Greenland halibut (*Reinhardtius hippoglossoides*) spawn annually but successive cohorts of oocytes develop over 2 years, complicating correct assessment of maturity

James Kennedy, Agnes C. Gundersen, Åge S. Høines, and Olav S. Kjesbu

**Abstract:** Ovary development in Greenland halibut (*Reinhardtius hippoglossoides*) is complex, with several cohorts of developing oocytes present during vitellogenesis; this is unusual for a determinate spawner. There are also speculations that Greenland halibut are not capable of spawning every year. To investigate this possibility, ovaries from Greenland halibut caught throughout the year were examined histologically, and successive cohorts of oocytes were tracked through development. Results showed that the initial maturation of the ovaries from immature to spawning takes more than 1 year. The ovary initially develops as far as early vitellogenesis; however, the time scale for this is unclear. During the final year of development, the cohort of vitellogenic oocytes splits to form two cohorts; the larger cohort increases in size and is spawned in the coming spawning season. The smaller cohort also continues to develop, but at a much lower rate, in preparation for development for spawning in the following year. Within each month, there is a large range of oocyte sizes between fish; this leads to the extended spawning season that is known in many populations of this species. This complicates the assessment of maturity, and a more accurate microscopic maturity scale is proposed.

**Résumé :** Le développement ovarien chez le flétan du Groenland (*Reinhardtius hippoglossoides*) est un phénomène complexe car il y a plusieurs cohortes d’oocytes en maturation durant la vitellogenèse; cela est inhabituel chez un poisson à reproduction déterminée. On soupçonne aussi que le flétan du Groenland n’est pas capable de frayer chaque année. Afin de vérifier cette possibilité, nous avons étudié l’histologie des ovaire de flétans du Groenland capturés au long de l’année et nous avons suivi les différentes cohortes d’oocytes au cours de leur développement. La maturation initiale des ovaire du stade immature à la fraie requiert plus de 1 année. L’ovaire se développe initialement jusqu’au début de la vitellogenèse, mais l’échelle temporelle de cette étape n’est pas claire. Durant la dernière année du développement, la cohorte des oocytes vitellogènes se divise en deux cohortes; la cohorte des oocytes plus grands augmente en taille et est pondue durant la période de reproduction suivante; la cohorte des oocytes plus petits continue aussi à se développer, mais à un rythme beaucoup plus lent, en vue d’une maturation pour la fraie des années subséquentes. À chaque mois, il y a une grande étendue de tailles d’oocytes chez les divers poissons, ce qui a pour conséquence la saison de reproduction prolongée qu’on connaît chez plusieurs populations de cette espèce. Cela complique l’évaluation de la maturité et nous proposons une échelle microscopic de maturité plus précise.

[Intraduit par la Rédaction]

**Introduction**

Greenland halibut (*Reinhardtius hippoglossoides*) is found throughout the North Atlantic and is divided into several management units. The unit considered in this study, the Northeast Arctic stock, is distributed along the continental slope of Norway from 62°N to the regions north of Svalbard and in the Barents Sea. It inhabits depths between 200 and 1500 m where water temperatures are between –1 and 4 °C (Bowering and Nedreaas 2000). Length at 50% maturity is generally around 60 cm (Morgan et al. 2003), and peak spawning is believed to take place from November to January; however, females are found in spawning condition throughout the year (Fedorov 1968). Greenland halibut is a determinate spawner (the maximum fecundity of an individual female is determined before the onset of spawning; Hunter et al. 1992) and has a low fecundity in comparison with other determinate spawners (Kjesbu et al. 1998; Gundersen et al. 2000; Kennedy et al. 2007), and eggs collected in the field are documented as being as large as 3.9–4.7 mm (Magnússon 1977; Stene et al. 1999).

