Age determination of Atlantic halibut (*Hippoglossus hippoglossus* L.)

- Size at age and regional growth differences along the coast of Norway

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Front page photo: Stine Karlson

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Abstract

The traditional method for estimating age of Atlantic halibut (*Hippoglossus hippoglossus*) is through surface readings. 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 study also show a significant difference between age interpretations made after the former method previously used by the Institute of Marine Research (IMR), and the updated procedure, indicating either under- or overestimation of ages respectively.
The timing of seasonal zone formation varies between species. This study confirms that in Atlantic halibut, the opaque increments are deposited during summer, while the translucent increments are formed during winter.

The length and weight relationship, and the relationship between size and age, are described using data collected along the Norwegian coast. The differential growth between male and female halibut that has been found in previous works is confirmed. Regional size differences, consistent with previous findings, are demonstrated for different latitudes, with larger individuals distributed at the higher latitudes.

The expenses and difficulties related to age determination of fish make the application of length and weight distributions for age estimation an attractive choice. This study attempts to construct an age-length-weight key based on length and weight data collected in the time period 2004-2006 and 2008-2010, and on the ages interpreted for the corresponding otoliths.

1. Introduction

1.1 Biology

1.1.1 Taxonomy

The Atlantic halibut, *Hippoglossus hippoglossus*, has been classified under the superorder Acanthopterygii as a member of the order Pleuronectiformes, by Linnaeus in 1758. It belongs to the family Pleuronectidae and subfamily Hippglossinae (Roje, 2010).

1.1.2 Distribution

Atlantic halibut inhabits the boreal waters (Haug, 1990), and is distributed in large parts of the North Atlantic Ocean (Figure 1). It is numerous off Newfoundland and Labrador, on the west side of the Atlantic. The halibut also occur from Cape Cod and far north along the coast of Greenland. It is further distributed from the east of Greenland and Iceland, beyond Svalbard to Novaja Zemlja and down south to Biscay (Michalsen, 2010).

In our waters the halibut has been found along the entire Norwegian coastline, in south-western parts of the Barents Sea to Bear Island, and in some cases up to Svalbard (Haug, 1990).

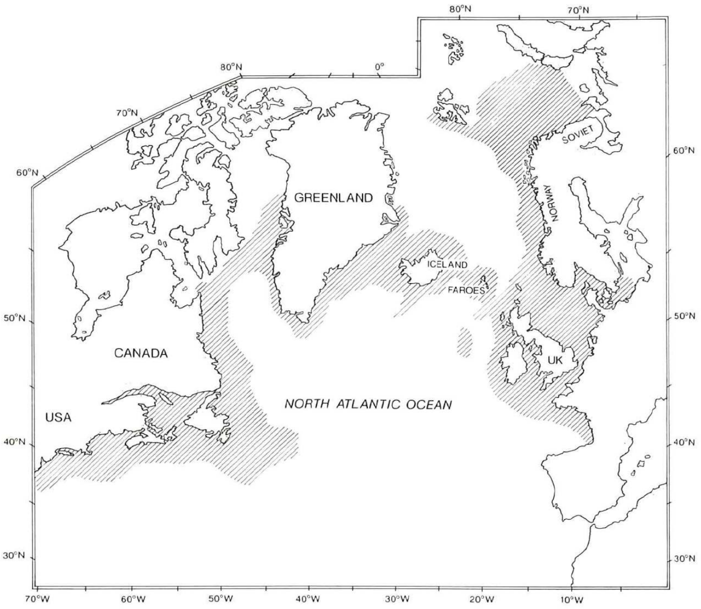

Figure 1: The distribution of Atlantic halibut (Haug, 1990).

Choice of habitat differs between immature and mature halibut. A tagging and recapture study of Atlantic halibut in Norwegian waters, suggests that during the first 4-6 years, young halibut remain stationary in coastal nursery areas (Godø and Haug, 1988). The tagging experiments also indicated long-distance migrations from nursery areas for the young halibut, in all directions and over deep waters (Godø and Haug, 1988). In the same study, large mature halibut were recaptured at both coastal banks where depths ranged from 13-400 m, and in inshore waters (Godø and Haug, 1988).

1.1.3 Characteristics

The Atlantic halibut, *Hippoglossus hippoglossus,* is the largest of all flatfishes. With a maximum length of at least 3.5 m and a weight close to 300kg for females (Michalsen, 2010), the Atlantic halibut also ranges as the largest of the teleost fishes in Norwegian waters (Høines et al., 2009). The halibut is a right side flat fish. The eyed side is dark brown, while the blind side is normally white. The eyed side is darker in adult halibut and lighter in young individuals (Haug, 1990).

Halibut in Norwegian coastal waters spawn during the winter in deepwater spawning locations over a soft clay, or mud bottom (Haug, 1990). As spawning season approaches, sexually mature fish seek deep towards the spawning grounds. Tagging experiments have shown that halibut show a remarkable “homing” response, returning to the same spawning ground for several years (Godø and Haug, 1988). When halibut aggregate to spawn on the spawning grounds, they are an easy target for fishermen. Heavy fishing on these grounds can cause catastrophic damage 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 (Anonymous, 2011). The mortality of halibut is most likely dominated by fishing, seeing as they rapidly reach a size evading most predators (Haug, 1990). The Atlantic halibut 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). The minimum size for halibut was therefore recently raised from 60 to 80 centimetres (Anonymous, 2011). Male and female halibut have different age and size at first maturity. The male halibut are both younger and smaller than the female at this stage. Results from different periods and areas have indicated variations in age at first sexual maturity. The most conspicuous changes in age at maturity seem to have occurred in the northern Norway. It has been suggested that a reduction in age at maturity here has been due to an increased growth rate and a decline in halibut density following from exploitation (Haug and Tjemsland, 1986).

1.1.4 Growth of Atlantic halibut

Growth rates of fish are highly density dependent, and vary with a number of factors such as availability of food, temperature and exploitation (Godø and Haug, 1999). 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). Heavily exploited stocks of halibut in Norwegian waters have demonstrated a noticeable growth variation over time (Godø and Haug, 1999). Male and female halibut differ in growth rate (Haug and Tjemsland, 1986). A study performed in Faroese waters show that in all age classes, females are larger than males, with a significant difference in both length and weight. The difference increased after the first 6 years, when average males had reached a length of less than 80 cm, and the average female a length of 85 cm (Jakupsstovu and Haug, 1988). In a recent study on halibut collected from the Scotian shelf and southern Grand Banks, a similarity between lengths at age was observed for males and females up to about 5 years. Divergence in growth was increasingly after this age (Armsworthy and Campana, 2010). The study showed that females reached a larger asymptotic length than males. It was also found that a declining growth rate followed from the presumed onset of sexual maturity, and that females had a faster growth rate than males after this event, enabling them to reach a greater size (Armsworthy and Campana, 2010). This is in accordance with the results found in Norwegian and Faroese waters (Haug and Tjemsland, 1986, Jakupsstovu and Haug, 1988).

1.2 The Norwegian halibut fishery

The Atlantic halibut has been an attractive target species for Norwegian fishermen for a long time because of its high market price (Godø and Haug, 1988). The fishery was traditionally based on the use of long lines on coastal banks and in fjords, and later also on the trawlers operating on the banks (Haug and Tjemsland, 1986). With the introduction of special deepwater halibut nets in 1936, the Norwegian halibut fishery was revolutionized, almost doubling the catches (Devold, 1938). Annual exploitation using the large-mesh gillnets continued on the deepwater spawning areas in the fjords (Mathisen and Olsen, 1968). Due to the high vulnerability to exploitation, the halibut catches soon declined again due to the efficiency of the new fishing gear (Haug and Tjemsland, 1986). During World War 2 (WW2), fishing intensity was low, and halibut were allowed to accumulate on the fishing grounds again (Mathisen and Olsen, 1968). After the WW2, the eastern Norwegian and Barents Seas, and the Icelandic and Faroese grounds, were the most important fishing areas in the northeast Atlantic (Haug, 1990). Fishing with both gillnets and long lines was continued and expanded after WW2 (Mathisen and Olsen, 1968). The results from the halibut fishery were good during the first post-war years, but due to the vulnerability to exploitation, the stock abundance and catches declined again and decreased gradually from 1948 to the end of the 20th century (Haug and Tjemsland, 1986). 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 (Høines et al., 2009). Data received from the Directorate of Fisheries in Norway indicate that while catches in the south of Norway has remained at low level, the total landing of halibut north of 62 ºN have increased considerable in the years between 1998 and today (Høines et al., 2009, Popper and Lu, 2000). Reasons for this variation in development between these two regions may be the result of a combination of factors. The increase observed in the north could be explained by an increase in stock size following from an increased effort in the fisheries, or from the introduction of the shrimp grids designed to let larval halibut escape, and/or from the restrictions in fishing periods. In the south the decrease in catches may be a result of a decreased stock size, a reduced effort in the fisheries and/ or antropogenic activity in the fjords (Høines et al., 2009).

An understanding of fish biology is essential in fisheries, and age estimates are highly fundamental in the understanding of fish biology and the dynamics of their populations. It is therefore essential that ages are validated, i.e. proving the accuracy of a method (Beamish and McFarlane, 1983).

1.3 Age determination

Information regarding age is a prerequisite for calculations of numerous biological variables such as growth rate, productivity and mortality rate (Campana, 2001). 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). The estimation of age is in most cases done by counting periodic growth increments, and several calcified structures in fish have proven useful in this field of study. Although otoliths is applied in most cases, other calcified structures that are useful for age estimation includes scales, fin rays, vertebrae, opercula and cleithra (Campana, 2001).

The determination of fish age occur over two scales. To support population studies and harvest calculations, annual aging is used (Campana, 2001), while for studies of young fish and recruitment, daily aging based on otolith microstructure is used (Campana and Neilson, 1985). Different methods for determining age exist for otoliths. Some of the methods most commonly used today include estimation of age from the whole otolith (Albert et al., 2009), breaking and burning of the otoliths (Blood, 2003a), and preparation of thin cross-sections (Armsworthy and Campana, 2010). In a study performed on three flatfish stocks inhabiting the Seto Inland Sea in Japan, otoliths were sectioned and etched with hydrochloric acid (HCl) before examination (Katayama et al., 2010).

Age determination from otoliths are complicated by discontinuous structures and false increments corresponding to non-seasonal events (Katayama et al., 2010). Validation of annual periodicity can be achieved from mark-recapture experiments, while precision can be obtained by duplicate readings of the same otoliths (Forsberg, 2001). A study performed on age validation of the Greenland halibut, *Reinharditus hippoglossoides*, using bomb radiocarbon assays and oxytetracycline (OTC) recaptures, indicate underestimation of age for whole otoliths and cross-sections, as well as for scales (Treble et al., 2008). Beamish and McFarlane (2000) re-evaluated the interpretation of annual increments from sablefish otoliths, studying otoliths from sablefish after tagging with OTC, release and recapture after liberty for 13-20 years. A general agreement was found between the years at liberty and the number of increments identified after OTC marking (Beamish and McFarlane, 2000). The ages of close to a million fish every year are determined using otoliths, and based on otolith increments, age estimates of at least 100 years have been recorded for some deepwater fishes (Campana and Thorrold, 2001).

1.3.1 Otoliths

The inner ear containing the semi-circular canals are found at the bottom of the cranial cavity, positioned lateral to the main axis of the fish in the posterior part of the brain cavity (Panfili et al., 2002). The fascinating construction with clusters of hair cell bundles differently oriented in the macula, enable perception of frequency, direction and amplitude of sound. In addition, the fish perceive static and dynamic position (Mosegaard and Moreales-Nin, 2000).

There are three otolithic organs in the inner ear, the saccule, utricle and lagena, which each contain a calcareous otolith. The sagitta is found in the saccule, the lapillus in the utricle, and the asteriscus lies in the lagena. Otoliths vary in shape and size (Popper and Lu, 2000). The sagittal otoliths are normally much larger than the lapillus and asteriscus, and is therefore the choice for most age determination studies (Forsberg, 2001).

Otoliths are mineralized, acellular structures (Campana and Neilson, 1985). They are formed extracellularly, when the aragonite form of calcium carbonate is crystallized onto an organic matrix template, mainly composed of a keratin-like protein, otolin (Degens *et al.*, 1969; Watabe *et al.*, 1982; Morales-Nin, 1987a). With the addition of concentric layers of calcium carbonate and proteins, the otolith accretes or grows, and gives a structure comparable with an onion (Panfili et al., 2002). Because of the concentric deposition of mineral rich and matrix rich areas on the otolith, they can be used to study daily, seasonal and annual growth cycles. Age estimations can be made either by observing the whole otolith or after preparation, depending on the aim of the study (Panfili et al., 2002). Because otoliths are thought to grow continuously, and have been shown to be metabolically inert, not likely to be resorbed or reworked like scales or other skeletal tissue, they have many applications (Campana and Neilson, 1985).

The application for otoliths in fisheries biology studies has expanded over the recent years. The discovery of daily increments in otoliths made by Panella (1971) enabled scientists to study the early life history of fishes, and the discovery that chemical elements from the environment was incorporated into the otoliths made it possible to mark otoliths for age validation studies, trace movements in fish, discriminate between fish populations, reconstruct life history events, and to study environmental changes over time (Panfili et al., 2002). Physiological and environmental variables such as temperature, photoperiod, growth and feeding fluctuate cyclically, and the otolith deposition are potentially influenced by these factors (Campana and Neilson, 1985). The deposited increments have alternating optical density, appearing either translucent or opaque when viewed under reflected light. The differences in opacity is a result of differences in protein amount between the zones and also the shape of the aragonite crystals (Forsberg, 2001). In periods when deposition is fast, there is a high production of organic fibres. Calcification is even higher in this period, giving a ratio of organic to inorganic fibres that strongly favours the inorganic portion, making up over 90 percent. When deposition is slow, few organic fibres are produced and calcification is almost absent, producing a ratio that favour the organic portion (Pannella, 1971).

An opaque and a translucent zone equal one year’s growth in an otolith (Forsberg, 2001).

The relative width of the increments decrease as the fish grows older. Broad opaque zones occur in the first few years when the otolith growth is rapid. The increments become narrower as the fish ages, almost matching the width of the translucent increments (Forsberg, 2001). The onset of reproductive activity may be a reason for the decrease in increment width, affecting efficiency and continuity of otolith calcification (Pannella, 1971). Interpretation of age and growth from otoliths is based upon assumptions such as a constant frequency of formation and proportionality between fish growth and increments (Campana and Neilson, 1985). Certain environmental conditions can disrupt annual increment formation and lead to formation of non-annual additional opaque or translucent increments, causing erroneous age estimates. An understanding of factors affecting increment formation and pattern in otoliths are therefore important. Different explanations have been proposed to explain increment formation, including the physiological and environmental changes, and endogenous control, but the true reason may be a combination of these explanations (Neat et al., 2008). Knowledge of factors influencing increment formation is still poor (Høie, 2003), and the timing of this zone formation have been shown to vary between species. For the management of fish stocks, invalid age estimation can lead to severe implications (Høie, 2003). For most fish species inhabiting subpolar waters, opaque zones are formed during spring and summer seasons (Høie and Folkvord, 2003), which is periods with faster growth, and the translucent zones are formed in winter, when slower growth is typical (Forsberg, 2001). Temperature has been found to effect otolith accretion rate as well as optical density, where otoliths become more translucent with higher temperatures (Neat et al., 2008). The relative incorporation of the oxygen isotope $\delta^{18}O$ is dependent on temperature, while metabolism and feeding pattern influence carbon isotope $\delta^{13}C$ in otoliths (Høie, 2003). Increment formation can be related to annual temperature cycles by relating the otoliths optical properties to stable isotope composition (Høie, 2003). If temperature and feeding activity is plausible causes for the variability of opaque or translucent increment formation during the seasons for different species, it is important to be aware of migration patterns for all species considered and temperature fluctuations in their migration paths.

In Atlantic halibut, the opaque zones in the otoliths are believed to be formed during summer, while the translucent zones are formed during the winter (Olsen, 1956).

1.3.2 *Age determination of Atlantic halibut*

The saggitae otoliths of Atlantic halibut are medium sized in relation to the fish length and has a skewed oval shape (Härkönen, 1986). The age information needed to develop age-structured population models for management improvement, and to estimate growth and mortality rates, recruitment, age at maturity and longevity, is today still not available (Armsworthy and Campana, 2010). In a study performed in 1956 by the Institute of Marine Research (IMR), the procedure used for determining the age of Atlantic halibut was based on the same method employed in Devolds work on "The North Atlantic Halibut and Net Fishing" (1938). The otoliths were broken in two across the core, and the increments were viewed under a microscope with the use of transmitted light (Olsen, 1956). Given the differences in the inner structure of the left and right otolith, it was found that the left otolith was preferred as the zones were more easily traced. Specimens younger that 6-7 years were not broken as the growth zones were found to be easily countable on an unbroken otolith under transmitted light (Olsen, 1956). In a study performed on age determination using prepared cross-sections of otoliths, and validating age by using bomb-radiocarbon, it was found that Atlantic halibut could be aged up to 40 years without strong bias (Armsworthy and Campana, 2010). They also found that the reference radiocarbon chronology and the otolith core $\Delta^{14}C$ values corresponded, which indicated that growth increments are formed annually in this species (Armsworthy and Campana, 2010).

