Title: Age determination of Atlantic halibut (*Hippoglossus hippoglossus* L.) along the coast of Norway - status, and future potentials

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Abstract

The Atlantic halibut, *Hippoglossus hippoglossus,* is known as the largest of the teleost fishes inhabiting North-Atlantic waters, and its ability to attain great ages is well known. Because the halibut is highly vulnerable to exploitation, and accurate age estimates are fundamental in the understanding of fish biology and the management of fish populations, this study re-evaluates the current aging methodology. The traditional method for estimating age of Atlantic halibut is through surface readings of otoliths. Previous studies have not been compared to find the best practice for surface reading techniques. Based on experiments with different preparation treatments and techniques, this study establishes an updated procedure for the age determination of Atlantic halibut. The present study suggests significant underestimation of ages have occurred using previous protocols. The potential for using age determination to investigate spatial population structure parameters such as size- and sex differences is also shown.

Keywords: aging methodology, Atlantic halibut, *Hippoglossus hippoglossus*, otoliths, size at age

Introduction

The Atlantic halibut is distributed throughout the boreal waters in large parts of the North Atlantic Ocean (Godø and Haug, 1988 a, b). The Atlantic halibut has been an attractive target species for fishermen for a long time because of its high market price. Today, the stock size of Atlantic halibut is low in the entire North-Atlantic ocean. Fisheries are not regulated by quotas, and halibut are most often caught as bycatch in other fisheries. Data received from the Directorate of Fisheries in Norway indicate that while catches in the south of Norway has remained at low levels, the total landings of halibut north of 62 ºN have increased considerably in the years between 1998 and today (Høines et al., 2009). The mortality of halibut is most likely dominated by fishing, seeing as they rapidly reach a size evading most predators (Haug, 1990). They reach sexual maturity relatively late in life, and is as a consequent vulnerable to even moderate levels of fishing pressure as many of them are harvested before they have the chance to reproduce (Sigourney et al., 2006). Also, when halibut aggregate to spawn, they are vulnerable to fishing as they concentrate in a small area and therefore become an easy target for fishermen. Heavy fishing on these spawning grounds can thus cause catastrophic damages to the stock (Høines et al., 2009). Due to the vulnerability of the spawning stock, fishing for halibut in the time period between December 20 and the 31 of March is today prohibited in Norwegian waters and the minimum size for halibut has also recently been raised from 60 to 80 centimetres (Anonymous, 2011).

Male and female halibut has been found to differ in growth rate. Previous studies show a declining growth rate following from the onset of sexual maturity, and a faster growth in females than males after this event, enabling them to attain a greater size (Armsworthy and Campana, 2010, Jakupsstovu and Haug, 1988, Haug and Tjemsland, 1986). Although the age and growth of Atlantic halibut has not been rigorously validated, they are presumed to be long-lived, reaching an age of at least 50 years (Armsworthy and Campana, 2010). The estimation of age is in most cases done by counting periodic growth increments in otoliths.

In the management of fish stocks and stock assessment, invalid age estimation can lead to severe implications (Høie, 2003, Bermejo, 2007) and knowledge of age is therefore one of the most important issues to consider in order to construct efficient management plans and strengthen the basis for recovery strategies. Information regarding age is a prerequisite for calculations of numerous biological variables such as growth rate, productivity and mortality rate. Because all rate calculations demand an elapsed time term or age, the need for age data is found in everything from simple growth rate calculations to more complex analysis such as virtual population analysis (Campana, 2001). Different methods have been tested and used for aging Atlantic halibut in recent years. The procedure commonly used today e.g. by the Institute of Marine Research in Bergen (IMR), involves reading whole otoliths, immersing both left and right otolith in water and photographing both using transmitted light. The method is basically the same as the one used for Greenland halibut, *Reinhardtius hippoglossoides,* where the right otoliths (from the dark side of the fish) is interpreted because it is the one with the longest readable axis (Kvalsund and Solbakken, 2008). Previously the otoliths from the left (white) side was used for age determination on halibut ((Haug and Tjemsland, 1986). Given the characteristic differences between Atlantic and Greenland halibut otoliths, there is some uncertainty whether or not the same interpretation approach can be applied for the Atlantic halibut. It is suspected that the former method underestimates the true age of halibut and the method for aging Greenland halibut is also currently under re-evaluation.

The main objective of this study was thus to compare different approaches of age determination for the Atlantic halibut, and to establish a new and improved procedure for aging Atlantic halibut. In order to improve the utilization of the information that the otoliths can provide in future management of the species, we also describe the relationship between age, length and weight, spatial size variations, and provide a preliminary age-length key.

Materials and methods

Material

Halibut were sampled in the years 2004-2006 and 2008-2010 along the coast of Norway (Figure 1, Table 1). All otoliths collected between 2004 and 2006 had been stored dry in paper envelopes and been aged either directly through a stereomicroscope or from digital images.

Additional sampling

Halibut were sampled on board the research vessel “Johan Hjort” during the annual coastal cruise in October 2010. The procedure for collecting otoliths has remained the same for all samples collected in all years included in this study. Trawl hauls were mainly performed on regular stations along the coast using the Campelen 1800 shrimp trawl. All trawling procedures were carried out according to standard procedures (Anonymous, 2008). The halibut caught in each haul were weighed, and both individual and total weights of halibut were recorded. The total length was measured on an electronic fish measuring board, the fish meter (Scantrol). The left and right saggital otoliths were removed from their position directly behind the brain by making a frontal section of the skull of the halibut. The otoliths were immediately put into small lidded cups, and partly filled with seawater to prevent them from drying out. After marking the cups with station number and fish number, they were put into plastic bags and frozen.

