J. Northw. Atl. Fish. Sci., Vol. 40: 81–95 Publication (Upload) date 14 Oct 2009

Towards Accurate Age Determination of Greenland Halibut

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Albert, O. T., M. Kvalsund, T. Vollen, and A.-B. Salberg. 2009. Towards Accurate Age Determination of Greenland Halibut. *J. Northw. Atl. Fish. Sci.*, **40**: 81-95. doi:10.2960/J.v40.m659

Abstract

Based on tag-recapture experiments, this paper shows that previous age determinations of Northeast Atlantic Greenland halibut from whole otolith surfaces greatly underestimates the age of older individuals. It also shows that the mean individual annual growth of adults is slightly below one cm per year. Surface methods are much more effective than other more time-intensive methods, which is an important consideration for use in stock assessment. The paper describes a new surface method that is in accordance with growth increments from tag-recaptures. The method relies on improved protocols relating to storing, imaging, choice of reading axis, and definition of annuli. The definitions of the first two annuli were validated by length frequencies of juveniles. The new reading axis and annuli of older otoliths were validated by tagging experiments involving injection of OTC, a chemical tag that incorporates into the otolith as a visual band marking the otolith size at time of release. With the new method, 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.

Keywords: age validation, growth, otoliths, OTC, morphometry, tagging, *Reinhardtius hippoglossoides*

Introduction

Age determinations of Greenland halibut (*Reinhardtius hippoglossoides* (Walbaum)), an arcto-boreal deep-water flatfish, have long been considered highly uncertain (ICES, MS 1997; Alpoim *et al.*, MS 2002; Treble and Dwyer, 2008) and recent results indicate a tendency to underestimate the age of older individuals (Gregg *et al.*, 2006; Treble *et al.*, 2008). Some laboratories have therefore ceased to age this species and this has hampered analytical age structured assessments in several regions of the Northeast Atlantic (ICES 2008a, b). Serious concerns have been raised that the annual production estimated with the current age estimates (and typically restricted to a maximum age well below 20 years) is too high (ICES, 2003; Gregg *et al.*, 2006; Cooper *et al.*, 2007; Treble and Dwyer, 2008; and O. T. Thomas, unpublished data). There are many examples from other species where systematic underestimation of age has resulted in failure to realise the stock's vulnerability to exploitation (Campana, 2001), and for many deep-water species this has led to sudden declines of stocks as well as the fishery that they supported. The question of correct age determination of Greenland halibut is therefore important for development of sustainable management of the fish stocks.

The traditional ageing method for Greenland halibut is to examine the pattern of assumed seasonal zones (annuli) on the surface of the whole left otolith (sagitta). The method has been widely used and was to some extent standardised through international ageing workshops (ICES, MS 1997). Although between-reader comparisons gave highly variable results, and in spite of the new concerns of under-ageing, several laboratories continue to use the traditional method (Treble and Dwyer, 2008). But at the same time efforts are being made to critically evaluate and validate the methodology. The most rigorous work in this respect to date is based on analyses of the dated chemical tag of radiocarbon (14C) that was released during atomic bomb testing in the 1960s and incorporated into the otoliths of fish born at that time (Treble *et al.*, 2008). By comparing the 14C content in otolith cores of Greenland halibut from historic samples with a constructed reference curve of ambient radiocarbon levels, the authors showed that in the Northwest

Atlantic, Greenland halibut probably reach an age of more than 30 years and that the traditional ageing method underestimated the true age by up to 15 years. Gregg *et al.* (2006) showed that by counting zones in transverse sectioned otoliths the maximum age of their particular sample increased from 28 to 36 years (as opposed to 21 years used as maximum age in the relevant stock assessment (Cooper *et al.*, 2007)).

Rather than developing new and time consuming sectioning and staining techniques, we explored the potential of refining the traditional surface method. As for most flatfishes, the pattern of zones on the surface varies between the left and the right otoliths. The left otolith of right-turned flatfishes, like Greenland halibut, has a more centrally placed nucleus, whereas the a-centric right otolith possesses the longest growth axis. Our experience with otoliths of several deep-water species is that the longest growth axis is often associated with checks and false annuli for the younger ages (the first several years of otolith growth), whereas for older ages this axis may be the only one that possesses visible zones (O. Thomas, unpublished observations). Therefore, it is common practice in our laboratory to evaluate zones along different axes for younger and older ages respectively. Although the right otolith of Greenland halibut has generally been considered as not possible to interpret, this has not been well-documented in the literature. Based on promising results from an initial Institute of Marine Research workshop (unpublished), we therefore chose to investigate the zone pattern on the right whole otolith. To facilitate interpretation, efforts were made to improve image quality by careful handling and storing, and by using digital images and image enhancement techniques.

The purpose of this paper is to describe a new surface method and compare it both with the traditional method and with available information on individual growth of both fish length and otolith size. We used three different methods to validate or evaluate the new method, namely: (1) growth increments in total length from tag-recapture experiments, (2) growth increments in OTC-marked otoliths, and (3) changes in otolith morphometry. It is reasonable to assume that mean annual length increment of individual fish should be similar to difference in mean length at age for succeeding age groups. Therefore, to evaluate the two ageing methods, mean individual growth increments were compared to mean length at age in survey data, with age determined by both methods. OTC is a chemical tag that incorporates into the otolith as a visual band marking the otolith size at time of tag release. The OTC-marked otoliths may thus present a more direct way of age validation, by showing if the zones that are interpreted as annuli, are really marks of time elapsed between tagging and recapture. Finally, it is expected that some otolith morphometrics should be correlated with age, even after de-trending with fish length (Boehlert, 1985; Reznick *et al.*, 1989; Pawson, 1990; Worthington *et al.*, 1995; Choat and Axe, 1996; Butler and Folkvord, 2000; Cardinale *et al.*, 2000; Labropoulou and Papaconstantinou, 2000; Pilling *et al.*, 2003; Lou *et al.*, 2005; Steward *et al.*, 2009). This requires of course that the age estimates be correlated with the true age. Morphometric analyses were therefore also included to compare the two age reading methods and to relate them to previous findings from other species.

Materials and Methods

Traditional and new ageing methods

A new method is presented in this paper and compared to the traditional method of ageing Greenland halibut. Both methods were based on counting of assumed annuli visible through the surface of whole sagitta otoliths. An annulus is here defined as a pair of translucent and opaque zones.