The procedure used today by the Institute of Marine Research in Bergen (IMR), involves reading whole otoliths, immersing both left and right whole otolith in water and photographing both using transmitted light. The method is basically the same as the one employed for Greenland halibut, where they read the right otolith because it is the one with the longest readable axis (Kvalsund and Solbakken, 2008). Given the featural 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 also suspected that the former method underestimates the true age of halibut.

1.4 Objectives

This study aims to compare different approaches of age determination for the Atlantic halibut, and to establish a new and improved procedure for age determination of Atlantic halibut.

Sub-goals are to:

- Compare previous results obtained by other age readers after the former procedure, with results obtained in this study.
- Establish some updated reading rules.
- Perform the preferred method on otoliths collected in the years 2008-2010, where age has not previously been determined.
- Validate timing of seasonal zone formation.
- Study the relationship between age, length and weight, and attempt making an age-length-weight key.
- Compare size at different locations along the Norwegian coast.

2. Materials and methods

2.1 Material

The material available for this study has been collected in the years 2004-2006 and 2008-2010 at sampling locations indicated by Figure 2.

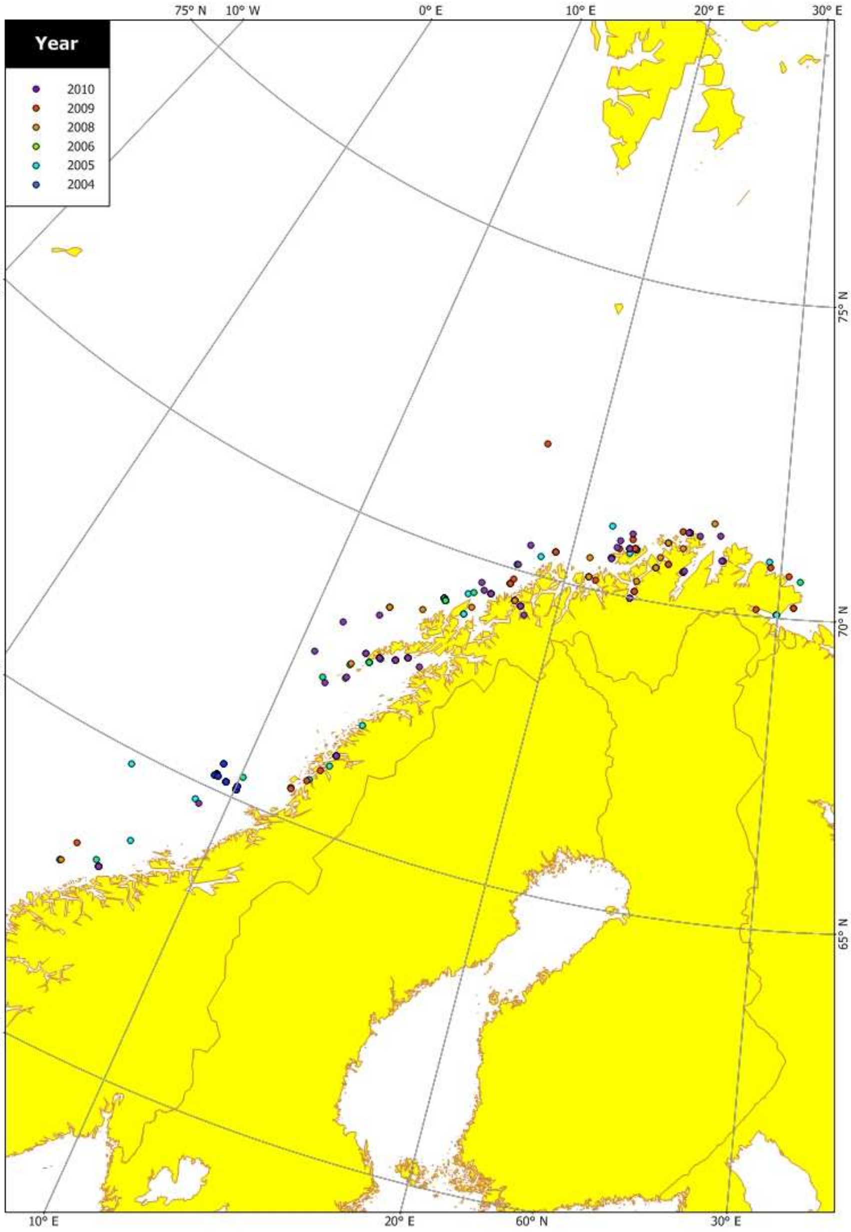

Figure 2: Sampling localities of Atlantic halibut along the Norwegian coast. The color of the dots indicate sampling year.

The material available for this study is listed in Table 1. Otoliths which are not available for this study are otoliths that have already been sectioned by lab technicians at the IMR. All otoliths collected between 2004 and 2006 have previously been aged either through a stereomicroscope or from digital images. Of the material available in this study, sex has been determined for 186 halibut. Otoliths collected between 2008 and 2010 were all kept in the freezer at IMR, and had no previous work done on them.

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	Johan Hjort	14.10-10.11	31	0
	Jan Mayen	22.10-07.11	16	0
	Førde Jr	19.09-03.10	48	44
	Mac Galben		1	1
	Fishermen		9	0
2005	"Eggakanten"	24.02-25.02	8	0
	G.O. Sars	27.02-17.08	7	2
	Reference fleet	27.04-22.08	10	2
	Johan Hjort	23.10-04.11	17	17
	Jan Mayen	26.10-07.11	11	11
	Amigo	26.11-30.11	6	6
2006	Johan Hjort	12.02-16.11	23	23
	Amigo	1.08	22	22
2008	Johan Hjort	03.10-14.11	21	21
	NIFES	20.02-11.12	23	23
2009	NIFES	21.01	1	1
	Johan Hjort	06.10-25.10	17	17
	Jan Mayen	04.10-24.10	17	17
2010	G.O. Sars	24.08	1	1
	Johan Hjort	03.04-03.11	56	56
Total			345	264

2.2 Field sampling

2.2.1 Trawling

In the current study, halibut were sampled on board the research vessel Johan Hjort during the annual coastal cruise in October 2010, together with the IMR in Bergen. 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, and for this, the standard survey trawl, the bottom trawl (Campelen 1800 shrimp trawl) was used (see Appendix 1). When performing reference trawls both the bottom trawl and the pelagic Harstad trawl was used (Anonymous, 2008). Halibut were only caught using the bottom trawl. The IMR have since 1981 been using the Campelen 1800 shrimp trawl annually in bottom trawl surveys for cod and haddock. Since 1981, many changes and modifications have been made in equipment and methods for bottom trawl surveys. Today, the trawl has 40 m sweeps with rockhopper gear. The mesh size in the front part of the trawl measures 140 mm. At the mouth opening of the trawl, the circumference measures 72 m, and the mesh size is 80 mm. The minimum mesh size in the codend has been reduced to 22 mm. (Anonymous, 2008). All trawling procedures are described thoroughly in the manual “Handbook for vitenskapelig tråling” by the IMR (Anonymous, 2008).

2.2.2 Sampling

After hauling the trawl, the catch was transferred to a shaft leading it into the wet lab on board. All species were sorted, measured and weighed. The halibut caught in each haul was weighed, and both individual and total weight of halibut was recorded. The total length was measured on an electronic fish measuring board, the fish meter (Figure 3) (Scantrol).

Figure 3: The total length of a halibut measured on a fish meter® (Scantrol).

Both saggital otoliths were removed for age determination by the procedure illustrated in Figure 4.

Figure 4: Otolith removal with frontal head sectioning of the halibut. a, b, c: frontal head section of the skull. d, e, f: localization and removal of the otolith pair directly behind the brain.

By making a frontal section of the skull of the halibut, the left and right saggital otolith were removed from their position directly behind the brain. 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 together with the corresponding trawl sheet and frozen. Fin clips of the halibut were also taken for a later DNA analysis, but these data were not included in this study.

2.3 Digital images

In this study, the otoliths that had not yet been sectioned, collected in the time period 2004-2006, were photographed with a NIKON Stereoscopic Zoom Microscope SMZ 1500, objective HR Plan Apo 0.5x. Calibration represents the real length of one pixel in the image, and is important for correct measuring in Photoshop. The magnification “1x” was chosen on the microscope, and a micrometer was put under the stereomicroscope. The image was adjusted so that the micrometer was focused and captured (Appendix 2). The command “New Optical Configuration” was chosen and the optical configuration name “1x” was typed in. “Create and Calibrate New Objective” was chosen and 10 mm was defined on the micrometer as the length unit.

Otoliths collected in the years before 2008 had been stored dry in paper envelopes. To test what method that give the clearest images, the otoliths collected in 2004 were first photographed directly, with no previous treatment. The same otoliths were also photographed after being immersed in water for 24 hours, and after being stored in a mixture of 60% glycerol for 24 hours. The otoliths were placed in plastic trays with 24 wells. One in each well, marked with right or left otolith, station number or serial number and fish number. When photographing the otoliths directly, one pair was placed in a Petri dish filled with water, under the microscope. Eclipse net software® was opened, and the button for "live-pictures" was switched on. To achieve a high quality of the pictures, some time was spent adjusting lighting, white balance and focus. Translucent light was used while adjusting the light intensity. By pressing the "Automatic White Balance" button, the white balance was taken. After adjustment, the first picture was taken. The right and left otoliths with their concave side facing the objective were placed in the image. (For the 2004 and 2005 otoliths, the left and right otolith is placed in opposite position). For an optimal focused image, the magnification was increased, depth sharpness was adjusted and exposure-time increased. Fine adjustments were made to achieve a clear image of the otolith. The magnification was set back to 1x and depth sharpness adjusted. A directory where all captured images was to be saved was chosen by pressing the "Capture and Save Options" button.

Two pictures were taken of each pair of otoliths. The first picture was taken with translucent light and a white background. The second picture was taken with reflected light. The light was switched off in the room where the photographing took place. The pictures taken with dark background became clearer if taken in a dark room. After being photographed, the otoliths were returned to their wells. When all dry otoliths had been photographed, the wells were filled with water, and the otoliths were left immersed for 24 hours. After being stored in water they were photographed again after the same procedure as for the dry otoliths. The next step was to empty the water in the wells and fill them with a mixture of 60% glycerol. This mixture was made by diluting 87% glycerol with water. The otoliths were left in the wells with glycerol for approximately 24 hours. New photos were taken. The same procedures were performed on the otoliths collected in 2005 and in 2006.

2.4 Preparation of otolith sections

Otoliths from 20 fish collected in 2006 were chosen for sectioning. By studying the pictures taken in whole mount style, 10 pairs that showed clear annual increments and 10 pairs with relatively unclear increments that appeared difficult to read were chosen. The purpose was to compare both clarity and number of annual increments in the sectioned otoliths with the whole mount images.

2.4.1 Embedding

The otoliths were embedded in a mixture of epofix resin and hardener at the proportion 9:5 by weight. Epofix resin and hardener were weighed and stirred together for at least 5 minutes to ensure complete mixture. A thin layer of Vaseline was applied to the rubber mould and the mixed epoxy resin was poured into it, forming a bottom layer. The mixture was left for 24 hours in the ventilation hood to harden. After 24 hour a small amount of epoxy resin was prepared to attach the otoliths to the hard bottom layer. The otoliths were placed in a row in the mould with the concave side facing up. After approximately 3 hours, giving the epoxy resin some time to set, a new mixture was made and poured into the moulds, forming the top layer completely covering the otoliths. The mould was again left for 24 hours to harden.

2.4.2 Sectioning

Transversal cuts were made of the otoliths using the Isomet 1000 low speed saw (Figure 5).

Figure 5: The Isomet 1000 low speed saw, with a block of hard epoxy resin containing otoliths to be sectioned attached. To the right is a rubber mould used for embedding.

The embedded otoliths were attached to the saw, and the midline of the core of the otolith was located and placed directly over the blade. Some extra weight was mounted on top of the blade to make the cutting more efficient. Care had to be taken choosing the amount of weight, as to much weight could shatter the otolith. The otolith was moved 400 microns to the right, and a cut was made. The otolith was then moved 800 microns to the left and sawed, producing approximately 400-600 µm thick sections. The sawing was conducted at low speed and the lower part of the blade was immersed in osmotic water during sawing to avoid breaking and heating of the fragile otoliths. Two or three sections were made of each otolith to ensure a good section through the core.

2.4.3 Slide mounting

After cutting, the sections were studied under the stereomicroscope to determine which section from each otolith that had the least breakage and that was cut closest to the core. The best section was chosen, and the least favorable side was polished gently with four different grit abrasive papers and tap water on a grinding and polishing mechanical rotating disk (Figure 6) (Buehler Phoenix beta). Grinding and polishing this side was done by placing the section on the tip of a finger, carefully moving it in a manner that would equally grind all parts of the section.

Figure 6: The Buehler Phoenix beta rotating disk used for grinding and polishing.

The section was measured with a micrometer and attached to an object-glass, polished side facing the glass, using a clear Crystalbond^{TM} adhesive preheated to approximately 135 °C. The section was then polished again with four different abrasive papers and tap water, starting with 600 µm grit, then using 1000 µm, 2500 µm and finally 4000 µm grit. The section thickness was measured using the micrometer during the polishing to prevent the otolith section from becoming too thin. The resulting thickness of the sections was somewhere between 200 and 400 µm.

2.4.4 Digital images of the sections

Digital images were taken of the prepared sections for both left and right otolith from all 20 halibut. The Nikon DS 2 camera was connected to the stereomicroscope, Leica MZ 9.5 (Figure 7).

Figure 7: The Leica MZ 9.5 stereomicroscope connected to a camera, for capturing images of the sectioned otoliths.

The image software NIS-Elements F version 3.0 was opened, and before the otoliths were photographed, a micrometer was placed under the stereomicroscope for calibration. A picture was taken of the micrometer using translucent light, and the program Image J was opened to set the scale. After calibrating, the prepared object-glass was placed under the stereomicroscope. A new calibration was done in the beginning of each session and with the change of magnification. The different magnifications used to photograph sections are presented under Appendix 3. The magnification that fitted the whole section was chosen. The button for “automatic exposure” was pressed, and the automatic white balance adjusted. The section was further magnified to focus the image and subsequently returned to the original magnification. High contrast was chosen, and the button for “manual exposure” was pressed to regulate gain and exposure further. After achieving the best possible light conditions and contrast, a picture was taken and saved in a designated folder. The magnification was increased on the same section to get a picture of half the section including the core, with the purpose of obtaining greater detail. This procedure was performed on all otoliths sectioned.

2.5 Age determination

The different methods for photographing whole otoliths collected in 2004 - 2006 were compared by studying the clarity achieved either by photographing the otoliths directly, after 24 hour immersion in water or after 24 hours in 60% glycerol. After choosing the best method, an Action script was performed in Photoshop on all otoliths photographed, with both reflected and translucent light. Images of Otoliths from 2004, immersed in water, taken with translucent light were saved in a folder named JPG. A new folder was created and named PSD. The first picture was opened in Photoshop. The action used today by the IMR for the age interpretation of Greenland halibut, *Reinhardtius hippoglossoide,* was received and saved in Photoshop. The Action palette was opened and “Load action” was typed and the action was loaded. A Batch action was run in order to apply the same settings to the whole “batch” of images, and to make the process more efficient. “File” was pressed and “Automate” and “Batch” was chosen. The preferred action was chosen and the “source folder” was set to the JPG folder containing the images, and “destination folder” was set to PSD. The recording started automatically and all files ended up in the destination folder. A new reading layer was created for this study. This procedure was repeated for all images taken after immersion in water, with both translucent and reflected light, for the years 2004 - 2006. This procedure was also performed on the images of the sectioned otoliths.

Age was interpreted for all photos in Photoshop. Color and size of the brush was chosen, as well as the interpretation layer of choice. Two interpretation layers were used as the otoliths also were to be interpreted by an experienced lab technician at the IMR. The brush was used to mark the annual increments, and the marks were afterwards counted. For convenience, the 1st of January has been accepted as the date of birth for the entire population. Before marking the final annual band, one needs to consider the date of capture in order to decide whether or not the final increment is fully formed and can be counted as one year (Figure 8).

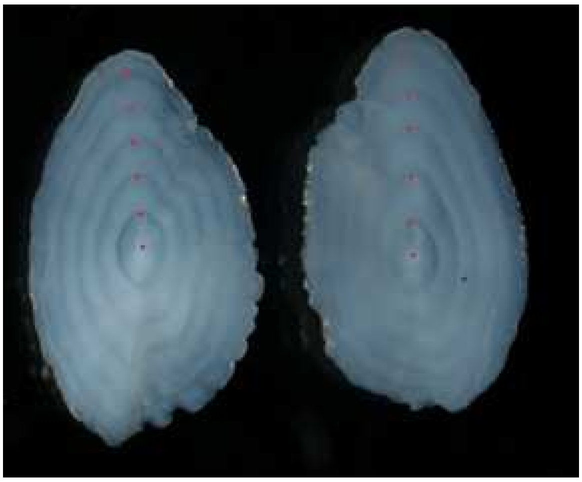

Figure 8: Halibut caught on the 16th of November. The annual increments are marked with red dots. Because it was caught in November, the final band is not marked.

