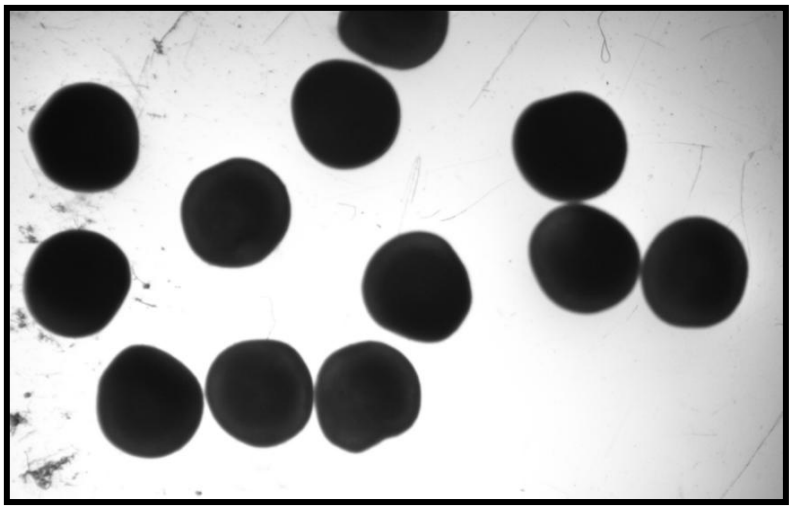
UNIVERSITY OF TROMSØ UIT

NORWEGIAN COLLEGE OF FISHERIES SCIENCE

Reproductive potential and maturity staging of Greenland Halibut (*Reinhardtius hippoglossoides*, Walbaum)



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Summary

Greenland halibut (*Reinhardtius hippoglossoides*, Walbaum) is a commercially important fish species in the Barents Sea. This fishery has been strongly regulated, including a fishing ban from 1992 to 2009. Studies of fish reproduction are needed to extend the knowledge about this species in order to improve management and to avoid another decay of the stock.

This Master Thesis describes the relationships between fecundity (thousands oocytes per female) and length (cm) ($\text{Fecundity}=2\times10^{-6}\times\text{Length}^{3.9418}$) and fecundity (thousands oocytes per female) and weight (g) ($\text{Fecundity}=0.003\times\text{Weigth}^{1.1251}$) for Northeast Arctic Greenland halibut based on 138 females taken in November-December 2011 on the continental slope of the Barents Sea. Fecundity was compared to previous data from the same area and it was found to be in the same range as data from 1996, 1997 and 1998. Maturity stages were stated using the new scale proposed by Kennedy et al. (2011) based on oocyte diameter measurements. These data were compared with the maturity stages given at sea, using both a standard macroscopic scale and the macroscopic scale special for Greenland halibut. Differences were found, both with regard to stating of maturity stages and the boundary between mature and immature individuals. Spawning stock size and total egg production were calculated using both methods, and an overestimation of the spawning stock size, as well as of the total egg production, was found when the macroscopic scale was used. Due to the fact that it is difficult to implement the microscopic scale at sea, it is proposed that when using the special macroscopic scale for Greenland halibut females, the boundary of immature females is moved from stage 1 to also include stage 2.

Contents

Introduction

Greenland halibut (*Reinhardtius hippoglossoides*, Walbaum) is an arctic-boreal species distributed along the North Atlantic. Although the genetic homogeneity for the entire population is still unclear, the Northeast Arctic (NEA) stock constitutes a separate management unit in the International Council for the Exploration of the Sea (ICES) management system (Gundersen et al. 1999, Knutsen et al. 2007). The current study is focused on this stock, which is distributed along the continental slope of Norway from 62ºN to the regions north of Spitsbergen and in the Barents Sea (Albert et al. 2001).

As a deep water species, Greenland halibut inhabits depths between 200-1500 m where water temperatures range between -1 and 4º C (Bowering and Nedreaas 2000). This species has a slow growth and late maturation and, as other species of the order Pleuronectiformes, has significant distinctions between males and females in rates of sexual maturation (Figure 1), growth dynamics and life span (Albert et al. 2010). Males mature at lengths around 40-50 cm, while females mature later, at lengths around 50-60 cm (Morgan et al. 2003; Albert et al. 2010).

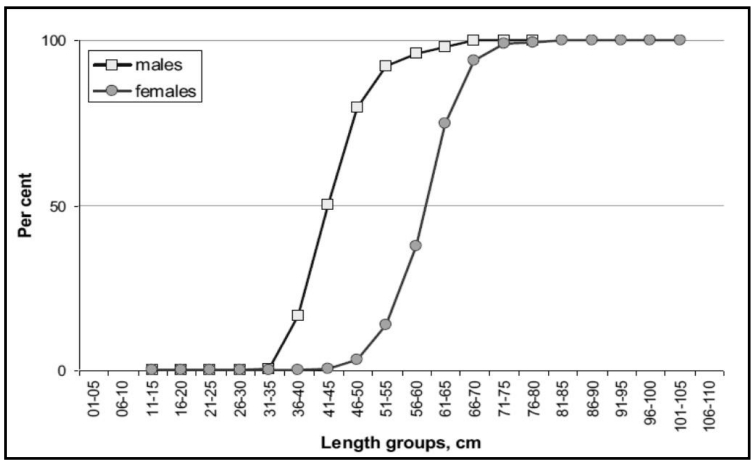

Figure 1. Percentage of mature specimens among males and females of NEA Greenland halibut by length groups in 2007-2009 (Albert et al. 2010)

Greenland halibut is a determinate spawner, which means that total fecundity before the spawning period is equivalent to the potential annual fecundity (Murua and Saborido-Rey 2003). It has a low fecundity (number of eggs) in comparison with other determinate species and eggs can be larger than 4 mm (Stene et al. 1999). The contribution of older females to the total egg production is proportionally higher compared to the younger females (Gundersen et al. 2000). There are evidences that the

ovarian development of Greenland halibut is prolonged, and oocytes take more than one year to complete the maturation process (Junquera et al. 2003; Kennedy et al. 2011).

Earlier studies conclude that the main spawning season for the NEA G. halibut stock occurs from November to mid-January, with a peak in December (Albert et al. 1998). However, recent studies have indicated that Greenland halibut spawning season is more extended (Kennedy et al. 2011).

Greenland halibut is a commercially important fish species in the Barents Sea. Before the 1960s, the fishery was mainly a longline fishery with landings of 3 000 tonnes, those landings increased up to 80 000 tonnes with the introduction of international trawlers in the mid-1960s (Høines and Korsbrekke 2003). As shown in Figure 2, landings decreased to a level of 20 000 tonnes during the early 1980s.

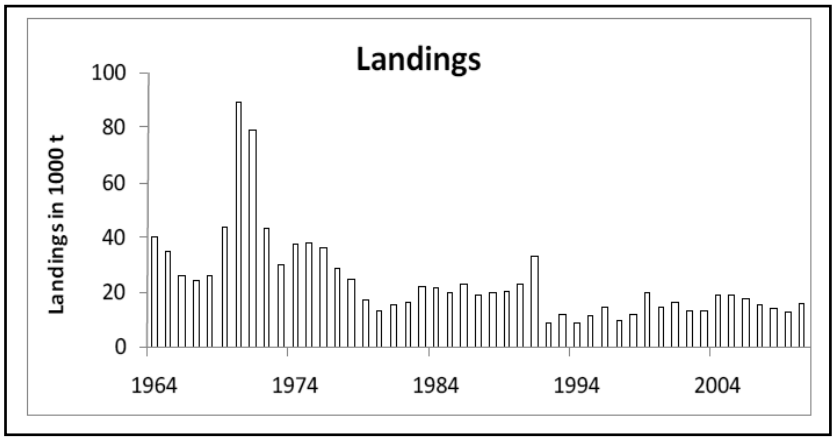

Figure 2. Historical landings of NEA Greenland Halibut (ICES 2011).

A decay in the spawning stock biomass was observed in the 1990s (ICES 2011) and together with a reduction in the commercial catch per unit of effort (CPUE) led the stock to strong regulations in later years, including a fishing ban from 1992 to 2009 north of 71º30N (Gundersen et al. 2000; ICES 2011). In 2009, the 38th session of the Joint Norwegian-Russian Fisheries Commission, decided to cancel the ban against targeted Greenland halibut fishery and established a total allowable catch of 15 000 tonnes for the following three years. The quota for 2012 was later increased to 18 000 tonnes (ICES 2011).

Studies on fish reproduction, including reproductive potential and maturity staging, are needed to extend the knowledge about this species in order to improve its management. Fecundity of Greenland halibut is an important component of the stock

reproductive potential (Gundersen et al. 2009), and the study of fecundity and ovarian growth dynamics is essential in order to be able to estimate the total fecundity of a population, which is also one of the main issues in fisheries management.

This Master Thesis includes two main studies in relation to the reproduction of Greenland halibut based on fish samples taken in the Barents Sea area in November-December 2011.