Digital images

Otoliths were photographed using a NIKON Stereoscopic Zoom Microscope SMZ 1500, objective HR Plan Apo 0.5x. Otoliths collected in the years prior to 2008 had been stored in paper envelopes. To determine what method of photographing gave the clearest view of increments, the otoliths collected in the years between 2004 and 2006 were photographed after receiving different treatments, using Eclipse net Software®. The otoliths were first photographed directly by placing them with their concave side facing the objective, in a Petri dish filled with water. The same otoliths were also photographed after a 24 hour immersion in water and after storage in 60% glycerol for 24 hours. The otoliths were photographed using both transmitted and reflected light.

Preparation of otolith sections

With the purpose of comparing both clarity and number of increments between sectioned otoliths and whole mount otoliths, 10 pairs that showed clear and 10 pairs that showed relatively unclear increments were chosen for sectioning. The otoliths were embedded in a mixture of Epofix resin and hardener at the proportion 9:5 by weight, and left for 24 hours to harden. Transversal cuts were made using the Isomet 1000 low speed saw, producing sections measuring approximately 500 µm. To ensure a representative section through the core, two or three sections was made from each otolith. The sections that were cut closest to the core and had the least breakage were chosen. The least favourable side was polished gently using four different grit abrasive papers and tap water on a grinding and polishing mechanical rotating disk (Buehler Phoenix beta), starting with 600 µm grit, then using 1000 µm, 2500 µm and finally 4000 µm grit. The section was measured using a micrometer and attached to an object-glass, polished side facing the glass, using a clear Crystalbond™ adhesive, preheated to approximately 135°C. The section was polished again and the resulting section thickness was somewhere between 200 and 400 µm.

Digital images were taken of the prepared sections for both left and right otolith from all 20 halibut using a Nikon DS 2 camera connected to a stereomicroscope, Leica MZ 9.5, and the image software NIS-Elements F version 3.0. Calibration was performed in the beginning of each session and with the change of magnification.

Age determination

All images captured of the whole otoliths were viewed in Photoshop using the action script used by IMR for the age interpretation of Greenland halibut, *Reinhardtius hippoglossoides* (Kvalsund and Albert, 2007, Kvalsund and Solbakken, 2008). A new interpretation layer was created and a brush of defined color and size was used to mark annual increments. Before marking the final annual band, the date of capture was considered in order to decide whether or not the final increment was fully formed and could be counted as one year (with the 1st of January accepted as the birth date of all fish). The different methods for photographing whole otoliths collected in the years between

2004 and 2006 were compared by studying the clarity of increments achieved either by photographing the otoliths directly, after 24 hour immersion in water or after 24 hours in 60% glycerol, using both transmitted and reflected light. Ages were interpreted for all otoliths photographed by counting the marked increments on both left and right otolith, using transmitted and reflected light, and on sectioned otoliths. All otoliths were assigned an age and a readability ranging from 1-4, depending on the quality of the otolith. The results were compared across treatments and light sources.

The choice of light source was based upon which of the two otoliths that consistently revealed the highest number of distinct increments, and the preferred otolith was the one that revealed more and clearer increments. Comparing sections and whole mount images of both left and right otolith gave an indication of coherence of interpretation between the two methods. Comparisons between the different methods gave a preferred approach for reading the otoliths, and this approach was performed on all otoliths collected in the years between 2008 and 2010.

Statistical Analysis

The data analysis software system Statistica, version 10 (StatSoft inc., 2010), was used for all graphical illustrations and statistical analyses.

The regression of the relationship between length and weight, as well as length and weight at age, were analyzed using generalized linear models (GLM) analysis (StatSoft inc., 2010). The effects of sex were tested by including sex as a factor in these analyses, and excluded if the interactions or main effects were insignificant.

Results

Effect of otolith treatment and source of light on numbers and quality of increments

When comparing the images of otoliths exposed to different treatments (Figure 2), photographing after a 24 hour immersion in water proved to be the best approach for achieving the most defined increments (Figure 2b and e). Otoliths photographed directly after dry storage gave a matt surface (Figure 2a and d), whereas otoliths photographed after 24 hours in glycerol produced a refringent surface (Figure 2c and f). The otoliths in Figure 2a, b and c are photographed using transmitted light, while the ones in Figure 2d, e and f are photographed with the use of reflected light. Separating true increments from false increments (non-annual additional opaque or translucent increments) was found to be more difficult on images captured using transmitted light. Using reflected light revealed a higher number of distinct increments, as well as more equivalence between left and right otolith interpretation. There was no significant difference between the numbers of increments counted for the two light sources (paired t-test, Table 2, $p > 0.05$).

The ages interpreted on the left and right otolith were in most cases the same. In a few cases the age was interpreted as being higher on the right otolith, but mostly it was the other way around. The most common difference between left and right otolith was one increment (year) but in some cases it was even more. There were a higher number of increments on the left than on the right otoliths (paired t-test, Table 2, Figure 3, $p < 0.001$). The left otolith also showed the better readability.

Interpreting whole otoliths and their sections gave the same ages in most cases. For otolith pairs where the age estimated differed between left and right whole mount otolith, their sections showed equivalence in 69.2 % of the cases. The interpretation was also more comparable with the left whole otolith. However, comparing the age interpreted for the left and right whole otolith with the sections revealed no significant difference (paired t-test, Table 2, $p > 0.05$).

Comparing former and current estimates of age

The ages estimated for halibut in this study were higher in almost all cases compared to former estimates of the same otoliths. Both the left and right otolith resulted in higher age estimates in this study compared to previous estimates (paired t-test, Table 2, $p << 0.001$, Figure 4). This difference increased with age and was also in a magnitude of several years for many of the cases.