Greenland halibut has a cohort of cortical alveoli oocytes (CAO) present in the ovary alongside the developing vitellogenic oocytes (VO) (Fedorov 1968; Junquera and Saborido-Rey 1995; Rideout et al. 1999), a pattern not typical of determinate spawners with a synchronous or group-synchronous spawning event.
pattern of oocyte development. The fate of these CAO have been considered, and theories propose that they are reabsorbed (Fedorov 1968), may be used for a second spawning season in the summer in addition to the main one in winter (Fedorov 1968), are residual (Junquera and Saborido-Rey 1995), are “fast-tracked” through development when the fish is close to spawning to supplement the current year’s fecundity (Rideout et al. 1999), or are spawned in subsequent seasons (Gundersen et al. 2000), which is what occurs in the Atlantic wolffish (Anarhichas lupus) (Beese and Kändler 1969). The fate of the CAO in Greenland halibut, however, remains unresolved.

There are indications that ovarian development in Greenland halibut could last more than 1 year, which also implies that the Greenland halibut does not spawn every year (Albert et al. 2001; Junquera et al. 2003). If this is true, then estimations of the spawning stock biomass (SSB) could be overestimated through the wrong use or interpretation of present maturity scales. Incorrect classification of maturity status can affect the formulation of a maturity ogive and the estimation of SSB (Nielsen and Boje 1995). To prevent this, a reliable method for mature classification, based upon sound knowledge of ovary development, is required. A standardized scale is also essential to allow comparisons between data sets. In the literature, there are several maturity scales for Greenland halibut that differ in many aspects, including the number of stages (Walsh and Bowering 1981; Junquera and Saborido-Rey 1995; Albert et al. 2001). These scales are based upon macroscopic observations, as they are cheap, quick to perform, require no specialist equipment, and can be performed at sea. However, macroscopic staging alone results in variable and inaccurate results, both for Greenland halibut (Nielsen and Boje 1995; Albert et al. 2001) and other species (Claereboudt et al. 2005; Gerritsen and McGrath 2006; Vitale et al. 2006).

The aim of this study was to investigate the indications that ovary development in Greenland halibut may take more than 1 year and whether this would result in individual fish being capable of only spawning every 2 years, which to our knowledge would be a unique reproductive strategy for a marine determinate spawner. Ovaries were examined from Greenland halibut caught on the spawning grounds in the Barents Sea once a month over a period of 1 year. The development of the various cohorts of oocytes was tracked through the year to clarify their fate. The results of this were used to evaluate both the methods currently used to assess maturity and also the maturity scales used for this species.

### Materials and methods

A total of 128 Greenland halibut ovaries from fish between 51 and 90 cm in total length were collected at known spawning locations along the continental slope of northern Norway between January and December 1997 (Fig. 1). Ovaries were macroscopically staged using a seven-stage key (Table 1) by experienced research personnel aboard the ship. The ovaries were then preserved in buffered 3.6% formaldehyde. Total fish length (1 cm) was measured at sea.

In the laboratory, samples of tissue from both ovarian lobes in each fish were embedded in Technovit (resin) following standard protocols. Four histological sections (3 μm) were then prepared for each lobe from the embedded tissue and stained with 1% toluidine blue and 2% sodium tetraborate. The sections taken were considered to be representative of the whole ovary, as Greenland halibut ovaries have been shown to be homogenous in respect to development stage and oocyte diameter (Nielsen and Boje 1995; Rideout et al. 1999). The diameter of all oocytes that were sectioned through the nucleus was measured manually from images using ImageJ (ImageJ Processing and Analysis in Java, http://rsbweb.nih.gov/ij/). The number of oocytes measured in each sample was not equal because of an unequal number on the slides; however, all samples contained a minimum of 20 oocytes. No correction was made for shrinkage of the oocytes, as all samples were treated in the same manner.
Preliminary investigation showed that cortical alveoli began to appear in oocytes when they were approximately 180 μm, and true vitellogenesis began when the oocytes were around 480–520 μm (Fig. 2); this agrees with the results of Rideout et al. (1999) (with slight discrepancies being attributed to the use of resin vs. paraffin embedding media). These size ranges were subsequently used to assign oocytes in whole mounts to developmental categories. Hydrated oocytes (Fig. 2) were noted when present but were not measured because they collapsed during the dehydration process. The leading cohort oocyte diameter (LC) was defined as the average diameter of the largest 10% of oocytes from the largest cohort. The LC was used to indicate the progression of ovary development. The presence of postovulatory follicles (POF) was noted (Fig. 2).