The first task is to measure the oocyte size distribution in the ovaries, which indicates the oocyte growth pattern and maturity stage of the fish, while the second task is to estimate the fecundity of the population. The distribution of maturity stages in the population, as well as the fecundity, could then be compared with past data (Gundersen et al. 1999, 2000). The second objective of this study is to establish a female maturity scale for the NEA Greenland halibut stock based on microscopic examination and oocyte diameter, and compare maturity staging by this scale to maturity staging by two different macroscopic maturity scales that are used in the sampling at sea. Swept area abundance estimates by length from survey are used; examining how the use of the different maturity scales can affect estimates of mature female abundance.

Materials and methods

Sampling

Ovary samples of Greenland halibut were taken in an Institute of Marine Research (IMR) in Norway bottom trawl survey that took place between the 20th of November and the 6th of December 2011 at the continental slope west of Norway, Bear Island and Spitzbergen (approximately 68°N-78°N) (Figure 3).

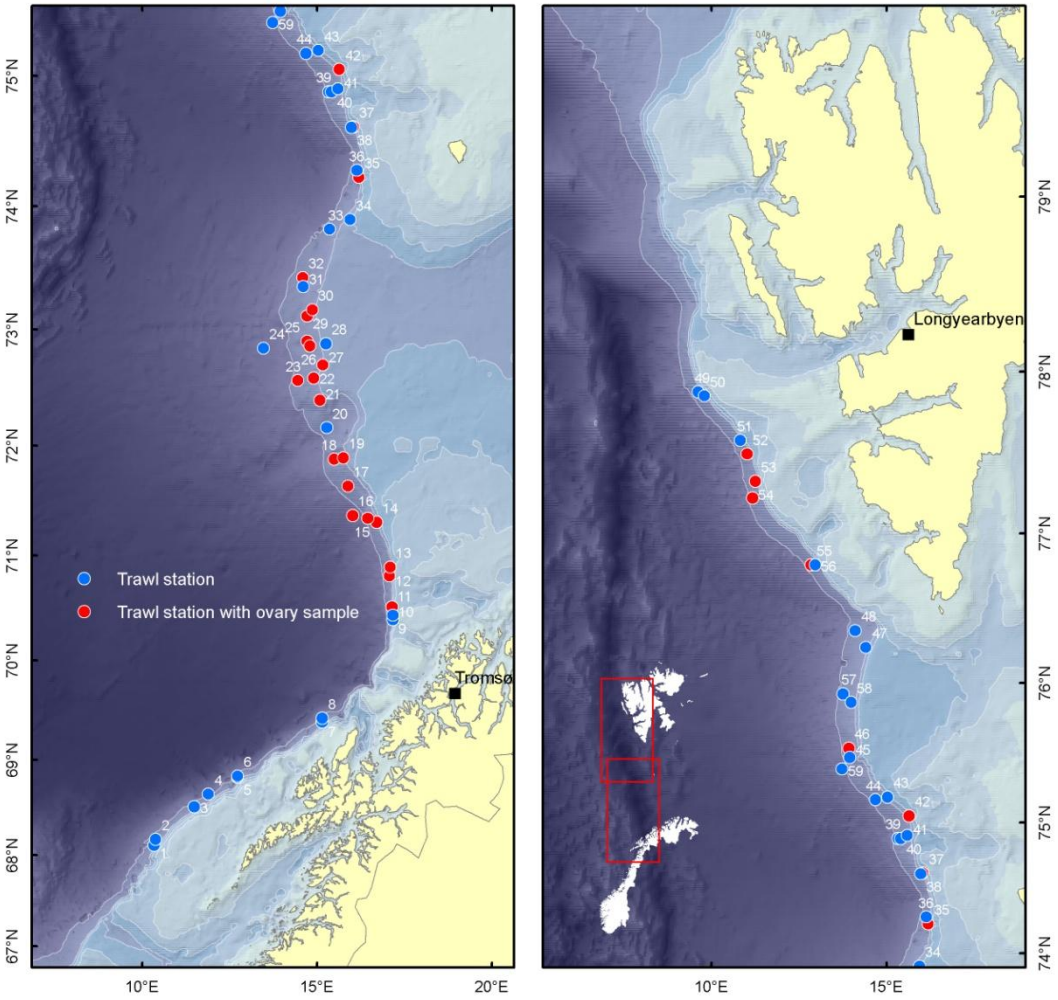

Figure 3. Sampling area for the IMR Greenland halibut survey in Nov./Dec. 2011. Location for all sampling stations is shown, with the stations used in this study marked in red.

For length measurements of Greenland halibut at the survey all catches per station, or alternatively a subsample of 200 representative individuals of the catch, were collected and divided by sex. For each sex, a subsample of a maximum of two fish per 5

cm length group was selected for biological sampling, where e.g. length, weight, sex and maturity stage were registered. For the present study, the total sample consisted of ovaries from 138 females collected among the ones selected for biological sampling at 26 stations (Figure 3). Samples were organized on a length-stratified basis with respect to female size (max 10-15 individuals in total per 5 cm-groups) (Table 1). Length was preferred because of problems related to age reading (ICES 2011).

Table 1. Number of ovaries collected within each length range.

Length (cm)	**<30**	**30-34**	**35-39**	**40-44**	**45-49**	**50-54**	**55-59**
Number	0	7	10	14	15	12	13
Length (cm)	**60-64**	**65-69**	**70-74**	**75-79**	**80-84**	**85-89**	**90**
Number	14	12	12	10	13	4	2

Weights of ovaries were measured fresh at sea and then samples were preserved in 3.6% buffered formaldehyde and stored in plastic containers. In the case of small ovaries (less than 34.4 grams in this study), whole ovaries were stored intact. However, from the large ovaries a slice was cut and weighed in order to have a better preservation and an easier handling. In earlier studies (Gundersen et al. 1999) homogeneity of the oocyte distribution within the ovary was confirmed. Still, in order to have the best comparison, samples were taken from the middle section of the right lobe of the ovary in the present study.

At sea, maturity was macroscopically staged by experienced research personnel aboard the ship using two different scales presented in Table 2 and Table 3. First, using a standard scale with a general description of maturity stages used for many species of fish, such as haddock, salmon or cod, by IMR (Mjanger et al. 2011). Additionally, in the case of Greenland halibut females, they were labeled under another scale, called special stages for Greenland halibut, which is a seven-stage key more detailed and accurate for this species (Fotland et al. 2000, in Norwegian. Translation from Kennedy et al. 2011).

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Table 2. General scale for description for maturity stages (Mjanger et al. 2011).

Stage	Description
Blank	Undecided/not checked.
1	Immature. Gonads are small. No visible eggs or milt.
2	Maturing. Gonads are larger in volume. Eggs or milt are visible but not running.
3	Spawning. Running gonads. Light pressure on the abdomen will release eggs or milt.
4	Spent/Resting. Gonads small, loose and/or bloody. Regeneration starting, gonads somewhat larger and fuller than stage 1. No visible eggs or milt.
5	Uncertain. Use only when difficult to distinguish stages 1 and 4.

Table 3. Special scale for maturity staging used for Greenland halibut (Fotland et al. 2000, in Norwegian. Translation from Kennedy et al. 2011).

Stage	Description
1	Immature. Ovaries are small. No oocytes are visible to the naked eye.
2	Early maturing. Oocytes visible to the naked eye, but less than 1 mm in diameter.
3	Maturing. Oocytes are 1–2 mm in diameter.
4	Late maturing. Oocytes are 2–4 mm in diameter.
5	Oocytes are hydrated and in spawning condition.
6	Spent. Oocytes are released. Ovary may be red.
7	Uncertain.

Laboratory analysis

Fixation and storage of ovaries in formaldehyde can cause small changes in their weight and volume (Witthames et al. 2009). This will not affect fecundity estimations in this study because individual fecundity is measured in oocytes per female, but it can affect work on maturity stages. However, the shrink factor is not going to be taken into account in this study. Measurements at sea may be uncertain if they are taken, as in many cases, under bad weather conditions with the boat rolling. In the case of the large whole ovaries, where a slice had to be cut, the changes in weight (fresh-fixed) were found to be erratic. Consequently, for the fecundity analysis, gonad weight after fixation was not taking into account, and fresh weight of ovaries was used instead.

Before laboratory analyses, subsamples were taken and weighed with the intention of establishing the raising factor, which is defined as:

$$(Equation\ 1) \quad R_{xy} = \frac{GW_x}{SW_{xy}}$$

R_{xy}= raising factor for ovary x and subsample y, GW_x = gonad weight before fixation (fresh) of ovary x, and SW_{xy} = subsample weight after fixation of ovary x, subsample y.

The gonadosomatic index (GSI) is used to describe the maturity stage of the female; in this case, GSI is defined as the ratio between the fresh ovary weight (OW) (g) and the total weight (W) (g) of the fish in percentage.

$$(Equation\ 2) \quad GSI = \frac{(OW\ \times 100\%)}{W}$$

For the fecundity estimations, a combination of the gravimetric method (used by Gundersen et al. 1999, 2000, 2009) and a particle analysis system (Thorsen and Kjesbu 2001) was used. The gravimetric subsampling method described by Bagenal and Braum (1978) consists on weighing the ovary and taking subsamples with known weight to count the eggs in each subsample in order to estimate the total number of eggs in the whole ovary. In the present study, image analysis software is used to count and measure the oocytes.