Size and growth of Atlantic halibut

The relationship between log total length and log wet-weight for Atlantic halibut was close to allometric (Figure 5). The slope (3.21) was significantly different from 3 (GLM, $p << 0.001$), indicating a non-isometric relationship. There were no differences in the length-weight relationship between male and female halibut in the size range studied (GLM, $p > 0.05$).

The measured length at age was found to differ for males and females. The weight and length appeared to increase continuously for both sexes as they grew older, and females were generally heavier and longer at a given age than males (Figure 6, GLM, $p < 0.05$).

Spatial variation in sizes and in sizes at age for males and females

Dividing our sampling sites into northern and southern regions gave us latitudinal locations ranging from 62.9°N to 71.2°N with 66.5°N as the north/south boundary. The halibut caught in northern latitudes were larger in size than the ones sampled further south (GLM, $p < 0.05$). The lengths and weights at age in the northern latitudes were also significantly greater than those in southern (GLM, $p < 0.05$, Figure 7).

Discussion

Aging procedure for Atlantic halibut

The traditional method for aging halibut is by otolith surface readings (Armsworthy and Campana, 2010). In the present study, surface readings after different treatments were performed. Glycerol was expected to enhance the contrast of the growth increments (Forsberg, 2001). After 24 hours storage in 60% glycerol the results showed otoliths with a certain transparency, where increments were almost erased. It was found that a 24 hour immersion in water is the best approach for enhancing the incremental structure of the otoliths. The choice of reflected light over transmitted light also proved to be preferable. The choice of left or right otolith has previously been determined based on which of the two that has the longest readable axis (Kvalsund and Albert, 2007). Even though the right otoliths have the longest readable axis in halibut, this study concluded that the left otolith showed clearer growth increments, as well as a significantly higher number of distinct increments than the corresponding right otolith. This is in accordance with (Haug and Tjemsland, 1986), which also found that the otoliths collected from the left side of the fish show clearer growth increments. Sectioning of otoliths reinforced the conclusion that the left otolith is preferable, also the left whole otolith was more comparable with the section than the interpretation when using the right otolith.

No significant differences were found between the surface readings and the cross section readings in this study. Sectioning of otoliths was found to be a useful tool in cases where the whole otoliths are damaged above or below the core as it can still be interpreted. Previous aging studies performed for a number of species have found that otolith surface readings underestimate age (Albert et al., 2009, Blood, 2003, Lee et al., 2009). Although no evidence of underestimation was found in our study, this might be a result of the lacking number of older individuals. If the fish is old it can be difficult to interpret the outer most increments of the otolith. A cross section reveal greater detail and can then give a more reliable estimate of the age. A study comparing surface reading and break and burn methods for Pacific halibut, found a divergence of the two aging methods beginning at age 7, with the break and burn method yielding a higher age (Blood, 2003). From images captured in this study, it is clear that the quality of the otoliths vary greatly. When the interpretation of annual increments exceeds nine, the difference between left and right otolith sometimes become more than one. In cases where this difference occurs, it is often related to the readability of the otolith. In this study, otoliths that were given a readability of three or four, meaning either very difficult to read or broken respectively, occasionally had age differences between right and left of more than one year. With poor readability, sectioning was found to be a preferable method for age interpretation as the visibility of increments was improved and the difference between the otoliths were reduced.

The current study showed that the number of increments recorded for both left and right otolith was significantly higher than the number of increments recorded by the method previously used by IMR, indicating a possible previous underestimation of the age in Atlantic halibut. The severity of underestimation is related to the resulting overestimation of growth rates, mortality rates and reduction in stock abundances (Bertignac, 2007). Long term management and recovery plans will therefore be hampered. The current study also found that aging became more difficult for presumed older individuals. Errors related to accurate aging of older fish are not random and are biased towards younger ages. Subjectivity is an element that is difficult to avoid in age interpretation, and therefore a possible source of error (Haug and Tjemsland, 1986). The only way to prove that an age is accurate is through validation (Beamish and McFarlane, 1983). It is therefore not possible to conclude with absolute certainty which of the two methods give the correct estimates of age without proper validation. However the occurrence of the opaque character of the outer increment for halibut caught during summer and translucent increment for individuals caught during winter, indicate annual increment deposition in halibut otoliths. Recently developed validation methods that have proven to be rigorous include bomb-radiocarbon assays (Armsworthy and Campana, 2010) and chemical tagging of otoliths using oxytetracycline (OTC) followed by recapture (Treble et al., 2008).

Size and age relationships

This study found a similar relationship between length and weight for males and females for the size range studied. The relative growth appeared greater at younger ages and decreased with age. During the first 4-6 years there was no pronounced difference in length or weight between the sexes. Male sizes at age appeared to level out some at an age of 10-12, while female growth seemed to accelerate at this point. Females were found to become significantly longer and heavier with age than males. These observations are consistent with previous findings (Jakupsstovu and Haug, 1988, Haug and Tjemsland, 1986, Sigourney et al., 2006, Devold, 1938, Armsworthy and Campana, 2010).