With the traditional method, dissected otoliths were put in paper envelopes and stored dry. After a few weeks or months, they were submerged in water and read under stereomicroscope with transmitted light. Fish length, weight and sex were written on the envelopes in the sampling phase, and were often consulted in the process of age interpretation. The basis for the interpretation was the pattern of zones on the whole left otolith.

With the new method, otoliths were put in plastic trays together with surrounding tissue (the labyrinth organ), labelled with station and fish number and stored frozen. After thawing the still wet otoliths were submerged in water and photographed under a stereomicroscope with transmitted light, using a digital camera with 5 or 12 MP resolution. The images were imported to Photoshop, calibrated to mm-scale and improved by standard image analyses tools. The sizes of the otoliths were therefore directly available (measurable) to the age reader, but not fish length, sex etc. Since the concern was that the traditional method possibly underestimated the true age, it was decided to focus on the longest growth axis in order to increase the probability of discerning as many presumed annuli as possible. To reduce the common problem of many checks and "false" annuli in regions of fast growth, the first several (approximately five) annuli were often counted along an axis oblique to the longest growth axis.

Data sets

For the purpose of this paper, three datasets with age estimates of Greenland halibut otoliths were considered. They are hereafter referred to as the re-analysed samples, the juvenile samples, and the adult samples, respectively.

The re-analysed samples. The traditional and the new methods were mutually exclusive due to different storing and preparation of the otoliths. It was further considered appropriate to let each age reader use only one of the methods, in order not to confuse those doing the production ageing. In order to get a sample for direct comparison of the two methods, a subset of 586 otoliths that were initially dried and read with the traditional method, were later stored in glycerin for at least 24 h in order to enhance contrasts, before the right otoliths were photographed and re-interpreted according to the new method. Glycerin was used because of its clearing properties, which enhances the visibility of growth increments as it permeates the micro-channel architecture of the otolith (Gauldie *et al.*, 1998). The re-analysed samples were selected from among all otoliths collected during surveys in August 2003 that had already been aged with the traditional ageing method. The first ten females and five males were randomly selected from each of the age-groups according to the traditional method. Then, to get enough large fish, all remaining aged samples of fish larger than 70 cm were also included in these re-analysed samples.

The juvenile samples. To validate the first several zones, samples of juvenile Greenland halibut were analysed according to the new method. These juvenile samples consisted of 384 individuals taken from a bottom trawl survey in the main nursery area in Svalbard waters in September 1998–2005. These samples were used to validate the starting point for counting of annuli, by comparing the size of otoliths from young fish (age 1–5), as well as the size of the first few annuli of older fish (see morphometry section below), to the modal groups from the length frequency distributions of Greenland halibut in the nursery area.

The adult samples. The main material for this study comes from surveys of the main adult distribution area along the shelf break between Northern Norway and the Svalbard archipelago in August 2006 and 2007, and consists of 1 948 individuals with readable otoliths. At each trawl catch, two individuals of each sex were randomly selected from each five-cm length interval. Samples were kept frozen and treated and analysed according to the new method. For a subsample, a between-reader bias plot was constructed and mean coefficient of variation (CV) calculated, as recommended by Campana *et al.* (1995).

Morphometry

A random subsample of adult fish that were aged to 10-20 years using the new method was taken from the 2006 survey in the adult distribution area. For this subsample the longest diameter (across the nucleus) of each of the 1, 2, 3, and 5 annuli were measured. These zone diameters correspond presumably approximately to the longest axis of the otoliths at 1 January as the individual fish grow past the corresponding juvenile ages. These zone diameters were then compared to the measured longest axis of otoliths for each inferred age group from a subsample of the juvenile samples.

The silhouette and dry weight (dried at room temperature) of both the left and right otoliths of the re-analysed samples were recorded. Silhouettes were extracted from the digital photographs and otolith weights were measured to nearest mg. From the adult samples, silhouettes were extracted for 510 pairs of otoliths. These include all samples read by the most experienced age reader, excluding all pairs where either of the otoliths was broken or deformed. For the adult samples, otolith weight was not available, but due to the higher image quality, it was possible to record the length of the longest interpretation axis in the right otoliths with acceptable precision.

The otolith silhouette was extracted using an intensity segmentation procedure, where the threshold was calculated by Otsu's method (Petrou and Bosdogianni, 2000). The image was subjected to morphological filters to remove salt-and-pepper noise, and the circumference, area, and longest axis, were calculated from the silhouettes. An index of the degree of lobes was calculated as the ratio between the circumference and the square root of the area. The effect of fish length on each of the otolith variables was removed by fitting a linear model with fish length as explanatory variable. The residuals from these models were then tested for age dependencies. Before testing, residuals were plotted both against fitted values and fish length, and when necessary, variance was stabilised by log-transformation and a second order term was included to account for curvilinear relationships. The resulting residuals were normally distributed (Kolmogorov-Smirnov tests), with constant variance and no trends.

Tag recaptures

Approximately 30 000 Greenland halibut were tagged in the adult distribution area in several traditional tagging experiments since 1995. Until March 2009, 1 450 recaptures were recorded from these experiments. Since length increment is crucial for this paper, we only considered those 512 recaptures for which length measurements were made by our own scientific staff onboard hired commercial vessels. Ninety-one percent of the 512 recaptures were from tagging experiments in 2001–2006, and for calculation of growth increment per year, only the 259 individuals that had been at sea for at least 300 days were used.

The fish were caught either by long-line or bottom trawl, and the fishing operations were made as gentle as possible. At recapture, the total length was measured and sex, maturity, and weight were recorded and in most cases the otoliths were also sampled. Since the recaptures were made during commercial fishing operations, the tagged fish were usually found during processing of the catch and not within the smaller subsample selected for biological sampling. Length at recapture was therefore often measured several hours after the fish was dead. This time period was not recorded, but crew members estimate it to be typically between 1 and 3 hours, and occasionally up to 6 or 7 hours. To estimate how this may have influenced the resulting growth increment, an experiment was done with a sample of 20 Greenland halibut. All individuals were numbered and measured by two experienced scientific crew members just after catch and again after one, three and seven hours.