Age was estimated for all otoliths. Both left and right saggital otolith were interpreted with both translucent and reflected light. When interpreting the images, the images were magnified two times, and all otoliths were assigned an age and a readability ranging from 1-4. A readability of 1 was given to the otoliths that are easily interpreted; a readability of 2 was given to the ones that are a bit more difficult, with false or spilt increments and discontinuity complicating the interpretation. A readability of 3 were given in cases were this problem is further enhanced, and readability 4 was assigned to otoliths that are broken or where crystallization have occurred. After all otoliths had been interpreted, the ages were compared between left and right, translucent and reflected light. These ages were then compared with the interpretation of the sectioned otoliths. The sectioned otoliths were also assigned an age and a readability ranging from 1-4. A readability of 1 meaning the section is easily interpreted, 2 was assigned to those more difficult. Sections that had not been made precisely through the core of the otolith are difficult and were given a readability of 3, while the sections with too many cracks obscuring the increments seriously were assigned a readability of 4.

Comparing images taken with translucent and reflected light gave a preferred lights source, while comparing left and right gave a preferred otolith to trust. The choice of light source was based upon which of the two that revealed the highest number of increments, and the preferred otolith was the one that indicated a higher age as well as the best readability. Comparing sections and wholemount 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.

2.6 Timing of seasonal zone formation

The outer edge of a number of otoliths photographed in this study was analyzed in Photoshop. The character of the final seasonal increment deposition was determined, and the date of capture was related to the optical density of the edge. An attempt was made to determine what season halibut deposit opaque and translucent increments.

2.7 Statistical analysis

The data analysis software system Statistica, version 10 (StatSoft inc., 2010), was used for all graphical illustrations and statistical analyses performed in this study. In order to illustrate the differences between the ages interpreted from the use of images taken with either reflected or translucent light, frequency scatterplots were made for both left and right otolith. The results were analyzed performing a paired t-test, testing whether the two methods give the same mean for the same otolith. The same type of graph was made and the same analyses was performed when comparing left and right otolith, wholemount otoliths and the sections of the same otoliths, the left and right section, and for testing the differences between the results obtained from the new and the previous method for age determination. For t-test results, see appendices 14-18.

In an attempt of making an age-length-weight key, cumulative histograms were made, indicating percent distribution of age groups within given weight- and length intervals for both sexes.

Scatterplots were made to illustrate the relationships between lengths and weights, age and size, and size at age at locations for both males and females. The regression of the relationship between length and weight were analyzed using generalized linear models (GLM) analysis (StatSoft inc., 2010). This type of analysis was also used to analyze length and weight at age. The effects of sex were tested by including sex as a factor in these analyses, and excluded if the interaction were insignificant. To compare growth at different locations for males and females, longitude and latitudes were included in the GLM analysis as factors. Non significant higher order interactions were removed. The results obtained in the GLM analysis are presented under appendices 19-22.

3. Results

3.1 Comparing images photographed after different treatments

When comparing images taken of otoliths directly after dry storage, after 24 hours in water and after 24 hours in 60% glycerol, using two different light sources (Figure 9), it is clear that photographing after a 24 hour immersion in water is the best approach for achieving the most defined increments (Figure 9b and e). Otoliths photographed directly after dry storage gives a matt surface (Figure 9a and d), whereas otoliths photographed after 24 hours in glycerol produce a refringent surface (Figure 9c and f). The otoliths in Figure 9a, b and c are photographed using transmitted light, while the ones in Figure 9d, e and f are photographed with the use of reflected light. For more examples of images indicating differences between the treatments see Appendix 4.

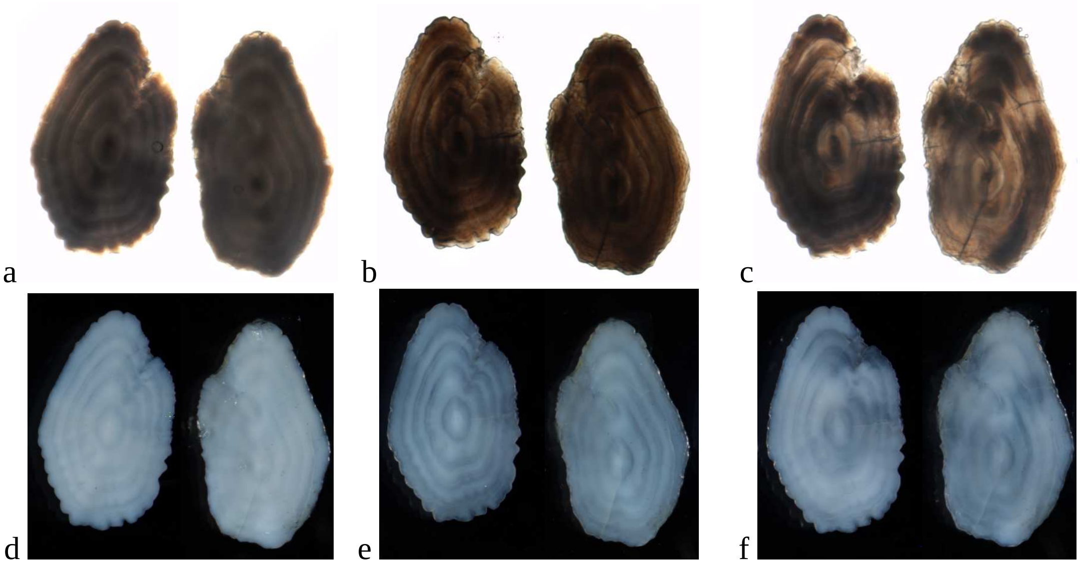

Figure 9: Examples of otolith pairs photographed after different treatments, and with different light sources. Images in the upper panel are photographed using transmitted light, while the ones in the lower panel are photographed with the use of reflected light. Images a, d) otoliths are photographed 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.

3.2 Comparing transmitted and reflected light

When comparing otoliths photographed with transmitted and reflected light (Figure 10) it is apparent that reflected light show the most pronounced increments. It also appears that it is harder to separate true increments from false increments on images taken with transmitted light. The images from 2004-2006 showed that reflected light indicated a higher age, as well as more equivalence between left and right otolith interpretation. For more examples of images taken using transmitted and reflected light, see Appendix 5.

Figure 10: Example of an otolith pair, where a) the image is taken with transmitted light and b) Image is taken with reflected light.

The ages interpreted from the left otoliths photographed using reflected light and the ages interpreted from photos of the same otoliths using transmitted light, differ for several otoliths. The slope of the regression line in Figure 11 is higher that 1, indicating that reflected light show a higher number of increments. The $y = x$ line lie within the confidence interval, indicating that the difference between the lines are not significant. The regression line is indicated by a black line, together with a broken line indicating the confidence interval. The $y = x$ line go through the origin and is illustrated by a bold grey line. Even though the two methods give different means, the difference between the two is not statistically significant ($p > 0.05$, Appendix 14). The same trend is apparent for the right otoliths photographed with the same two light sources. There is a high equivalence between ages interpreted for both methods. Still, the age interpreted differ in a number of cases, and a higher age estimate is more frequently found using reflected light. The slope of the regression line in Figure 12 shows that reflected light show a higher number of increments. The $y = x$ line lie within the confidence intervals, and the difference between the two is not significant ($p > 0.05$, Appendix 14).

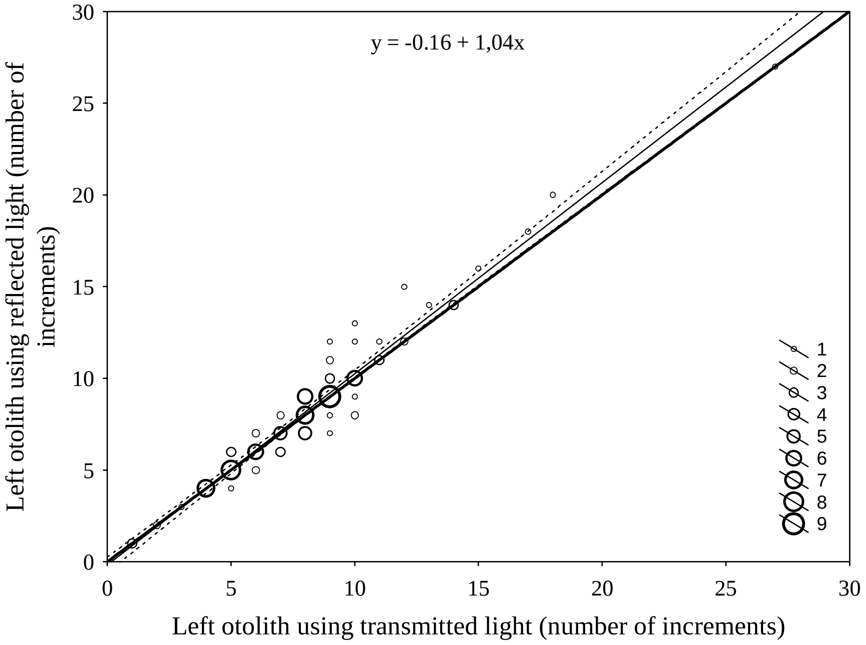

Figure 11: The relationship between the ages interpreted for left otolith using reflected and transmitted light. The regression line, confidence interval and the y = x line are indicated by different shape and thickness. Frequencies of observations are indicated by the size of the dots.

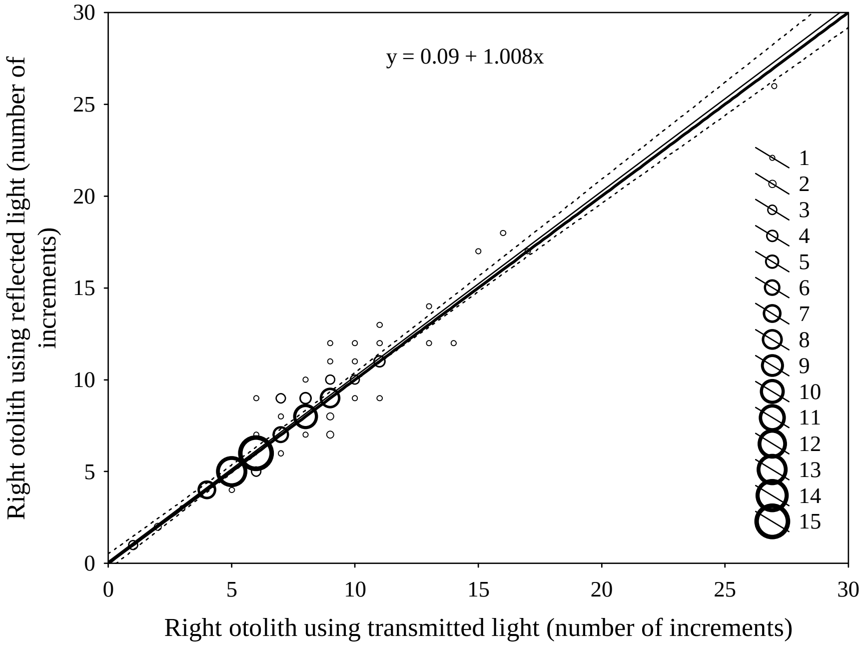

Figure 12: The relationship between the ages interpreted for the right otolith using reflected and translucent light. The regression line, confidence interval and the y = x line are indicated by different shape and thickness Frequencies of observations are indicated by the size of the dots.

3.3 Comparing left and right otolith

Comparing left and right otolith for all images showed that the age interpreted on left and right otolith are in most cases the same. Nevertheless, in some cases the ages differ between the two. In a few cases the age is interpreted as being higher on the right otolith, but mostly it is the other way around. In figure 13, the numbers of increments are indicated by red dots on the translucent bands of the otolith. The center mark indicate year zero. The most common difference between left and right otolith is one year (Figure 13a, b, d, f), but in some cases it is even more (figure 13d, e).

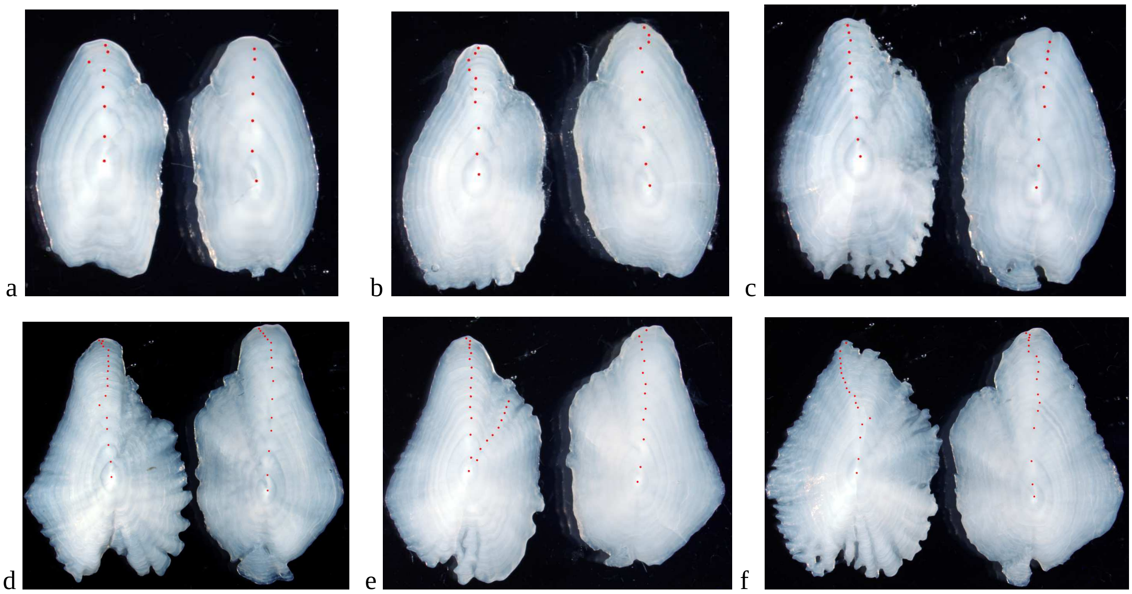

Figure 13: Examples of otolith pairs photographed after frozen storage in seawater. Defrosted and immersed in a petri dish filled with water. The numbers of increments are indicated by red colored dots in the translucent increments.

The regression line in figure 14 has a higher slope than the y = x line, indicating a higher number of increments on the left otoliths. Results show a statistical significant difference between the ages estimated for the two (paired t-test, $p < 0.001$, Appendix 15), (Figure 14).

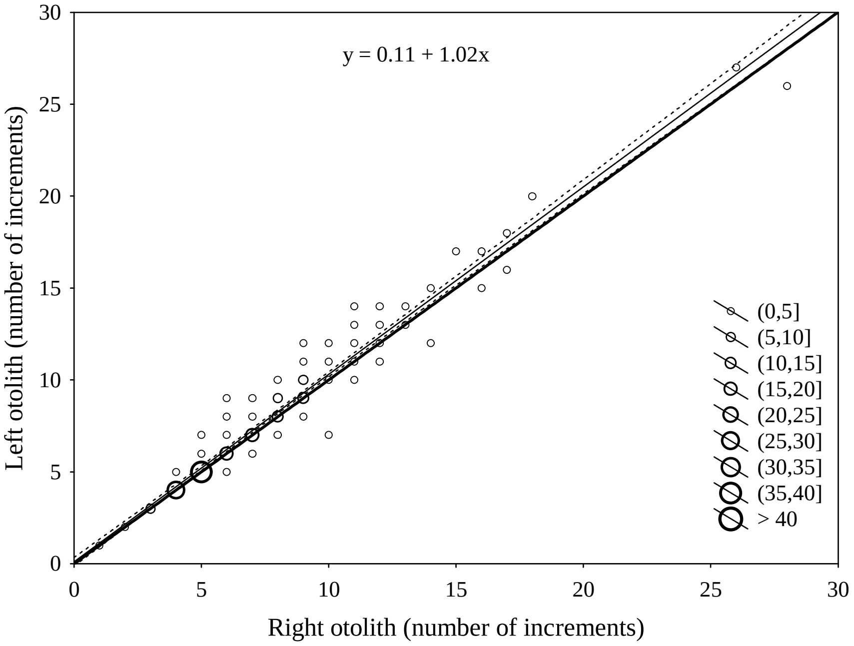

Figure 14: Scatter plot of the relationship between ages of the left and right otolith photographed using reflected light. The regression line, confidence interval and the y = x line are indicated by different shape and thickness. The size of the dots indicate frequency of observations.

3.4 Comparing whole mount otoliths and their sections

After aging all sectioned otoliths, both left and right, and comparing these results with the results obtained by interpreting whole mount images, it was found that the two methods gave the same ages in most cases (Figure 15). For some otolith pairs, in which the age estimated differs between left and right whole mount otolith, the section showed equivalence between left and right otolith (Figure 16). Examples where sectioning proved to be necessary, are illustrated under Appendices 6-9).

Figure 15: Example of an otolith pair aged to 3 years on a) both left and right whole otolith and the b) left and c) right sections of the same otoliths showing the same amount of years. The red dots indicate increment counts.

Figure 16: Example of an otolith pair where the a) whole mount image show an age of 17 and 15 for left and right otolith respectively and a section of the b) left otolith and the c) right where the age has been interpreted to 16 years. Age is indicated by red dots.

The age estimated for the whole otoliths and the sections of the same otoliths appear to be equivalent in most cases. The slope of the regression lines in Figure 17 and 18 is lower than the y = x line, indicating a slightly higher number of increments interpreted on the whole otoliths than the sections. The y = x line is within the confidence interval, indicating an insignificant difference between the lines. When the section indicates a higher age, the difference is less than a year. In the few cases where the whole otolith exhibit the highest age, the estimated age can differ with up to two years. Comparing the age interpreted for the left and right whole otolith with the sections of these showed no significant difference (paired t-test, $p > 0.05$, Appendix 16).