In addition, to be able to know which oocytes were going to be spawned in the coming spawning season, the new maturity scale (Table 4) proposed by Kennedy et al. (2011) was used. They state that only fish in stages 4 or 5 were capable of spawning within the nearest season. For this reason, in this study, only oocytes larger than 1 300 µm were counted in order to estimate the number of pre-spawning oocytes in the whole ovary and thus, the individual fecundity.

Table 4. Maturity scale for Greenland halibut proposed by Kennedy et al. (2011).

Stage	Description
1	Immature. Only previtellogenic oocytes present.
2	Cortical alveoli. LC < 500 µm.
3	Vitellogenesis 1. LC > 500 µm but there is no hiatus present within the cohort of vitellogenic oocytes.
4	Vitellogenesis 2. LC > 1300 µm and a hiatus is present in the cohort of vitellogenic oocytes.
5	Spawning. Hydrated oocytes are present in the ovary.
6	Spent. Oocytes have been released. Ovary may be red.

Of the 138 females used in the present study, 45 individuals were used for the fecundity estimation, the ones accomplishing the requirements (oocytes larger than 1300 µm). Four subsamples of each sample were weighed and stored in 3.6% buffered formaldehyde in small containers. Two of these subsamples were analyzed and the number of oocytes larger than 1 300 µm was counted. Fecundity was estimated by:

$$(Equation\ 3)\quad F_{xy} = R_{xy} \times N_{xy}$$

F_{xy}= fecundity of ovary x, subsample y, R_{xy}= raising factor for ovary x and subsample y, and N_{xy}= number of oocytes larger than 1300µm counted in ovary x and subsample y.

As explained in Gundersen et al. (1999), the mean fecundity of the individual female was estimated with two subsamples; if the coefficient of variation (CV) (Equation 4) between the two samples exceeded 5%, the other two subsamples were counted and used in the analysis. The CV is, expressed as percentage, the standard

deviation (std) of the estimates divided by the mean fecundity (F_{mean}) (Sokal and Rohlf, 1995 in Gundersen et al. 1999)

$$(Equation\ 4)\ \ CV\ = \frac{(std\ \times 100\%)}{F_{mean}}$$

Subsamples were between 1 and 6 grams, in order to get a CV below 5%. The number of oocytes in each sub-sample was higher than 200, in order to make precise estimations, but lower than 500 to reduce the analysis time.

For the maturity stage analysis all the ovarian samples were used. To state the maturity stage of the ones used for the fecundity estimates, notes were taken to distinguish the hydrated oocytes (stage 5) from the non-hydrated oocytes larger than 1300 µm (stage 4). However, to stage the rest of the samples ca. 5 pictures were taken from each sample (93 individuals) in order to observe and measure the immature oocytes, since this study will follow the new scale proposed by Kennedy et al. (2011), in which the maturity stage is deduced from the measurement of the oocyte size distribution (Table 2). In the case of the spent ovaries, they were identified according to the macroscopic scale when ovaries were fresh (at sea). A Leica binocular stereomicroscope with Nikon Digital Sight DS-5M-U1 microscope camera system connected to a computer was used to take the pictures.

Computer analysis

Oocyte diameter and oocyte numbers were measured from the photos with the ImageJ software (Rasband 1997-2011), using the plug-in ObjectJ (http://simon.bio.uva.nl/objectj). This software enables the user to measure and count round and oval oocytes both automatically and manually (A.Thorsen, IMR, pers.comm.)

To be able to run the software, in the case of the fecundity study, oocytes were separated manually from other oocytes and ovarian tissue. As the purpose of the study is to count and measure all the oocytes in the sample, since a modification of the gravimetric fecundity counting method is being used, this step is very important in the process and has been done carefully. Once all the oocytes in the subsample were split, they were placed in a photographic chamber before the picture was taken. This chamber has the same size as the area of the view in the picture, so all the oocytes in each picture can thus be counted.

In this type of image analysis, it is also important to have a light source underneath the sample that results in a high contrast between the oocytes and the light background (Figure 4).

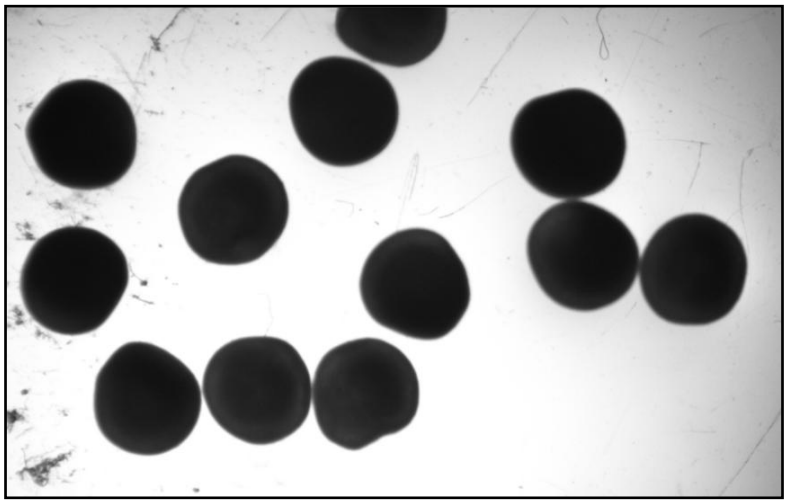

Figure 4. Picture of large vitellogenic oocytes, showing the contrast between the oocytes and the background.

Pictures of all the oocytes in each subsample were taken with Eclipse programme, stored in the computer and transfer to ImageJ/ObjectJ software, in order to run such programme. Once the automatically measurements were finished (Figure 5), every picture was checked and manually corrections were made if needed (Figure 5).

The results obtained by ImageJ were exported to Microsoft Excel for further statistical analysis.

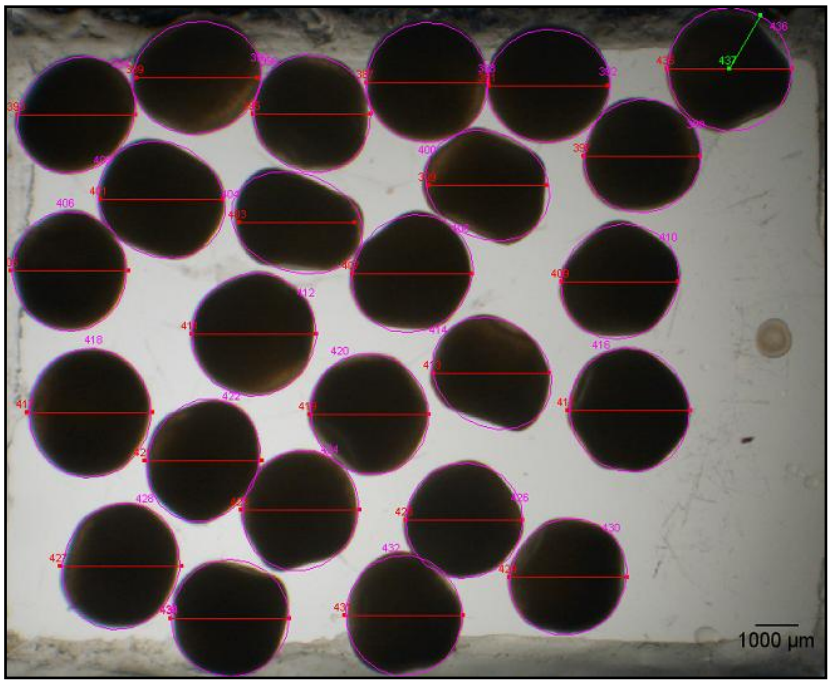

Figure 5. Example of a finished analyzed picture using ImageJ/ObjectJ. Oocyte in the top right of the picture with the green line is a manually measurement, all the other were done automatically.

Data analysis

Relations between fecundity and length and fecundity and weight were established using regression analysis as done in Gundersen et al. (1999) and Gundersen et al. (2000).

The maturity ogives by length and length at 50% maturity (L50) were calculated using macroscales separately for all data of the survey and only the data that were used in this study, as well as using the microscopic scale with data that are included in the study. A function was fitted using a generalized linear model (GLM) with binomial error distributions in the statistical package R (R development Core Team 2011).

The number of mature individuals, biomass and total egg production (TEP) were determined using data from the survey. The three parameters were calculated separately for each maturity scale presented in this study in order to compare them. The number of individuals was estimated using the swept area method. Therefore, there might be errors in the calculations in relation with the errors of the own method. Total biomass was calculated by parameterization of the relationship (Equation 5) between length and weight commonly used for many fish species:

$$(Equation\ 5)\ W = a \times L^b$$

W = weight, L= length; a = 0.0004 and b = 3.7732 in this case.