The possible effects of environmental variation on fish size should be considered as the halibut included in this study was sampled at different times of the year. Evidence of the occurrence of post-spawning recovery growth in cod, *Gadus morhua*, has been established, and the condition of fish has been found to have an influence on size and growth (Pedersen and Jobling, 1989). Previous studies have found that the mean relative liver weight is reduced throughout the spawning season in Atlantic halibut, and depending on their state of maturity, both male and female halibut gonads had different weight proportions, affecting the total weight of the fish. In spite of these findings, the general body weight loss has been found to be low during spawning (Haug and Gulliksen, 1988). The use of relative weight measurements as indices of growth, should perhaps be reconsidered, as it might be a more robust predictor of fecundity

Size distribution of halibut along the Norwegian coast

This study found latitudinal differences in the size distribution of Atlantic halibut along the Norwegian coast where both males and females collected in the more northern latitudes were significantly larger. Also, the lengths and weights at age were significantly greater in the ones collected at the higher latitudes. Distinct variations in life history strategies and biological characteristics have been documented in species inhabiting wide latitudinal ranges (Boehlert and Kappenman, 1980). The Atlantic halibut has a large north - south distribution range in the North Atlantic Ocean. For several species, it has been shown that northern fish populations can have a higher growth potential than populations further south (Conover and Present, 1990). In a study performed on geographic variation in growth of juvenile Atlantic halibut, it was found that high latitude populations of juvenile halibut displayed a higher growth rate at all temperatures compared to lower latitude populations (Jonassen et al., 1999). Whether the observed growth patterns are due to genetic population differences among northern and southern halibut populations along the Norwegian coast remains to be documented.

Age-length-weight keys

Given the established differences in growth rates between males and females, an age key has to be separate for the sexes. The scarcity of material available in the different age groups sampled in this study prevents the construction of a valid age-length-weight key, and leave us with a very rough overview of what ages to expect for the different length classes (Table 3 and 4). The primary determinant of weight for fish is length (Anderson and Neumann, 1996). The allometric relationship that was found to exist between length and weight, indicate a change in body shape with increasing age. Halibut included in the current study were sampled at various times of the year, and weight has been shown to vary with condition, which again vary with season (Pedersen and Jobling, 1989). For this reason, including weight in an age key may introduce certain potential problems since weight (and condition), fluctuates seasonally, making it a less suitable indicator of age. Mean length in combination with age are often used by fisheries biologists to assess fish growth (Bettoli and Miranda, 2001). The expenses and difficulties related to age determination of fish, makes the application of length distributions for age estimation an attractive choice (Kimura and Chikuni, 1987). Also, measuring the length of a large number of individuals is relatively easy compared to the rather tedious assessment of the ages of each and every individual (Ogle, 2008). In our preliminary age-length key, a halibut of 50 cm is around 5 years of age (5.0 and 5.4 for females and males respectively). At this size there is a large overlap in age-at-size between males and females, but a further divergence in age-at-size for older individuals is indicated (e.g. on average 8.3 and 9.1 years of age for 80 cm females and males). However, more data is required for the larger size classes to construct a reliable age-length key.

A new aging procedure is proposed for Atlantic halibut, and comparison with previous age estimates, indicate that a significant age-underestimation has taken place for older individuals. Regional comparisons further indicate a faster growth of halibut caught in the northernmost areas along the Norwegian coast compared to further south.

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References

ALBERT, O. T., KVALSUND, M., VOLLEN, T. & SALBERG, A. 2009. Towards accurate age determination of Greenland halibut. Journal of Northwest Atlantic Fishery Science, 40**,** 81-95.

ANDERSON, R. O. & NEUMANN, R. M. 1996. Length, weight, and associated structural indices. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland, 5**,** 447-482.

ANONYMOUS 2008. Håndbok for vitenskapelig tråling, versjon 3.0. p1-94.

ANONYMOUS 2011. Kysten er din- regler for fritidsfiske i sjøen. Bergen Fiskeridirektoratet.

ARMSWORTHY, S. L. & CAMPANA, S. E. 2010. Age determination, bomb-radiocarbon validation and growth of Atlantic halibut (*Hippoglossus hippoglossus*) from the Northwest Atlantic. Environmental Biology of Fishes, 89**,** 279-295.

BEAMISH, R. J. & MCFARLANE, G. A. 1983. The forgotten requirements for age validation in fisheries biology. Transactions of the American Fisheries Society, 112**,** 735-743.

BERMEJO, S. 2007. Fish age classification based on length, weight, sex and otolith morphological features. Fisheries Research, 84**,** 270-274.

BERTIGNAC, M. D. P., H. 2007. Consequences of bias in age estimation on assessment of the northern stock of Eurpoean hake (*Merluccius merluccius*) and on the management advice. ICES Jornal of Marine Science 64**,** 981-988.

BETTOLI, P. W. & MIRANDA, L. E. 2001. Cautionary note about estimating mean length at age with subsampled data. North American Journal of Fisheries, 21**,** 425-428.

BLOOD, C. L. 2003. Comparison of surface and break- and -burn otolith methods of aging Pacific halibut. Technical report**,** 20-31.

BOEHLERT, G. W. & KAPPENMAN, R. F. 1980. Variation of growth with latitude in two species of Rockfish (*Sebastes pinniger* and *S. diploproa*) from the Northeast Pacific Ocean. Marine Ecology-Progress Series, 3**,** 1-10.

CAMPANA, S. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59**,** 197-242.

CONOVER, D. O. & PRESENT, T. M. C. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. Oecologia, 83**,** 316-324.

DEVOLD, F. 1938. The North Atlantic halibut and net fishing. Fiskeridirektoratets skrifter, HavUndersøkelser 5**,** 1-47.

FORSBERG, J. E. 2001. Aging manual for Pacific halibut: procedure and methods used at the International Pacific Commission (IPHC). Technical report, 46.

GODØ, O. R. & HAUG, T. 1988a. Tagging and recapture of Atlantic halibut (*Hippoglossus hippoglossus*) in continental shelves off eastern Canada, and off western and eastern Greenland. Journal of Northwest Atlantic Fishery Science, 8**,** 25-31.

