WKARGH REPORT 2011

ICES ADVISORY COMMITTEE

ICES CM 2011/ACOM:41

Report of the Workshop on Age Reading of Greenland Halibut (WKARGH)

14-17 February 2011

Vigo, Spain

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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Recommended format for purposes of citation:

ICES. 2011. Report of the Workshop on Age Reading of Greenland Halibut (WKARGH), 14-17 February 2011, Vigo,Spain. ICES CM 2011/ACOM:41. 39 pp.

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Executive summary

Several age reading methods for Greenland Halibut were described and evaluated together with available validation and corroboration results. The different methods can be classified into two groups: A) Those that produce age-length relationships that broadly compare with the traditional methods described by the joint NAFO-ICES workshop in 1996 (ICES, 1997), typically indicating age around 10-12 years for 70 cm fish; and B) Several recently developed techniques that provide much higher longevity and approximately half the growth rate from 40-50 cm onwards compared to the traditional method. These typically produce age estimates around 20 years or more for 70 cm fish.

All available validation and corroboration results, both several published and a few unpublished, were in favour of group B methods. There are still validation works to be done in order to fully appreciate the full range of variability in the formation of annuli in otoliths from different stocks within different environmental regimes. There is also a need for improved precision, especially for the group B methods. Based on the review in this report, the relevant assessment working groups are advised to seriously consider how to proceed with age reading of their stocks.

1 Abstracts of presentations

1.1 Ole Thomas Albert - Introduction: The present unresolved situation and our expectations for progress during the meeting

There are three key tasks for developing an appropriate methodology for age estimation: 1) We have to find a structure (e.g. otolith, scale, vertebrae) and an axis within that structure with continuous life-long growth; 2) Then we have to see if it is possible to distinguish zones along that axis and to verify that those zones are formed consistently every year; 3) Finally, it is time to develop reading rules according to the validation results and to train age readers to produce age estimates that on average is consistent with the true age (un-biased) and with as low uncertainty (random variability) as possible.

For Greenland halibut, as for many other species, traditional ageing methods are not based on results from validation studies (points 1 and 2 above) and the last workshop in 2006 concluded that it underaged old fish. Since then this conclusion has been validated and supported by several independent publications (Gregg *et al*., 2006; Cooper *et al*., 2007; Treble *et a*l., 2008; Albert *et al*., 2009). Some labs have developed alternative methods that give very different views on population growth rates than what is generally accounted for in assessment models.

The present workshop does not start from scratch, but should build on all previous validation results and fill in with new information. It is validation that is our first priority, not precision, and we should try to make concrete recommendations with an applied attitude, as e.g. answering the following questions: "What is the probable age range for the size groups that constitute the bulk of the fishable stock?" and "What portion of the traditional ageing can be considered as reliable?"

1.2 Delsa Anderl (presented by Ole Thomas Albert) - Description of the "Stained Section" method, including bomb radiocarbon validation

This method is based on bisecting the thickened area near the sulcus on the left otolith, followed by polishing and staining, before annuli are counted along either side of the sulcus using the structure along the dorsal or ventral margins to assist in age interpretation. The method was described in detail at the 2006 workshop (Treble and Dwyer, 2008) and is documented by Gregg *et al* (2006). It is currently used for production ageing of Greenland halibut at Alaska Fisheries Science Center (AFSC). For smaller individuals whole otoliths also may be read using reflected light. The method provides results indicating large variability in individual growth, with 60-70cm fish varying in age from 10 to at least 30 years.

Thirty-two $\Delta^{14}C$ values from the Greenland halibut samples were compared to a loess curve (reference curve) calculated from known age Gulf of Alaska Pacific halibut $\Delta^{14}C$ values. In the analysis of the ^{14}C results, it is important to note that Greenland halibut specimens falling low and to the right of the reference curve does not definitively suggest under ageing. Under ageing cannot be separated from the possibility that some of the Greenland Halibut resided in a water mass with lower bomb $\Delta^{14}C$, i.e., below the mixed surface layer. The two possible reasons for these results are confounded and cannot be separated, so comparisons of ageing error between Greenland Halibut and the reference curve should be made with these caveats in mind. In most cases, prior to validation, the stained section method estimated birth years that were within 3 years to the left or right of the reference curve. No samples showed estimated birth yrs >3 years to the left of the reference curve but there were at least 9 cases where birth years were >3 years to the right of the reference curve. Several Greenland Halibut samples were used for illustration in Presentation no. 2. Six samples, ranging in length from 62 cm to 91 cm, were from the bomb ^{14}C study. Their $\Delta^{14}C$ values when compared to the reference curve estimated birth yrs that would suggest ages of 15 years to 33 years, which was greater than previously estimated by counting presumed annual zones. In two cases, the birth years fell at the bottom of the reference curve between 1950 and 1955. In the other four cases, pre validation birth year estimates fell 3-7 years to the right of the reference curve. Retrospective examination of the growth pattern from these four samples subsequently led to a slight revision in the ageing criteria most notably after an observed transition zone where more densely packed annuli are observed in outer regions of sections from older specimens. A transition from faster growth to slower growth has been identified as young as 7 years and as old as 12 years. The cause of this change in growth pattern is not known but may indicate maturity (Cooper *et al* 2007). The oldest cross-section age was approximately 31-34 yrs and corresponded to $\Delta^{14}C$ values from the pre-bomb era.

1.3 Merete Kvalsund - Description of the "New Whole Right" method

Institute of Marine Research (IMR) in Norway uses digital images to age the right, whole otolith with transmitted light. Both otoliths are collected and put into plastic trays that have 24 wells. Salt or fresh water is added to the wells to prevent the otoliths from drying, then they are stored frozen until starting to photograph them. After thawing and cleaning, the otoliths are submerged in water and photographed under a stereomicroscope with transmitted light, using a digital camera. To achieve a high quality image it is important to be accurate with the lighting, white balance and focusing. After the otoliths are photographed they are stored in standard otolith envelopes. The images are imported to Photoshop, calibrated to a 10 mm-scale and an enhancement layer and several layers for individual interpreters are added. Finally, the Norwegian “new whole” method uses 5 rules for age interpretation:

- Rule 1: The age reader does not know the fish size during interpretation. The only available information is date of catch and sex.
- Rule 2: Count the hyaline (winter) zones. The right otolith is preferred, and counted along an axis from the center and out to the longest point on the anterior margin or “fingertip”.
- Rule 3: There is very little overlap in the otolith length composition of age 0 and age 1. The inner edge of the first translucent zone defines a shape with a longest diameter of 2.0 (± 0.5) mm.
- Rule 4: The first several opaque (summer) zones are relatively wide, and much wider than the translucent ones. On average, these zones gradually decrease in width. The outer zones are narrow and of more similar width. The transition between the two growth patterns may be gradual or abrupt, and varies between interpretation axes. Any zone that does not fit with such an *a priori* growth pattern is considered to not represent a seasonal zone, and should not be counted.
- Rule 5: By definition, change in age occurs on 1 January.

1.4 Margaret Treble - Description of the "Traditional Whole" method

Age determination methods from North Atlantic Fisheries Centre-Canada-Prepared by K. Dwyer, R. Burry and B. Greene

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

1.5 Rick Wastle - Description of the "Hydrochloric Acid and Lateral Grind" method

Researchers at Fisheries and Oceans Canada's Freshwater Institute (FWI) noted impressive ring patterns seen on laterally ground strontium chloride marked Greenland Halibut otoliths which led to the exploration of the lateral grind technique and later a hydrochloric acid treatment method, to help with age interpretation. Due to the curvature of the distal reading surface of these otoliths, the lateral grind technique was often limited in how it could be applied. A 20% hydrochloric acid treatment method was found to expose the rings regardless of the otolith shape. The HCl treatment worked on both left and right otoliths, but had limitations for the smaller and larger ones. On small otoliths, the HCl treatment caused weaker rings (presumably false) to be exposed along the longest reading axis, leading to over-ageing. On the large otoliths, irregular exposure of the reading surface could lead to one area looking over treated by the acid, while another area on the same otolith could still appear to require more exposing. By applying the HCl treatment followed by a lateral grind, it was often possible to expose all the rings, including close together edge rings, on large otoliths. Three preferred ageing zones were identified depending on otolith size, and potential problems with these procedures were addressed.

1.6 Oleg Smirnov - Brief description of the methods used at PINRO

Age reading methods of Greenland halibut by scales and otoliths used by Knipovich Polar Research Institute of Marine Fisheries and Oceanography (PINRO, Murmansk) are described.

<u>PINRO scale method</u> used by PINRO lab for a long time is based on early research comparing various ageing structures, including scales, otoliths, vertebrae and fin rays from Milinsky (1944) and Krzykawski (1976). Scales are removed from the dorsal area above the lateral line. Scales are dried flat in paper envelopes at sea at moderate temperature (approx. 20°C). In the lab the scales are soaked in ammonium hydroxide to remove any mucous membrane that may be left on them. Scales selected for age reading are not damaged and of uniform size. After selection several scales are pressed between two glass slides and read with a microfiche using transmitted light. A com-

bination of widely spaced and narrowly spaced (seen as a dark band on the scale) circuli, are considered an annulus.

PINRO otoliths methods is the same as old (traditional, production) Norwegian method. The left whole otolith used for age reading. Otoliths are stored dry in paper envelopes. Prior to age reading procedure otoliths are soaked in water and then investigated in transmitted light with 9-16 magnification.

The results were compared and conclusion was made on their consistency. Annual length growth at age 1-4 years is 6-7 cm, 4-5 cm at age 5-9 years, 2-4 cm at age 10-20 years.