The macroscopic stage was reassessed for each fish in light of the results from the histological sections.

Results

Individual pattern of oocyte development

Starting at the previtellogenic stage, a cohort of oocytes would progress to the cortical alveoli stage with CAO being present throughout the entire development of the ovary (Fig. 3). A group of oocytes would then begin true vitellogenesis, with oocytes being around 480–520 μm in diameter at this stage. These VO were termed vitellogenic cohort 0 (VOC0). As ovary development continued, the average diameter of the VO increased in size, but no hiatus formed between the CAO and VOC0; the range of oocyte sizes simply increased (Figs. 3b–3c). When the LC reached around 1150–1300 μm, a hiatus forms within VOC0, resulting in two distinct cohorts of VO (the larger (in diameter) cohort was termed VOC1 while the smaller was termed VOC2) (Fig. 3d). The VOC1 increased in size over time until hydration (Figs. 3d–3h). The maximum average diameter of VOC1 was 2525 μm; however, the average diameter of VOC1 in one individual where many oocytes had begun hydration (the oocytes that were measured had not begun hydration) was 2320 μm. The largest unhydrated oocyte measured was 2936 μm.

As LC increased, in ovaries containing VOC1 oocytes, the average diameter of VOC2 increased (linear regression; \( r^2 = 0.44, p < 0.0001 \)) (Fig. 4), showing that VOC2 continues to develop at the same time as VOC1 but at a much lower rate. During the development of VOC1 (which increases from an average of about 1000 to 2500 μm), VOC2 increases from approximately 600 to 800 μm, making the growth rate of VOC1 about 7.5 times greater than that of VOC2. The average diameter of the CAO also increased with LC; however, this was only until LC was about 1400 μm (linear regression; \( r^2 = 0.30, p < 0.0001 \)) (Fig. 4). There was a weak neg-

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**Table 1.** Maturity stage used for Greenland halibut from Fotland et al. (2000).

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

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**Fig. 2.** Light micrographs of different stages of oocyte development from Greenland halibut: (a) cortical alveoli stage with previtellogenic (PVO) and cortical alveoli oocytes (CAO); (b) early vitellogenesis showing PVO, CAO, and vitellogenic oocytes (VO); (c) late vitellogenesis; (d) hydrated oocyte (HO); (e) postovulatory follicle (POF). Bars for panels a, b, c, and e = 500 μm; bar for panel d = 1000 μm.
Fig. 3. Oocyte size distributions of Greenland halibut at different stages of ovary development. Open columns = cortical alveoli oocytes, grey columns = vitellogenic oocytes in VOC₀, hatched columns = vitellogenic oocytes in VOC₁, black columns = vitellogenic oocytes in VOC₂ (see text for explanation of VOC₀, VOC₁, and VOC₂). Month refers to the month in which the fish was caught; LC is leading cohort oocyte diameter. Hydrated oocyte sizes are not shown on panel h because these were not measured. The purpose of the figure is to examine cohort formation rather than to accurately quantify the number of oocytes represented in each cohort; therefore, the bias due to the decreased likelihood of smaller oocytes being sectioned through the nucleus in comparison with larger oocytes has not been corrected for.
ative relationship between the diameter of CAO and LC when LC was greater than 1400 μm (linear regression; $r^2 = 0.16, p = 0.005$) (Fig. 4).

Five individuals were caught with hydrated oocytes in the ovaries. Two of these were caught in January, one in May, and two in August and were between 61 and 73 cm in length. POFs were present in the ovaries of 15 fish that were caught in January, February, August, November, and December. All of these individuals had vitellogenic oocytes with an LC between 600 and 1100 μm, i.e., there was only a single cohort of vitellogenic oocytes.