GODØ, O. R. & HAUG, T. 1988b. Tagging and recapture of Atlantic halibut (*Hippoglossus hippoglossus*) in Norwegian waters. Journal Du Conseil International Pour I`Exploration de la Mer, 44**,** 169-179.

HAUG, T. 1990. Biology of the Atlantic halibut, *Hippoglossus hippoglossus* (L, 1758). Advances in Marine Biology, 26, 1-70.

HAUG, T. & GULLIKSEN, B. 1988. Variations in Liver and Body Condition During Gonad Developmentof Atlantic Halibut, *Hipoglossus Hipoglossus* (L.). Fiskeridirektoratets skrifter, 18, 351-363.

HAUG, T. & TJEMSLAND, J. 1986. Changes in size-distributions and age-distributions and age at sexual maturity in Atlantic halibut, *Hippoglossus hippoglossus*, caught in Norwegian waters. Fisheries Research, 4, 145-155.

HØIE, H. 2003. Stable oxygen and carbon isotopes in fish otoliths as environmental recorders. Dr. Scient. thesis, University of Bergen.

HØINES, Å., BJELLAND, O., MICHALSEN, K., VØLSTAD, J. H., HELLE, K., VOLLEN, T., K, N. & ALBERT, O. T. 2009. Kveite i norske farvann, status og utfordringer for forvaltning og forskning. Rapport fra havforskningen, 1, 1-15.

JAKUPSSTOVU, S. H. & HAUG, T. 1988. Growth, sexual maturation, and spawning season of Atlantic halibut, *Hippoglossus hippoglossus*, in Faroese waters. Fisheries Research, 6, 201-215.

JONASSEN, T. M., IMSLAND, A. K., FITZGERALD, R., BONGA, S. W., HAM, E. V., NÆVDAL, G., STEFANSSON, M. O. & STEFANSSON, S. O. 1999. Geographic variation in growth and food conversion efficiency of juvenile Atlantic halibut related to latitude. Journal of Fish Biology, 56, 279-294.

KIMURA, D. K. & CHIKUNI, S. 1987. Mixtures of empirical distributions: an iterative application of the age-length key. Biometrics, 43, 23-35.

KVALSUND, M. & ALBERT, O. T. 2007. Age estimation of Greenland halibut (*Reinhardtius hippoglossoides*). Technical report, Institute of Marine Research, 1-9.

KVALSUND, M. & SOLBAKKEN, L. 2008. Prosedyre for aldersbestemmelse av kveite (*Hippoglossus hippoglossus*). Technical report**,** Institute of Marine Research, 2-8.

LEE, J. H., KODAMA, K., KUME, G., OYAMA, M., KATAYAMA, S., TAKAO, Y. & HORIGUCHI, T. 2009. Comparison between surface-reading and cross-section methods using sagittal otoliths for age determination of the marbled sole *Pseudopleuronectes yokohamae*. Fisheries Science, 75**,** 379-385.

OGLE, D. 2008. Assigning individual ages with an age-length key. assessment**,** 1-8.

PEDERSEN, T. & JOBLING, M. 1989. Growth rates of large, sexually mature cod, *Gadus morhua*, in relation to condition and temperature during an annual cycle. Aquaculture 81**,** 161-168.

SCANTROL, A. *Scantrol AS* [Online]. Bergen. Available: www.scantrol.no/marineresearchproductguide.cfm?pArticleId=8945&pArticleCollevtionId=447 [Accessed March 2011].

SIGOURNEY, D. B., ROSS, M. R., BRODZIAK, J. & BURNETT, J. 2006. Length at age, sexual maturity and distribution of Atlantic Halibut, *Hippoglossus hipoglossus* L., off thee Northeast USA. Journal of Northwest Atlantic Fishery Science, 36**,** 81-90.

TREBLE, M. A., CAMPANA, S. E., WASTLE, R. J., JONES, C. M. & BOJE, J. 2008. Growth analyses and age validation of a deepwater Arctic fish, the Greenland halibut (*Reinhardtius hippoglossoides*) Canadian Journal of Fisheries and Aquatic Sciences, 65**,** 1047-1059.

Table 1. Halibut otoliths collected in the years 2004-2006 and 2008-2010, indicating how many pairs of otoliths that have been collected and how many of these which are available for further study.

Year	Source/ vessel	Date	No. Otolith pairs	Available for the study *
2004	RV Johan Hjort	14.10-10.11	31	0
	RV Jan Mayen	22.10-07.11	16	0
	FV Førde Jr	19.09-03.10	48	44
	Fishermen		10	1
2005	Fishermen	24.02-25.02	8	0
	RV G.O. Sars	27.02-17.08	7	2
	Reference fleet	27.04-22.08	10	2
	RV Johan Hjort	23.10-04.11	17	17
	RV Jan Mayen	26.10-07.11	11	11
	FV Amigo	26.11-30.11	6	6
2006	RV Johan Hjort	12.02-16.11	23	23
	FV Amigo	1.08	22	22
2008	RV Johan Hjort	03.10-14.11	21	21
	NIFES**	20.02-11.12	23	23
2009	NIFES**	21.01	1	1
	RV Johan Hjort	06.10-25.10	17	17
	RV Jan Mayen	04.10-24.10	17	17
2010	RV G.O. Sars	24.08	1	1
	RV Johan Hjort	03.04-03.11	56	56
Total			345	264

* Some otoliths were not available due to prior sectioning

** National Institute of Nutrition and Seafood research

Table 2. Overview of mean increment number counted when comparing across methods.