1.7 Maria Dolgikh - Traditional method of age determination with use of two registering structures (scale and otoliths)

Experts from Russian Federal Research Institute of Fisheries and Oceanography (VNIRO, Moscow) read Greenland halibut age using cross-sectioned otolith, as otoliths allow easier identification of annuli, especially for older fish. Cuts are ground and age is read without staining in reflected light. Comparison of results of age reading by scale and by otolith demonstrated that both structures qualify for age reading and the methods demonstrate corroboration for fish that constitute the majority in the catch structure (<60 cm length) (2010 data, n=350). The difference in age determined by scale and otolith is insignificant. It implies that the traditional methods of age reading by scale (used in Russia) and by otolith are equally applicable. Age reading based on assessment of two independent registering structures allows objective determination of age and minimize the possibility of bias. Taking into account the predominance of fish with size of up to 60 cm in catches, the age reading by scale in previous years may be considered as rather objective. Determinations by cross section of otolith showed the pronounced annual rings. The new proposed methodology results cannot be compared with results of other existing age reading methods.

1.8 Margaret Treble - Within-lab comparisons of five methods

See section 2.2 below

1.9 Margaret Treble - 2010 Otolith Exchange comparing "Stained Sections", "New Whole Right", and "Traditional" methods

See section 2.2 below.

1.10 Steve Campana - Age validation and corroboration methods

Although many otoliths can be aged precisely, and although many experienced age readers are very consistent in their ages, consistent ages are not necessarily accurate ages. There have been many high-profile examples of stock assessment models failing, or even fisheries collapsing, after tens of thousands of otolith readings by experienced age readers which were subsequently determined to be wrong. Confirmation (or validation) that an ageing method is correct does not necessarily mean that all of the individual ages are correct; only that the method produces correct ages on average over the entire age range of the species or stock. Of the available methods, the following are most accurate: 1) release of known-age and marked fish into the wild; 2) bomb radiocarbon to identify fish born in the 1950s and 1960s; and 3) mark-recapture of chemically tagged wild fish after more than 1 yr. While widely used, marginal increment analysis is best suited to very young fish, and often yields unreliable results for older fish. Bomb radiocarbon assays are best suited for old fish born in the 1950s and 1960s, but can be extremely useful in that they validate the actual age of the fish, not just the formation of otolith annuli near the edge. Carefully selected samples for bomb radiocarbon assay can produce age estimates which are accurate to within 2-3 yr, with no ambiguity. Various other methods can provide age corroboration; although they do not actually validate the age of the fish, they can provide strong supporting evidence. Examples of age corroboration methods include modal length analysis, tag recapture analysis and progression of strong year-classes. On the other hand, there are several methods which provide indicators of ageing consistency, but do not provide any confirmation or corroboration of ageing accuracy. Such methods include age comparisons among age readers, growth back-calculation, comparisons among multiple structures within a fish (eg- scale vs otolith), and consistency with published papers that did not actually conduct age validation. Once an ageing method has been validated, it can often be made more precise through subtle refinements.

1.11 Ole Thomas Albert - Results of OTC experiments related to the three methods: "Stained Sections", "New Whole Right", and "Traditional"

Almost 25 000 juvenile Greenland Halibut were tagged and injected with Oxytetracycline (OTC) in Svalbard waters during surveys in September 2006-2008. Since these remote juvenile areas are far from the major fishing grounds, few recaptures are expected until the fishes enter the fishable portion of the stock. Also, data analyses depend on return of the whole fish, or at least the otoliths, which also limits the return rate. However, there are regular surveys with hired commercial trawlers in the Barents Sea and the Svalbard areas, and these provide some recaptures of younger fish.

So far 12 OTC otoliths in good condition have been collected from fish that have been at large for 1-4 years with growth rates ranging from 0.0 to 5.4 cm/year. They ranged in length at release from 33 to 48 cm, and in length at recapture from 39 to 51 cm. The three slowest growing (0.0-0.5 cm/year) were on average 44 cm at time of release (range 39-46 cm), and the three fastest growing ones (3.1-5.4 cm/year) were on average 34 cm at release (range 33-36 cm). These 12 otoliths were prepared and analysed according to three different age reading methods that are commonly in use at present: 1) The traditional whole left otolith method, 2) The new whole right otolith method, and 3) The stained section method.

All twelve otolith pairs showed growth increments beyond the OTC time stamp representing time at release of tagged individuals. The growth increments were not evenly distributed along all axes, indicating that some reading axes are more appropriate than others. For method 2 above, the increments were consistently found along the preferred reading axis from the nucleus of the acentric right otoliths and along the fastest growth axis towards the anterior finger-like extentions. For all otoliths it was possible to distinguish the expected number of annuli between the fluorescent band and the outer margin. For method 3, growth increments were also seen consistently for all otoliths, although it was a bit unclear whether the expected numbers of annuli were present on all samples (this may be due to preparation by inexperienced personnel). For method 1 above, no additional growth was seen for the three slowest growing individuals, making accurate age estimation impossible. For another three relatively slow growing individuals, some additional growth was seen, but not the expected number of annuli. For the three fastest growing ones similar patterns of surface growth were seen on both the left and right otolith (methods 1 and 2 respectively).

It is concluded that the whole left otolith, which is the main basis for the traditional method, is unsuited for identifying annuli of slow growing individuals. Since the OTC experiment was made on juveniles, no data are so far available for old individuals. The results show however, that the problem of missing annuli in left otoliths may arise for fish down to 39 cm, and for immature and mature individuals of both sex.

1.12 Margaret Treble - Results of $SrCl_2$ marking

Use of strontium-chloride and oxytetracycline marked otoliths to examine otolith growth and validate ages– by Tracey Loewen, Margaret Treble, Norm Halden, Rick Wastle, Jim Reist and Kaj Sünksen - presented by Margaret Treble

One of the recommendations from the Greenland Halibut age determination workshop held in St. John's in 2006 was that tagging and mark-recapture studies be conducted for each stock area in an effort to document otolith growth and validate age methods. In 2007 such a study was undertaken by the Greenland Institute of Natural Resources (GINR) for NAFO Division 1A and Fisheries and Oceans Canada (DFO) for Division 0A. Fish were tagged with Floy tags and approximately half were also injected with a solution of Strontium Chloride (**$SrCl_2$**) which binds with calcium leaving an elevated **$SrCl_2$** signal in the otolith at the time of tagging. In 2008 otoliths from several fish that had been marked with **$SrCl_2$** were returned to GINR. These otoliths were subsequently sent to DFO for analysis at the University of Manitoba using a scanning electron microscope and electron microprobe. Results from two samples caught 1 year and 2 months after being marked were presented. One was a 43 cm fish that had grown to 45 cm since tagging. The right otolith growth was greatest (approximately 250 microns) in the rostrum, anterior to the nucleus and was considerably reduced as you moved to the posterior margin. The presumed annulus corresponding to the time since marking was observed. The age had been estimated at 13 years by examining the distal surface using reflected light prior to preparation for **$SrCl_2$** analysis and a minimum age of 10 was determined following analysis using a modification of this surface method involving a lateral grind treatment. The central region of the otolith was still covered with epoxy after the lateral grind procedure, making annuli in that area difficult to interpret. The second sample was a 46 cm fish that had not grown in length since tagging. The slower growing ventral region of the right otolith was examined and the **$SrCl_2$** mark located less than 100 microns from the edge and declining to less than 50 microns in the area posterior to the nucleus. The right otolith was initially aged at 12 years (distal surface, reflected light), and after the lateral grind preparation for **$SrCl_2$** analysis was re-aged at 13 years (one extra annulus near the edge). The surface (posterior to ventral region) of the corresponding left otolith was examined. Growth was greatest in the posterior margin, declining towards the ventral margin. The presumed annulus corresponding to time since marking could be observed at the posterior margin. The age had been estimated at 12 years prior to the lateral grind/ **$SrCl_2$** analysis (distal surface, reflected light) and 13 years following. The left cross-section was also examined and exhibited maximum growth zones on the ventral and dorsal margins and on the centrally thickened region of the proximal margin on either side of the sulcus. This section was initially aged at 12 years (reflected light, no staining), and still aged at 12 years after the **$SrCl_2$** analysis. The last annulus counted for the initial ageing, corresponded to the expected last annulus determined after examining the **$SrCl_2$** indicated growth. Upon review, one more possible annulus was identified further interior from the margin which could indicate an age of 13 years.

Oxytetracycline marked otoliths from Cumberland Sound, off Baffin Island in NAFO Div. 0B, presented at the 2006 workshop and published in Treble *et al*. 2008 indicated active growth zones on the anterior margin of the right otolith after preparation by the lateral grind technique. The samples were from two females (64 cm, 2 years 11 months at large and 66 cm, 3 years 10 months at large) aged 20-22 years using the whole surface method with reflected light. These growth zones were examined for this meeting. They were found to be very compact and required higher magnification to locate the presumed annuli. Although this preparation didn't help to identify annuli prior to the OTC mark, it did show very slow growth after the mark for every year these fish were out after tagging. Handling stress may influence growth in the short term, however, these fish were at large for 3-4 years and the results indicate that the annual growth beyond one year post-marking was small and annuli could not have been seen on the surface of the otoliths without applying higher magnification and this lateral grind treatment.

1.13 Ole Thomas Albert - Growth analyses from Norwegian tag recaptures

There are mainly three series of recent tagging experiments on Greenland Halibut in Norway: 1) In 1995-1997 there were several small scale tag releases as part of regular trawl surveys; 2) In 2002-2005 there was a large scale tagging program for adult fish in the main fishing areas based on fish caught with long-lines; 3) In 2006-2008 a large-scale OTC tagging program was accomplished during three dedicated surveys to the juvenile areas north and east of the Svalbard archipelago using bottom trawls with a specially designed cod-end aquarium.