TEP was obtained by using a combination of the fecundity-length relationship with estimations of the number of mature individuals for the different lengths, similar to what was done in Gundersen et al. (2000).

$$(\text{Equation 6})\ \text{TEP} = \sum \text{F}_\text{i} \times \text{SSS}_\text{i}$$

$$(\text{Equation 7})\ \text{SSS}_\text{i} = \text{TF}_\text{i} \times \text{MF}_\text{i}$$

F_i = fecundity- length relationship estimated, SSS_i = spawning stock size for length i, TF_i = total number of females for length i and MF_i =ratio of mature females for length i.

Results

Total length and total weight of the 138 females of Greenland halibut used for this study ranged between 31 - 90 cm and 235 - 10 350 g respectively (Table 5). All of them were used in order to state maturity stages, while 45 individuals were used for fecundity estimations; these individuals were in the size range between 60 - 90 cm.

Table 5. Mean, standard deviation (Std) and range values for the different parameters measured for the 138 Greenland halibut females.

	Mean	Std	Minimum	Maximum
Total length (cm)	59.1	15.7	31	90
Total weight (g)	2 756.8	2 460.3	235	10 350
Ovary weight (g)	299.4	548	0.5	3 410
GSI_t (%) all females	5.3	7.4	0.1	33.1
GSI_m (%) females used for fecundity	14.4	6.2	3.1	33.1
Potential fecundity (oocytes per female)	51 670	23 401	13 821	104 030

Ovary weight was mainly in the range 0.5 – 2 140 g (Table 5), with the exception of one large ovary of 3 410 g. The relationship between ovary weight and total length and total weight of the individuals is shown in Figures 6 and 7 respectively.

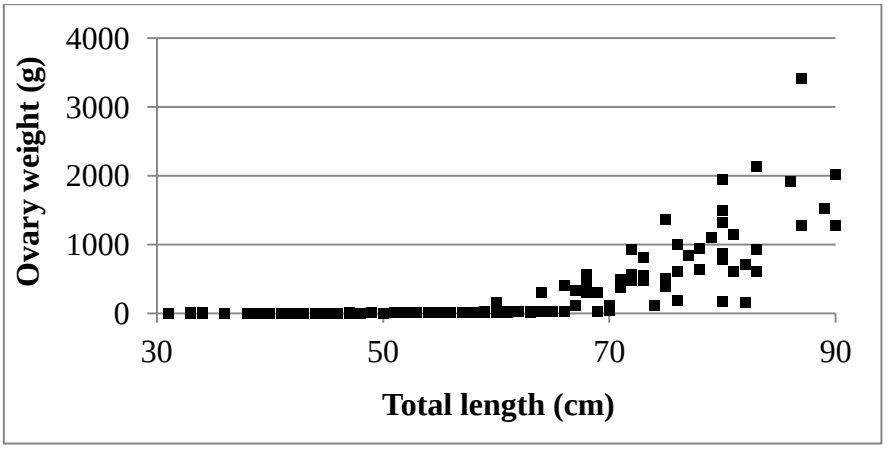

Figure 6. Relationship between total length of Greenland halibut females and ovary weight.

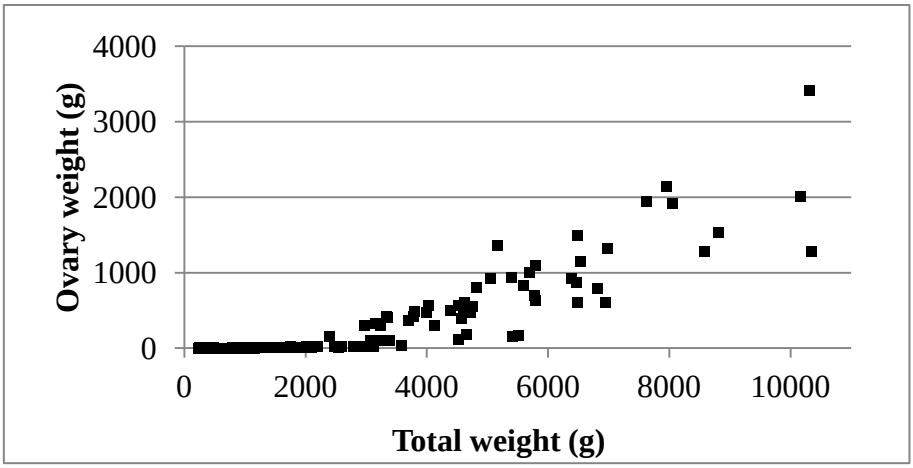

Figure 7. Relationship between total weight of Greenland halibut females and ovary weight.

As shown in Figures 6 and 7, ovary weight starts to increase when total length is around 60 – 70 cm, and total weight is around 3 000 – 4 000 grams.

Gonadosomatic indices of the 138 females (GSI_t) (Figure 8) were in the range 0.1 – 33.1 % (Table 5). However GSI_m, the GSI of those females who were used to estimate fecundity, ranged between 3.1 – 33.1%. The mean GSI increased from 5.3% (GSI_t) to 14.4% (GSI_m). Moreover, GSI_m is positively correlated with total length (Linear regression; $p < 0.05$).

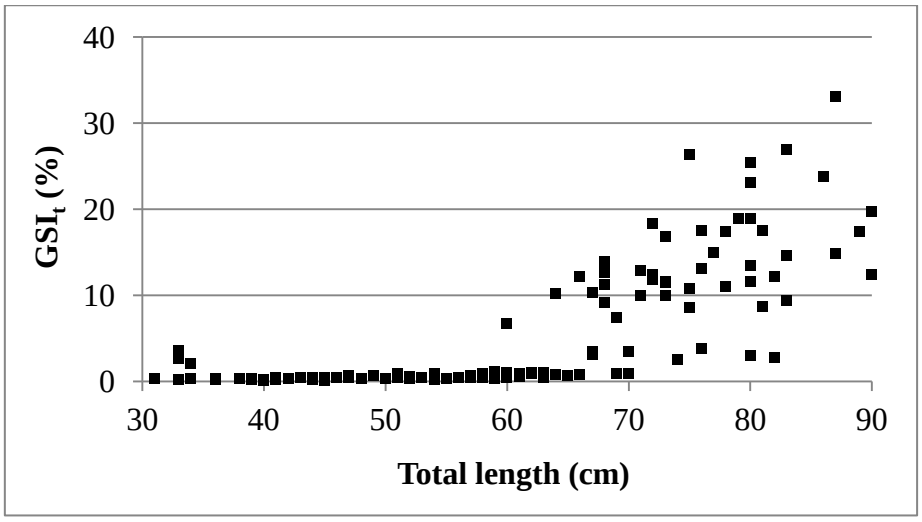

Figure 8. Gonadosomatic index (GSI) against total length for the 138 Greenland halibut females used in the present study.

Fecundity

The potential fecundity estimates ranged from 13 821 to 104 030 oocytes per female, with an average of 51 670 oocytes per female (Table 5).

The relationship between fecundity (F) and length (L) (Figure 9) is:

$$F = 2 \times 10^{-6} \times L^{3.9418} \quad (r^2 = 0.5798)$$

The relationship between fecundity (F) and weight (W) (Figure 10) is:

$$F = 0{,}003 \times W^{1.251} \quad (r^2 = 0.6473)$$

Fecundity estimations are expressed in thousand oocytes per females, total length in cm and total weight in grams.

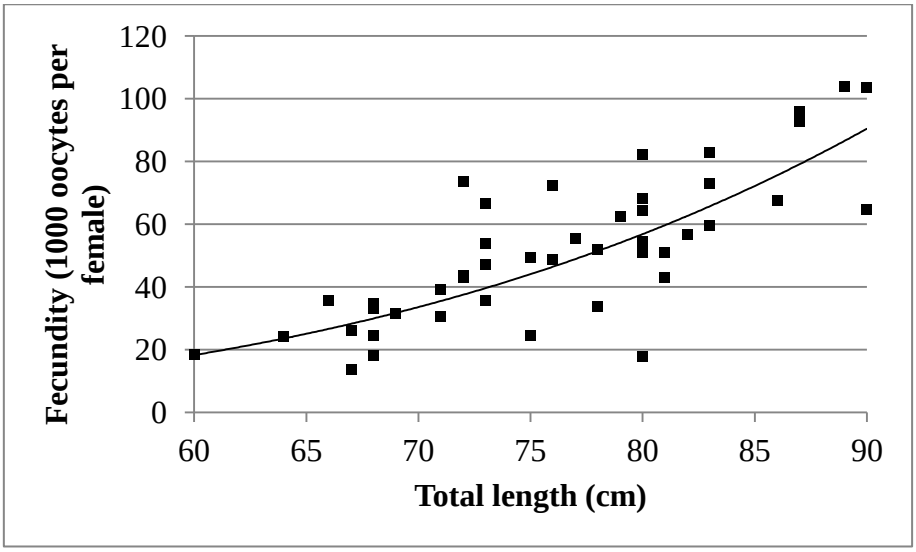

Figure 9. Fecundity estimations (in thousands) of Greenland halibut related to total length (L) (cm), given as $F = 2 \times 10^{-6} \times L^{3.9418}$ (r^2 = 0.5798).