	Gr 1 Mean	Gr 2 Mean	p-value
Left otolith, Reflected light vs. Transmitted light	8.16	7.99	0.054
Right otolith, Reflected light vs. Transmitted light	7.70	7.54	0.077
Left whole otolith vs. Right whole otolith	7.45	7.19	< 0.001
Left section vs. Left whole	8.79	8.88	0.571
Right section vs. Right whole	8.91	8.68	0.128
New age estimate vs Previous age estimate	8.09	6.08	<<0.001

Table 3. Distribution of Females within each age and length class. Because of low numbers of large halibut, individuals estimated to an age of 12 years old or older, and 100cm long or longer are pooled together.

	Age												
LClass	1	2	3	4	5	6	7	8	9	10	11	12+	Row
20	**1**	0	0	0	0	0	0	0	0	0	0	0	1
30	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	**1**	**5**	**3**	0	0	0	0	0	0	0	9
50	0	0	**2**	**13**	**14**	**4**	**6**	0	0	0	0	0	39
60	0	0	0	**1**	**4**	**6**	**2**	**2**	**2**	0	0	0	17
70	0	0	0	0	**1**	**3**	**1**	**1**	**3**	0	0	0	9
80	0	0	0	0	0	**1**	**2**	**2**	**2**	**1**	**1**	0	9
90	0	0	0	0	0	0	**1**	**1**	**2**	**1**	0	0	5
100+	0	0	0	0	0	0	0	0	0	**2**	**3**	**4**	9
All Grps	1	0	3	19	22	14	12	6	9	4	4	4	98

Table 4. Distribution of males within each age and length class. Because of low numbers of large halibut, individuals estimated to an age of 12 years old or older, and 100cm long or longer are pooled together.

	Age												
LClass	1	2	3	4	5	6	7	8	9	10	11	12+	Row
20	**1**	0	0	0	0	0	0	0	0	0	0	0	1
30	0	**3**	0	**1**	0	0	0	0	0	0	0	0	4
40	0	0	**3**	**2**	**5**	0	0	0	0	0	0	0	10
50	0	0	0	**4**	**8**	**9**	0	**1**	0	0	0	0	22
60	0	0	0	**1**	**3**	**5**	**4**	**3**	**1**	**1**	0	0	18
70	0	0	0	0	**3**	**1**	**1**	**4**	**1**	0	**1**	0	11
80	0	0	0	0	0	0	**1**	**1**	**3**	**1**	0	**1**	7
90	0	0	0	0	0	0	0	0	**1**	0	0	**1**	2
100+	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	**6**	10
All Grps	1	3	3	8	19	15	6	10	7	3	2	8	85

Figures

Figure 1. Sampling localities of Atlantic halibut along the Norwegian coast. The different symbols indicate sampling year.

Figure 2. Examples of otolith pairs photographed after different treatments, and with different light sources. Images in the upper panel are photographed using transmitted light, while the ones in the lower panel are photographed with the use of reflected light. Images a, d) otoliths are photographed directly, displaying a rather matt surface, b, e) otoliths photographed after a 24 hour immersion in

water, were increments are pronounced and clear, and in c, f) otoliths are photographed after 24 hours in glycerol, producing a refringent surface.

Figure 3. The relationship between ages of the left and right otolith photographed using reflected light. The regression line, confidence interval and the y = x line are indicated by different shape and thickness. The size of the dots indicate frequency of age observations.

Figure 4. The difference in age interpreted for the same otoliths using the former and current method (regression line (solid line), confidence interval (dashed line) and the y=x line (thick solid line)).The size of the dots indicate frequency of age observations.

Figure 5. Regression of the relationship between log transformed length in cm and log transformed weight in grams. N= 247

Figure 6. Regression of the relationship between length and age, categorized by sex. Males and females are indicated by different color and symbols. N= 183

Figure 7. Regression of the relationship between length and age observed for male and female halibut caught at different latitudes. N=174

Figure 1

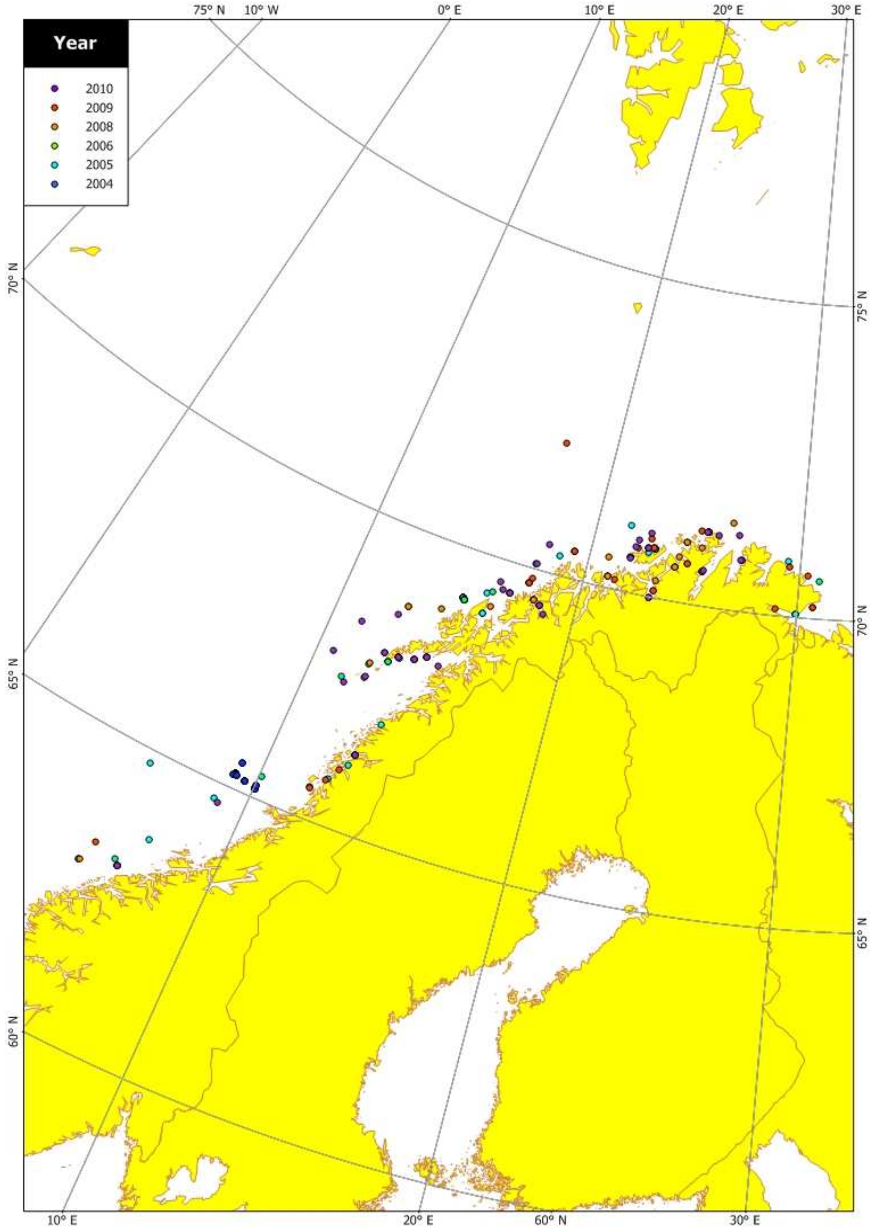
Year
2010
2009
2008
2006
2005
2004
75° N
10° W
0° E
10° E
20° E
30° E
70° N
75° N
65° N
70° N
60° N
65° N
10° E
20° E
60° N
30° E