Until December 2010 a total of 1565 recaptures were recorded. Due to a large field program on Greenland halibut up to 2009, a relatively large portion of the total Norwegian catch was taken by vessels carrying IMR personnel. Therefore as many as 525 of the recaptured fish were measured and sampled by trained personnel using electronic measuring board and other standard equipment, making estimates of growth increment during time at large more accurately than in most other tag recapture experiments. For estimation of growth only those recaptures that had been at large for more than one year (N=239) and that were not considered as outliers (N=9) were used, leaving 230 accepted individual growth estimates. Of these, 116 individuals had been at large for more than two years, and 49 individuals for more than three years.

To further improve accuracy of growth estimates during time at large, possible post mortem shrinkage of fish, as described in Albert *et al.* (2009) was accounted for by a model describing shrinkage as a linear function of length at recapture for fish larger than 50 cm (Shrinkage 0 cm at 50 cm and 1 cm at 80 cm). A rough growth model was fitted to the estimated mean growth rate during time at large for each 5 cm length group of fish measured at release. Due to lack of time for preparation, the model was not based on any elaborated fitting procedure, and the resulting estimated length at age curve was without confidence intervals and should only be regarded as an example and as information about a work in progress. Even with the analytical shortcomings it is still worth noting that the length at age curve described quite well the age-length data based on the new whole right otolith method, with 70cm as the mean length at age 30, and with mean annual growth of 1 cm per year for fish between 50 and 70cm.

1.14 Kaj Sünksen - Growth analyses of tag recaptures from NAFO SA0 and 1

Greenland Institute of Natural Resources has in the period from 2007 to 2009 released 12 500 Greenland halibut tagged with conventional floy tags. Of these 2000 were tagged in Canadian waters and 9000 in the offshore Greenlandic area while the rest were from inshore areas in Greenland. In 2007 3000 of the fish that were tagged with the conventional floy tag were also injected with a $SrCl_2$ solution in order to set a mark in the otolith.

Until February 2011 208 of these tags were returned with information on the recapture area. 33 of these fish had been at sea more than 300 days and had information on length at both release and recapture. Of these 9 were frozen fish returned to the lab at Greenland Institute of Natural Resources where they were measured. In addition to this another 9 fish were measured by either trained or trusted persons. Estimated mean growth rate were in the order of 2 cm/yr for a 45 cm fish.

1.15 Steve Campana - Statistical methods for estimating age composition

The age composition (proportions at age in a sample or population) can be estimated in two ways: directly, through age determination of a random sample of fish; or statistically, via either an age-length key or mixture analysis. Typically, mixture analysis is used to estimate age composition based on a large number of fish length measurements, and a smaller subsample of direct otolith ages. However, a method has been developed which allows otolith weights to be included in the process, which can improve accuracy. Otolith weights usually increase with fish age, as do fish lengths. However, otolith weights often differ more among older age groups than do fish lengths. Log-log plots of otolith weight vs fish length often show clear groups corresponding to age groups, at least for the younger age groups. These age groups can be defined using bivariate ellipses and then estimated using a mixture model described in Francis *et al* (2005). The benefits of this approach are primarily one of cost, since large numbers of direct age determinations using a validated and precise ageing method will yield a more accurate age composition than will mixture models. However, a similar age composition can be produced much more quickly and inexpensively using a small number of age determinations, a larger number of quickly-acquired otolith weights, and an even larger number of fish length measurements incorporated into a mixture model. This approach assumes that the age determinations are accurate (on average), and needs to be repeated for each stock or year where the mean growth rate differs. A commonly-used variation of mixture analysis (based initially on Macdonald and Pitcher 1978) uses only a subsample of direct ages (no otolith weights) to decompose a catch or population length frequency into a catch at age matrix. This approach is well documented, requires only accurate estimates of mean length at age (and accompanying standard deviations), and is an excellent approach for estimating catch at age in a stock where there is an accurate underlying growth model and where routine ageing is problematic.

1.16 Alf Harbitz - Morphometric analyses of Greenland Halibut otoliths

The major goal of the presented work was to explore the ability to estimate growth of Greenland Halibut in terms of fish length, based on otolith growth from OTC otolith images. First about 800 right otolith images from the Barents Sea and Norwegian Sea were analyzed (10-90 cm fish), and a close to proportional relationship between fish length and maximum otolith diameter was found. Then the found relationship was applied to the maximum otolith diameter at capture as well as the estimated maximum otolith diameter at release from the OTC marks in 12 images of OTC marked otoliths (33-48 cm fish at release). The estimated growth of fish length was finally calculated as the difference between the predicted fish lengths at release and capture. By comparison with observed growth (0-15 cm), a mean difference of -0.036 cm and a standard deviation of the differences equal to 0.87 cm was found.

1.17 Yvan Lambert - Growth rate of Greenland Halibut in experimental conditions: Can results be useful in interpreting size structure and growth patterns of different stocks in their natural environment

In the absence of age determination, the use of growth rate estimates as a means of determining mean size-at-age of Greenland Halibut was examined using the northern Gulf of St. Lawrence population. Growth rates were estimated from both the natural environment and experiments conducted in controlled conditions. The examination of seasonal and annual size frequency distributions of Greenland Halibut in the Gulf of St. Lawrence were used to determine sizes-at-age from modal lengths for juvenile fish while previous work conducted in the 1980’s was used as an indication of the mean sizes-at-age of adult fish. Different growth experiments conducted at temperatures between 2 °C and 6 °C on fish ranging in size between 15 cm to 63 cm were used to obtain a relationship between specific growth rate and fish length. Comparisons between mean sizes-at-age observed in the Gulf of St. Lawrence and estimated sizes-at-age from size-specific growth rates in controlled conditions demonstrate the possibility of deriving a growth model that could potentially be used as the basis for the age-structured decomposition of the population length frequency using conventional mixture models.

1.18 Kaj Sünksen - Temperature effects on juvenile growth

(*Based on Sünksen et al. (2010) Journal of Sea Research 64:125-132*)

The relationship between temperature and growth of juvenile Greenland Halibut from 1993 to 2003 was studied in West Greenland offshore waters. In 1993–1996 the average temperature was 2.0 °C but it increased to 3.4 °C in 1997 and remained at this level for the rest of the study period. Concurrent with the temperature increase, mean total length of 1-year-old Greenland Halibut increased significantly in all areas and depth strata. For example, mean length of 1-year-old halibut in Disko Bay at depths from 201 to 400 m increased from about 13 cm to 15 cm when temperature rose from 1.5 °C to 3 °C. On average the mean length of the 1-year-old Greenland halibut increased 1.6 cm °C^{-1} and, similarly, the increase in length from 1 to 2 years old increased on average 1.5 cm °C^{-1}. Absolute growth of Greenland halibut 1 to 2 years old varied between 6 and 10 cm depending on temperature.

2 Evaluation of the different methods (ToRs a, b and c)

The following Terms of Reference (ToRs) were considered during the workshop and are addressed below:

a) Review information on age estimations, otolith exchanges, workshops and validation work done so far;

b) Evaluate all available information on individual growth patterns in order to achieve a general consensus about the most probable levels of longevity and growth rates for the different stocks; and

c) Report on progress in studies of otolith growth axes based on samples from Greenland halibut injected with OTC or similar substances that makes a mark in the ageing structure.

2.1 List of methods evaluated by the Working Group

Underlined methods are the preferred methods currently used by one or several labs for production ageing.

1) **Cross-sections** of left otoliths bisected through the nucleus along transverse axis:

   a) **<u>Stained</u>** with interpretation of zones down the central bulb and use of digital images as applied by Alaska Fisheries Science Center (Presentation no. 1.2) and described by Gregg *et al.* (2006);

   b) **Unstained thick sections** (approx. 2 mm) with interpretation of zones down the central bulb as applied by Alaska Fisheries Science Center and described by Gregg *et al.* (2006). Used by FWI-Canada;

   c) **Unstained** with interpretation of zones using section method as described by Alaska Fisheries Science Center Gregg *et al.* (2006), but with reading axis towards the dorsal and ventral edges. Also prepare right cross-section if left otolith is broken or missing. Used by VNIRO-Russia (Presentation no. 1.7).

2) **Whole right otolith viewed with transmitted light:**

   a) **<u>Using digital images</u>** and interpreting seasonal zones along the longest growth axis, as applied by Institute of Marine Research (Presentation no. 1.3) and described by Albert *et al.* (2009);

   b) **<u>Using polarized light</u>** and interpreting seasonal zones along the longest growth axis, if in doubt aided by the left otolith. Used by Greenland Institute of Natural Resources.

3) **<u>Traditional method</u>** using whole left otoliths viewed with reflected light, as well as other variants described by the 1996 joint ICES-NAFO workshop in Reykjavik (ICES, 1997) giving broadly similar results. Most laboratories dealing with routine ageing of Greenland halibut have used this method, or some of its variants (e.g. transmitted light) for many years both before and after the 1996 workshop.

4) **Right Otolith Hydrochloric Acid and Lateral Grind at Margin**

Left and right otoliths viewed with reflected light along the longest growth axis after treating with hydrochloric acid, as described in the present working group (Presentation no. 1.5). Lateral grind treatment could be applied to expose edge growth in largest samples as done for **$SrCl_2$** marked samples (Presentation no. 1.12). Trials conducted by FWI-Canada.

5) **Scales** as used in production ageing of the Barents Sea and NAFO area stocks by PINRO and described in Presentation no. 1.6.