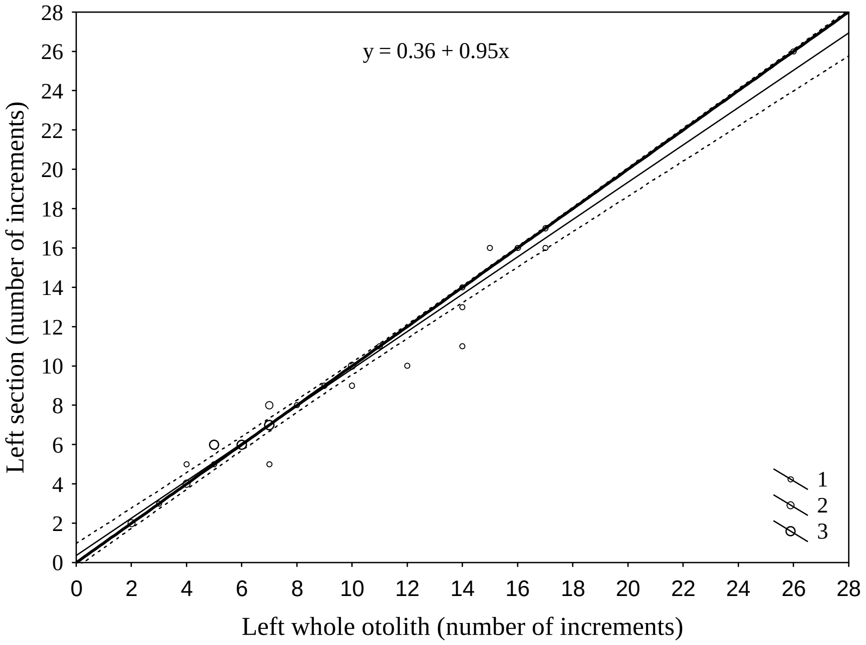

Figure 17: Scatter plot of the relationship between ages interpreted on the left section and on the left whole otolith. The regression line, confidence interval and the y = x line are indicated by different shape and thickness. The size of the dots indicate the frequency of observations.

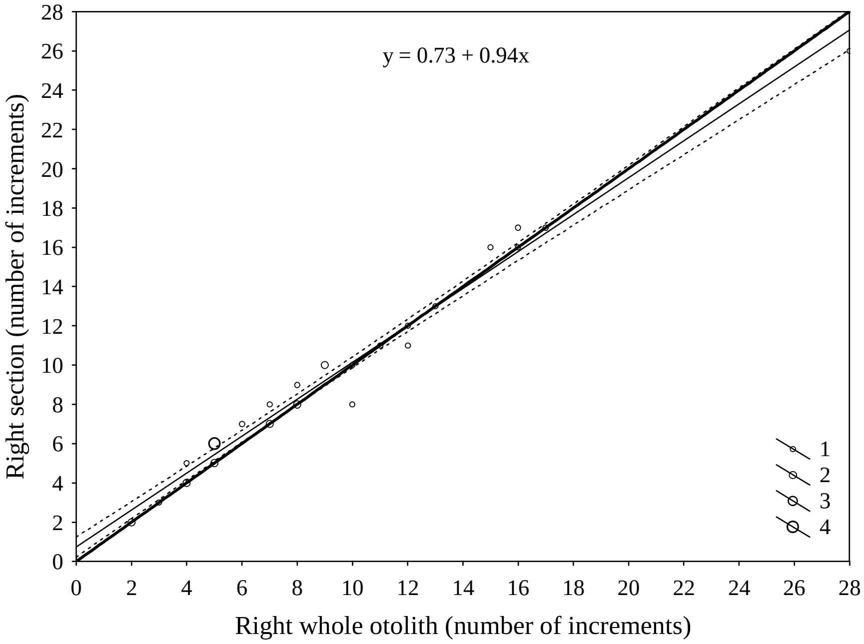

Figure 18: Scatter plot of the relationship between ages interpreted for the section of the right otolith and for the whole right otolith. The regression line, confidence interval and the y = x line are indicated by different shape and thickness. The size of the dots indicate the frequencies of observations.

3.5 Comparing left and right section

When comparing the left and right sectioned otoliths (Figure 19) it was found that they give the same ages more frequently than whole mount otoliths.

Figure 19: Example of a sectioned otolith, where the a) left and b) right otolith, both show the age of 16. Age is indicated by red dots.

Age interpreted for the right and for the left sectioned otolith are the same in almost all cases. The regression line and the y = x line in Figure 20 is close to overlaid, indicating high equivalence between left and right section. Of the 34 otolith pairs included in the comparison, only seven pairs differ in age. The age difference between the right and left sections is not more than one year for these seven pairs, and there is no apparent trend enabling us to conclude which of the two give the higher age estimate. There is no significant difference in the age interpreted for left and right otolith section (paired t-test, p > 0.05, Appendix 17).

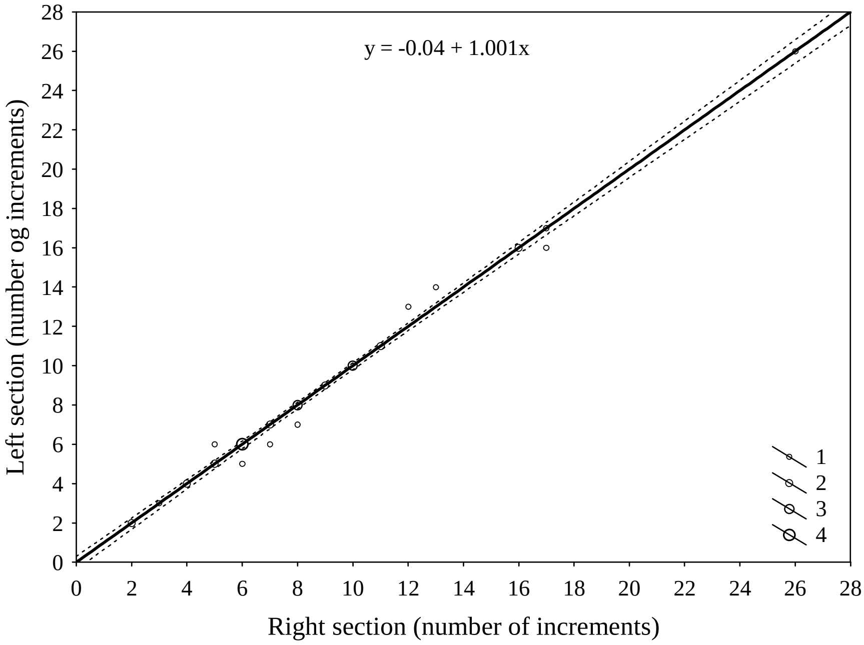

Figure 20: Scatter plot illustrating the relationship between ages interpreted for the left and right sections. The regression line, confidence interval and the y = x line are indicated by different shape and thickness. Frequency is indicated by the size of the dots.

3.6 Comparing old and new method

When comparing the old and new method, it was found that the ages estimated for halibut in this study were higher in almost all cases studied compared to the estimates made for the same otoliths by the previous method. The regression line in Figure 21 has a very low slope compared to the y = x line, indicating a higher number estimated by the new method. This difference increase with age and is also in a magnitude of several years for many of the cases. The y = x line is also far from within the confidence interval indicating a significant difference between the lines. Both left and right otolith result in higher age estimates in this study compared to previous estimates (Figure 22 and 23).

When comparing the age estimated for the left otoliths after the former and current method (Appendix 18) it was found that the difference between the former and the current estimates were statistically significant (paired t-test, p << 0.001).

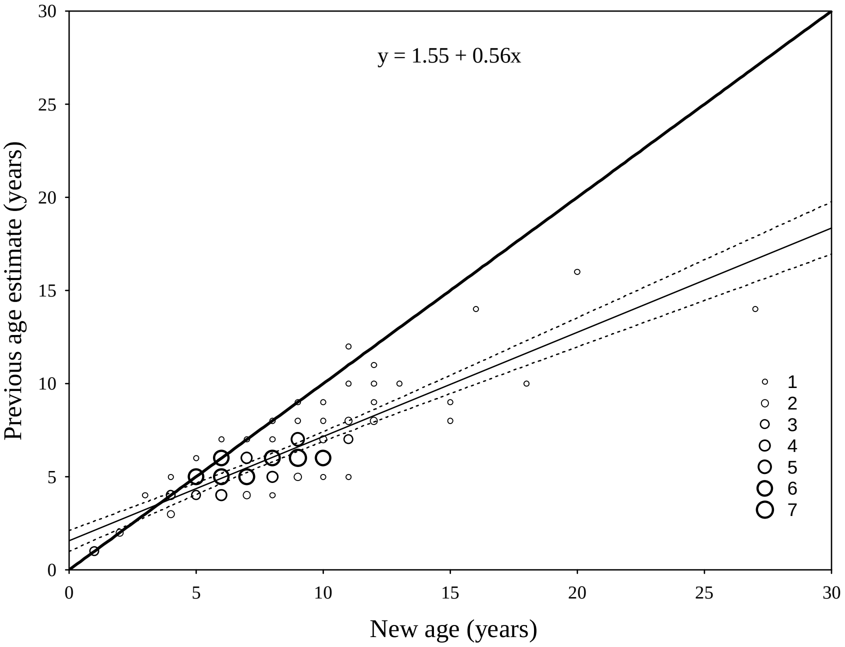

Figure 21: Scatter plot illustrating the difference in age interpreted for the same otoliths using the former and current method. The regression line, confidence interval and the y = x line are indicated by different shape and thickness. Frequency is indicated by the size of the dots.

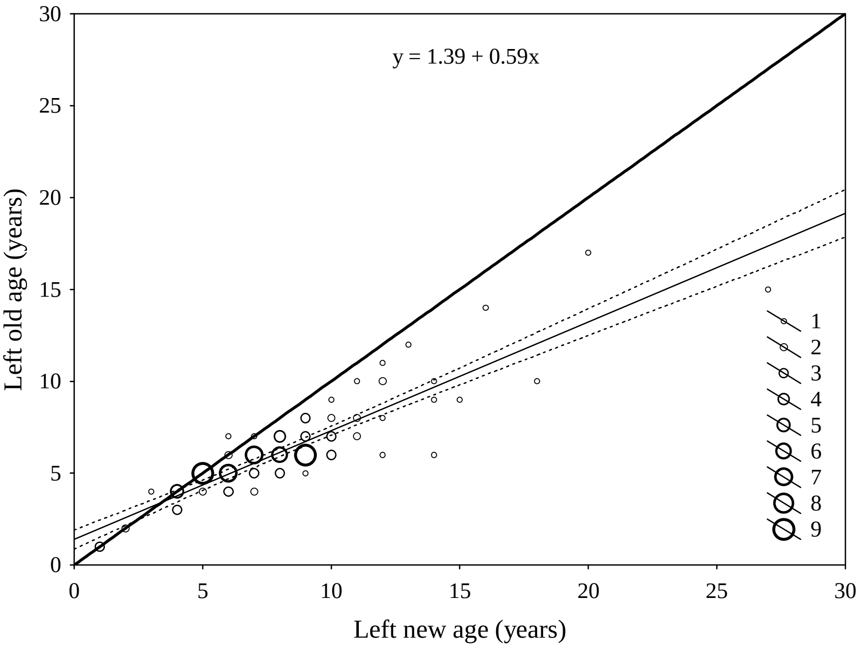

Figure 22: Scatter plot indicating differences between the ages interpreted for the left otolith using the former and the current method. The regression line, confidence interval and the y = x line are indicated by different shape and thickness. Frequency is indicated by the size of the dots.

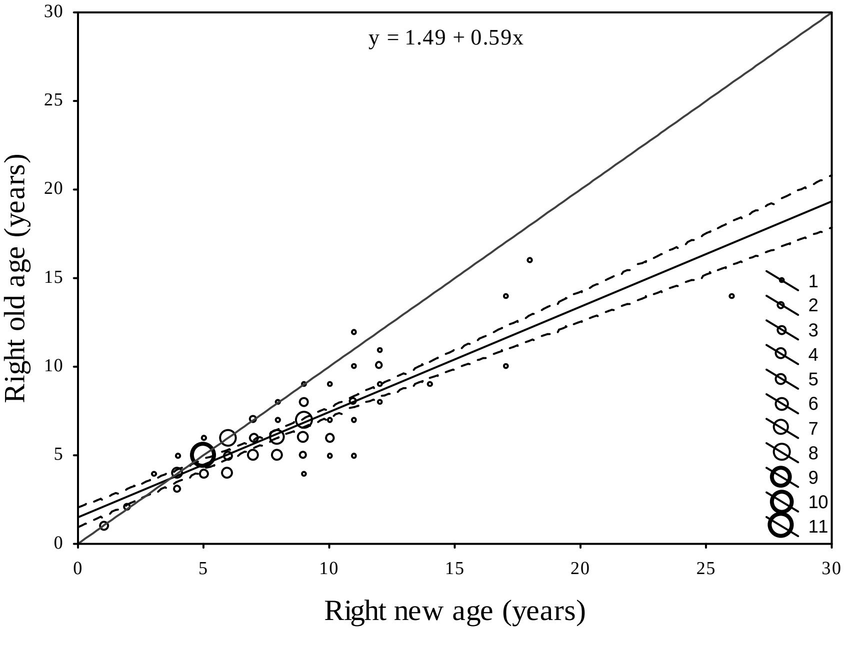

Figure 23: Scatter plot indicating differences between the ages interpreted for the right otolith using the former and the current method. The regression line, confidence interval and the y = x line are indicated by different shape and thickness. Frequency is indicated by the size of the dots.

3.7 Season for translucent and opaque zone formation

When studying the outer edge of the otoliths photographed in this study, it was found that otoliths sampled in April appear to have a fully formed translucent zone, and in many cases the opaque zone has started to form (Figure 24). In October, the opaque "summer" zone is completed and the formation of the translucent "winter" zone has begun (Figure 25). For more examples, see Appendix 10.

Figure 24: Otolith pair from a halibut sampled on the 4th of April. The translucent zone seems to be completed, and an opaque zone is about to be formed. The red arrow indicates the translucent zone.

Figure 25: Otolith pair sampled on the 25th of October. The opaque zone is completed, and a translucent zone is being formed. The red arrow indicate the complete opaque zone.

When studying the optical character of the final increment in 20 random otolith pairs collected in October and November, 18 pairs showed a complete opaque final increment and/or the start of translucent increment formation.

These findings leads to the conclusion that the opaque and translucent zones are, in fact, laid down during the summer and winter season respectively.

3.8 Size and growth of Atlantic halibut

The relationship between log length and log weight for Atlantic halibut is close to allometric (Figure 26). A general linear regression analysis (GLM) indicates that the relationship between the two variables is close to linear. There were no differences in the length-weight relationship between male and female halibut (GLM, $p > 0.05$, Appendix 19). This indicates that the relationship between length and weight is just as similar for both sexes.

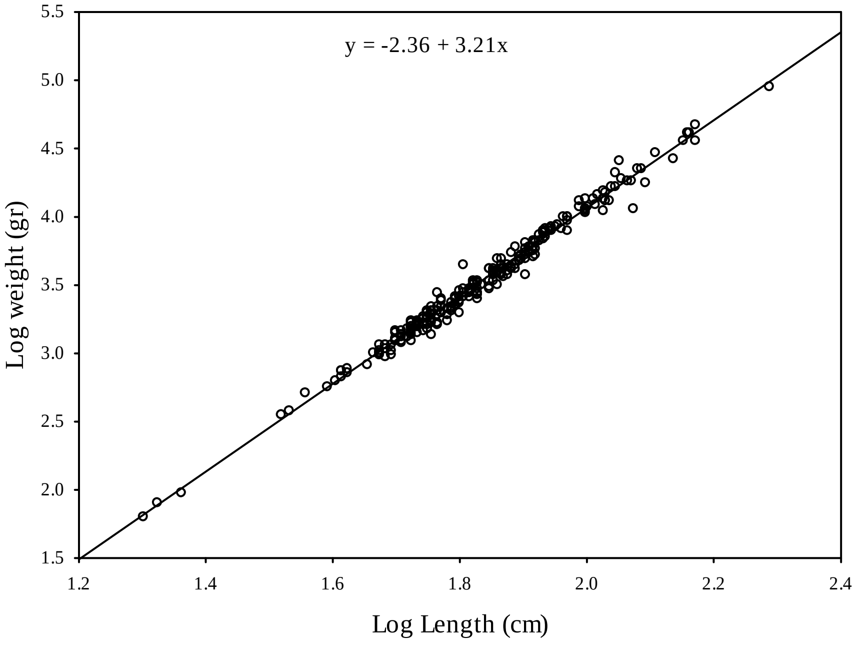

Figure 26: Regression of the relationship between log transformed length in cm and log transformed weight in grams

3.8.1 Size at age for males and females

In this study it was found that the measured length at age for males and females differs, and length appears to increase continuously for both sexes as they grow older. Females are generally longer at a given age than males (Figure 27). The difference in length at age between males and females are significant (GLM, $p < 0.05$, Appendix 20).