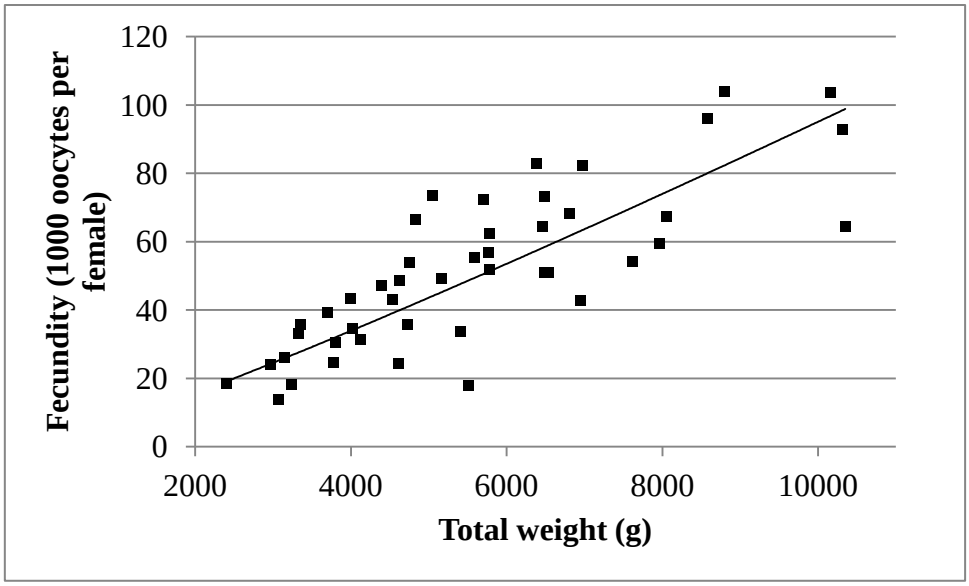

Figure 10. Fecundity estimations (in thousands) of Greenland halibut related to total weight (W) (g), given as $F = 0.003 \times W^{1.1251}$ ($r^2 = 0.6473$).

Maturity stages

In connection with description of maturity stages of the ovaries, the new scale proposed by Kennedy et al. (2011) was followed as explained in the Material and Methods chapter.

In stage 1 there are only immature previtellogenic oocytes, which stuck together by the ovarian tissue; therefore, it is difficult to spread them as shown in figure 11.

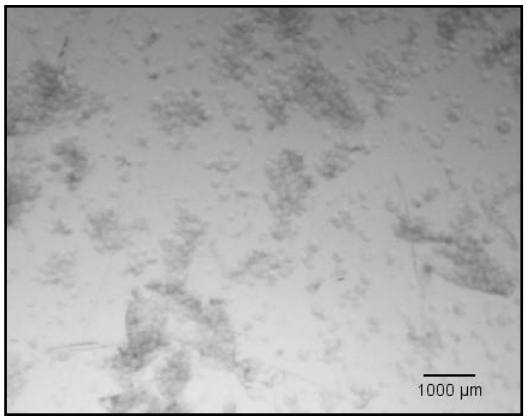

Figure 11. Picture of oocytes from an ovary in stage 1, only previtellogenic oocytes.

Stage 2 is characterized by containing previtellogenic oocytes but also cortical alveoli oocytes that are not larger than 500 µm (Figure 12).

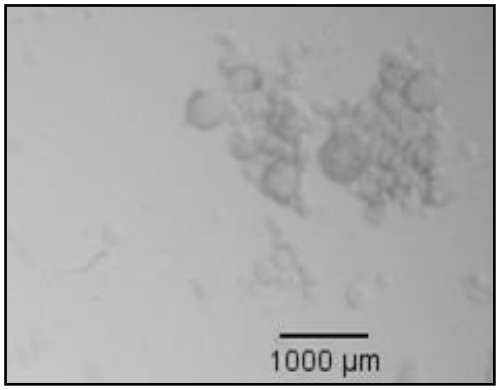

Figure 12 Picture of oocytes from an ovary in stage 2, previtellogenic oocytes and small cortical alveoli oocytes.

Vitellogenesis starts in stage 3 (Figure 13), however, the vitellogenic oocytes are smaller than 1 300 µm. Therefore, the individual has started its maturation process but these oocytes are not going to be spawned in the coming season.

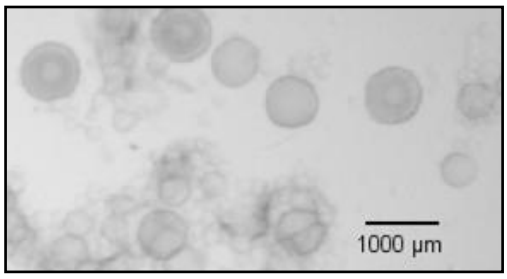

Figure 13. Picture of oocytes from an ovary in stage 3, small vitellogenic oocytes.

Stage 4 is distinguished due to the fact that through this point of maturation of the oocytes, the individual starts to be a part of the spawning stock. Vitellogenic oocytes are larger than 1 300 µm (Figure 14) and oocytes in earlier maturation stages are also found.

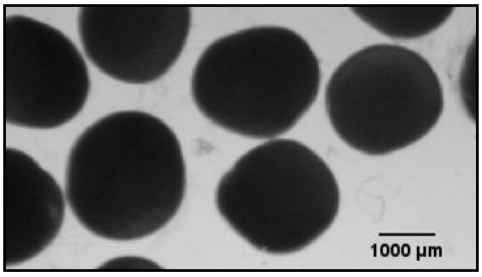

Figure 14. Picture of oocytes from an ovary in stage 4, large vitellogenic oocytes.

Stage 5 indicates the full maturation of the oocytes, they become hydrated and transparent (Figure 15). Usually, all the oocytes become hydrated at the same time, but some individuals were found with large vitellogenic oocytes mixed with the hydrated ones, showing that the process does not happen at once for all the cohort of mature oocytes.

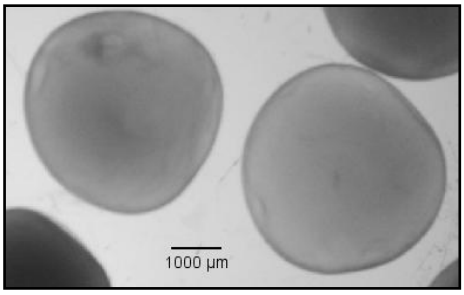

Figure 15. Picture of oocytes from an ovary in stage 5, hydrated oocytes.

As mentioned earlier, oocytes that are going to be released in the coming spawning season are the ones in stages IV and V. The largest oocyte in stage IV had a diameter of 3 662 µm (Table 6) while the largest oocyte in stage V, the hydrated ones, was 4 609 µm.

Table 6: Mean, standard deviation (Std) and range diameters for oocytes in stages IV and V.

Oocyte size (µm)	Mean	Std	Minimum	Maximum
Stage IV	2 559	242	1 356	3 662
Stage V	3 706	303	3 259	4 609

The relationship between the total length of Greenland halibut females and ovarian maturity stage is clear (Figure 16). Earlier stages are found in small individuals and, while length increases the individuals mature. However, length ranges for each maturity stage overlap with the others, showing that maturation does not only depend on length, but also on other factors.

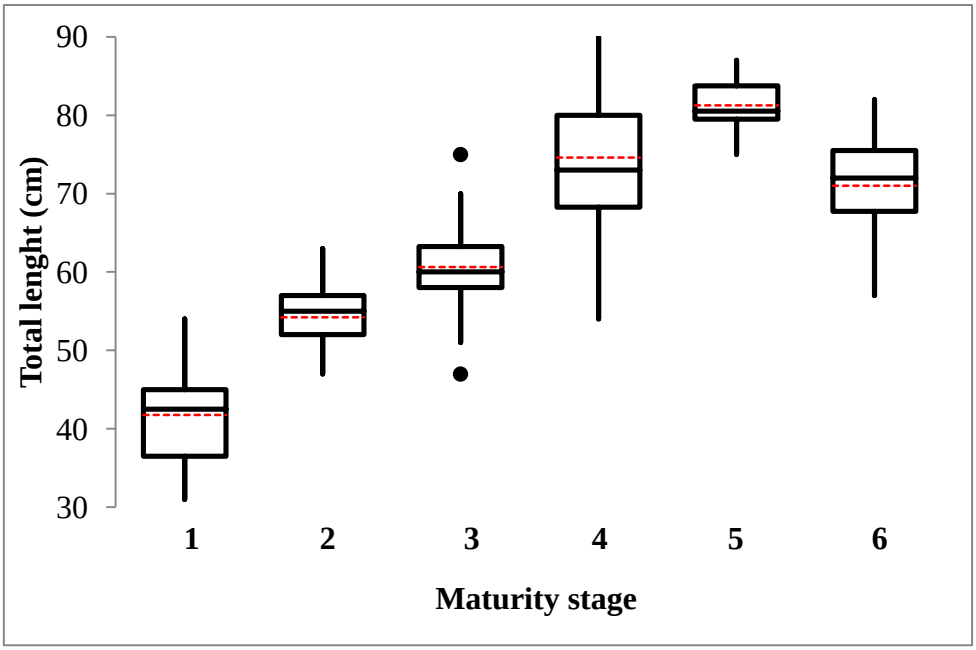

Figure 16. Box Plot showing the total lengths of Greenland halibut females within each maturity stage. Vertical lines indicate the range of data, the solid black line indicates the median of each category and the dashed red line indicates the mean value. Black dots indicate moderate outliers.