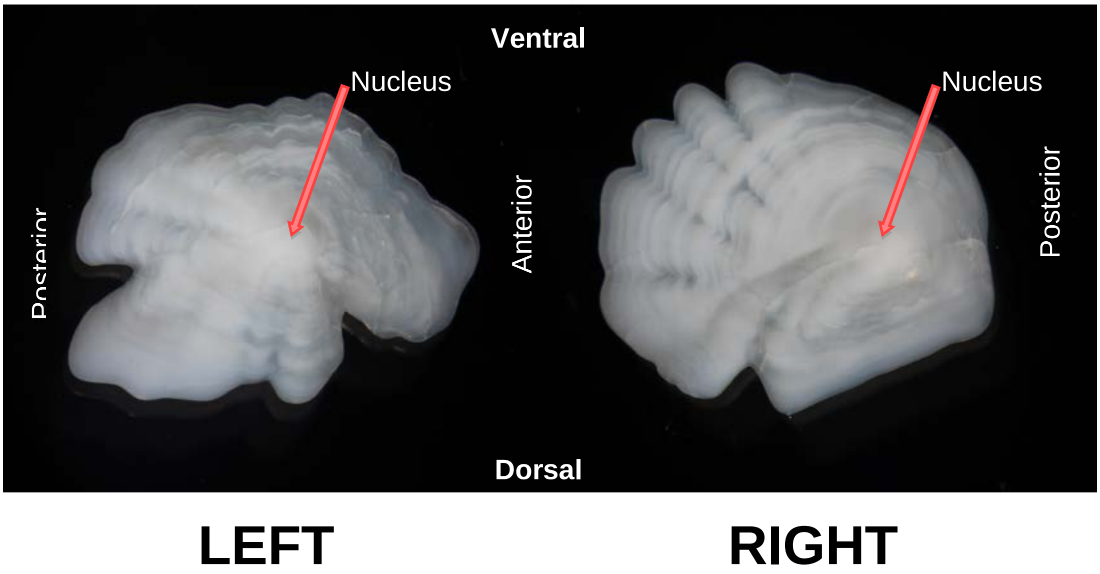

Figure 1. Greenland Halibut otoliths. The typical section plane on the left otolith runs slightly oblique to the transverse plane (ventral to dorsal margin), bisecting the nucleus and passing through the thick perisulcular region (see Gregg *et al.* 2006 for more detail).

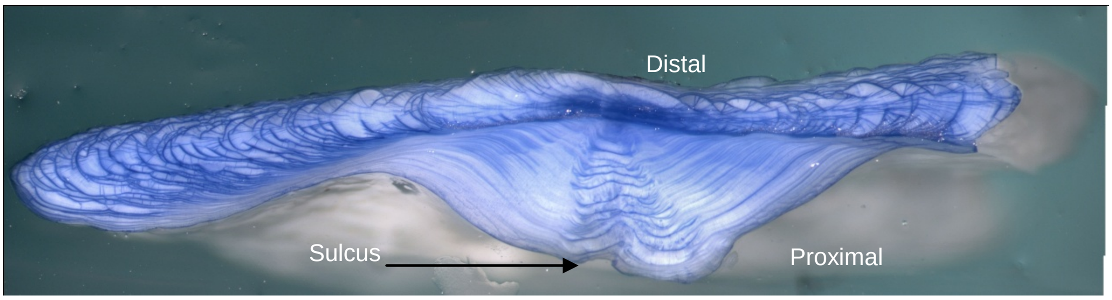

Figure 2. Stained section of the left otolith of a Greenland Halibut from NAFO Division 3KL (see Presentation no. 1.9). Note the area of increased growth on the proximal surface near the sulcus. This is the area that contains the preferred reading axes when ageing using the section method.

2.2 Comparison of methods

Results from comparisons of structures and methods can indicate the level of precision and relative bias between methods, which can be helpful in comparing criteria used by different readers and laboratories. The results do not indicate which methods if any are accurate. However, they do indicate which methods tend to produce older ages than others.

2.2.1 Review of 2005-2006 exchange

Age bias plots produced from the 2005-06 exchange of otoliths and scales were reviewed. Samples were collected on the Flemish Cap and were limited to a size range of 15 cm to 57 cm (n=100). The samples were aged by labs in Canada, Spain, Portugal, Russia, Greenland and Norway. The otoliths were read whole using the traditional method, without any additional preparation. Following the workshop labs in Norway and Portugal (INIAP/IPIMAR) re-aged the samples using their typical method, right whole using transmitted light and baked using reflected light, respectively. Ages ranged from 1 to 10 years, coefficient of variation between readers varied between 3.5% and 17.8% (Treble and Dwyer 2008). Age bias plots from this study were reviewed at this meeting. Two transmitted light methods, Greenland (method 2b. above) and Norway (method 2a. above), and the baking method applied in Portugal, produced ages which were 1-2 years greater than the traditional method. This bias between the methods began at age 4 to 5 using the traditional left whole otolith method (Fig. 3 below). In addition the transmitted light method applied in Norway had increasing bias beyond age 5. This method has subsequently been revised based on OTC marked fish. The scale method produced ages which were lower than the traditional method beginning at age 3 with the amount of bias increasing with age.

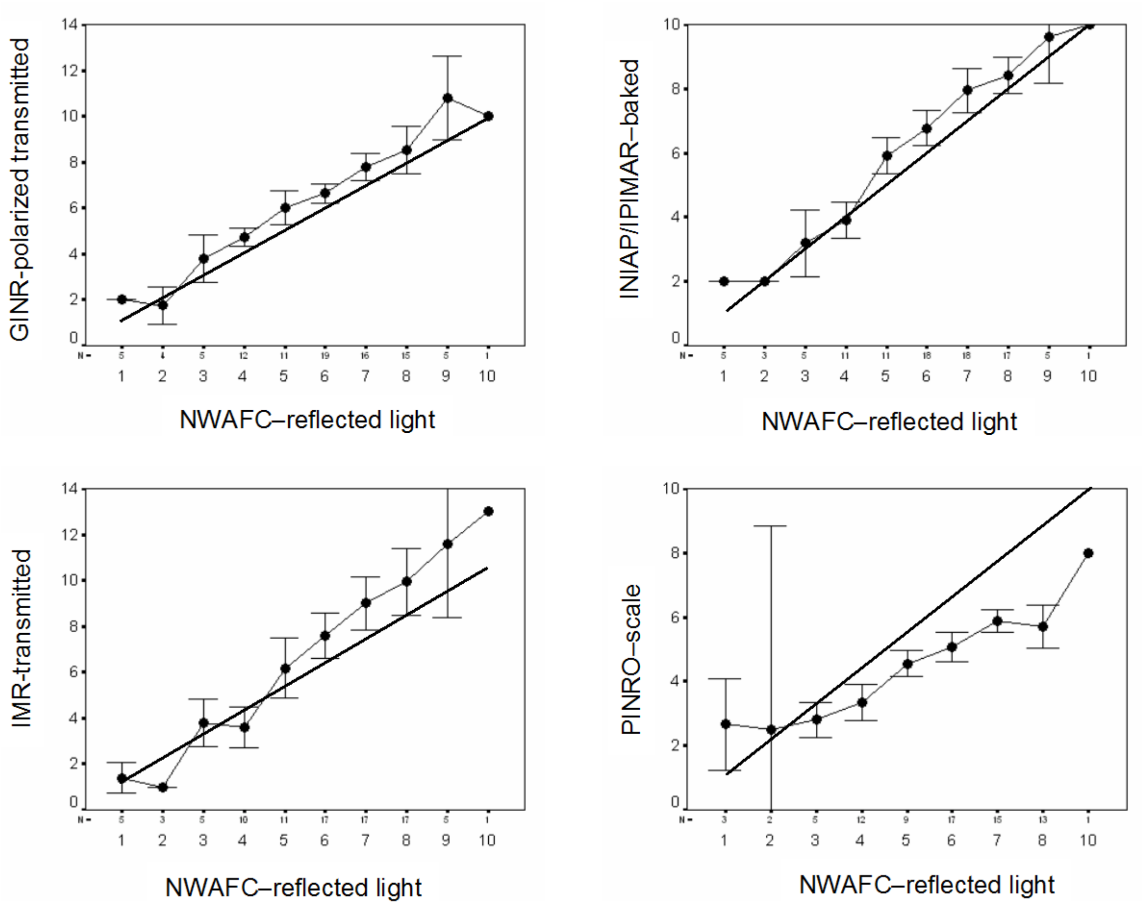

Figure 3. Age bias plots from 2005-06 exchange samples of small Greenland Halibut (15-57 cm) from Flemish Cap.

2.2.2 Review of 2010-2011 exchange

Information was reviewed for the most recent exchange of otoliths collected from the fall trawl survey of NAFO Division 3KL and frozen at sea (n=184). Three different methods were applied by the labs experienced in these methods. However, the preparation was not ideal for the application of the IMR method due to the frozen samples drying out during shipping. There was also damage to the samples during shipping and the Alaska Fisheries Science Centre was only able to age 96 samples using the left cross-section method, with very few greater than 50 cm. Samples ranged in size from 12 to 57 cm and the maximum overall age assigned was 11 years. Coefficient of variation between methods ranged from 11% to 13%. The IMR method (no. 2a) produced older ages compared to the NWAFC method (no. 3) after age 5 and the AFSC method (no. 1a) after age 3 (Fig. 4). The bias is increased with age in both cases. The NWAFC method produced older ages compared to the AFSC method by about 1 year beginning at age 2 and increasing to about 2 years by age 8. No biases were observed between methods up to age 3. Biases are observed between all methods from age 5 onwards.