It is also apparent that the weight of females is generally higher than the weight of males at a given age (Figure 28). For both sexes, the weight appears to increase with increasing age. A GLM analysis of weight at age, with sex as a factor, shows that there is a significant difference between the weight at age for females and males (GLM, $p < 0.05$, Appendix 20).

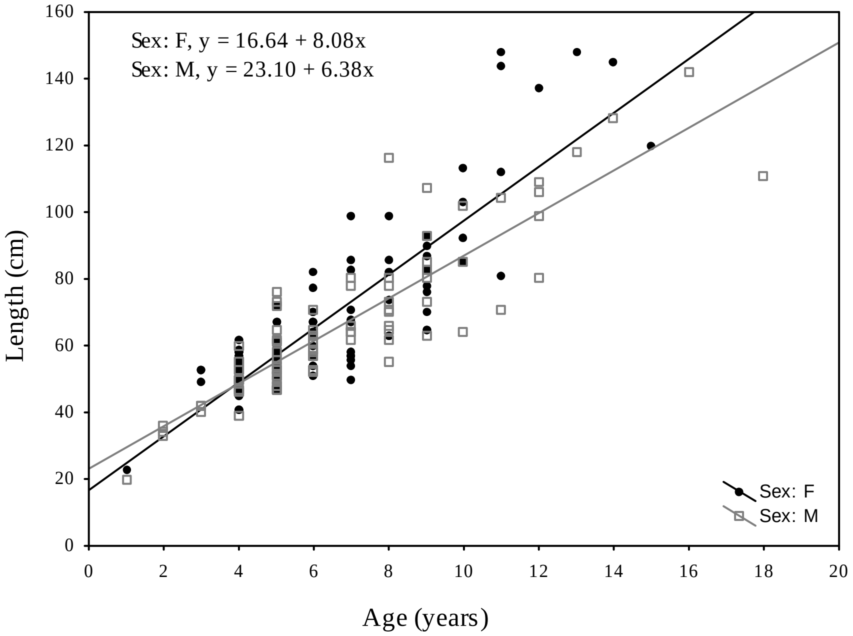

Figure 27: Regression of the relationship between length and age, categorized by sex. Males and females are indicated by different color and symbols.

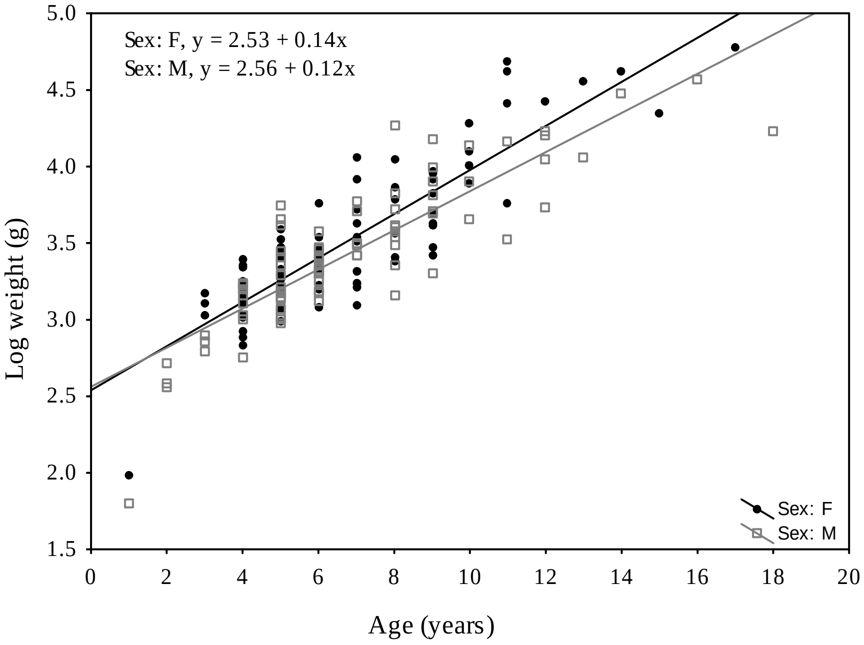

Figure 28: Regression of the relationship between log weight and age for males and females. The males and females are indicated by different color and symbols.

3.8.2 Comparison of size at different locations for males and females

Comparing growth at different sampling locations along the coast (Figure 29) shows that the interaction between length, sex and latitude has a significant effect on weight (GLM, $p < 0.05$). The halibut caught in northern latitudes are larger in size than the ones sampled further south. There is no significant effect of different longitudes on the relationship between length and weight for the sexes (GLM, $p > 0.05$). The GLM results are presented under Appendix 21. Sizes at different longitudes sampled are illustrated in Appendix 12.

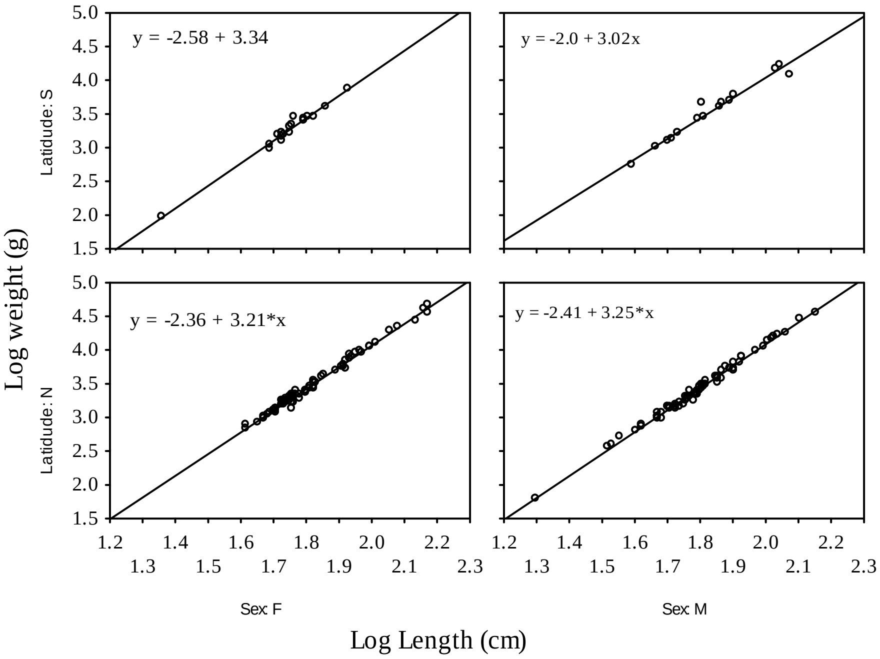

Figure 29: Regression of the relationship between length and weight with latitude and sex as factors.

3. 8. 3 Size at age at different locations for males and females

The observed lengths at age for male and females at different latitudes are illustrated in Figure 30. The lengths at age in the northern latitudes are significantly greater than those from southern areas (GLM, $p < 0.05$, Appendix 22). Comparing the weights at estimated ages for male and female halibut sampled at different latitudes (Figure 31) showed that halibut in northern latitudes are significantly heavier at age (GLM, $p < 0.05$) than halibut further south. Length and weight for male and female halibut do not show any significant differences between longitudes (GML, $p > 0.05$). Size at age for halibut caught at different longitudes is illustrated in Appendix 13.

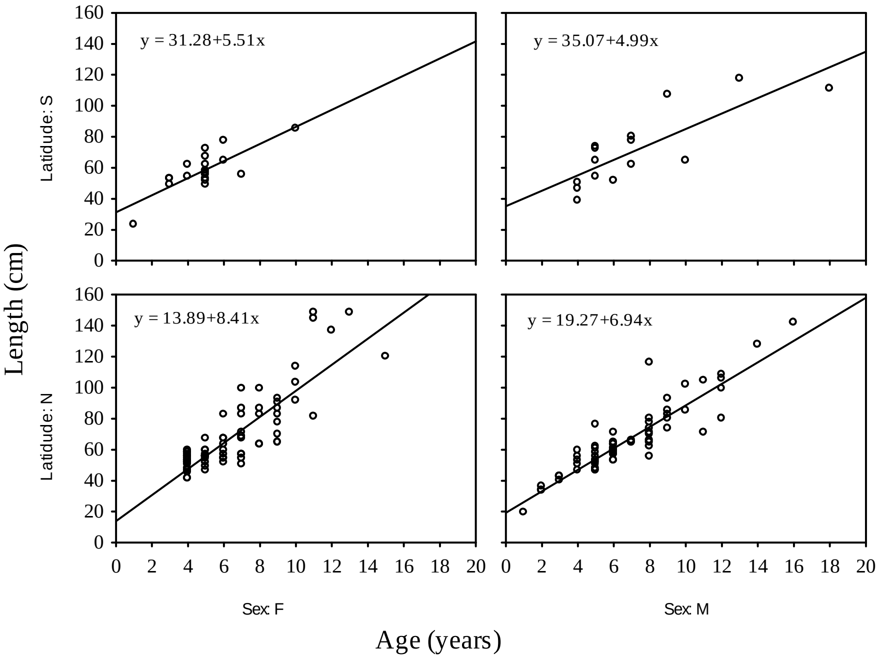

Figure 30: Regression of the relationship between length and age observed for male and female halibut caught at different latitudes.

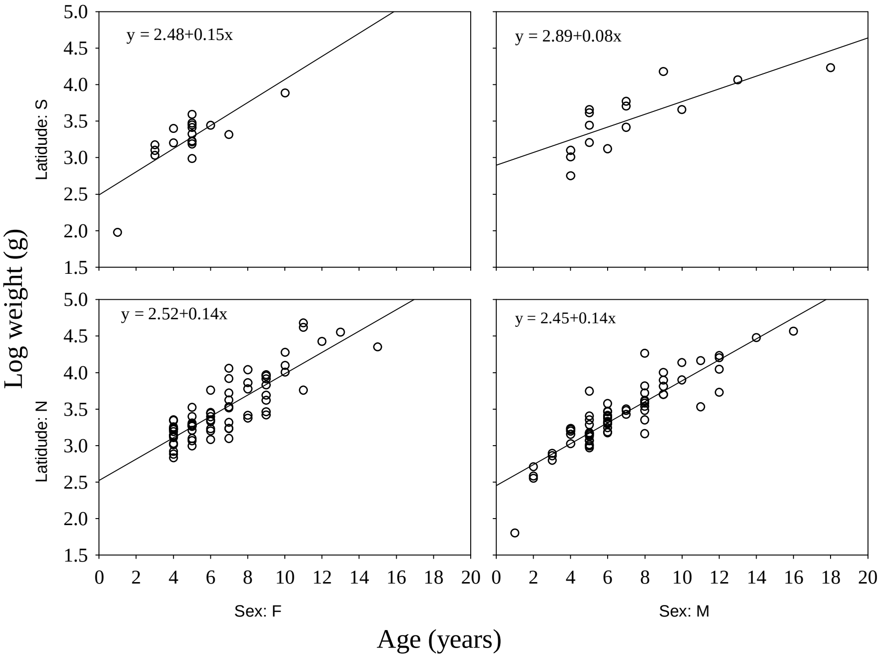

Figure 31: Regression of the relationship between weight and age for males and females at different latitudes.

3.9 Age-length-weight key

Because the number of individuals representing some of the ages were scarce, ages recorded for halibut were pooled together in age groups. The number of individuals in each age group, and their maximum and minimum lengths and weights are listed under Appendix 11. For several of the halibut included in this study, sex is not determined. Because male and female size at age has been found to differ, these can not be included in an age-length-weight key. The number of male and female halibuts within the different age groups found in this study is listed in Table 2 and Table 3 respectively, together with minimum and maximum lengths and weights measured for the groups. Missing values indicate no information. A few of the halibut collected by fishermen do not have both length and weight measurements. For halibut estimated to be older than 14 years, the material available is too scarce for making an age key.

Table 2: Number of male halibut within the different age groups registered in this study, and minimum and maximum lengths and weights found within the respective age groups.

Age	Number of ind.	Min length (cm)	Max Length (cm)	Min weight (gr)	Max weight (gr)
<= 2	4	20	36	64	517
(2-4]	11	39	60	567	1731
(4-6]	34	47	73	940	4540
(6-8]	17	55	116	1460	18400
(8-10]	11	63	102	2020	13670
(10-12]	5	71	109	3395	17080
(12-14]	2	118	128	11660	30000
(14-16]	1	142	-	36800	-
(16-18]	1	111	-	22490	-

Table 3: Number of female halibut within the different age groups registered in this study, and minimum and maximum lengths and weights found within the respective age groups.

Age	Number of ind.	Min length (cm)	Max Length (cm)	Min weight (gr)	Max weight (gr)
<= 2	1	23	23	95	95
(2-4]	22	41	62	685	2516
(4-6]	36	47	82	975	6750
(6-8]	17	50	99	1250	11485
(8-10]	13	65	113	2649	19000
(10-12]	5	81	148	5750	48000
(12-14]	2	145	148	36000	41850
(14-16]	1	120	-	22490	-
(16-18]	1	-	-	60300	-

The cumulative histograms in Figures 32a and b indicate which lengths we can expect within the different age groups for females and males respectively. If the length of a female is measured to be 60 cm, the probabilities are ~40%, ~30% and ~20% of it being in the age groups (<= 4], (4,6] and (6,8] respectively. For 70 cm long females, the probabilities are ~40%, ~30% and ~10% of being within the age groups (4,6], (6,8] and (8,10] respectively. For 80 cm long females, the probabilities are ~30% for being in the age group (4,6], ~40% of being within (6,8] and ~30% of being within (8,10]. For a 100 cm long female, the probabilities are ~70% for being in (8,10] and ~20% of being within (10,12] (Figure 32a).

If the length of a male halibut is measured to be 40 cm, the probability is ~75% that it is within the age group (<= 2]. A 60 cm male halibut is ~40% likely to be in the group (2,4] and ~35% likely to be in the group (4,6]. If it is measured to be 70 cm long, the probabilities are ~50% and ~25% that it is within the groups (4,6] and (6,8] respectively. A length of 80 cm gives a ~35% probability of being within age group (4,6], ~35% probability of age group (6,8], ~20% and ~15% probabilities of being within age groups (8,10] and (10,12] respectively. If it is measured to be 100 cm long, the probabilities are ~25% and ~60% of being within the age groups (8,10] and (10,12] respectively (Figure 32b).

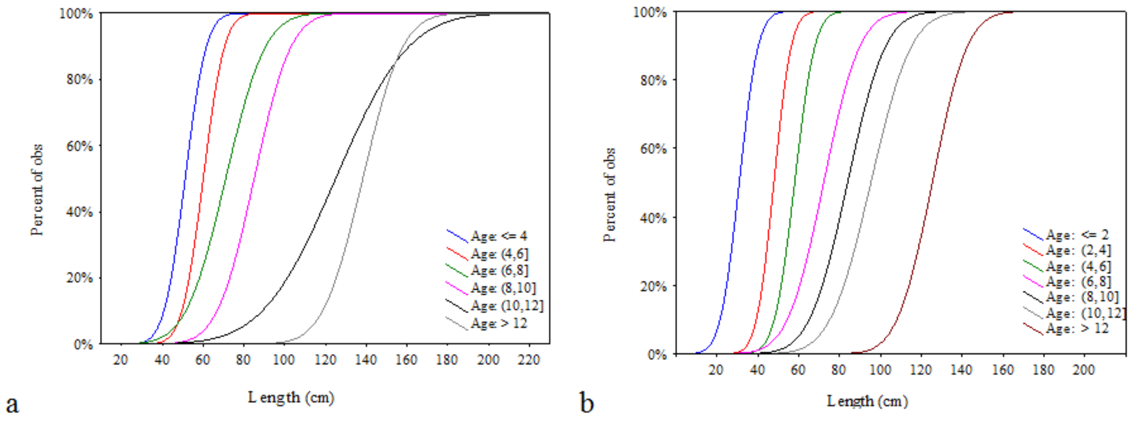

Figure 32: Cumulative histogram of length in cm for a) female halibut, and for b) male halibut categorized by age groups. Age groups are indicated by different colors.

For female and male halibut that is weighed to a certain weight, the probabilities of being within the different age groups are indicated by Figure 33a and b respectively.

For a female halibut weighed to be 2kg, the probabilities are ~30% that it is within the group (<= 4], ~30% that it is within (4,6] and ~20% that it is in the age group (6,8]. If it is weighed to be 5kg, the probabilities are ~30%, ~40%, and ~25% of being within the age groups (4,6], (6,8] and (8,10] respectively. A 10kg female has a ~20% probability of being in age group (6,8], ~65% of being within (8,10] and a ~15% probability of being in (10,12]. A weight of 30kg for females, indicate a ~40% probability of it being within (8,10], ~40% of being within (10,12] and a ~25% probability of being > 12 years old (Figure 33a).

A male that is weight to 2kg, has a ~40% probability of being within age group (2,4] and ~45% of being within (4,6]. If a it is weighed to be 5kg, the probabilities are ~30%, ~40%, ~15% and ~15 % that it is within the age groups (4,6], (6,8], (8,10] and (10,12] respectively. For a 10kg male, the probabilities are ~20% of it being in the group (6,8], ~25% that it is (8,10], ~40% that it is within (10,12] and ~10% that it is within (12,14]. A male weighed to be 30kg has ~20% probability of being within age group (10,12], ~10% probability of being within (12,14] and ~65% probability of being >14 years old (Figure 33b).