Growth estimates from OTC marked otoliths

In addition to the traditional tagging mentioned above, a few thousand Greenland halibut were tagged and injected with OTC (Oxytetracycline). A 100 mg/l solution of Aquacycline ® Vet. (commercial OTC solution) was injected into the intraperitoneal cavity of the fish immediately after was measured and tagged with a plastic Floy tag. The dosage rate was 50 mg OTC/kg, as used by Treble *et al.* (2008). Fish weight was approximated using a length-weight relationship for Greenland halibut determined from previous surveys in the Barents Sea. The OTC forms chemical bonds to calcified structures like otoliths, and emit fluorescence under ultraviolet light (McFarlane and Beamish, 1987). This way, a time stamp is incorporated in the otolith so that the growth increment between release and recapture may be identified.

Until March 2009, 28 recaptures were recorded, three of which had been at large for approximately two years and were returned with the otoliths intact. For the other recaptures, otoliths were either not recovered, or they had been at sea for less than three months. The three samples were photographed both with translucent light and with UV-light with filter set for tetracycline.

Results

Comparisons with traditional method

The new and traditional methods differed markedly in the quality of the otolith images that were interpreted. The otolith readers trained with the new method found that the quality after glycerol treatment was still largely inferior to those that were stored frozen. With the traditional method the dried otoliths appeared grey and with little contrast compared to otoliths photographed after thawing, with near natural level of water content. Fig. 1 shows the same sample photographed after thawing and after a subsequent period of two weeks stored dry in an envelope. All the settings of light, microscope and camera were similar in the two examples. In this particular example, the effect of drying is especially visible in the lower right portion of the left otolith images. Here a clear, regular zone pattern, most likely attributable to annuli, is apparent in the fresh version, whereas after drying, these zones are blurred and fused, resulting in a pattern of much lower frequency. With the traditional ageing method, two independent age readers interpreted the dried left otolith to eight and nine years respectively. The new method resulted in an age estimate of 16 years, based on presumed annuli in the right otolith before drying.

This is consistent with the overall differences between the two methods used on the re-analysed samples (Fig. 2, top). From age five onwards, the two growth curves separated with a slope of 4.0 and 2.5 cm/year for the traditional and new methods respectively. With the new method, the maximum age of these samples was about double of that indicated by the traditional method.

The new method used on high quality images resulted in an even higher maximum age and lower growth rate (Fig. 2, middle). For fish lengths between 40 and 60 cm, mean annual increase in length at age was only 1.5 cm. With the traditional method, fish within this length range were almost exclusively interpreted as 5–9 years old. With the new method, the corresponding age range was 8–27 years. Thus, the new method not only results in higher mean age of adult Greenland halibut, but also indicates considerable variability in individual growth trajectories. It should be noted that the data used in the two lower panels of Fig. 2 are from the surveys in the adult distribution area only. Therefore, length com-

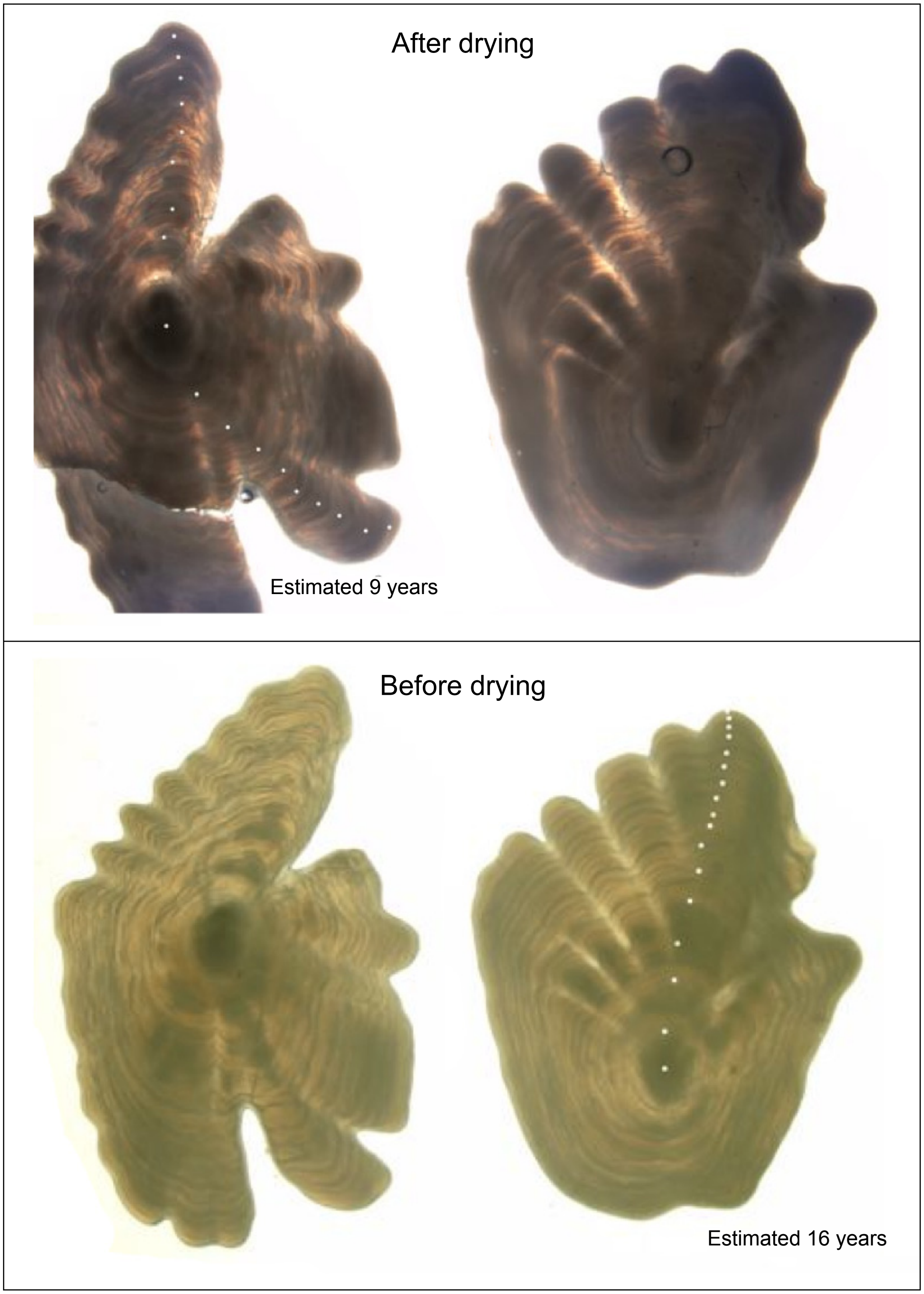

Fig. 1. Whole Greenland halibut otoliths used for age interpretation by counting zones on the surface. Left: the centric left otoloth. Right: The a-centric right otolith. Top: Dried otoliths submerged in water used with the traditional method; Bottom: The same otoliths before drying, still wet after being frozen, used with the new method. The fish was 60 cm and caught in March, and markings denote typical interpretations of annuli with the traditional method (top left, estimated age to be 9 years) and with the new method (bottom right, estimated age to be 16 years).