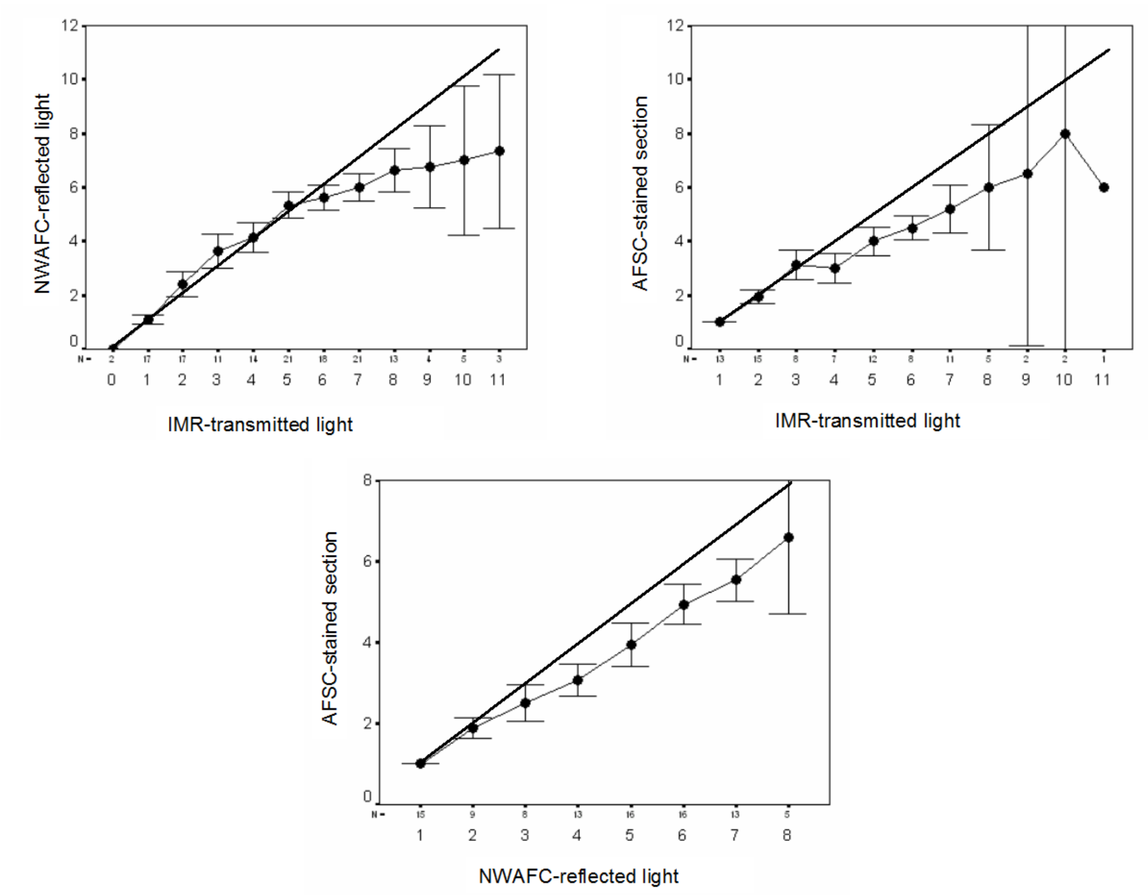

Figure 4. Age bias plots from 2010-11 exchange samples of small Greenland halibut (mostly below 50 cm) from NAFO Division 3KL.

2.2.3 Fisheries and Oceans Canada, Central and Arctic Region within lab comparison of methods (1a, 1b, 2a, 3 and 4)

Following the 2006 workshop we decided to apply four methods to a set of otolith samples to assess their ability to improve age determination for Greenland Halibut. The methods were: 1) the left whole otolith using reflected light (reading along the longest growth axis); 2) fresh frozen right whole otolith using transmitted light (IMR method); 3) transverse cross-section of the left otolith with no stain applied; and 4) left cross-section with stain applied, using the AFSC method (Gregg *et al.* 2006). A fifth method using hydrochloric acid applied to the right otolith, developed recently by our lab (see above), was also included. The sample set was comprised of 100 pairs of otoliths collected and frozen at sea during multi-species trawl surveys in 2007 in Divisions 1D (fish >25 cm) and 0B (fish <25 cm). An equal number of males and females were included and overall size ranged from 6 cm to 89 cm. Maximum ages ranged from 22 years for the unstained section to 27 years for the stained section, left whole reflected and right whole HCl and to 37 years for the right whole transmitted method. For many methods the growth in length at age slowed after approximately age 5. There were challenges in applying methods that were not familiar (i.e. right transmitted and left stained section) which could have influenced the results. Most methods presented difficulties in identifying the first few annuli in young fish (less than approximately age 10) and it was often difficult to resolve the middle ages (approximately 3-8 years) in larger/older fish. This may explain why the precision in assigned ages (coefficient of variation) for each combination of these methods were high, ranging from 12.69 for the two left cross-section methods to 31.18 for the two right whole methods. Bias plots for several combinations of the methods were examined at this meeting: the left stained section and left whole reflected both gave older ages than the left unstained section; the right whole HCl and left unstained section gave similar ages; and the right whole transmitted method gave greater ages compared to both the left unstained section and the HCl methods. In conclusion we are still not confident in recommending one method over another. It may be that a combination of methods would be required to help resolve the difficulties in interpreting the annuli (e.g. a whole method applied to small/young fish paired with a section method applied to larger/older fish). Also, the HCl method, with the possible addition of a lateral grind treatment at the edge for larger/older fish (see Strontium Chloride validation study presentation above), shows improved clarity in the structure of the whole otolith and warrants further investigation.

2.2.4 Within lab comparison of methods 1c, 3 and 5

In 2003 PINRO conducted an experiment on comparative reading by scale and otolith of the same individuals. The sampling was conducted for 200 individuals (length range 22-83 cm, age range 2-14 years). The results demonstrated minor underestimation of age by otoliths especially for fish >10 years (Presentation 1.6).

In 2010 VNIRO conducted an experiment on comparative reading by scale and cross-sections of otoliths of the same individuals (methods no. 1c and no. 6). The sampling was conducted for 350 individuals (length range 30-85 cm, age range 3-14 years) (Presentation 1.7).

Traditional methodology of age reading is based on assessment of two registering structures – scale and otolith. The difference in age determined by scale and otolith is insignificant. It implies that the traditional methods of age reading by scale (used in Russia) and by the traditional otolith method (no. 3 above) give similar results. Age reading based on assessment of two independent registering structures indicates good agreement between methods.

By Russian data maximum observed length was 123 cm aged 25 by traditional method.

2.3 List of results from validation and corroboration studies

1) **Validation**

a) **OTC – Fisheries and Oceans Canada-FWI** - n=3 samples from Cumberland Sound (inshore area of NAFO Div. 0B) were examined to determine otolith growth zones and validate age methods; 1 was a female that was 66 cm at time of re-capture and at large 3 years 10 months. The OTC mark was visible at the edge of the longest growth axes on the right otolith, also on left surface at the anterior margin (Treble *et al* 2008 and Treble and Dwyer 2008). However, growth was minimal. On the left section the greatest growth was in the thickened perisulcular region and then in the dorsal and ventral margins. Growth rate was approx. 1.5 cm yr^{-}. The results of this study indicated that the appropriate number of annuli formed after the chemical mark as viewed along an axis in the actively growing perisulcular region on the left otolith section. Assuming that earlier annuli were also formed annually, this suggests that accurate age determination should be possible from otolith sections up to a size of at least 66 cm.

b) **OTC – IMR Norway -** n=12 samples from Svalbard waters; 33-48 cm at release – 1-4yrs at large– (Presentation no. 1.11) There was little or no growth visible on the left otolith surface for slowest growing individuals (39-48 cm at release). Growth was visible on the right whole otolith out along the anterior margin or "fingers" and in the thickened perisulcular region and dorsal and ventral margins of the left otolith cross-section. For faster growing individuals (33-36cm at release), all three methods show growth increments along the observed growth axis with zones that are possible to count. The results of this study indicated that the appropriate number of annuli formed after the chemical mark as viewed along the longest growth axes of the right otoliths as well as on the sections. Assuming that earlier annuli were also formed annually, this suggests that accurate age determination should be possible from whole right otoliths and left otolith sections up to a size of at least 48 cm. Annuli were not always visible outside of the chemical mark in left whole otoliths, suggesting that the traditional ageing method may underestimate age in some slow-growing fish.

c) **$SrCl_2$ – Fisheries and Oceans Canada-FWI and Greenland Institute of Natural Resources -** n=2 samples from NAFO Div. 1A (see Presentation no. 1.12); The chemical mark could be detected at the edge on the whole right and left otoliths and the left cross-sections. The appropriate number of annuli formed after the mark as viewed along the longest growth axes on the right and left whole otoliths and the left cross-section. Assuming that earlier annuli were also formed annually, this suggests that accurate age determination should be possible on these three structures up to a size of at least 46 cm. This validation is preliminary as we only have 1 yr of growth beyond the mark. Active growth zones seen with **$SrCl_2$** correspond to what has been shown previously for samples from Cumberland Sound and at this workshop for the Barents Sea. This study is consistent with the Norwegian study in that growth zones appear to be formed annually in whole right and sectioned left otoliths.