The percentage of each maturity stage found within the different length-groups (10 cm) of Greenland halibut females is shown in Figure 17. Stages 1, 2 and 3 (blue colors) correspond to individuals that are not going to spawn the nearest season, and stages 4, 5 and 6 (red colors) are individuals that are going to spawn or have spawned already. Sexually mature individuals are present from 50 cm total length, increasing in percentage with length. On the other hand, immature individuals are present in all the length ranges under 80 cm.

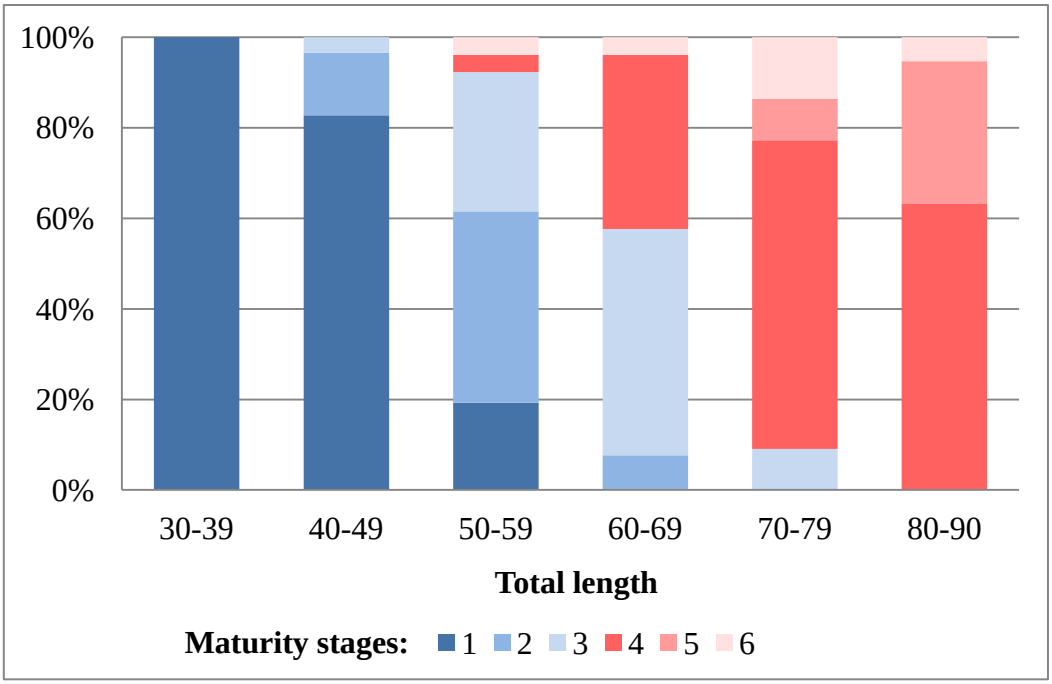

Figure 17. Percentage of each maturity stage for 10 cm length groups of females of Greenland halibut. Blue colors represent the individuals that are not going to spawn and red colors represent individuals that are going to spawn or have spawned already.

With this study we also wanted to check the accuracy of the macroscopic ovarian scale used at sea, compared with the new one based on oocyte measurements. It is difficult to compare stage by stage since the scales are not equal. However, an approximate comparison has been made in order to evaluate both scales (Table 7).

Table 7. Number of females assigned to each maturity stage. Comparison of the maturity stages assigned to Greenland halibut at sea using the macroscopic special scale for Greenland halibut with the ones assigned in the lab by using the new scale based on oocytes diameter measurements.

Field	Laboratory					
	1	2	3	4	5	6
1	46	17	16			
2			7			
3			1	2		
4				34	1	
5				1	7	1
6						5

The main difference between the two scales is found at the lowest maturity stages, as shown in Table 7. Ovaries stated at stage 1 at sea are stated in the lab as stage 1, 2 or 3. Ovaries that were stated as stage 2 at sea were finally classified on stage 3.

Another difference between scales is where the researchers placed the separation between mature and immature individuals. IMR uses two macroscopic scales, as explained before, in both cases, classifying stage 1 as immature and the others as mature individuals. They calculated the L50, equal to 61.5 cm, with these data from all the survey (Figure 18).

Since this study states the maturity stages based on the new scale proposed by Kennedy et al. (2011), where the separation between spawners and not spawners is based on oocyte diameter measurements; two new L50 were calculated using only the samples of this study but based on both methods (macroscopic and microscopic), to be able to compare them. With the macroscopic scale (>1 mature), L50 is 62.37 cm (SE:1.04), less than one centimeter higher than the same calculation with all the individuals of the survey. On the other hand, by separating the mature individuals by diameter measurement of the oocytes, L50 is 65.61 cm (SE: 1.11), more than three centimeters higher than using the macroscopic method.

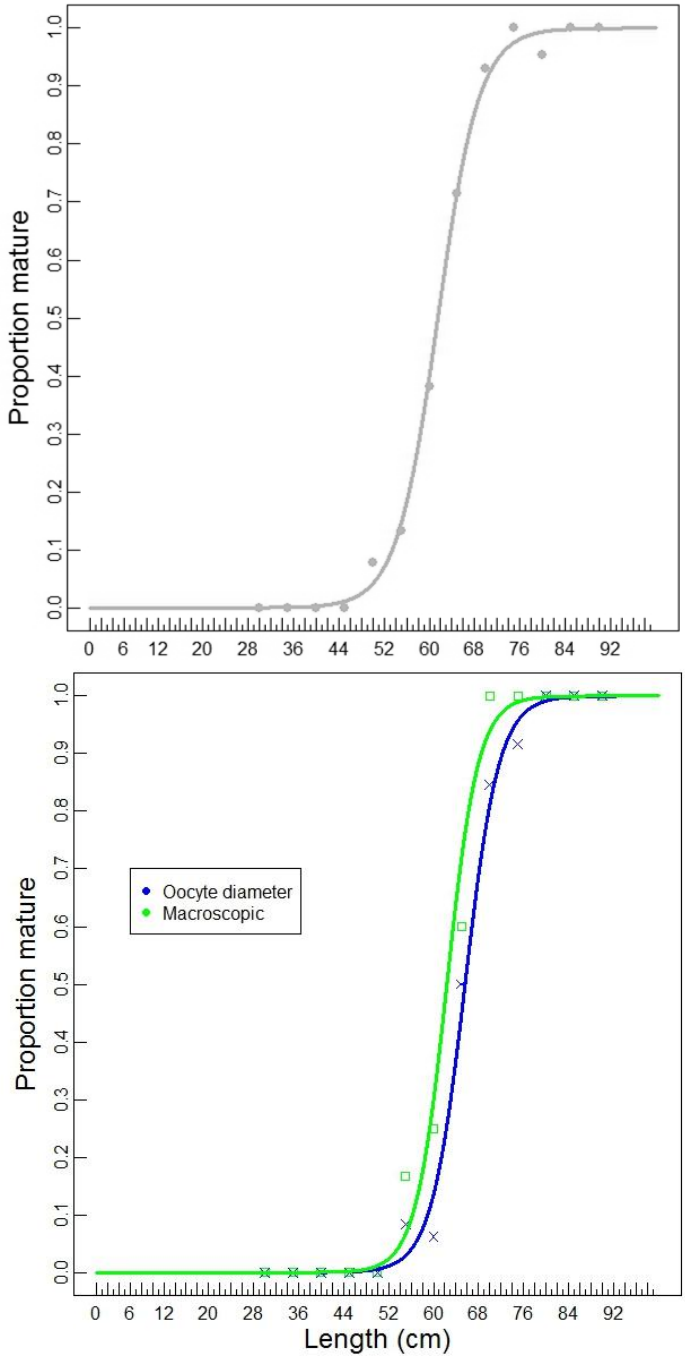

Figure 18. Maturity-length for Greenland halibut females. Top: all females of the survey based on macroscopic staging (>1 is mature). Bottom: 138 females used for the present study based on macroscopic staging (>1 is mature) (green) and based on new scale measuring oocyte diameter (blue).

Total egg production (TEP)

For this purpose, three different methods were used to make the calculations:

Mature 1: all females of the survey were used for the calculations. Maturity stages were stated using the macroscopic scale at sea (Special stages scale for Greenland halibut (Table 3)).