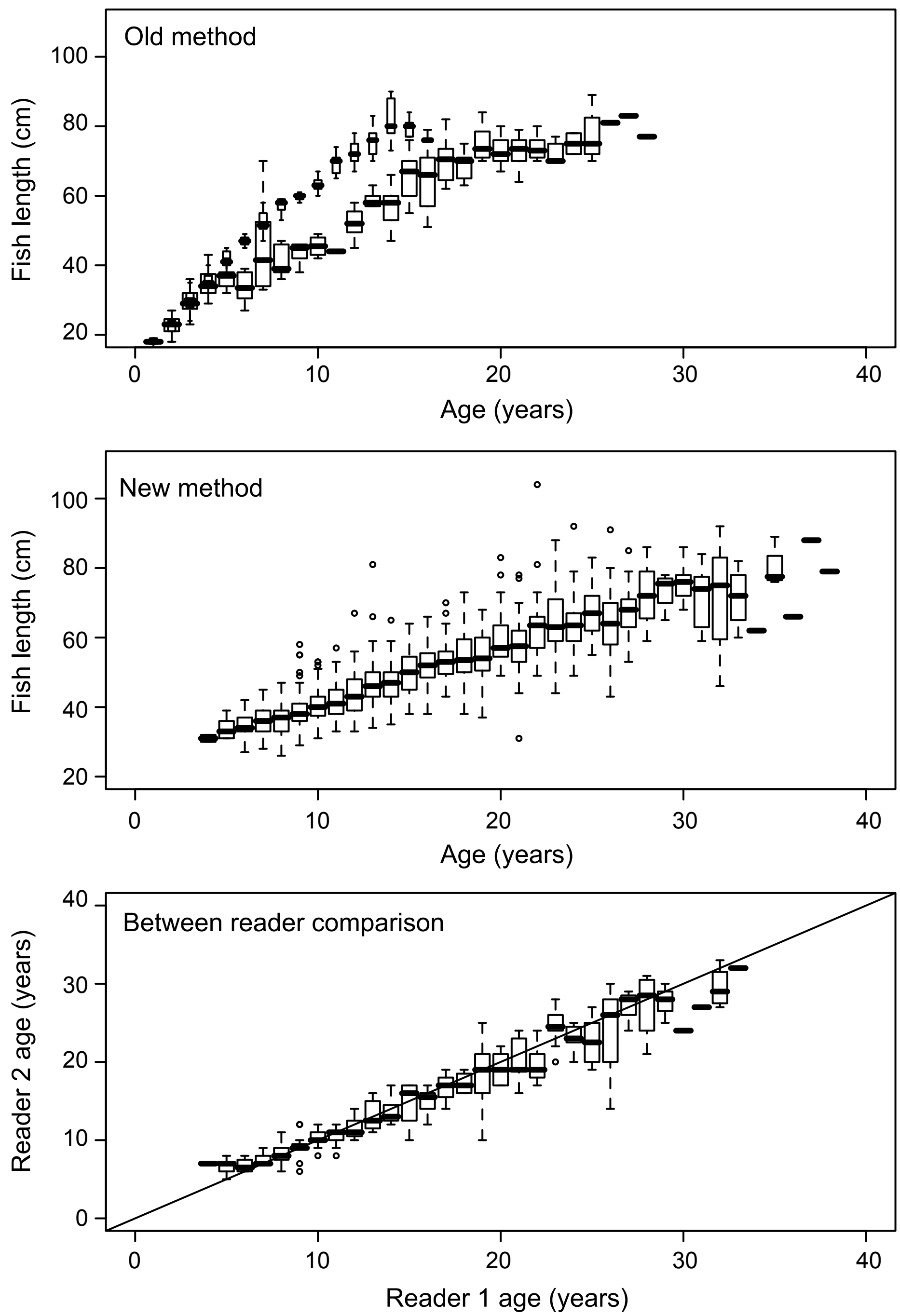

Fig. 2. Top: Fish length at age from the re-analysed samples. Age based on old age determination method (narrow boxes) and on the new method applied on the same samples cleared in glycerol (wide boxes). Middle: Fish length at age based on the new method used on frozen-stored samples from surveys in the main adult distribution area in 2006 and 2007. Bottom: Between-reader comparisons of age estimates based on the new method applied on a subsample of the data included in the middle panel. Each box-plots shows the modal, inter-quartile range, main range and outliers.

positions of the youngest age groups are biased towards larger sizes (Albert, 2003). This is seen in Fig. 2 (middle) as an almost constant minimum length for all age groups less than 10 years.

The lower panel of Fig. 2 compares age estimates of the adult sample made by the two age readers that were most experienced with the new method. Even though this method has so far only been applied for two surveys in the adult distribution area, there was no general between-reader bias of the age estimates. The mean coefficient of variation (CV) was 12%.

Validating the new method

Comparisons with length frequencies of young fish. Length frequency distributions from survey trawls in the juvenile distribution area north and east of the Svalbard archipelago in September typically consist of three well-defined modal groups with mean length at approximately 14, 22 and 31 cm respectively (Bowering and Nedreaas, 2001). Fig. 3 shows this distribution from the survey in 2005, together with length distributions of individuals with estimated age of 1 to 5 years respectively. The figure shows that the new ageing method is consistent with the assumption that the two first modal groups represent one and two year old fish respectively. According to the new method, the third modal group does apparently not represent a single yearclass, but consists of 3–5 year old fish. Further length modes are usually not apparent in the trawl catches.