d) **^{14}C - Fisheries and Oceans Canada-FWI**- n=10 samples from NAFO Divisions 0B, 1C and 2G assessed for $\Delta^{14}C$ values to determine accurate ages and validate age methods; The oldest was a 74 cm female with a ^{14}C age of 27 years. Ages assigned prior to validation were; 16 years for the left whole with reflected light, 22 for the right whole with reflected light, and 12 years for the left section. For lengths of approx. 70-80 cm ^{14}C validated ages varied between 20-27 years. The ^{14}C age exceeded the whole otolith age by a range 3 to 11 years and the section age by a range of 1 to 15 years, with an average difference of 6 years for both methods. Because ^{14}C ages represent the minimum possible ages consistent with the radiocarbon data, these results indicate that the age readings from the whole otolith and left otolith sections underestimated the actual age of these fish. Age underestimation was most pronounced in the oldest fish. The maximum observed age from whole and section ages of this subset of otoliths was 22 years and 20 years, respectively, while the maximum ^{14}C age was 27±3 years.

e) **^{14}C – Alaska Fisheries Science Center -** n=32 samples from the Bering Sea; Several Greenland halibut samples were used for illustration in Presentation no. 1.2. Six samples, ranging in length from 62 cm to 91 cm, were from the bomb 14C study. Their $\Delta^{14}C$ values when compared to the reference curve estimated birth years that would suggest ages of 15 years to 33 years, which was greater than previously estimated by counting presumed annual zones. In two cases, the birth yrs fell at the bottom of the reference curve between 1950 and 1955. In the other four cases, pre validation birth year estimates fell 3-7 years to the right of the reference curve. Retrospective examination of the growth pattern from these four samples subsequently led to a slight revision in the ageing criteria most notably after an observed transition zone where more densely packed annuli are observed in outer regions of sections from older specimens. A transition from faster growth to slower growth has been identified as young as 7 years and as old as 12 years. The cause of this change in growth pattern is not known but may indicate maturity. The oldest cross-section age was approximately 31-34 years and corresponded to $\Delta^{14}C$ values from the pre-bomb era.

2) **Corroboration**

a) **Growth information from Tagging –** Mark-recapture experiments can provide information on growth rates in fish if you can obtain length information at time of re-capture. These data can be used to corroborate ages and assess age determination methods.

Norway (Presentation no. 1.13) – Based on the best quality length measurements at both release and recapture, estimated mean growth rate for time at large was 4 cm for 25-30 cm fish and 1 cm for fish around 50 cm and above. By comparison, age-length data based on the new whole right otolith method (method 2a) gives mean annual length increase of 1,5 cm for fish between 40 and 50 cm (the "adult samples" from Albert *et al.* (2009)), whereas the traditional method indicates annual length increment of approximately 4 cm per year for fish of similar sizes.

DFO and GINR – historic tagging data were examined and Gulland and Holt analyses indicated a mean annual growth of 2 cm (Treble *et al* 2008). The GROTAG model estimated growth for fish 50-75 cm at approx. 3.2 cm/yr although it was associated with considerable and un-quantified uncertainty.

GINR – recent data, n=9 (frozen) and additional samples from other labs and observers; growth in the order of 2 cm/yr for 45 cm fish was measured on samples after freezing.

Note: Due to increased stress at time of tagging, it is possible that the post-release growth rate has been underestimated in these tagging studies. Also, there may be differences in length when measuring live and dead fish, as well as fresh or previously frozen samples and post mortem shrinkage with time since capture may increase with fish length (as shown in Presentation no. 1.13).

b) **Tracking year classes**- GINR shrimp survey tracks ages 1, 2 and 3 using length frequency. The growth increment from age 1 to age 2 is 6-10cm. The size of age 2 fish in June/July is 22 cm. Joint Russian-Norwegian juvenile Greenland Halibut survey north and east of Svalbard show modal progression of 1 and 2 year old fish in September, with modes around 14 cm and 22 cm respectively.

c) **Morphometry**- Used otolith morphometry data to compare the traditional method (no. 3) and the new whole right transmitted method (no. 2a). After fitting a model describing the morphometric variable as a function of fish length, the residuals were significantly correlated to age based on the new method, but not to traditional age estimates. This suggests that the new method has age information independent of fish length, whereas the traditional method captures little more than fish length (Presentation no. 1.16 and Albert *et al* 2009).

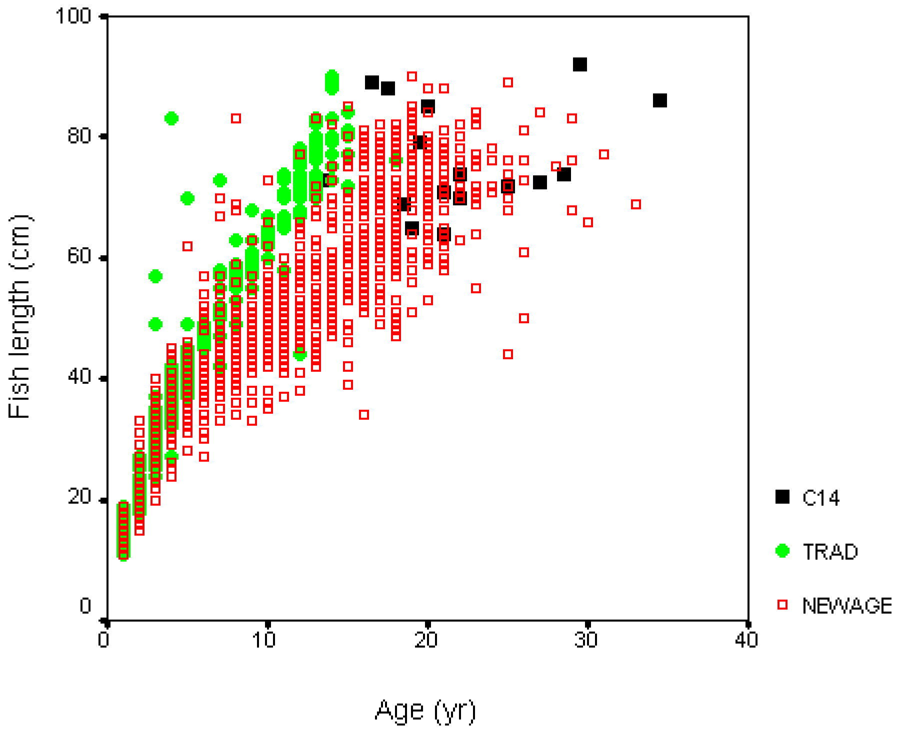

Figure 4. Growth rates for samples from the Barents Sea (the "revisited samples" from Albert *et al.* (2009)) aged using the traditional whole otolith method (green circles) and the new right whole otolith with transmitted light method (red open squares) compared to ^{14}C validated ages from west Greenland-eastern Canada area (NAFO Divisions 0B, 1C and 2G).

d) **Comparison between regions-** A comparison of length at age based on the traditional whole left otolith ageing method (no. 3) and that based on whole right otoliths (no. 2a) indicates that the latter tend to produce markedly older fish at lengths greater than about 40-50 cm (Figure 4). Traditional ages peak at a maximum age of about 15 years, while maximum ages based on the whole right method reach at least 35 years. Bomb radiocarbon ages from the west Greenland – eastern Canada area agree very well with the whole right ages, and are much greater than similarly-sized traditional ages.

2.4 Validation of each method

1) **Cross-sections**

a) **Stained** -The method and the interpretation criteria are validated up to age 34, corresponding to lengths of 70-80 cm. The variation around the ^{14}C estimates are greater for Greenland Halibut than for other species, but the results show that on average this method produced accurate age data. The preferred reading axis is also in a region of active growth as observed by OTC and SrCl analyses of otoliths from NAFO Subarea 0 and 1 and the Northeast Atlantic.

b) **Unstained thick sections**– The method and the interpretation criteria are validated up to 70 cm at ages of minimum 20-27 years using bomb radiocarbon (^{14}C) analyses. Also up to 46 cm and 12-13 years using the SrCl marking method. The preferred reading axis is also in the region of active growth as observed by OTC and SrCl analyses of otoliths from NAFO Subarea 0 and 1 and the Northeast Atlantic.

c) **Unstained** - The method and interpretation criteria are considered validated by correspondence with length frequency modes for ages up to 2-3 years, corresponding to lengths of 25-30 cm. The preferred reading axis is also in the region of active growth as observed by OTC and SrCl analyses of otoliths from NAFO Subarea 0 and 1 and the Northeast Atlantic. These zones are viewed in cross-section and under higher magnification compared to the surface view of the left or right otolith.

2) **Whole right otoliths viewed with transmitted light** (methods a and b)- Based on the OTC and SrCl analyses of otoliths from NAFO Subarea 0 and 1 and the Northeast Atlantic, these methods and interpretation criteria are capable of identifying annuli at the otolith edge for ages up to at least 10-15 years, corresponding to lengths of 40-50 cm. Assuming that annuli form prior to tagging have a similar appearance this ageing method would be accurate. The OTC results from NAFO Subarea 0 suggest that the method may as well be accurate also for older fish, if counting densely packed annuli at high magnification. Ages 1-3 are strongly corroborated using length frequency information.

3) **Traditional method**

The method and interpretation criteria are strongly corroborated by correspondence with length frequency modes for ages up to 3 years, corresponding to lengths of 25-30 cm. The preferred reading axis is in the region of slow or no growth as observed by OTC and SrCl analyses from NAFO Subarea 0 and 1 and the Northeast Atlantic. These studies suggest this method would not give accurate ages for older fish.

4) **Right and Left Otolith Lateral Grind at Margin**

Based on two individuals this newly developed method and interpretation criteria shows potential and is supported by the SrCl analyses for ages up to 10-13 years and 46 cm. The preferred reading axis is in the region of active growth as observed by OTC and SrCl analyses from NAFO Subarea 1 and the Northeast Atlantic.