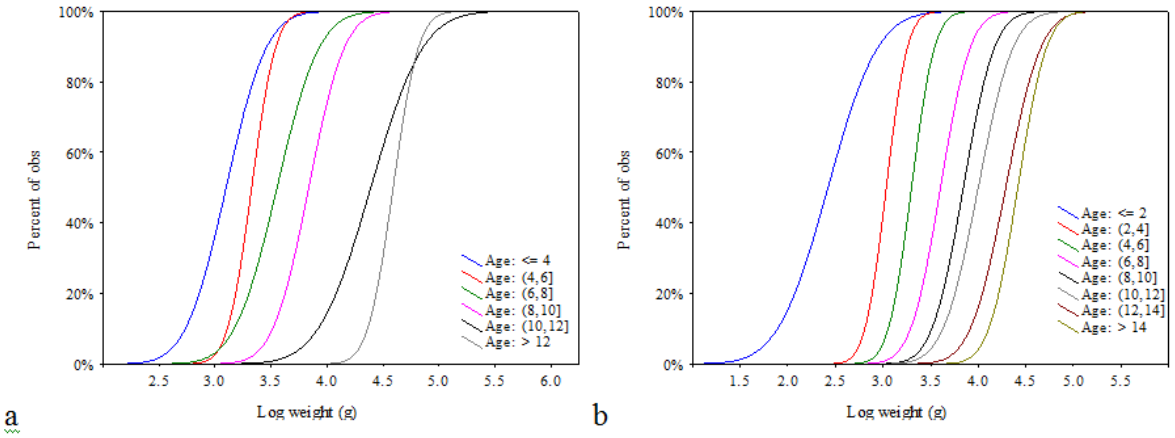

Figure 33: Cumulative histogram of log transformed weight for a) female halibut, and for b) male halibut categorized by age groups. Age groups are indicated by different colors.

4. Discussion

4.1 Aging procedure for Atlantic halibut

Different aging methods have been tested and used for Atlantic halibut in recent years, including breaking and burning (Blood, 2003a) and preparation of thin cross-sections (Armsworthy and Campana, 2010). The traditional method for aging halibut is by otolith surface readings (Armsworthy and Campana, 2010). However, previous studies have not been compared to find the best practice for these surface readings. In the present study, surface readings after different treatments were performed. Glycerol was expected to enhance the contrast of the growth increments. After 24 hours storage in 60% glycerol the results showed otoliths with a certain transparency, where increments were almost erased. It was found that for otoliths that have been stored in paper envelopes, 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.

In a study performed on Atlantic halibut caught in North Norwegian waters, it was found that the otolith collected from the left side of the fish show clearer growth increments (Haug and Tjemsland, 1986). In previous studies, the choice of left or right otolith is determined based on which of the two that has the longest readable axis (Kvalsund and Albert, 2007). The present study found that even though the right otoliths has the longest reading axis, the left otolith show clearer growth increments, as well as a significantly higher number of increments than the corresponding right otolith. A selection of the same otoliths was also sectioned for comparison. Sectioning of the same otoliths reinforces the conclusion that the left otolith is preferable, as the right and left section give an equal number of increments in most cases, and the left whole otolith is more comparable with the section than the interpretation when using the right otolith.

Previous studies on age determination performed for a number of species found that otolith surface readings underestimated age (Albert et al., 2009, Lee et al., 2009, Blood, 2003b). In the present study, no statistically significant differences were found between the surface readings and the cross section readings. Sectioning of otoliths was found to be a very useful tool in cases where the whole otoliths are damaged above or below the core as it can still be interpreted. 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. In 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, 2003b). From all images taken in this study, it is clear that the quality of the otoliths vary greatly. When the interpretation of annual increments exceeds the age of 9, the difference between left and right otolith sometimes become more than a year. In cases where this difference occurs, it is often related to the readability of the otolith. In this study, otoliths that are given a readability of three or four, meaning either very difficult to read or broken respectively, sometimes have an age difference between right and left that is more than one year. With poor readability, sectioning was found to be a preferable method for age interpretation. It should be taken into consideration that all otoliths should have been cleaned after observation in glycerol, using a solution of ether-alcohol (Panfili et al., 2002). This was not performed in the current study, and might have affected the otolith before sectioning. It is however unlikely that this has affected the comparison between left and right otolith.

Accurate ages are the key to determine life history traits such as growth rates, fecundity, number of annual reproduction, and age at maturity, which are all important for the management of fisheries (Beamish and McFarlane, 1983). The current study showed that the number of increments recorded for both left and right otolith is significantly higher than the number of increments recorded by the method previously used by IMR, indicating possible previous underestimation of the age of Atlantic halibut. Subjectivity is an element that is difficult to avoid in age interpretation, and therefore a possible source of error (Haug and Tjemsland, 1986). The current study 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. 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. 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).

4.1.1 Rules for age interpretation

One of the main objectives in this study was to establish a new and improved procedure for age determination of Atlantic halibut and to establish some updated reading rules.
The following rules for age interpretation are proposed on the basis of the experiments performed in this study.

1. If otoliths have been stored dry in paper envelopes they should be immersed in water for at least 24 hours before photographing.
2. Otoliths should be photographed with reflected light to enhance annual growth increments.
3. If both left and right otolith is available, they should both be photographed and aged.
4. The age should be interpreted on the anterior- posterior axis as this is the longest direction and therefore revealing all increments more easily.
5. An attempt to follow the increment around the otolith should be done to ensure that it is not in fact a split increment.
6. The distance between the increments should be considered when counting in order to avoid counting false increments.
7. The 1st of January has been accepted as the date of birth for Atlantic halibut. If the halibut is caught in December the final increment is not fully formed, and can not be counted as one year.
8. If there is a difference between the ages interpreted on the left and right otolith, the left otolith should be used to conclude an age if the difference is not more than a year.
9. If the difference between left and right otolith is more than a year they should both be sectioned.
10. If an otolith is estimated to be more than 9 years old it should be sectioned.
11. If an otolith has a very poor readability it should be sectioned.
12. If an otolith is broken above the core it should be sectioned.
13. Sectioned otoliths should be interpreted along the dorsal-ventral axis.

14. If both otoliths have been crystallized, they should be returned to storage for later studies.

A few of the otoliths in the current study had been crystallized and could therefore not be aged. Crystallization refers to otoliths that are composed of a structural variant of aragonite, vatarite. These otoliths have rougher surface textures, almost resembling sugar cubes (Forsberg, 2001). This occurs at different degrees, and if they are fully crystallized they cannot be aged as no increments are visible. When crystallization occurred in the current study it was usually only present in one of the otoliths and not the other.

4.2 Timing of seasonal zone formation

As an aging tool, fish otoliths must grow through the entire life of the fish, and display an internal increment structure, which forms on a determinable and regular time scale (Fowler, 1990). Even though most fish species inhabiting subpolar waters form opaque zones during spring and summer seasons and translucent zones in the winter (Høie and Folkvord, 2003), the timing for zone formation must be validated as seasonal zone formation is species specific. In a recent study on the temperature effects on otolith pattern formation in Atlantic cod, temperature was found to have a pronounced effect on the optical density of the otolith as well as on accretion rate. Rising the temperature was found to induce formation of translucent material, indicating that translucent zones can be deposited outside the winter months (Neat et al., 2008). In the current study it was found that the Atlantic halibut appear to deposit opaque zones during the spring/summer season and translucent zones in the winter.

The concluding timing of seasonal zone formation in the present study is based on the character of the outer edge of the otoliths, and it is assumed that the increments have been deposited on a regular time scale throughout the entire life of the fish. The timing and possible changes with age of seasonal zone formation can be validated by relating optical properties of otoliths to ambient temperature fluctuations across the lifetime of the fish (Høie and Folkvord, 2003). Suggestions for further studies is to validate timing of seasonal zone formation by marking and recapturing halibut, allowing at least a few years before recapture, and perform stabile isotope analyses of the seasonal increments formed in the period of liberty.

4.3 Size and age relationships

Atlantic halibut have been measured to a maximum length of at least 3.5 meters, a weight close to 300 kg for females (Michalsen, 2010), and have been found to reach an age of at least 50 years (Armsworthy and Campana, 2010). Previous studies have demonstrated that the growth rates of male and female halibut differ. In a recent study on the growth of Atlantic halibut caught in the Northwest Atlantic, a similar length at age was observed for male and female halibut up to about 5 years. The male and female growth diverged increasingly after this age, and the females were found to reach a substantially larger size than males (Armsworthy and Campana, 2010). In this study it was found that length and weight are just as correlated for males and females, but the females were found to become significantly longer and heavier with age than males. The relative growth appears greater at younger ages and decreases as they grow older. The male size at age appears to level out some at an age of 10-12, while the female growth appears to accelerate at this point. During the first 4-6 years there is no pronounced difference in length or weight between the sexes. After this the females become significantly longer and heavier than males. These observations are consistent with previous findings (Jakupsstovu and Haug, 1988, Haug and Tjemsland, 1986, Sigourney et al., 2006, Devold, 1938).

As spawning season approaches, many species undergo a starvation period. In a study on growth rates of sexually mature Cod, *Gadus morhua,* evidence that recovery growth occurred post-spawning was found (Pedersen and Jobling, 1989). It should be taken into consideration that the halibut included in this study are sampled at different times of the year, and the possible effect of environmental variations has to be considered. The condition of fish has been found to have an influence on size and growth, and poor feeding conditions before capture have been proven to affect the condition of fish (Pedersen and Jobling, 1989). The use of relative weight measurements as indices of growth, should perhaps be reconsidered, as it might be a more robust predictor of fecundity (Anderson and Neumann, 1996).

In order to draw any conclusions about individual growth, one needs to follow the same individuals for a considerable elapsed time. This was beyond the scope of this study. Suggestion for further studies includes the use of otolith increments. The repeatable relationship that has been found to exist between fish size and otolith size, together with the distance between otolith center and increment allows the estimation of size at a previous age (Chambers and Miller, 1995).

4.4 Size distribution of halibut along the Norwegian coast

Distinct variations in life history strategies and biological characteristics have been found frequently 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 (Jonassen et al., 1999). In a study on changes in size- and age-distributions in halibut caught in north Norwegian waters, a significant difference in the length distribution of females were found, where females collected from Malangen/Andfjord/Vesterålen were smaller than the ones sampled further north, at Sørøysund (Haug and Tjemsland, 1986). 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. This was explained by the possibility of adaptation to temperature and length of growth season, as a shorter growth season may lead to a greater growth capacity (Jonassen et al., 1999). The same study also found that Norwegian populations exhibited a higher efficiency in food conversion compared to Canadian populations, which may indicate that there could exist inter-population differences in energy utilization (Jonassen et al., 1999). In a study on intra- vs. interspecific latitudinal variation in growth of two silver side species, Atlantic silverside *Menidia menidia* and tide-water silverside *M. peninsilae,* the northern forms were found to accelerate their growth more rapidly than the southern form with temperature. They also found evidence of temperature adaptations at interspecific levels, where northern species maximized their growth rates at lower temperatures than southern species (Yamahira and Conover, 2002).

The current study found a difference between the size of males and females collected at different latitudes, where both males and females collected in the more northern latitudes were larger. The lengths and weights at estimated age for both males and females was found to be significantly affected by latitude, and the halibut caught at locations further north was larger at age that the ones caught further south. Even though the differences are not very large, they are still statistically significant.

Temperature is considered to be the main factor influencing growth (Campana and Hurley, 1989), followed by prey abundance (Suthers and Sundby, 1996). Climate changes such as global warming have been found to affect the growth rates of fish. Today, one of the main factors causing changes in marine ecosystems is the ocean warming (Pörtner and Peck, 2010). Even though slight increases in temperature has been found to be beneficial for growth rates (Jonassen et al., 1999), laboratory studies have demonstrated that raising the temperature can become a restrain on growth and lead to growth stagnation at a certain point (Neuheimer et al., 2011). The restraining effect of temperature was demonstrated in a recent study on the growth rates of two populations of the long lived Red Moki, *Cheilodactylus spectabilis*, inhabiting the warming waters in the Tasman Sea. The increasing temperatures were found to increase the growth rate for the cooler water populations near Australia, while an increase in temperature led to a decrease in growth rate for the warmer water New Zealand population (Neuheimer et al., 2011).

Another interesting factor to consider is the effect of the midnight sun on regional growth differences. In a study on the growth rate of early juvenile Arcto-Norwegian cod, it was found that juvenile cod inhabiting latitudes around 70°N, had ~48% more time for visual feeding during midnight sun conditions, than individuals further south, increasing potential food consumption (Suthers and Sundby, 1996). In a similar study on the effects of the midnight sun, it was found that juvenile cod can and will eat continuously at all hours of the day if visibility is sufficient (Helle, 2000). The positive effects of day-length on growth have been supported by laboratory experiments (Helle, 2000).

The low abundance of halibut in regions south of 62° N along the Norwegian coast compromise the strength of the current analyses, as all data in the this study is taken from halibut sampled north of 62° N. A better analysis of latitudinal differences in size and growth would be possible if halibut sampled further south was available.

4.5 Age-length-weight keys

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). 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). Constructing an age key is usually done by summarizing the relationship between length and age for a relatively small subsample of individuals, and applying the findings to the whole group of fish (Ogle, 2008). 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 leaves us with a very rough overview of what age to expect for the different lengths and weights. The current study had a maximum of 34 and 36 individuals for males and females respectively per age group and a minimum of 1 individual per age group. Such low numbers make it difficult to apply any summary to the entire population of Atlantic halibut. The age group containing the most representatives collected in this study is the 5-6 year olds. According to the current findings, a female halibut aged to be 5-6 years old, has a 30% probability of being 60 cm in length, 40% probability of being 70 cm in length, and 30% probability of being 80 cm long. A male estimated to the same age has a 35% probability of being 60 cm in length, 50% probability of measuring 70 cm, and a 35% probability of being 80 cm long. These are not very high probabilities, most likely due to the lacking number of individuals.

The primary determinant of weight for fish is length (Anderson and Neumann, 1996). The current study found an allometric relationship between the two variables, indicating a change in body shape as they grow older. Including weight in an age key, introduce certain potential problems. The halibut included in the current study are 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 Atlantic halibut, spawning takes place during winter, normally in January-February (Kjørsvik et al., 1987). The onset and peak of spawning activity has been found to vary between years, and also between locations (Kjørsvik et al., 1987). This means that condition, and therefore also weight fluctuates seasonally, making weight a less suitable indicator of age. Constructing an age-length key should be possible if enough halibut were available.

5. Summary

Based on a number of comparative experiments, an updated procedure for aging Atlantic halibut was established. The best procedure for surface reading of otoliths after storage in paper envelopes was found to be a 24 hour immersion in water with subsequent image analysis. Both otoliths should be photographed using reflected light. The left otolith is the preferred otolith in age estimation as it shows the most pronounced increments and also demonstrates coherence with sectioned otoliths. Sectioning is a useful tool in cases where the readability is poor, were a divergence in number of increments estimated for left and right otoliths occur, and for otoliths aged to be more than 9 years old.

The timing of seasonal zone formation is winter and summer for translucent and opaque zones respectively. This conclusion is drawn based on the character of the final increment deposited and the date of capture. However, without validating the regularity of seasonal zones through the entire lifespan of the fish, no absolute certainty can be achieved.

Due to the lack of enough individuals in each age group sampled, constructing a valid age-length-weight key is not possible in the current study. If enough material were available, constructing an age-length key would be feasible. Including weight in an age key, introduce certain potential problems, as weight is affected by seasonal variation in condition.

Atlantic halibut show an allometric growth, meaning that their relative body shape changes as they grow older. Females become significantly larger than males, and have a greater size at age. It should be taken into consideration that the halibut included in this study are sampled at different times of the year, and the possible effect of environmental variations has to be considered, as seasonal variation in weight might occur.

There is a difference between the size of halibut collected at different latitudes, where both males and females collected in the more northern latitudes are larger on average. The low abundance of halibut in regions south of 62° N along the Norwegian coast compromise the strength of the current analyses, as all data in the this study is taken from halibut sampled north of 62° N. A better analysis of latitudinal differences in size and growth would be possible if fish sampled further south was available.

