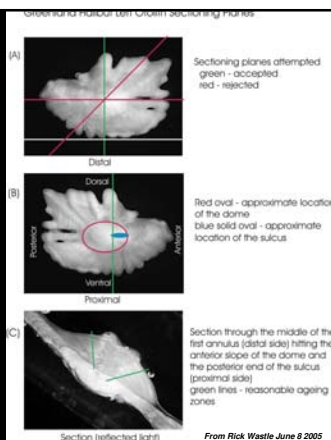


Fig. 5. Photos showing the entire otolith section under UV light for three Greenland halibut marked with OTC and recovered after: A) one year and 11 months; B) 2 years 11 months; and C) 3 years 10 months. The OTC mark shows up as a yellow band near the edge of the section.

Treble et al. 2005



Section reflected light

From Rick Wastle June 8 2005

## 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 -  $7.5 \pm 3.4$  degrees off the right angle to the long axis; a slightly oblique angle

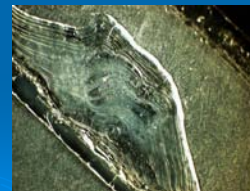
- Sectioned thickness: highly polished thin transverse slightly oblique angled sections ( $325 \pm 20$  microns), less preparation required if liquid immersion is used
- Zonation: zonation should not shift or move appreciably when focusing up and down; provides evidence about the orientation of the zonation 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 interpretation quality of section; maximum growth radii provide the best resolution for separating zonation

A detailed objective study with quantitative measures could provide necessary insights

## Discussion of Image Preparation and Interpretation Techniques

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

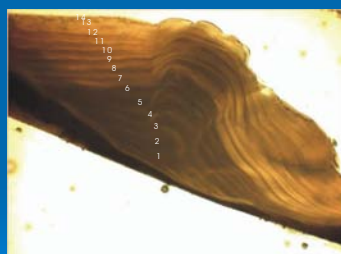
Age interpretation for Halibut Otoliths  
Thin Sectioning vs Acetate Replication



### Section Images

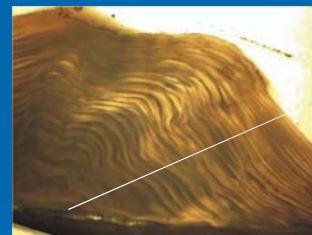
Acetate  
13.5 years

Section  
14.0 years

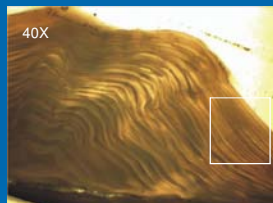


Acetate  
Mean 31 years

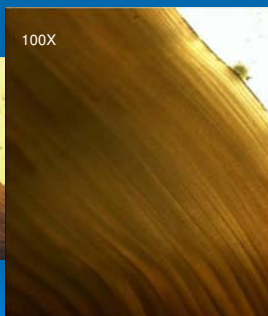
Section  
Mean 26 years



30 years



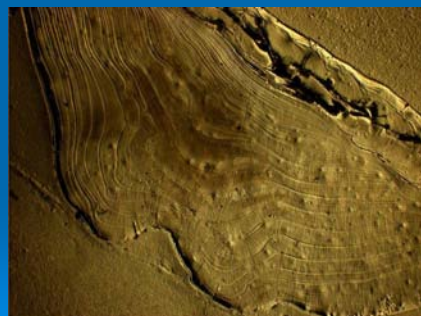
100X



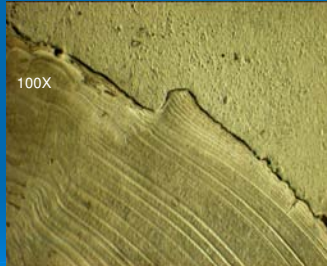
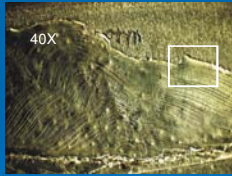
### Replicate images

Section  
Mean age 15

Acetate  
Mean age 14



20 years



#### Age Bias Plot Comparing all Sectioned and Acetate Ages

##### Paired t-test results

No overall difference  
between techniques  
N= 434 pairs,  $t = 0.128$ ,  
 $P = 0.899$ .

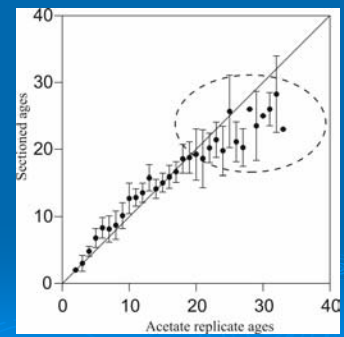
Arbitrary sub-groups based on  
acetate ages

##### Group 1

Age 1 to 20

##### Group 2

Age 21 to 40





## Appendix XIX

### Some comments concerning age interpretation of calcified structures of Greenland halibut

Prepared by Dr. John M. Casselman

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 unvalidated, 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 practised 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 tetracycline marking studies should be initiated. The real advantage of tetracycline labelling is that it provides considerable insights about structure growth. I would also recommend that a good comparative study be conducted to examine various interpretation procedures and that it include partly known-age samples (tag-recapture with tetracycline-labelling). "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 practised. Generally, the calcified structures of this species are very difficult to interpret, probably because the fish live in deep water, growth 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 otolitic 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.