Figure 2

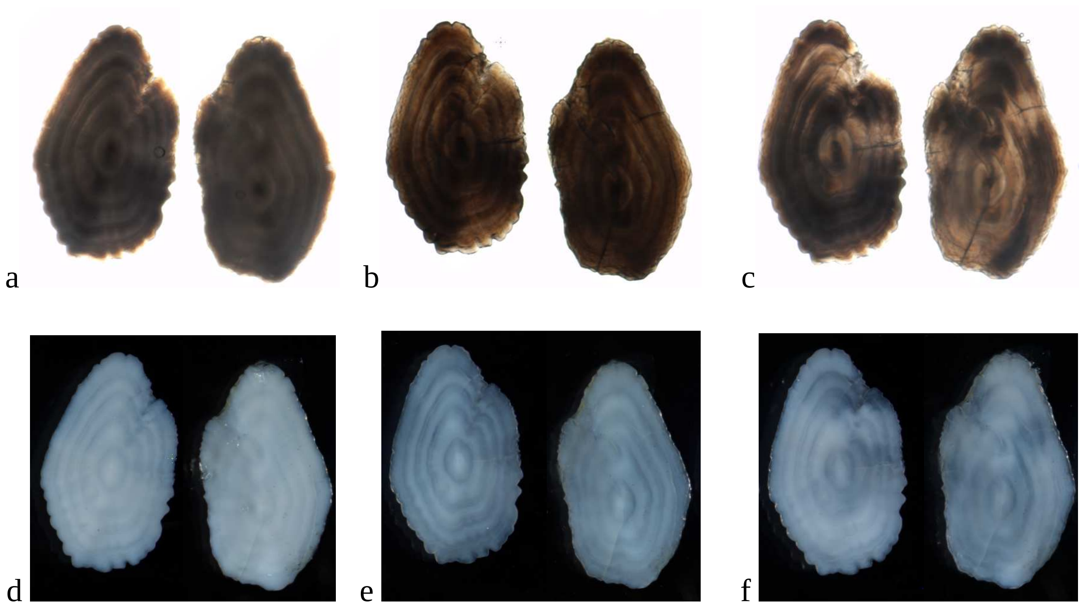

Figure 3

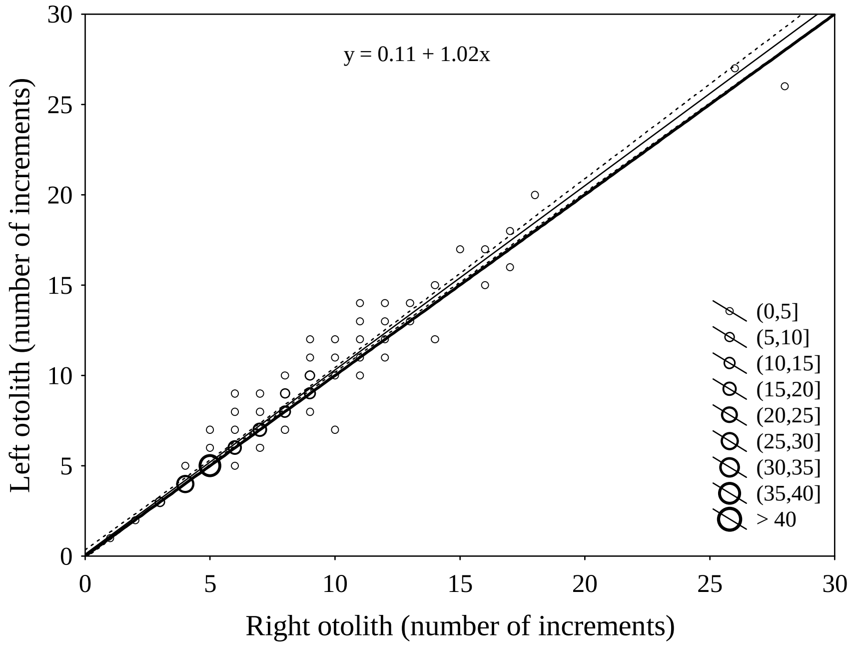

Figure 4