Mature 2: all females were used but they were stated based on the maturity stages given for the samples of the present study using the macroscopic scale at sea (Special stages scale for Greenland halibut (Table 3)).

Mature 3: all females were used; they were stated based on the microscopic scale (Table 4).

Total number of Greenland halibut females in the survey was 19.5 millions. The number of mature females was calculated by the different methods explained before, as it is shown in Table 8, was 5,7 mill, 5,3 mill and 4,1 mill respectively for Mature 1, Mature 2 and Mature 3 method. The number of mature females was lower when the new method was followed.

As it is possible to see in Figure 19, there is a peak of individuals with length 40-50 cm. With regard to mature individuals for all the methods, the peak is around 70 cm.

Table 8. Number of individuals (N ind) (in millions), biomass (in 1 000 tonnes) and total egg production (TEP) (in billions), calculated by the different methods (see text).

	Mature females			Total females
	Macroscopic scale		New scale	
	Survey (Mature 1)	Present study (Mature 2)	Present study (Mature 3)	Survey
N ind (mill.)	5.7	5.3	4.1	19.5
Biomass (1 000 t)	21.5	20.9	17.3	36.9
TEP (billion)	201.3	196.3	162.5	-

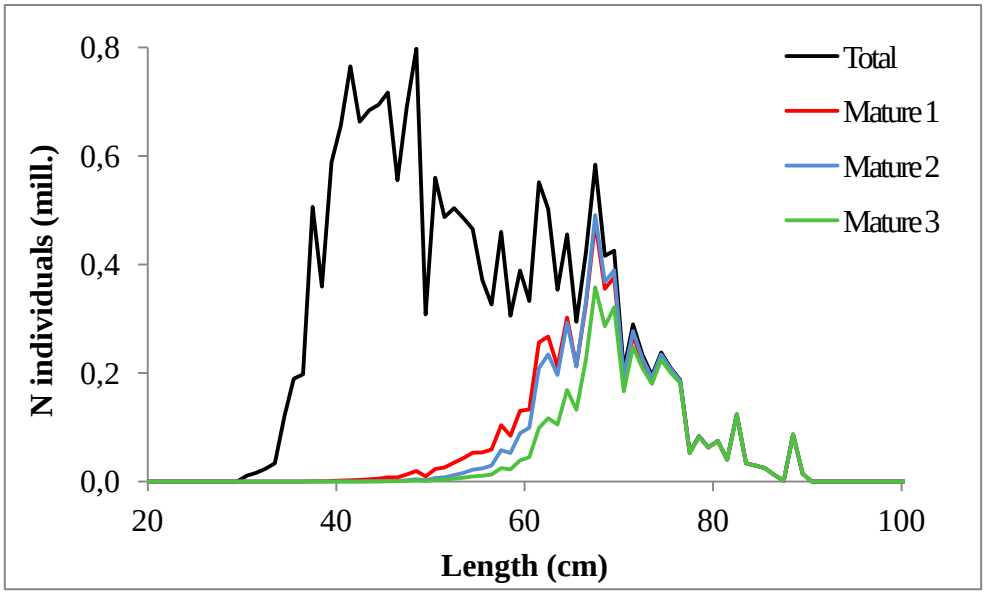

Figure 19. Number of females of Greenland halibut (in millions) by length (in cm). Black line represents total number of females in the survey, red line represents the number of mature females, following Mature 1 method; blue line shows the number of mature females following Mature 2 method and the green line, the number of mature females following Mature 3 method (see text).

Biomass was calculated using the formula explained in the material and methods chapter (Equation 5). Total biomass (Table 8) was equal to 36.9 thousand tonnes. For the mature females, biomass was 21.5 thousand tonnes when Mature 1 method was used and 17.3 thousand tonnes when using Mature 3 method. Biomass of mature females was lower when using Method 3, as it was the number of mature females.

Figure 20 shows a peak for total biomass around 70 cm for all the calculations, also for the total biomass. Small individuals contribute to increase the number of fish; however, the larger individuals are the ones contributing to the biomass.

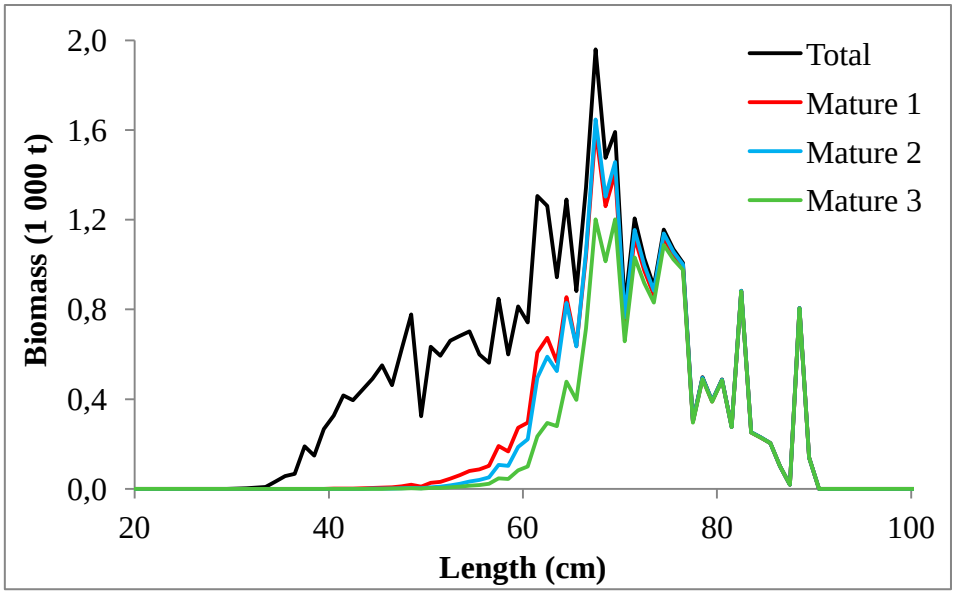

Figure 20. Biomass of females of Greenland halibut (in thousand tonnes) by length (in cm). Black line represents biomass of the total number of females in the survey, red line biomass of the mature females following Mature 1 method, blue line biomass of the mature females following Mature 2 method and the green line, biomass of the mature females following Mature 3 method (see text).

TEP (Table 8) was 201.3 billions calculated using Equation 6 and 7, with the Mature 1 method and 162.5 billions with the Mature 3 method. A decrease of almost 20% of the TEP takes place when using the new method.

Starting with individuals of 50 cm, TEP increases (Figure 21) to a maximum at 67 cm for the three calculations, the value is lower for TEP calculated with the microscopic scale (>3 spawners). After 70 cm, TEP decreases irregularly, with two peaks in larger individuals.

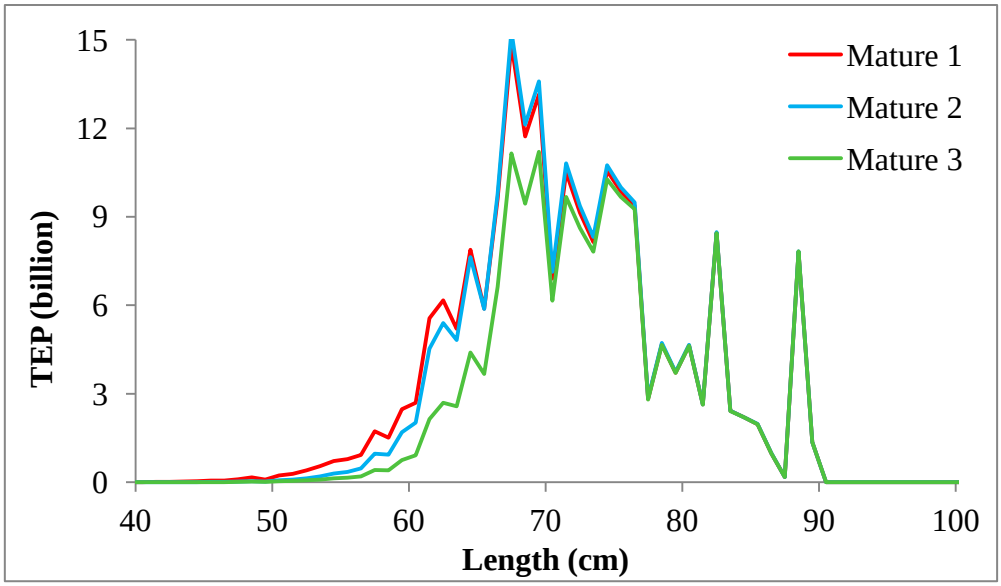

Figure 21. Total egg production (TEP) (in billions) for Greenland halibut shown by length (in cm). Red line indicates TEP calculated following Mature 1 method, blue line shows TEP calculated following Mature 2 method and the green line represents TEP calculated following Mature 3 method (see text).

Within the population, lengths from 66 cm to 80 cm contribute to the majority of the egg production (Table 9), producing 70% of the TEP.