Defining the first few annuli in older otoliths. Fig. 4 shows the mean zone diameter of the first few annuli in adult fish, which were aged at 10-20 years, from a random subsample of the 2006 survey in the adult distribution area. The annuli are considered as laid down at approximately 1 January and are plotted at age 1.0, 2.0, ..., etc. For comparison, the mean length of the right otoliths of juveniles from the survey in the juvenile area in September 2005 is plotted at age 1.75, 2.75, ..., etc, to account for the sampling time of the year. The figure shows that with the new age determination method the definitions of the first several annuli are consistent for both juveniles and adults, and that the identified annuli are consistent with the time of the year that these "winter-zones" are expected to be laid down.

Since only the first two modal groups can be considered as single age groups, only the first two annuli should be measured as guidance during age interpretation. The length of the longest axis across the annuli at the end of

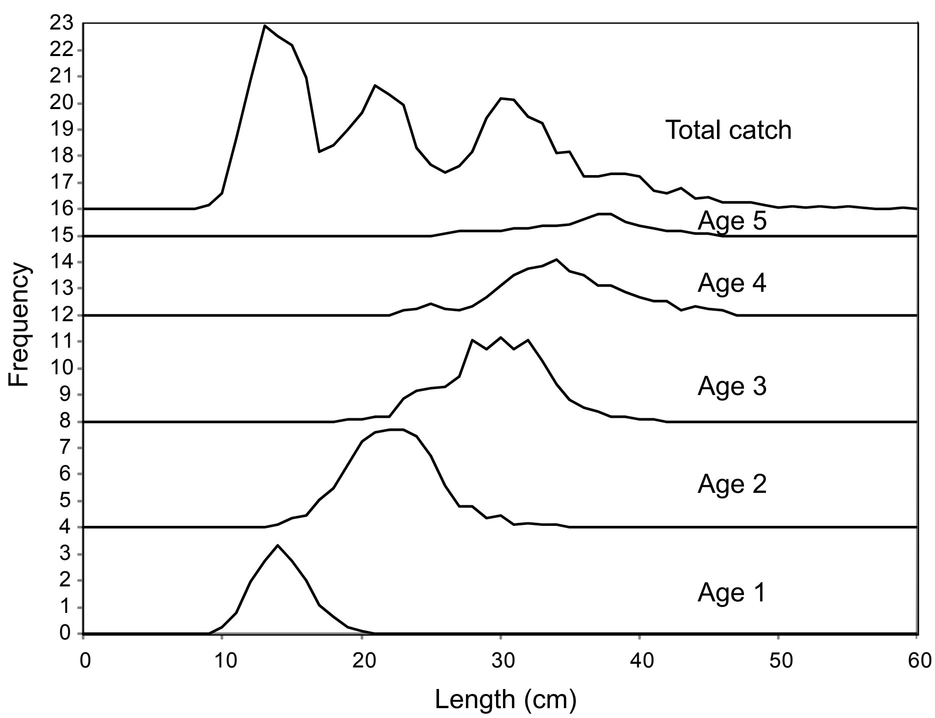

Fig. 3. Length frequency composition of each inferred age-group 1-5 compared with total length composition in nursery area.

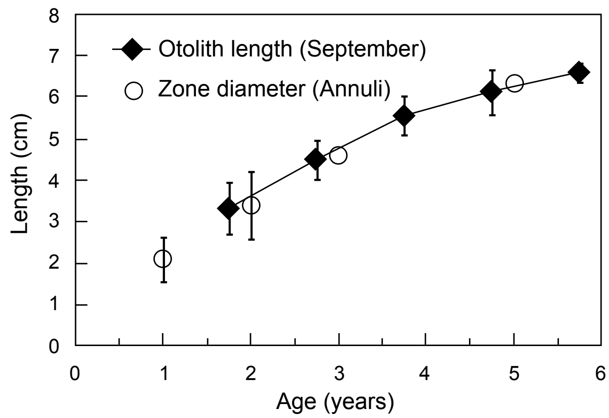

Fig. 4. Mean otolith length of ages 1–5 in September (solid diamonds) and mean zone diameter at ages 1–5 for fish aged to more than 10 years (open circles). Otolith lengths taken from the juvenile samples and zone diameter taken from adult samples. The bars indicate ± 2 SD. Age plotted as fraction of a year and annuli assumed laid down on 1 January.

the first and the second years (the end of 0-group and 1-group respectively) should be expected to be within 2 mm ± 0.5 mm and 3.5 ± 0.5 mm respectively.

Age information in otolith morphology of adult fish. For the re-analysed otoliths, age information on otolith morphometrics was evaluated for the traditional ageing method and the new method. Table 1 shows the results from significance tests for slope of the regression coefficient estimates after the removal of the linear effect of fish length. For the left otoliths, both the square root of area and the cubic root of weight were significantly correlated with the new methods age estimates ($p < 0.05$), but not with the traditional methods readings. The same was found with a slightly lower significance level ($p < 0.06$) for the length of the left otoliths and the cubic root of weight of the right otoliths. Thus, for the new method the null-hypothesis of no linear trend in the length corrected variables with respect to age could be rejected. For the traditional method, the null hypothesis could not be rejected. This means that if the new age estimates are used, the results show that the sizes of the otoliths are not only a function of fish size, but also of fish age. If the traditional estimates are used, there is no such independent age-effect.

The relationships of otolith morphometrics versus age were clearer when age estimates were based on the new method applied to samples stored frozen. The length of the reading axis of the right otolith, as well as otolith length and area of both otoliths, were significantly correlated with the new age estimates, after removal of the trend against fish length (Table 2). For all these measures of size of the otolith surface, the right otoliths contained more age information than the left otoliths. It is worth noting that the length of the reading axis was the metric that was most correlated with the age estimates, after removal of the trend against fish length. The index of lobes did not contain any age information apart from that explained by the fish length.

Annulus validation in OTC-recaptures. The left panels in Fig. 5 show fluorescence images of the left and right otoliths from the three recaptured Greenland halibut that were injected with OTC and that had subsequently been at large for about two years. The right panels show the right otoliths with translucent light and with identified seasonal zones (annuli) marked along the preferred reading axis. Table 3 summarises inferred age, sex, maturity, growth increment, and capture/recapture information for these individuals.