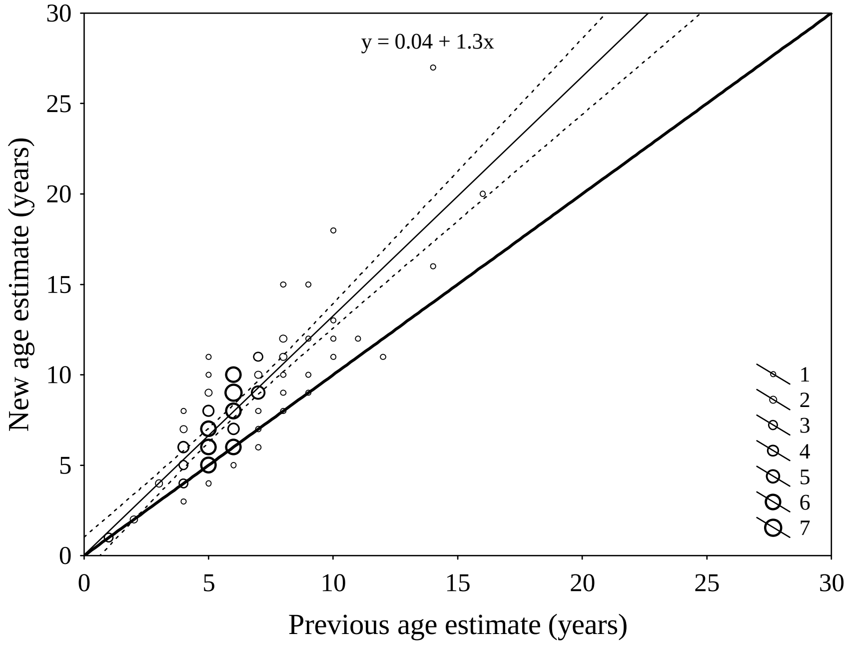

Figure 5

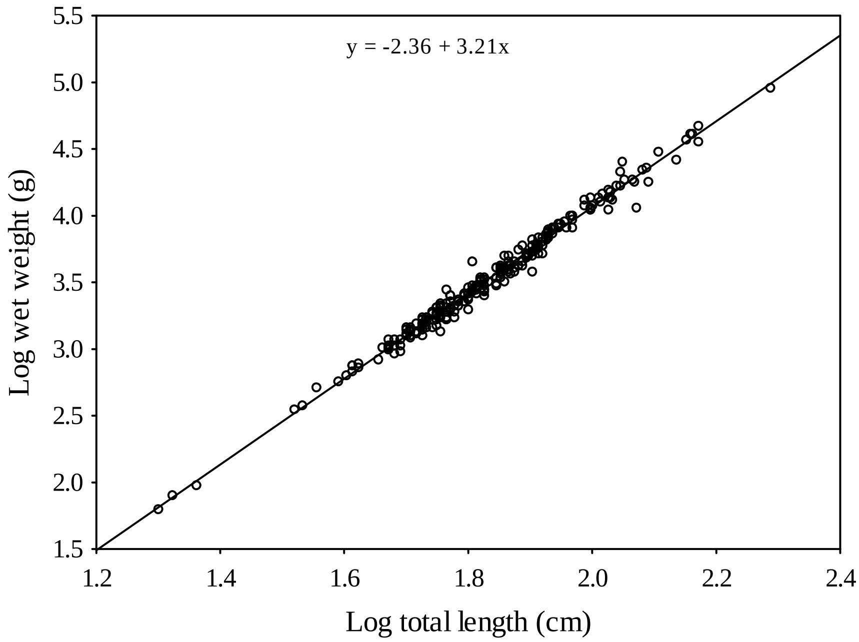

Figure 6

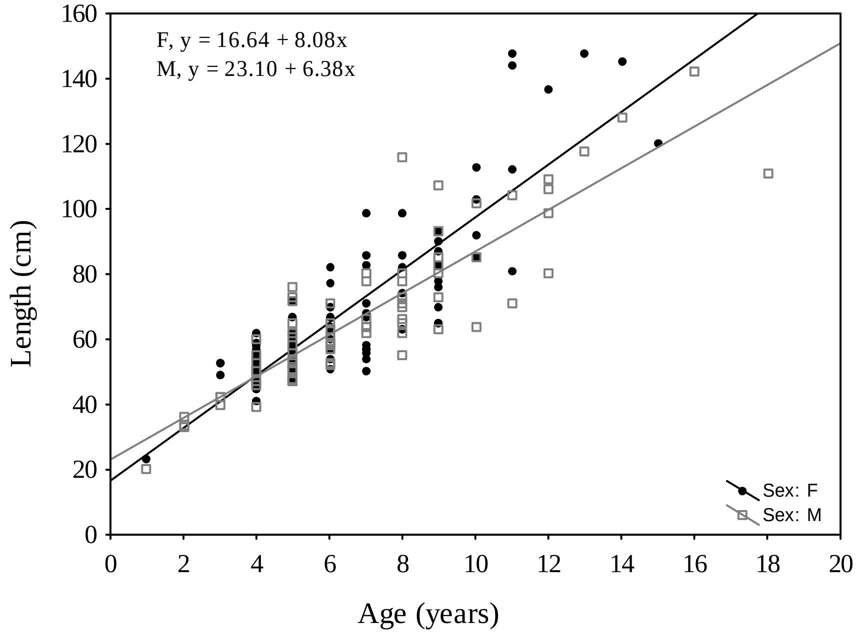

Figure 7