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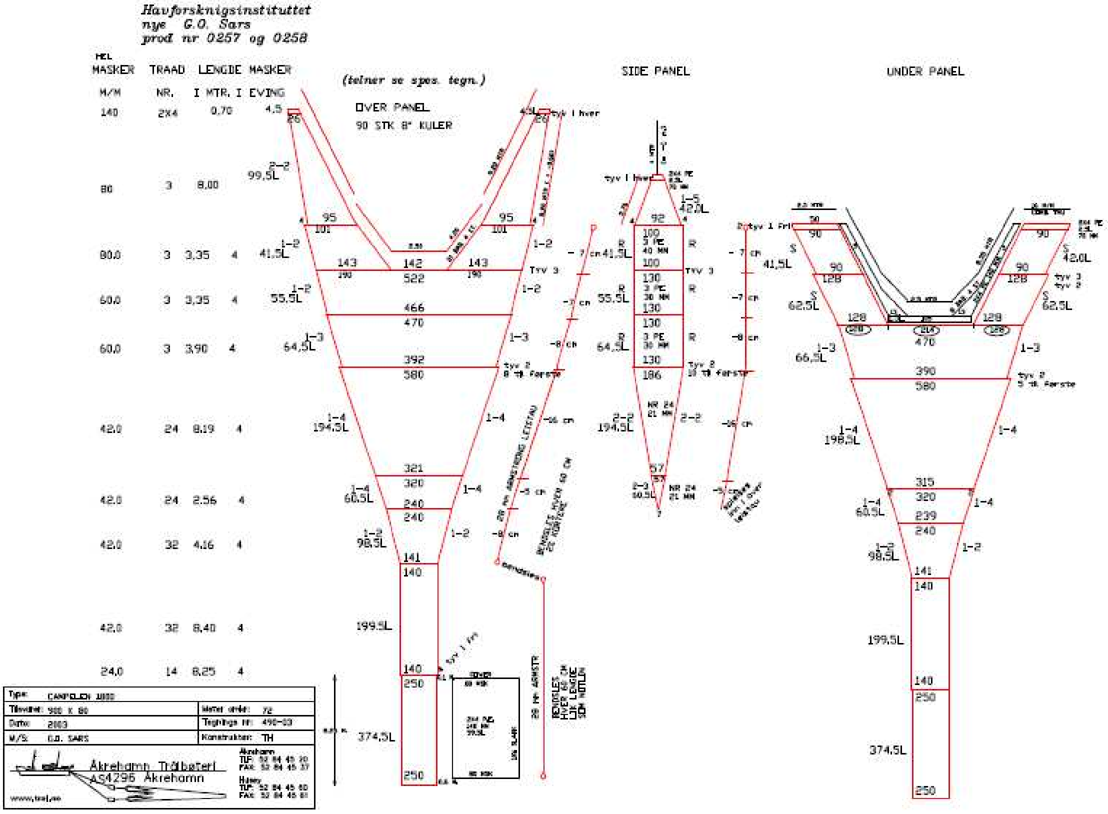
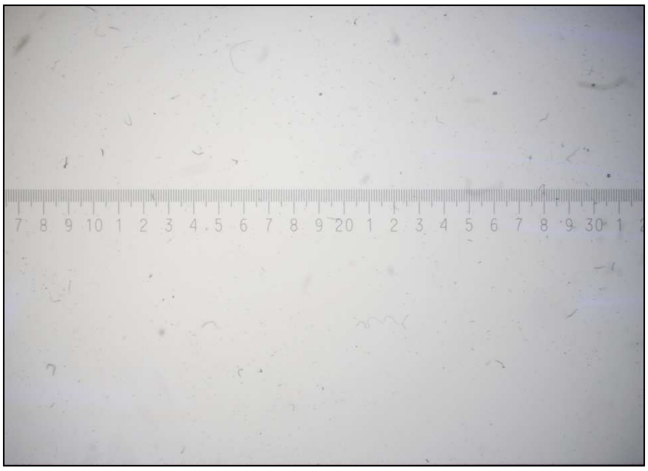
7. Appendices

Appendix 1: The design of Campelen 1800 shrimp trawl (Anonymous, 2008).



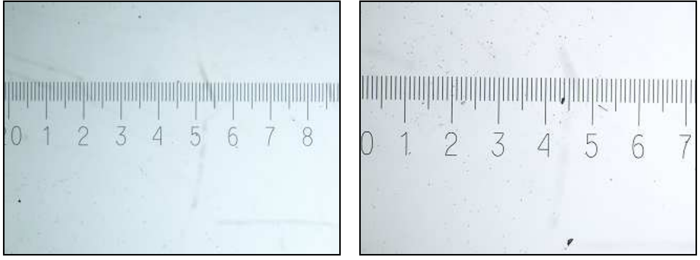
(Anonymous, 2008)

Appendix 2: Micrometer photographed with 1x magnification, used for calibrating images of whole mount otoliths.

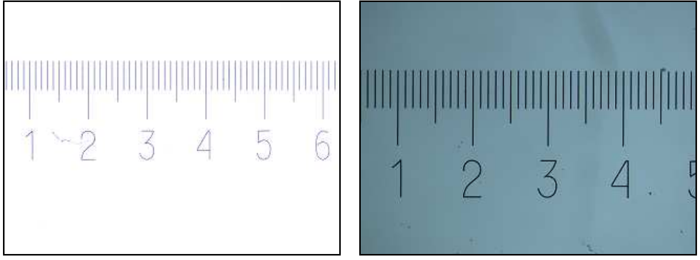


Appendix 3: Micrometers photographed with the magnifications used for calibrating images of sections.

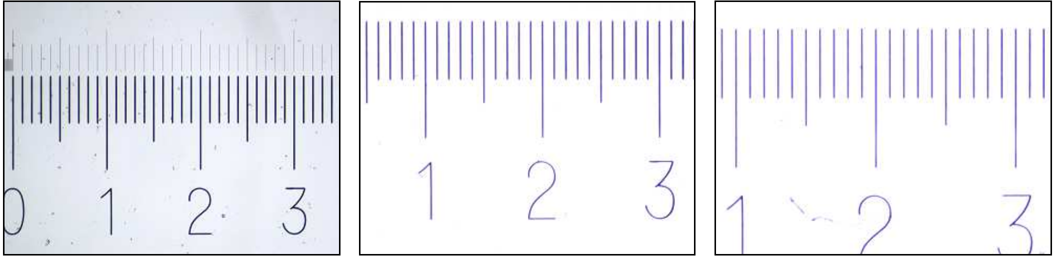
(1.6x magnification) (2.0x magnification)



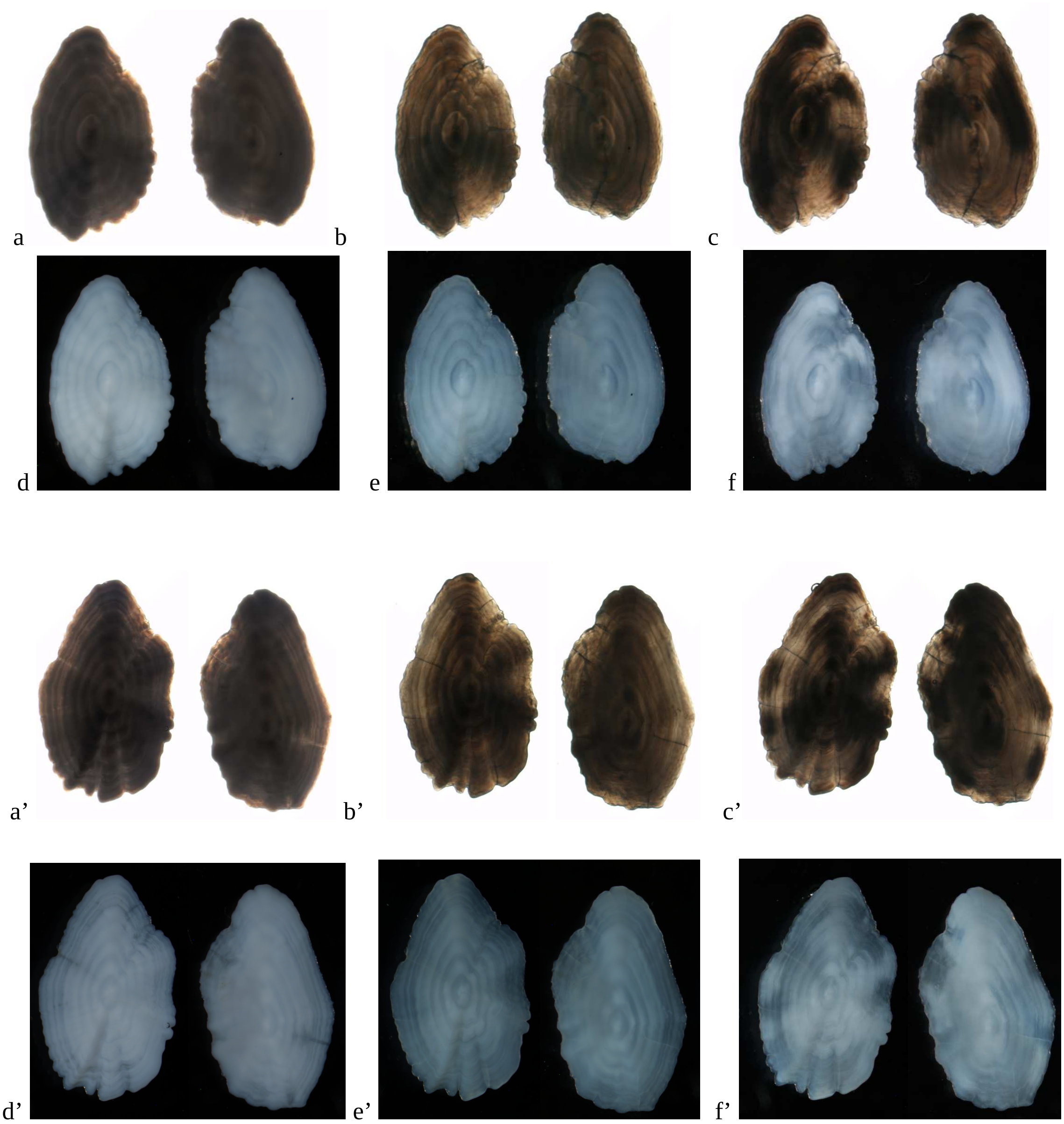
(2.5x magnification) (3.2x magnification)



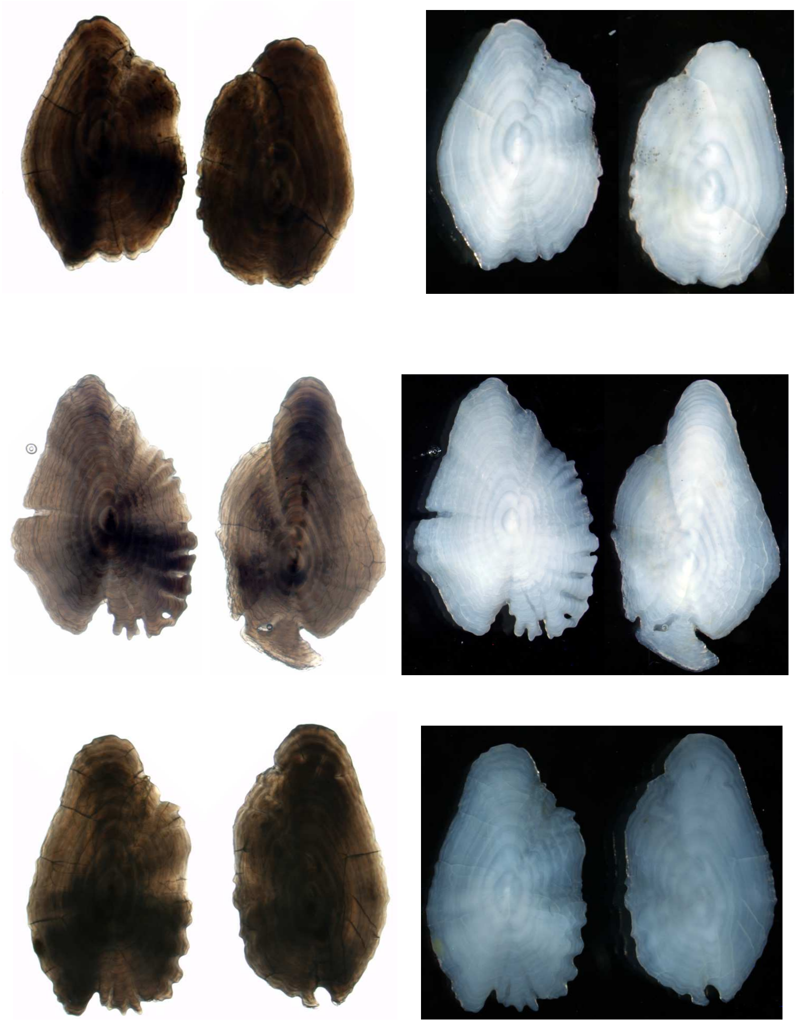
(4.0x magnification) (5.0x magnification) (6.0x magnification)



Appendix 4: Examples of otoliths photographed after different treatments. Otoliths in a, d, a’ and d’ are photographed directly after dry storage, otoliths in b, e, b’ and e’ are photographed after immersion in water, and otoliths in c, f, c’ and f’ are photographed after 24 hours in glycerol. 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.

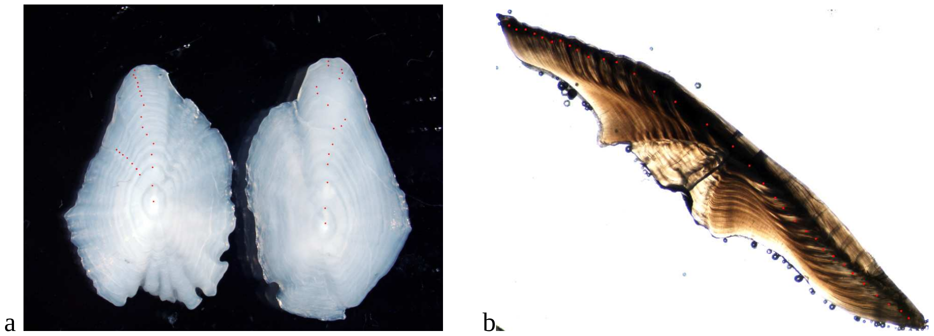


Appendix 5: Examples of otolith pairs photographed using transmitted and reflected light, after immersion in water. Otolith pairs to the left and right are photographed with transmitted and reflected light respectively.

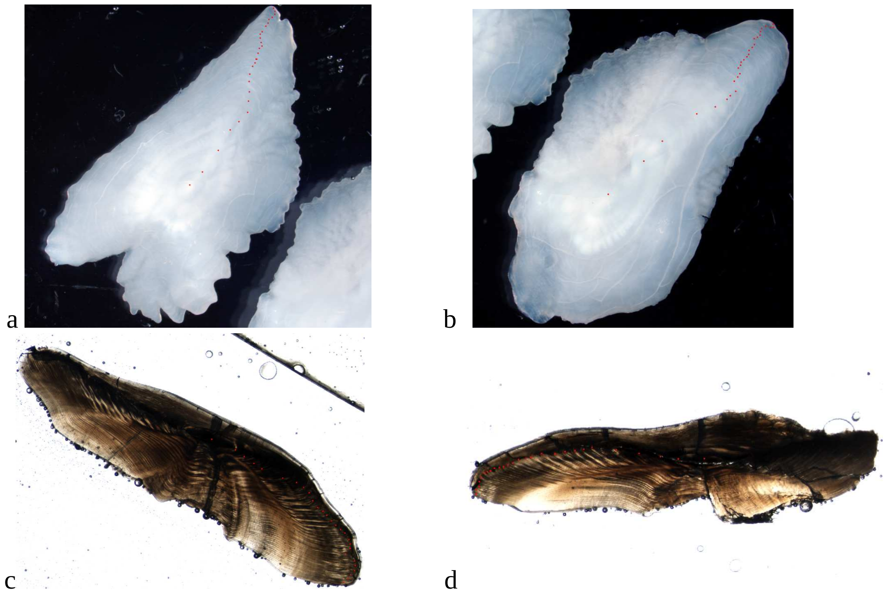


Appendix 6: Example of an otolith pair (a) where a part of the left otolith is broken and (b) the left and (c) right section of the same pair collected in 2008. The red dots are annual increment indications.