For the youngest of these individuals (Fig. 5, bottom), the pattern of fluorescence apparent through the otolith surface was diffuse, but with a relatively well-defined outer margin towards the top of both otolith images. For the right otolith, the area above this margin corresponds with the area spanned by the two outermost annuli, as shown in the right bottom panel. Also for the left otolith, two annuli were apparent outside the faint fluorescent area. This immature female had grown seven cm in length during the two years at large and had moved from the juvenile area northeast of the Svalbard archipelago towards the adult distribution area further south and west (Table 3). This individual was thus in a relatively fast growing phase, and age at time of release was estimated to seven years. However, the zonation pattern in the left otolith was clearer than in the right otolith, and this pattern indicated an age of five years at time of release.

For the two older individuals (Fig. 5, middle and top), the fluorescence pattern was very distinct and visible as a thin line towards the end of the preferred reading axis of the right otolith. These fluorescent bands were not visible on other parts of the surface of the right otoliths and they were not seen at all, or only at the very edge, on the left otoliths. The area outside of the fluorescent bands revealed two annual zones when inspected with translucent light, as shown in the right panels of Fig. 5.

Thus, in all three pairs of otoliths the expected seasonal zones where found along the preferred reading axis of the right otolith used by the new age determination method. For the smallest and most fast-growing indi-

TABLE 1. Age effects (linear regressions) on four measures of otolith morphometrics used on the re-analysed samples after the removal of the linear effect of fish length.

		Left		Right	
Method	Variable	R^2	p	R^2	p
New	Otolith weight$^{1/3}$	0.19	**<0.05**	0.15	0.06
	Area$^{1/2}$	0.26	**<0.05**	0.43	0.08
	Length	0.22	0.06	0.08	0.38
	Lobe-index	0.01	0.93	-0.01	0.95
Traditional	Otolith weight$^{1/3}$	0.07	0.45	0.10	0.20
	Area$^{1/2}$	0.13	0.24	0.37	0.09
	Length	0.11	0.32	0.11	0.27
	Lobe-index	-0.01	0.93	0.01	0.90

TABLE 2. Age effects (linear regressions) on different measures of otolith morphometrics used with the new method on the samples that were stored frozen after the removal of the linear effect of fish length.

	Left		Right	
Variable	R^2	p	R^2	p
Length of reading axis (Right otolith)			0.08	**<0.0001**
Length of otolith	0.01	**<0.05**	0.03	**<0.0001**
Area of otolith	0.01	**<0.05**	0.02	**<0.01**
Circumference of otolith	0.00	0.72	0.01	**<0.05**
Lobe-index	0.00	0.5	0.00	0.18

vidual, similar zones could also be found on the left otolith with the traditional method. It is further noteworthy that there was generally no abrupt change in the width of the inferred annuli when going from just before to after the time of tagging and release. This may indicate that the tagging procedures did not have any major effect on growth.

Growth increments in tagged fish. From the length at age analyses above it follows that if the traditional age interpretation method were reasonably accurate, we would expect that mean annual individual growth increments of the tagged fish should be around 4 cm. Based on the new method we would expect around 1.5 cm. Of the 259 marked and recaptured Greenland halibut that had been at sea for at least 300 days, 95% had grown less than 2.5 cm/year. The mean growth rate was 0.62 cm/year and the median 0.5 cm/year. Only two individuals had increased with as much as four cm per year, whereas 16% were measured to lower length at recapture than at release. Within the length range sampled, there was a significant ($p < 0.01$) linear reduction in growth rate with increasing fish length (Fig. 6, bottom).

Comparison of length of a live fish at time of release and of a dead fish after recapture may be biased due to post mortem biochemical processes. In the length measurement experiment, where 20 fish were repeatedly measured by two crewmembers over a period of seven hours, the length estimates varied both between crew members and declined over time after catch (Fig. 6, top). The mean difference between crewmembers was 0.5cm, and the mean reduction in length over seven hours was one cm. The reduction in length increased with fish length (linear regression, $p < 0.01$) from estimated 0.3 cm for a 40 cm fish to 1.9 cm for an 80 cm fish, and 75% of the reduction appeared within the first three hours.

The possible effect on annualised length increment of post mortem shrinkage should be less for individu-