5) **Scales**

The method and interpretation criteria are considered validated by correspondence with length frequency modes for ages up to 3 years, corresponding to lengths of 25-30 cm.

3 Potential usefulness of mathematical methods and supplementary information (ToRs d, e and f)

3.1 Term of Reference d) Report on progress of the compilation of biometrics data of Greenland halibut otoliths from all areas where such information has been collected and analysed.

To this point, there has been little in the way of routine otolith morphometric data collection for Greenland Halibut, despite the collection of many otoliths. However, some groups have begun to collect otolith weight data (i.e. in Winnipeg, Tromsoe). Some initial measurements of otolith thickness, area, maximum diameter, and perimeter have been completed by those in Tromsoe, and Albert *et al.* (2009) showed that with the new method (no. 2a), several measures of otolith size were correlated with age after correcting for fish length. This is expected for an accurate age determination method and was not apparent with the traditional method (no. 3). Although it is unclear if any other morphometric measurements have been made on Greenland Halibut in other laboratories, it appears that little has been done with respect to morphometric analysis on this species in any laboratory. Otolith weights have often been reported to be strongly correlated with age, although relatively few have used them to estimate age or improve ageing precision (Cardinale and Arrhenius 2004; Francis *et al* 2005; Lou *et al.* 2005).

3.2 Term of Reference e) To revise the age estimation procedures and explore the possibilities to use supplementary information to verify estimated ages, this includes: otolith weight and/or morphometry, as well as length distribution in surveys and catches.

It appears that no procedures have yet been developed to revise or modify age estimation based on otolith morphometrics. Some initial explorations have been made based on otolith weight and a dimensionless measure of otolith shape, but no applications to age determination have yet been developed.

Length frequency analysis of Greenland Halibut from research vessel surveys and shrimp commercial bycatch data from both the Grand Bank and the Svalbard area show strong length modes for young Greenland Halibut. Otoliths from these length modes were aged and provide good evidence of size at age for those young age groups (e.g.- ages 1-2 and possibly 3) (Bowering and Nedreaas 2001)

3.3 Term of Reference f) Exploring mathematical methods for estimating age composition of Greenland halibut catches to be used by ICES WG.

There are several mathematical methods available for estimating age composition, although all make assumptions above and beyond those required for direct age determination. These methods may also be appropriate for use in other assessment groups, e.g. NAFO.

a) **Using specific growth rates as a prelude to mixture models** (Presentation no. 1.17*)*: Greenland Halibut were reared under laboratory conditions for periods of up to a year, so as to measure size-specific growth rate at temperatures representative of the wild stock. Using the length modes of young fish as a starting point, the size-specific growth rate of the corresponding length modes was extrapolated over a year, then added to the preceding length mode to predict the size of the next age group. This process was repeated for all age groups, and an overall growth model developed. This growth model could then be used as the basis for the age-structured decomposition of the population length frequency using a conventional mixture model.

The advantage of this method is that no direct age determinations are required. The assumptions of the method include: 1) the size-specific growth of the lab-reared fish is comparable to that of fish in the wild, which would include knowledge of the effects of temperature and food availability; 2) the selectivity of the commercial fishing gear is constant (independent of age) or known.

This method is best suited for the age estimation of young fish in the population.

b) **Tag-recapture analysis to estimate a growth model** (Presentation no. 1.13): Annualized growth increments at size were estimated based on tag recapture measurements. Using the same basic approach described above, estimated annual growth increments were added to the observed length modes characteristic of young fish to predict the size of larger fish, and thus derive an overall growth model. This growth model could then be used as the basis for the age-structured decomposition of the population length frequency using a conventional mixture model.

The advantage of this method is that the annual length increments at size are based on wild fish. The central assumptions of the method are that: 1) tagging does not modify the growth of the tagged fish relative to untagged fish; and 2) the measurements at tagging and recapture are accurate.

This method could be improved with rigorous statistical estimation of growth after tagging. It is possible that measurements of otolith growth after tagging could be used to improve the precision of the post-tagging growth estimate.

It was commented that the resulting growth models based on the tag-recapture data indicate slow growth for fish above 40 cm. In the Barents Sea individuals above 80 cm are regularly caught, and the maximum observed size is 123 cm. If the growth model is reasonably accurate, this would imply that these individuals are either very old, or that they are faster growing than average fish from their cohorts.

Additional factors to consider when working with tag-recapture data, including OTC or SrCl marking for calculation of growth curves:

- Tagging and recapture results are challenged by accuracy of measuring length. The choice of outliers to be discarded is subjective.
- Possible body shrinkage after death was estimated, but there are still uncertainties in terms of the effect on the growth model.
- The influence of tagging itself on the growth rate of Greenland Halibut (physical injury, thermal and pressure change shock, loss of scales and mucus etc.) are known to occur in the short term. It is unknown to what extent this may also influence growth in the longer term.

- The additional influence of chemical markers on the growth rate is also not clear, but tank experiments suggest this will be of short term duration.
- The quantity of recaptured individuals marked by OTC (3 and 12 specimens) is useful to determine the appropriate growth axes and confirm annulus formation on those axes, but is too small to make conclusions about post tagging growth rate.
- Bomb radiocarbon analyses have not yet been applied to the Northeast Arctic stock of Greenland Halibut. Such studies will be useful.

c) **Mixture models using otolith weight**: Otolith weight is known to vary with age in a manner different than that of fish length. If a calibration sample of aged fish is available, it is possible to analyze a larger data set of joint measurements of fish length and otolith weight (and other otolith measurements, if appropriate) in a mixture model to estimate proportions at age in the population. These models have already been developed and tested (Francis *et al.* 2005), although they have not yet been applied to Greenland Halibut. If this model were to be used, aged calibration samples would be required for each fishery, area and year where length at age differed. The method does not assume that the calibration ages are correct, but it does assume that they are unbiased. This method is best suited for estimation of age composition where maximum age in the population does not exceed age 20.

d) **Variations of length frequency analysis:** There are existing mathematical methods for decomposing length frequencies into proportions at age. The most common examples of this approach are Multifan (Deriso *et al* 1985) and mixture models (Macdonald and Pitcher 1979, package mixdist in R). Most of these methods assume that there is an underlying accurate growth model or accurate estimates of length at age. In principle, Multifan can estimate proportions at age in the absence of a known growth model. However, it requires well-defined length modes to do so, which effectively limits the method to young fish.

There is considerable interest in methods for correcting a catch at age matrix based on inaccurate ages. In cases where the ageing errors are systematic (i.e. a missing annulus), the catch at age matrix is easily corrected. However, a nonlinear ageing bias (such as that evident in Greenland Halibut) is seldom correctable using mathematical methods (Melvin and Campana 2010). In such cases, subsamples of otoliths usually have to be re-aged using accurate methods in order to generate a revised catch at age.

Random ageing errors (due to imprecision) in a catch at age matrix are readily rectified using statistical methods, assuming that the ageing error matrix is known.

4 Next steps (ToRs g, h and i)

4.1 Term of Reference g) To join international experts on growth, age estimation and assessment in order to progress towards a recommended procedure for future age determination of Greenland halibut.

The meeting gathered people from most marine labs that do regular ageing of Greenland halibut, as well as experts in age validation studies, growth experiments, image analyses, statistics, and general population biology of Greenland Halibut. The group did not find reason to recommend one particular method, but noted that several of the recently developed methods provide age estimates that are supported by generally accepted methods of age validation and corroboration. By comparison, no results were presented for the group to provide validation or corroboration of the traditional method used for this species, or for other methods that provide comparable results.

4.2 Term of Reference h) Based on results, conclusions and recommendations from this workshop to initiate and design an international exchange of otoliths for age reading after the workshop

In order to improve both accuracy and precision of Greenland Halibut age estimates, it was decided to establish a reference collection of digital images of otoliths that have already been validated with either ^{14}C, OTC or SrCl methodology. This will include samples from the Barents Sea, Baffin Bay and Bering Sea, and they should be made available for use by all labs to assist in improving and calibrating the different age reading methods. The reference collections should include samples from across the size range and the distributional range of the species. Images should, as far as possible, be of both sections and whole otoliths, and a series of images should be taken with the best quality possible.

In a future exchange of otoliths for age reading the group recommends that the different labs used a standardized, agreed method in addition to their own preferred method. Perhaps all should read the ages on a Norwegian picture of the whole right otolith and an American picture of the unstained cross section of the left otolith? In this common reading of the pictures, the reading techniques on which axis to read, should be agreed upon.

There seems to be differences in age at maturity and growth patterns between stocks. Therefore it is recommended that this be reflected in the exchange material. For example material from both the Barents Sea and the western part of the Atlantic should be included. Also older and larger specimens should be represented in this exchange.

Since otoliths are destroyed in some labs the order that otoliths should be circulated are more or less locked at a route like:

Whole otoliths, frozen

1) Norway: Whole method on frozen right otolith. A reference picture is taken
2) Greenland: Whole method on frozen right otolith

Whole otoliths, dried

3) Spain: Dry left whole
4) Poland: Dry left whole

5) Canada, St. Johns: Dry left whole

Left otolith is cross-sectioned

6) USA: Left otolith is cut. A reference picture is taken

7) Russia, Moscow: Section on left otolith is read

8) USA: Section of left otolith is stained and read

Whole right otolith is acidified

9) Canada, Winnipeg: Right otolith treated with HCl and read whole

This route would lead to intensive travelling of the otoliths increasing the risk of breakage or loss of the otoliths. Alternatively a future exchange could operate with two sets of exchange otoliths. By doing so the Portuguese lab could also be included and should in that case be added at the end of the route since their method includes baking the otolith. Having two sets of exchange otoliths would also allow both labs that are using cross sections to apply their own cross section methods.