Table 9. Total egg production (TEP) (in millions) at length (cm), and proportion (%) of TEP for each length range.

Length (cm)	TEP (in millions)	%
46-50	0.05	0.03
51-55	0.36	0.22
56-60	1.93	1.19
61-65	12.72	7.83
66-70	42.09	25.91
71-75	42.54	26.19
76-80	30.10	18.53
81-85	20.33	12.51
86-90	12.31	7.58

Discussion

Sampling took place in December, corresponding with the peak of the spawning season (Albert et al. 1998), during a short period of time; therefore, the different parameters measured should not be influenced by growth of the individuals. Maturation of Greenland halibut based on GSI was described by Federov (1968) in Gundersen et al. (1999), and assumed that GSI prior to spawning reaches a level of 15-18%. GSI for this study was in the range of 0.1 – 33.1 %, with ten individuals over 18%, showing that the spawning season had begun. This was further confirmed by the observation of individuals that had spent ovaries.

Fecundity

There are several problems linked to fecundity estimations, one of them is atresia, or reabsorption of developing oocytes, which leads to an overestimation of fecundity. However, in this case, this problem is likely solved due to the fact that samples were taken in the middle of the spawning season, and the few atretic oocytes found were not included in the calculations. Another issue related with Greenland halibut spawning is that it is unclear if they spawn in a single batch or in several (Kennedy et al. 2009), which could lead to underestimation of the fecundity. Nevertheless, in this study, as Kennedy et al. (2009) and Stene et al. (1999) assumed, individuals were considered to be single batch spawners.

Even though the relationships between fecundity with weight and length were significant ($p<0,005$), the one with the total weight of the fish fitted better than with total length.

There are fecundity estimations for the past years in the same area (Gundersen et al. 1999, 2000). Individual fecundity estimations from this study ranges from 13 821 to 104 030 oocytes per female and are within the range of individual fecundity estimations from the other studies. If a further detailed comparison to earlier studies in the Barents Sea is made (Table 10), it is shown that fecundity estimations for 2011 are more similar to 1996 than to the other years, presenting 2011 as the year with a higher value for individuals of 70 cm.

However, these relationships do not explain the reason why some individuals of the same length are mature and ready to spawn, and others have not even start the

maturation process. This might be explained by a negative energy balance, it is known for other species such as cod, that liver condition index (hepatosomatic index) is positively correlated with recruitment (Marshall et al. 1999). Greenland halibut studies relating hepatosomatic index with fecundity were carried out (Gundersen et al. 1999) with no significant relationship observed. This is due to the fact that this species does not store the majority of the lipids in the liver, but, as other flatfish species, fat is stored in the muscle (Kennedy et al. 1999). Another reason may be the fact that Greenland halibut females do not spawn every year, as described by Kennedy et al. (2011).

Table 10. Comparison of fecundity of Greenland halibut in the Barents Sea area showing the year when the fish was caught, the reference of the data, length range of fish in each study and fecundity in thousands of an individual of 70, 80 or 90 cm calculated from the length-fecundity relationship.

				Fecundity (x 1000)		
Year	**Month**	**Reference**	**Length range (cm)**	**70**	**80**	**90**
1996	Jul-Aug	Gundersen et al. (1999)	60-80	35	65	112
1997	Aug-Oct	Gundersen et al. (2000)	52-91	29	55	97
1998	Sep	Gundersen et al. (2000)	54-95	31	52	83
2011	Dec	Present study	60-90	38	63	101

Maturity stages

In relation to the comparison of the maturity scales used, both macroscopic at sea and microscopic ovarian staging in the lab, the main differences were registered in the lower stages. However, the separation between mature and immature individuals is as important as the different descriptions for each stage given for the three scales are. An important goal of the IMR-survey was to estimate the number of females that are going to spawn in the nearest spawning season. For this reason it is important to highlight the difference between mature individuals and individuals that are going to spawn the coming season. Greenland halibut is a species with slow development and maturation, it is known that maturation of oocytes can last two years (Kennedy et al. 2011). Therefore, as they usually do in the IMR, they are including individuals that might be sexually mature but they are not going to spawn this season. This leads to an overestimation of the spawning stock biomass and the total egg production, as it was

shown in the results chapter (Figures 20, 21), where it was found a decrease in the TEP of 20% when using the new method.

When the standard macroscopic scale is used (Table 2), the division is made between immature (Stage 1) and the others (maturing, spawning and spent), the same is true for the macroscopic scale specially made for Greenland halibut (Table 3). In the case of the new scale (Table 4), the division is based on the diameter of the oocytes, stages 1, 2, 3 (<1 300 µm) and stages 4, 5 and 6 (>1 300 µm), that is, spawners or not spawners.

Even though the microscopic method is the most accurate one, it is expensive and limited since it requires time consuming work in the lab after the survey, and it is necessary to use formalin at sea for fixation, which is not allowed at all ships. Thus, instead of proposing the use of this new scale based on oocytes measurements, an alternative method is proposed in this study.

Since we are looking for a macroscopic method to be carried out at sea, in an easy way and more accurate than the present one, we decided to change the boundaries between immature and mature individuals to be close to the required boundary (between individuals that are going to spawn and the ones that are not going to spawn the nearest season). In order to find the best method, L50 was calculated with the same data but with different maturity scales and limitations for the immature stages (Table 11). It is assumed that the most accurate L50 is 65.61 cm, the one that was calculated following the microscopic method. So, we will try to get a value as close as possible to this one by changing the limit in the macroscopic scales.

Using the standard scale (Table 2), if we move the limit one stage further, only individuals with hydrated oocytes are included as spawners; therefore, this is not a valid scale, even after we move the boundary. For the special stage scale (Table 3) made for Greenland halibut, it was found that if the limit is moved one stage, including stage 1 and 2 as not spawners, the L50 changes from 62.37 cm to 65.21 cm, very close to the most accurate one (65.61 cm).

Table 11. L50 (cm) calculated for different maturity scales moving the limit between mature and immature (or individuals that are going to spawn or not).

Macroscales				Microscale	
Standard		Special			
Stage imm.	L50 (cm)	Stage imm.	L50 (cm)	Stage imm.	L50 (cm)
1	62.4	**1**	62.4		
		1,2	65.2	**1,2,3**	65.6
		1,2,3	66.5		
1,2	87.2	**1,2,3,4**	86.6		

When plotting the two curves (Figure 22), the one using the microscopic scale and the one using the macroscopic scale special for Greenland halibut but including stages 1 and 2 as individuals that are not going to spawn, it is demonstrated that if the boundary is moved, it is possible to have more accurate data using the macroscopic scale. For instance, if TEP is calculated using the special macroscopic scale with stages 1 and 2 as immature, the result is 166.3 billions, very similar to 162.5 billions which was the TEP calculated with the microscopic scale (Table 8); thereby, reducing the overestimation of TEP.

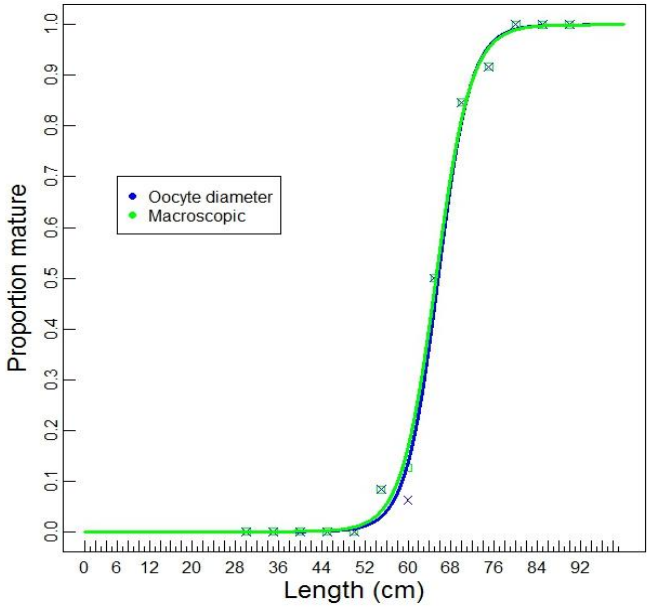

Figure 22. Maturity-length for Greenland halibut females. Green line shows the proportion using the microscopic scale and blue line shows the proportion using the macroscopic special scale, but adjusting the boundary between spawners and not spawners, from only stage 1 as not spawners to stage 1 and 2.

Conclusion

Greenland halibut fecundity for year 2011 was in the same range as estimations from previous years for the same area; however, further research is needed in order to understand the factors that affect potential fecundity. Regarding ovarian development and maturation, more information is needed to understand the growth dynamics and spawning which is still uncertain for this species.

In relation to the different maturity scales, it is proposed the use of the macroscopic special scale for Greenland halibut but adjusting the boundary that divided individuals from mature to immature. Moving this limit one stage further, it gets closer to the division between individuals that are going to spawn the nearest season and the ones that are not going to spawn, which is important for assessment; thereby, the spawning stock size is unlikely to be overestimated.

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