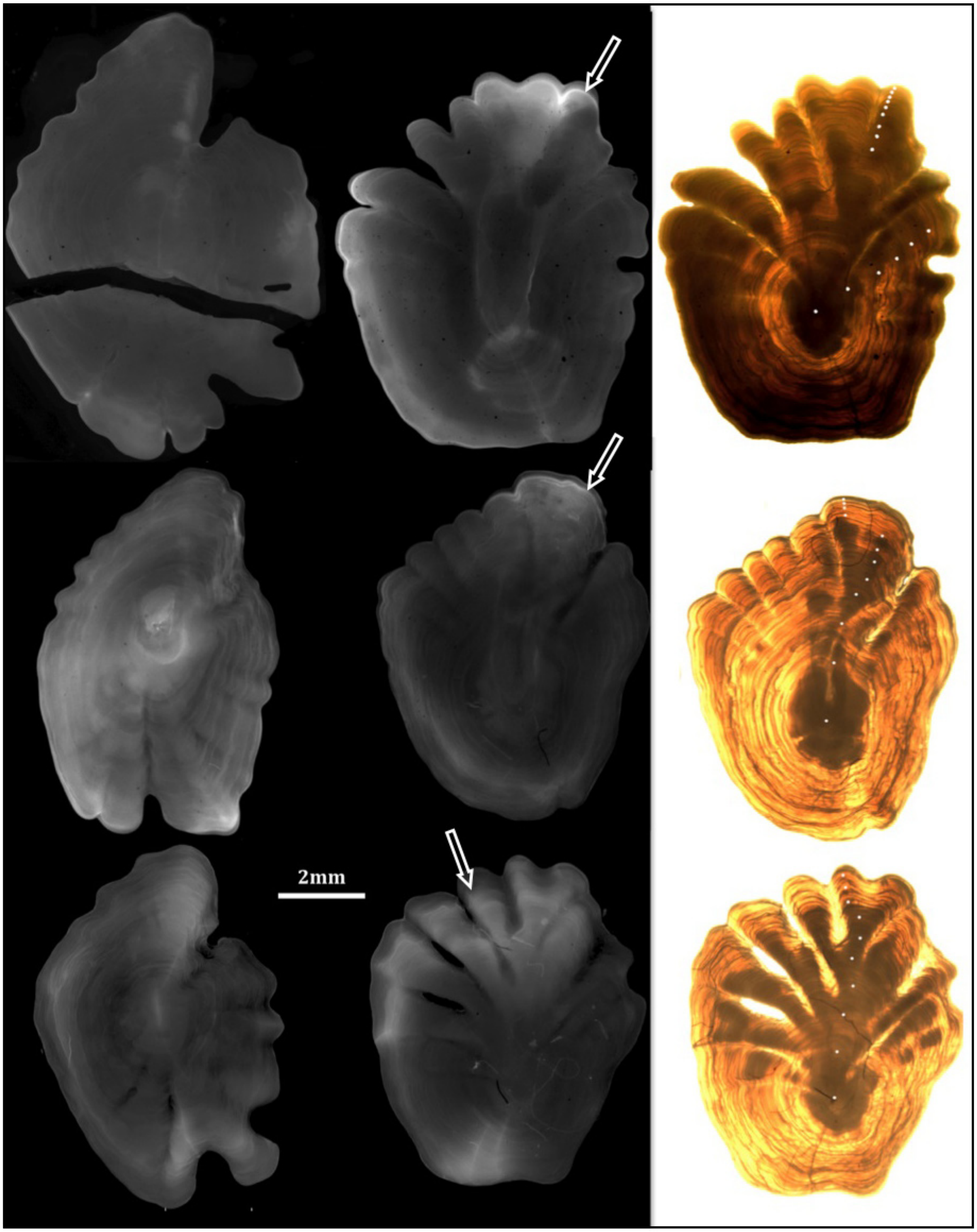

Fig. 5. Otoliths from three recaptured Greenland halibut injected with OTC and being at large for approx. two years. Left: OTC fluorescence of both otoliths seen as a bright band near the edge; Right: Age interpretations of the right otoliths. Arrows denote the outer edge of the OTC bands.

TABLE 3. Summary of the three recaptures of OTC injected Greenland halibut that had been at large for about two years.

	Tag no.		
	GH02207	GH09694	GH04158
Day of release (DDMMYY)	09.09.2006	16.09.2006	11.09.2006
Day of recapture (DDMMYY)	09.08.2008	07.08.2008	15.11.2008
Days at large	700	691	796
Release position (°N/°E)	79/27	79/26	80/17
Recapture position (°N/°E)	76/14	75/16	76/14
Inferred age (release/recapture)	7/9	9/11	13/15
Total length (release/recapture) (cm)	33/40	39/40	45/46
Sex (M or F) / maturity (I or M)	–	F/I	F/M
Annualised length increment (cm)	3.5	0.5	0.5
Fluorescence along reading axis: A distinct zone or a diffuse area	Area	Zone	Zone
Fluorescence visible on surface of left otolith	Yes	No	No

als that had been at large for longer time periods. In total, 36 recaptures had been at large for more than three years. They ranged in size at release from 41 to 84 cm. Those less than 50 cm at time of release had on average increased 0.9 cm per year. For those larger than 50 cm, the mean annual increase was 0.3 cm. Compensating for the linear length dependent shrinkage (Fig. 6, top) and assuming three hours between catch and length measurements, the mean annual increment of these 36 recaptures was 0.8 cm/year, with a confidence interval of 0.6–1.0 cm/year. This must be considered as the best growth estimate presently available for adult Greenland halibut in the continental slope area.

Discussion

Age estimation of Greenland halibut by use of the traditional method of counting annuli on the surface of the left otoliths results in underestimation of age for older specimens. The three OTC recaptures revealed consistent growth increments along the longest axis of the right otolith surface, but for the two larger individuals, no such growth appeared on the surface of the left otoliths. Moreover, in contrast to the new method based on right otoliths that had been stored frozen, the surface pattern on the dried left otoliths resulted in age estimates that were not correlated with fish length detrended measures of otolith size.

Since otoliths tend to grow even when the fish as such does not grow in length (Secor and Dean, 1989; Reznick *et al.*, 1989; Campana and Casselman, 1993; Cardinale *et al.*, 2000; FAbOSA, MS 2002), it should be expected that an accurate age determination method give age estimates that are correlated with otolith size, independently of the correlations between otolith size and fish length and between fish length and age. This has been called uncoupling between otolith and somatic growth (Mosegaard *et al.*, 1988; Wright *et al.*, 1990) and is found in a wide range of long-lived deep-water species (Talman *et al.*, MS 2003). Although this has not previously been shown for Greenland halibut, it is reasonable to assume that it might also be true for this species, and it is difficult to see an alternative explanation for the observed uncoupling when the new age estimates were used. It is therefore reasonable to consider these results as a further weakening of the traditional method.

The results of the morphometric analyses are fully compatible with the OTC results. The main difference between the two reading methods was the choice of which otolith to use. When the left otoliths were used (the traditional method), there was much less tendency of uncoupling than when the right otoliths were used (the new method). This is in accordance with the findings from the OTC experiments, that for older fish growth increments in surface area is most pronounced in the right

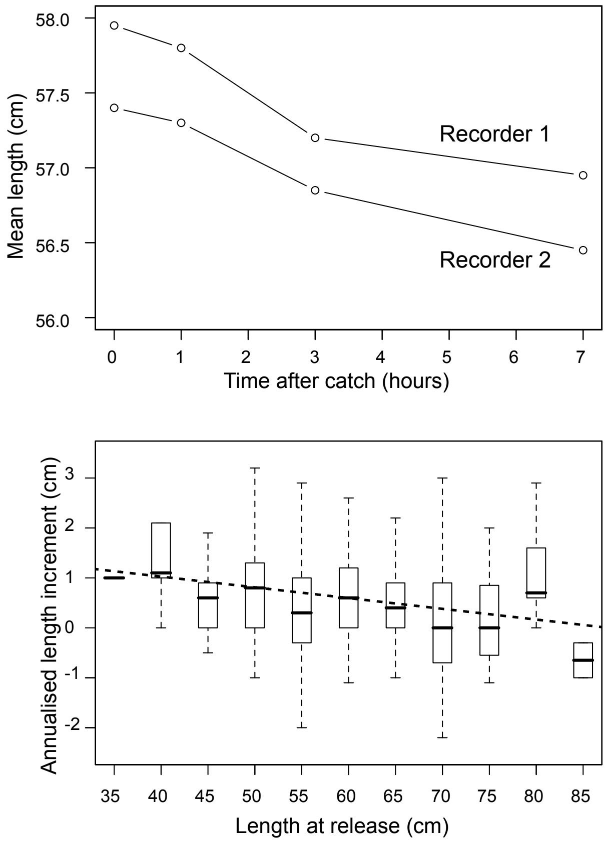

Fig. 6. Top: Mean length of a sample of 20 adult Greenland halibut measured right after catch and after one, three and seven hours respectively. The two lines represent length measurements recorded by two different scientific crew members. Bottom: Annualised length increments of Greenland halibut from release to recapture versus length at release. Only individuals that had been at large for more than 300 days were included and box-plot outliers excluded. The thick dotted line represents a linear regression of the data.

otoliths. The reason for no apparent uncoupling when age estimates were based on the traditional method is therefore probably the lack of contrasts in age estimates due to severe underestimation of older individuals.