A report should be prepared summarizing the results that includes calculations of coefficient of variation (Chang *et al* 1982). Age bias plots (mean and measure of uncertainty around the mean (e.g. 95% CI or SD)) should also be prepared in order to compare two methods and assess bias between methods.

4.3 Term of Reference i) Address the generic ToRs adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration')

The relevant generic ToRs are addressed in the different presentations above, as well as in the conclusions and recommendations sections.

5 Conclusions

1) From the information that was reviewed concerning validation and corroboration techniques and results, none of the methods stands out as being ideal. There is still work to be done to determine the best methods, although considerable progress has been made.

2) Bomb radiocarbon results from the Northwest Atlantic and from the Bering Sea shows that longevity of Greenland Halibut is much greater and growth rate less than half of that reported based on the traditional ageing method. Data on **$SrCl_2$**, OTC and tag recaptures from the Northwest Atlantic and from OTC and tag recaptures from both the Northwest Atlantic and Northeast Atlantic are consistent with the bomb radiocarbon results.

3) The OTC results show that the left whole otolith, which is commonly used as the basis for the traditional age estimates, apparently shows no growth in surface area for slow growing individuals. Without improved techniques, this prohibits accurate age estimation of these individuals by use of the whole left otolith surface. The results show that the stoppage in otolith growth is evident from lengths around 40cm and larger, and for both mature and immature males and females.

4) A variety of ageing methods were examined. Several showed improved clarity and interpretation compared to the traditional whole left technique. Most of the new methods that were examined resulted in older age estimates above approx. age 5 (approx. 45-50 cm) compared to the traditional method.

5) The only ageing axis on the whole otolith surfaces that consistently showed additional growth in the OTC collections were along the longest axis of the right otoliths. The sectioned left otoliths also showed consistent growth towards the proximal edge. Additional growth axes in right otolith section planes or other left section planes were not examined.

6) Based on the validation results, the OTC uptake and the methods comparisons, it appears that age determination using transverse sections of the left otoliths are likely to give the most accurate ages. This conclusion requires further confirmation.

7) Based on all available evidence it appears that the traditional method underestimate ages for ages above 5 years. A more accurate ageing method is currently under development and seems likely. Until such a method is accepted, stock assessments should note the likelihood that catch at age matrices based on the traditional ages are likely to be in error (too low ages).

8) Although a new age reading method would be expected to be applicable to all stocks of Greenland halibut, there may be differences in population growth between areas that would warrant use of different interpretation methods. The total age of Greenland halibut otoliths has so far only been validated in the Bering Sea and the Northwest Atlantic. Even though the results from the OTC experiments, tag-recaptures, as well as previous growth comparisons based on the traditional ageing method (Bowering and Nedreaas, 2001) indicate otherwise, it is still possible that e.g. the Northeast Arctic stock has a very different and much higher growth rate than the Northwest Atlantic stock. If the growth rate of a given stock is more than double of that documented for the Northwest Atlantic and the

Bering Sea for fish above approx. 40cm, the traditional ageing method may still be applicable for such areas. Validation of total age by bomb radiocarbon analyses is therefore warranted for all stock units for which required archived samples are available.

6 Recommendations

1) Based on present knowledge, identification of annual zones in Greenland Halibut otoliths should preferably be done along the longest growth axis of the whole right otoliths or towards the proximal edge of the sectioned left otoliths. Presumably the right otolith section would also show continued growth, but this was not evaluated at this meeting.

2) Identify archived samples from the Northeast Atlantic to be analyzed with the bomb radiocarbon method. They should have been born before 1960 and be as old as possible in order to provide information to validate the total age of the fish.

3) Given the nature of the Greenland halibut otoliths any ageing method is likely to be challenging. To reduce the number of fish that will have to be aged for stock assessments, we recommend that carefully constructed and validated growth models be developed for each stock. Using currently available and accepted mixture models, population numbers at age to be use in assessment working groups may be constructed by decomposing length frequency distributions. Such an approach would eliminate the need for routine annual ageing until such time as the population growth rate changes appreciably.

4) Establish a reference collection of digital images of otoliths, including those validated with ^{14}C, OTC and SrCl to be made available for use by all labs to assist in improving and calibrating age reading methods. Since the ^{14}C otoliths are effectively of known age, they can be used to test the accuracy of all new and existing methods and interpretations by age readers. The reference collections should include samples from across the size range and the distributional range of the species. Images should, as far as possible, be of both sections and whole otoliths, and a series of images should be taken with varying focus and light settings.

5) A new WKARGH meeting should be arrange within 4 years with the aim to improve precision of the new methods and to further standardize the approach.

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Annex 1 - Minority Statements

By the opinion of the Russian experts traditional methods of age reading (both Norwegian and Russian) were adopted by ICES and for a long time used as basis for assessment of North-East Arctic Greenland halibut stock. Based on combined Russian-Norwegian length-age matrix the assessment was carried out by VPA method that adequately reflected retrospective dynamics of the stock.

New Norwegian method is still under development. There is uncertainty as to the determination of the of intermediate growth zones. The growth curve based on tag-recapture data definitely distorts the real growth rate for older year classes at least for North-East Atlantic. It does not account for individuals of over 75 cm.

Since the new method is still being developed and have been neither validated nor approved by ICES it cannot be regarded as correct. Therefore the Russian delegation sticks to the opinion that traditional methods for the time being are the best for use in the Barents Sea and adjacent areas.

The chapter Conclusions of the Report (item 7 of Conclusions) states that underestimation of the Greenland halibut by traditional method is an established fact, though no conclusive evidence was presented to this end.

The traditional method used in Russia produces adequate estimation of the fish age. It proved right by ICES groups on other fish species (e.g. workshop on flounder age reading (WKAFLO 2007, 2008 reports)). Physiology and age structure formation of these bottom species is similar and exclusivity of Greenland halibut in this respect is not correct. With the view to the above we consider that the traditional method correctly reflects age pattern of the Greenland halibut stock.

Therefore scientists from VNIRO, Moscow, and PINRO, Murmansk, provide the following minority statements, not supported by the rest of the group:

1) The traditional method of the Greenland halibut age reading by unstained cross-section of otoliths and scale is adequate for the purposes of reading age of the Greenland halibut of the Barents Sea.

2) The new method shall not be recommended as appropriate until firm validation is in place.

Annex 2 – Workshop Terms of Reference

WKARGH

2009/2/ACOM44 The **Workshop on Age Reading of Greenland Halibut [WKARGH]** (Chairs: Ole Thomas Albert, Norway, and Margaret Treble, Canada), will be established and take place in Vigo, Spain, 14-17 February 2011, to:

a) Review information on age estimations, otolith exchanges, workshops and validation work done so far.

b) Evaluate all available information on individual growth patterns in order to achieve a general consensus about the most probable levels of longevity and growth rates for the different stocks.

c) Report on progress in studies of otolith growth axes based on samples from Greenland halibut injected with OTC or similar substances that makes a mark in the ageing structure.

d) Report on progress of the compilation of biometrics data of Greenland halibut otoliths from all areas were such information has been collected and analysed.

e) To revise the age estimation procedures and explore the possibilities to use supplementary information to verify estimated ages, this include: Otolith weight and/or morphometry, as well as Length distribution in surveys and catches.

f) Exploring mathematical methods for estimating age composition of Greenland halibut catches to be used by ICES WG.

g) To join international experts on growth, age estimation and assessment in order to progress towards a recommended procedure for future age determination of Greenland halibut.

h) Based on results, conclusions and recommendations from this workshop to initiate and design an international exchange of otoliths for age reading after the workshop.

i) Address the generic ToRs adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration')

WKARGH will report 1 March 2011 for attention to ACOM.

Supporting information:

Priority:	Essential. Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Assessment of Greenland halibut stocks using age structured models has proved useful in establishing a diagnosis on stock status. However, the approach has several limitations and shortcomings such as stock structure, natural mortality and growth. Age data is provided by different countries and are estimated using international ageing criteria which have not been validated. Therefore, a WK should be carried out in order to evaluate available information on otolith growth patterns, age determination issues and the current situation of age estimation of Greenland halibut which has been subject of concern of ICES AFWG and NWWG and make progress towards a solution.
Scientific justification:	Recently, several publications suggest that what is at present the most commonly used age interpretation method for Greenland halibut severely underestimates age of older specimens. The last workshop (St. Johns, 2006)

	demonstrated that there was no agreement or understanding of the underlying growth patterns of this species. Since then several institutions have conducted tagging programs, ageing structure comparisons, and other work in order to validate seasonal zones in otoliths. Since stock assessments are severely hamperd by this lack of clarification, it is appropriate to arrange a workshop where the results of these investigations can be presented and discussed. For the purpose of inter-calibration between ageing labs an appropriate exchange programme will be carried out **after** the workshop in 2011. This will include a set of otoliths (images) collected partially from tagging material and from previous WKs. The aim of the workshop is to identify the state of art of age estimation after validation studies conducted so far.
Resource requirements:	Before starting the exchange programme, the scientific institutions should make a concerted effort to compile the existing tagging material (digital otolith images) that can be used as a reference collection.
Participants::	In view of its relevance to the DCR, ICES NWWG and AFWG, and NAFO, the Workshop should try to include international experts on growth, age estimation and assessment in order to progress towards a solution. Participants should inform ICES secretariat and chairs no later than 1 November 2010 on their intention to attend the WKARG.
Secretariat facilities:	
Financial:	None
Linkages to advisory committee:	ACOM
Linkages to other committees or groups:	PGCCDBS, NWWG,AFWG and NAFO
Linkages to other organizations cost:	There is a direct link with the EU

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