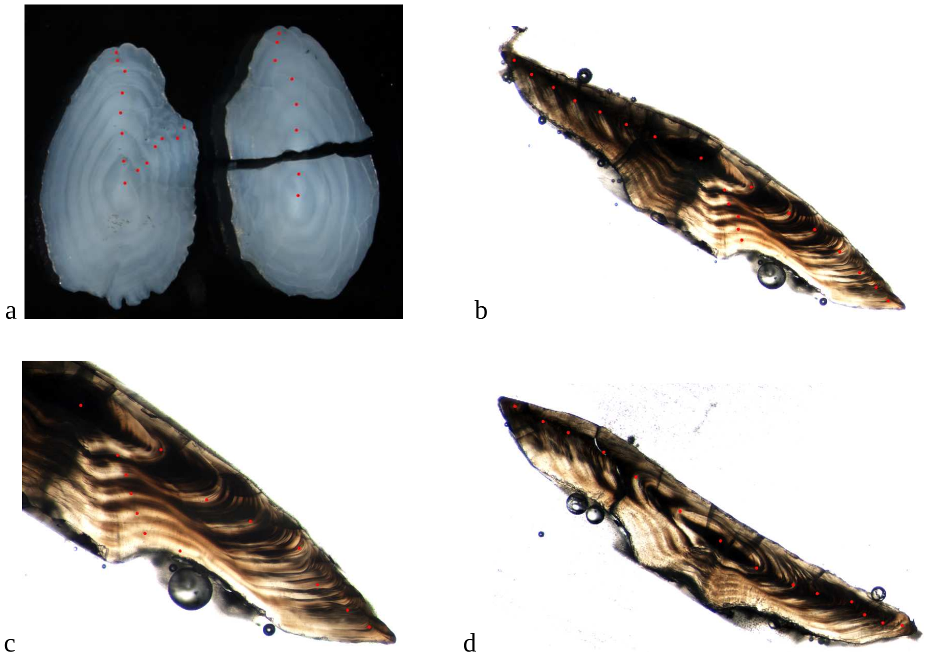
Appendix 7: Example of (a) an otolith pair and (b) the left section. Red dots indicate age interpreted on the otoliths



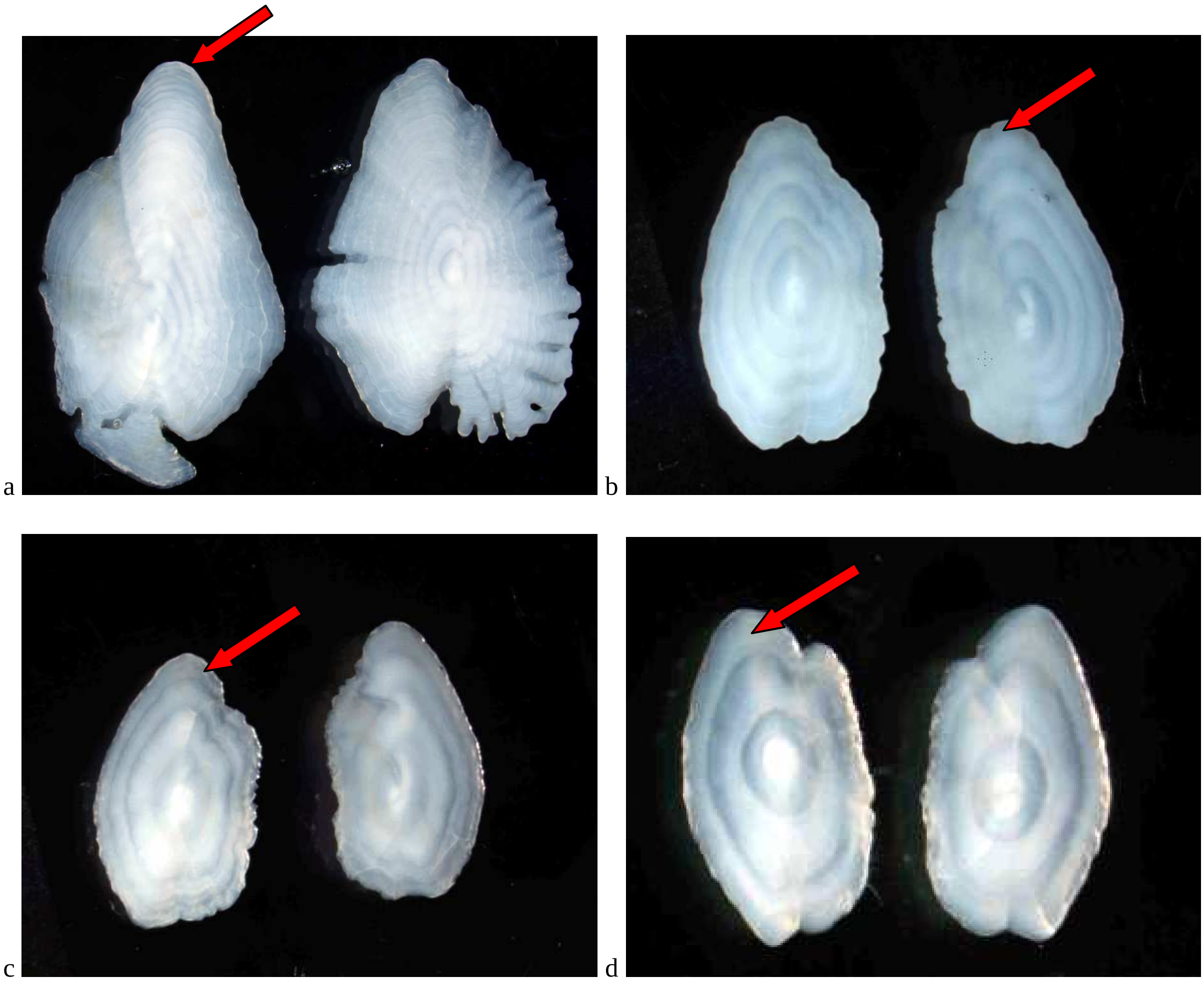
Appendix 8: Example of an otolith pair where the left (a) and right (b) otolith are very old and differing in age by two years, and the left (c) and right (d) section.



Appendix 9: Examples of an otolith pair where the right otolith is broken above the core (a) and the left (b, c) and the right (d) section of the same pair.



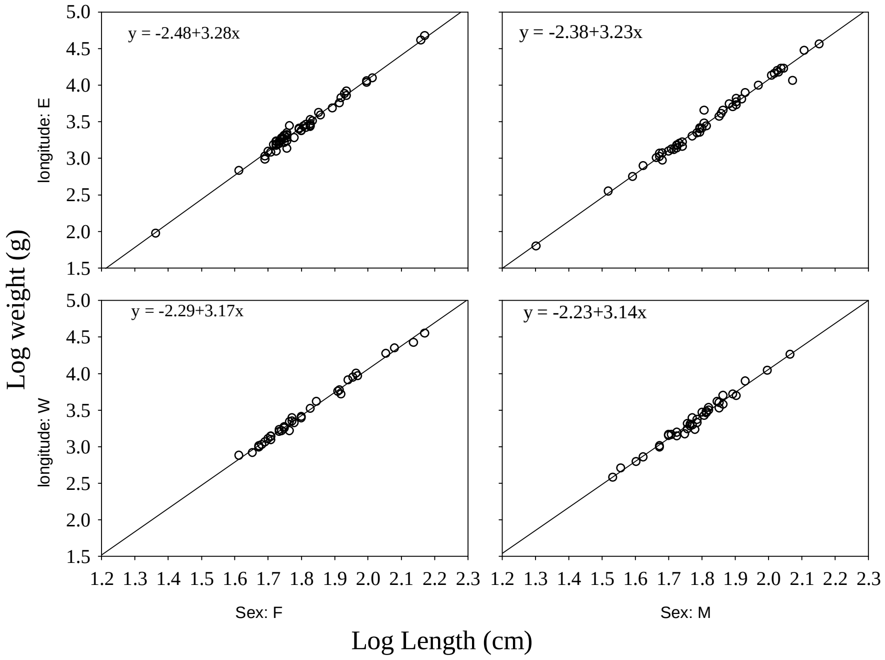
Appendix 10: Validating zone formation. Otoliths in image a, are sampled on the 24th of August. The final zone seems to be of opaque opacity. Otoliths in image b, are sampled on the 29th of March. The final zone seems to be a completed translucent band, indicating the beginning of opaque zone formation. In image c, which is otoliths sampled on the 11th of October, the opaque zone appears fully formed, and the translucent zone is being laid down. Otoliths in image d, is sampled on the 9th of October, and the opaque zone seems to be completed. Red arrows indicate the final zone.



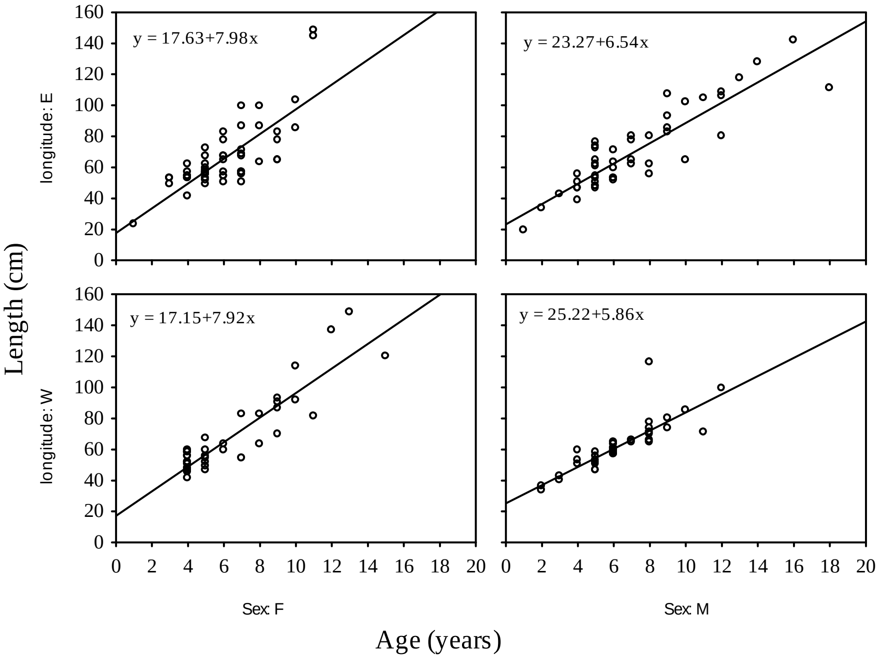
Appendix 11: Total number of aged individuals, both males and females, minimum and maximum lengths and weights found within different age groups.

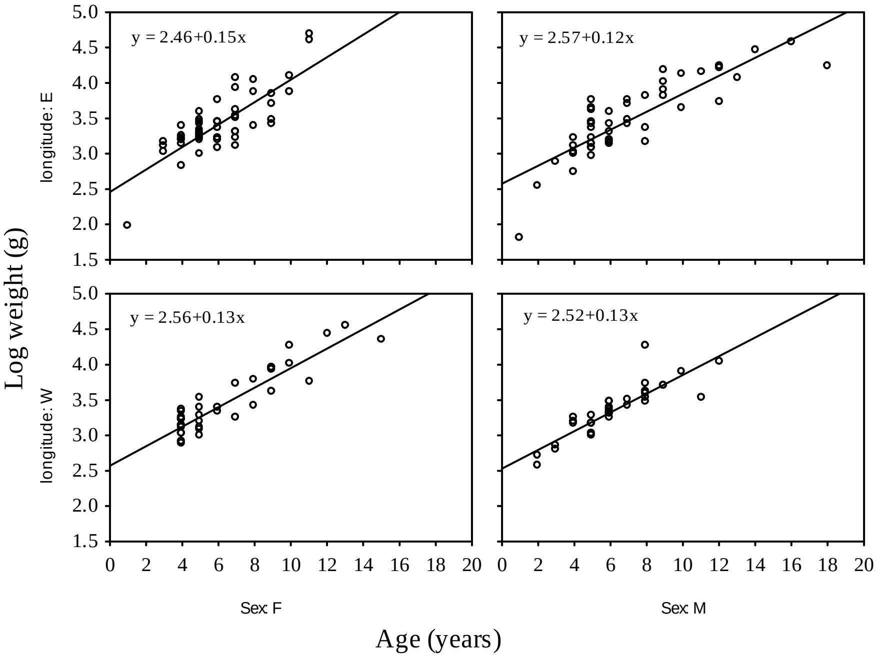
Age	Number of ind.	Min length (cm)	Max Length (cm)	Min weight (gr)	Max weight (gr)
<= 2	7	19	36	64	517
(2-4]	39	39	62	567	2505
(4-6]	74	47	80	991	5578
(6-8]	54	50	116	1250	18400
(8-10]	44	63	113	2020	19000
(10-12]	20	71	148	4230	48000
(12-14]	9	107	148	11660	45000
(14-16]	6	99	142	13680	70500
(16-18]	3	111	-	17000	53700
> 18	3	193	-	91600	190000

Appendix 12: Regression of the relationship between length and weight, with longitude and sex as factors.



Appendix 13: Regression of the relationship between length and age, and between weight and age with longitude and sex as factors.





Appendix 14: The results of the paired t-tests performed to compare the age interpreted for left and right otoliths using reflected and translucent light.

	Mean	Std. Dv.	N	Diff.	Std. Dv.	t	df	p
Left oto. R	8.160	3.884						
Left oto. T	7.990	3.629	106	0.169	0.899	1.9	105	0.054

	Mean	Std.Dv.	N	Diff.	Std.Dv.	t	df	p
Right oto. R	7.702	3.576						
Right oto. T	7.549	3.432	111	0.153	0.906	1.779	110	0.077

Appendix 15: The result of the paired t-test performed for the ages interpreted on the left and right otolith of the same fish.

	Mean	Std.Dv.	N	Diff.	Std.Dv.	t	df	p
Left	7.452	3.749						
Right	7.199	3.598	221	0.253	0.774	4.866	220	< 0.001

Appendix 16: The results of the paired t-test performed for the ages interpreted for the left and right whole otoliths and their sections.

	Mean	Std.Dv.	N	Diff.	Std.Dv.	t	df	p
Left section	8.794	5.079						
Left whole	8.882	5.272	34	-0.088	0.900	-0.571	33	0.571

	Mean	Std.Dv.	N	Diff.	Std.Dv.	t	df	p
Right section	8.906	5.189						
Right whole	8.687	5.462	32	0.218	0.792	1.561	31	0.128

Appendix 17: The results of the paired t-test performed for the ages interpreted for the left and right otolith sections.

	Mean	Std.Dv.	N	Diff.	Std.Dv.	t	df	p
Left section	8.794	5.079						
Right section	8.823	5.054	34	-0.029	0.459	-0.373	33	0.711

Appendix 18: The results of paired t-tests performed to compare age estimates obtained from the new method versus the previous estimates of the same otoliths, for left and right otoliths.

	Mean	Std.Dv.	N	Diff.	Std.Dv.	t	df	p
New age estimate	8.098	3.693						
previous age estimate	6.089	2.403	112	2.00	2.033	10.455	111	<< 0.001

	Mean	**Std.Dv.**	**N**	**Diff.**	**Std.Dv.**	**t**	**df**	**p**
Left new method	8.055	3.822						
Left old method	6.157	2.532	108	1.898	1.933	10.203	107	<< 0.001

	Mean	**Std.Dv.**	**N**	**Diff.**	**Std.Dv.**	**t**	**df**	**p**
Right new method	7.685	3.572						
Right old method	6.064	2.442	108	1.620	1.883	8.942	107	<< 0.001

Appendix 19: GLM analysis of length against weight for all halibut sampled, and for length and weight with sex as a second degree factor.

Log length	**SS**	**Degr. of**	**MS**	**F**	**p**
Intercept	2.285	1	2.285	8674.46	<< 0.001
Log weight (g)	4.799	1	4.799	18216.02	<< 0.001
Error	0.064	245	<< 0.001		

Log length	**SS**	**Degr. of**	**MS**	**F**	**p**
Intercept	1.722	1	1.722	6791.31	<< 0.001
Sex	< 0.001	1	< 0.001	1.17	0.280
Log weight	3.399	1	3.399	13398.05	<< 0.001
Error	0.046	181	< 0.001		

Appendix 20: GLM analysis of age at length and at weight, with sex as a second degree factors.

Age	**SS**	**Degr. of**	**MS**	**F**	**p**
Intercept	2.879	1	2.879	1.379	0.241
Length	1069.3	1	1069.3	512.175	<< 0.001
Sex	15.5	1	15.57	7.457	0.006
Error	375.8	180	2.088		

Age	**SS**	**Degr. of**	**MS**	**F**	**p**
Intercept	481.14	1	481.1	219.9	<< 0.001
Log weight	1161.4	1	1161.39	530.7	<< 0.001
Sex	13.09	1	13.09	5.982	0.015
Error	393.8	180	2.188		

Appendix 21: GLM analysis of length and weight with sex, latitudes and longitudes as factors.

Log weight	SS	Degr.of	MS	F	p
Intercept	2.841	1	2.841	1233.09	<< 0.001
Sex	0.009	1	0.009	3.918	0.049
Latitude	0.001	1	0.001	0.512	0.475
Log Length	17.057	1	17.057	7401.75	<< 0.001
Sex*Latitude	0.012	1	0.012	5.530	0.019
Sex*Log Length	0.008	1	0.008	3.766	0.053
Latitude*Log Length	0.001	1	0.001	0.442	0.506
Sex*Latitude*Log Length	0.012	1	0.012	5.624	0.018
Error	0.384	167	0.002		

Log weight	SS	Degr. Of	MS	F	p
Intercept	5.588	1	5.588	2384.57	<< 0.001
Sex	0.001	1	0.001	0.82	0.366
longitude	< 0.001	1	< 0.001	0.02	0.880
Log Length	33.710	1	33.710	14383.56	<< 0.001
Error	0.400	171	0.002		

Appendix 22: GLM analysis of age at length and at weight with sex, latitudes and longitudes as factors.

Length	SS	Degr. of	MS	F	p
Intercept	11489.74	1	11489.74	85.082	<< 0.001
Sex	197.44	1	197.44	1.462	0.228
Latitude	1063.21	1	1063.21	7.873	0.005
Age	40355.53	1	40355.53	298.837	<< 0.001
Sex*Age	613.80	1	613.80	4.545	0.034
Latitude*Age	921.00	1	921.00	6.820	0.009
Error	22686.99	168	135.04		

Log weight	SS	Degr. of	MS	F	p
Intercept	140.71	1	140.71	2663.62	<< 0.001
Sex	0.197	1	0.197	3.736	0.054
Latitude	0.295	1	0.295	5.591	0.019
Age	15.392	1	15.392	291.371	<< 0.001
Latitude*Age	0.308	1	0.308	5.831	0.0168
Error	8.874	168	0.052		

Length	**SS**	**Degr. of**	**MS**	**F**	**p**
Intercept	10688.22	1	10688.22	76.39	<< 0.001
Sex	227.54	1	227.54	1.626	0.203
longitude	112.48	1	112.48	0.803	0.371
Age	67287.70	1	67287.70	480.935	<< 0.001
Sex*Age	787.13	1	787.13	5.626	0.018
Error	23644.82	169	139.91		

Log weight	**SS**	**Degr. of**	**MS**	**F**	**p**
Intercept	173.881	1	173.881	3197.939	<< 0.001
Sex	0.281	1	0.281	5.179	0.024
longitude	0.004	1	0.004	0.084	0.771
Age	24.916	1	24.916	458.256	<< 0.001
Error	9.189	169	0.054		