The difference in age estimates between the traditional and the new surface method was near a factor of two, at least for larger specimens. The difference was larger with the adult sample, where image quality was better with the new method and the use of digital images tends to give higher age estimates (Fossen *et al.*, 2003). This is also in accordance with the reported underestimation of age of Greenland halibut from the Northwest Atlantic when using the surface of the left otoliths as compared to radiocarbon analyses of the core material (Treble *et al.*, 2008). It is also in accordance with Gregg *et al.*, (2006) who found that the left otoliths of larger specimens tended to grow in thickness rather than in surface area. Thus, even without a fully validated age determination method for Greenland halibut, it should be concluded that the surface of the left otoliths is not suited for age determination of larger individuals.

The individual growth increments from the tag-recapture experiments indicated annualized growth of slightly less than one cm per year for a 40–60 cm Greenland halibut. This was significantly less than the expected 1.5 cm/year from the increase in mean length at age based on the new method (although it is even further from the 4 cm/yr with the traditional method). This may indicate that the new method still underestimates age of older fish, or that fish growth is halted after tagging. Although the OTC-recaptures indicated similar growth in the otoliths after tagging as in the few preceding years, neither of these can be completely ruled out at present. But it is worth mentioning that there are some mechanisms that will tend to bias the two estimates of annual growth in opposite directions. Firstly, the OTC recaptures indicate that even the finest zones should be counted in the outer region of older otoliths, thus it seems probable that underestimation of the number of these zones may be a bigger problem than overestimation. This will tend to bias the slope of length at age in a positive direction. Secondly, the post mortem shrinkage of the fish total length will tend to underestimate the length increment in tagged fish. Thus, it seems probable that increased precision both in age reading and in measuring of fish in tag-recapture experiments (*e.g.* by recording fish weight in addition to length) would tend to reduce the observed difference between individual growth increments and the slope of length at age.

Both the new method of age interpretation and the growth estimates from tag-recaptures indicate that Greenland halibut grow much slower than previously believed. The difference in length-at-age based on the two methods was large for all size groups above 40 cm. These include almost the complete female population in the adult distribution area along the continental slope of North Norway and Svalbard (Høines and Gundersen, 2008). This will obviously affect stock assessments by changing estimates of mortality and production, and it may be that the species will be considered as more vulnerable to over-exploitation than previously anticipated.

The new surface method relies on improved protocols relating to storing, imaging, choice of reading axis, and definition of seasonal zones. The definitions of the first two zones were validated by length frequency analysis of juveniles. It appeared that the two first modal groups represent one and two year old fish respectively, whereas the third modal group does apparently not represent a single yearclass, as previously believed (Bowering and Nedreaas, 2001), but consists of 3–5 year old fish. The last few annuli of older individuals were validated by analyses of the fluorescence in the OTC recaptures. This showed that the longest growth axis in the right otoliths is the only axis on the otolith surfaces that consistently show pattern attributable to annuli.

The longest axis in the right otoliths was also the one morphometric variable that was most significantly correlated with the new age estimates after removal of the trend with fish length. Thus, it seems probable that this axis should be preferred when otolith surface methods are considered. However, this overall correlation with morphometry is not a validation of the age estimates. It only shows that older individuals were given higher age estimates and younger individuals lower estimates. The only validations reached in this work were for the two first annuli, and for the last few annuli of adult fish with slow growth. Between these annuli are a series of zones that will need to be validated before the life history of Greenland halibut can be fully understood.

Regarding the remaining uncertainty, we consider the age estimates between four and eight years as the most uncertain. In this adolescent phase there is no guidance from the length distributions, and the zonation pattern is typically characterised by several checks and false annuli, especially in the right otoliths. The smallest OTC-tagged and recaptured individual suggests that the zonation pattern may possibly be clearer in the left otoliths in these young fish. This phase also represents the ages of transition between the juvenile and adult distribution area, which is a length structured process involving the largest individuals of the younger age groups (Albert, 2003). It is also the size groups where the sampling trawl is clearly size selective (Albert *et al.*, 2003) thus further biasing any attempt to estimate growth in length from the catches. Unfortunately, these size groups were under-represented in the tag-recapture data. Future tagging experiments, shedding light on this adolescent phase, may therefore further improve age estimations of Greenland halibut.

Despite the above-mentioned uncertainty with the fourth to eighth annuli, the interpretation followed widely accepted interpretation rules (Williams and Bedford, 1974; Beamish and McFarlane, 1987), and the uncertainty within this section of the otoliths should probably not be expected to be more than a few years. But even if the new method can be considered as reasonably accurate, it is still relatively imprecise, with a mean CV of 12% between age readers. This is at the same level as that found for many other long lived fish species (Kimura and Lyons, 1991; Bergstad *et al.*, 1998; Stransky *et al.*, 2005) and experience has shown that this precision may be significantly improved by systematic exchange programs and agreement on specific interpretation principles (Bergstad *et al.*, 1998).

Future work should focus on increasing precision by finding aging structures, reading axes and preparation techniques that may enhance readability for intermediate ages. As more OTC marked fish are recaptured, it may be possible to better describe the full variability of otolith growth throughout the ontogeny of the fish. This may also warrant further exploration of axes in the 3D otolith structure (as well as of alternative structures such, as scales and vertebrae) that most consistently represent the true age of the individuals.

Acknowledgements

The paper has benefited from comments and suggestions from the Associate Editor and from three anonymous reviewers.

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