**Annual pattern of oocyte development**

Between January and April, the size range of LC between individuals was relatively similar; however, by about April–May two separate groups of individuals formed (Fig. 5). The LC of the group with the larger oocytes (termed group 1) increased through the year. The LC then reached a maximum in December–January, indicating that spawning took place around this time. The LC of the group with the smaller oocytes (termed group 2) remained relatively constant through the year. The average length of fish in group 2 was significantly smaller than the fish in group 1 (analysis of variance, ANOVA; $p = 0.001$). The length range of the fish in groups 1 and 2 were 59–90 cm and 51–73 cm, respectively. There are indications that group 2 may consist of two groups: one group having an LC between 500 and 1000 μm between June and December, and another group (group 3) having an LC between 250 and 500 μm. However, owing to the low number of samples, it is very difficult to discern with certainty whether this is one or two groups.

**Maturity scale**

Comparison of the results from assessing the maturity stages (listed in Table 1) macroscopically and histologically showed there was very little agreement between the two methods. Only 41% of the samples were correctly identified.

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**Table 2.** Comparison of the maturity stages assigned to Greenland halibut in the field macroscopically and in the laboratory using histology.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Field 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
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<td></td>
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<tr>
<td>2</td>
<td>18</td>
<td>11</td>
<td>2</td>
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<td>3</td>
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<td>6</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td>4</td>
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</tbody>
</table>

Note: Actual number of fish are shown; those correctly identified are shown in bold.

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Kennedy et al. 205

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in the field (Table 2). Ovaries in stages 2 to 4, which were wrongly assigned macroscopically, tended to be assigned to earlier stages.

**Discussion**

Previous studies on Greenland halibut have used the terms G1 and G2 to distinguish the different cohorts of oocytes present in the ovary (Gundersen et al. 1999, 2001). This terminology originated from a study on *Blennius pholis* by Shackley and King (1977), who believed each group represents a single egg batch; however, it is possible for a single “group” of vitellogenic oocytes to give rise to many egg batches, e.g., as seen in Atlantic cod (*Gadus morhua*) (Kjesbu et al. 1990). The current study introduces new terminology; the previous terminology proved unsuitable, as they did not distinguish between the different stages of oocytes, i.e., vitellogenic and cortical alveoli. The term VOC1 from the current study corresponds with the term G1. The term G2 was used to refer to the smaller cohort of oocytes, which may have encompassed both VOC2 and CAO oocytes. As previous studies have mostly focused on fecundity of individuals close to spawning, there is no term in the literature that corresponds with individuals that only contain VOC0.

**Individual cycle**

Greenland halibut appears to have a very unique ovary development pattern, which is unlike that of any previously documented teleost. As expected for teleosts, ovary development began with the oocytes recruiting into the cortical alveoli stage and then proceeding to an early stage of vitellogenesis. However, as ovary development proceeded, two distinct cohorts of vitellogenic oocytes formed, with the two cohorts developing at different rates. The larger (in diameter) cohort (VOC1) developed through the year and is spawned in the coming year’s spawning season. The second cohort (VOC2) developed at a much lower rate. These two cohorts are likely the result of two “pulses” of recruitment of vitellogenic oocytes from the stock of CAO.

While the VOC1 is developing, the size of the CAO increases; however, no hiatus forms between the previtellogenic oocytes (PVO) and the CAO. A similar pattern is seen between CAO and VOC2, with no hiatus forming even though the average diameter of VOC2 increases. This indicates there is a continuous recruitment of both CAO from PVO, and VOC2 from CAO, throughout the entire development of the ovary. When the LC reaches approximately 1400 μm, the diameter of the CAO decreases with further ovary development. This indicates that the rate of oocyte recruitment from PVO to CAO may not be constant, with an initial high pulse of oocyte recruitment followed by a lower rate of recruitment. When the LC reaches 1400 μm, this may mark the recruitment of the initial pulse of CAO recruiting to the VOC2.

Rideout et al. (1999) suggested that the CAO and early VO present during late vitellogenesis and spawning are oocytes that are being fast-tracked to supplement the current year’s fecundity, suggesting that Greenland halibut may have an indeterminate spawning strategy. It was also stated that a spent individual that contained no CAO or VO was proof that these oocytes are not used for the following year’s spawning. From the oocyte development rate, the VOC2 oocytes are unlikely to be matured in time for spawning. In addition, the average diameter of the VOC2 of individuals with hydrated oocytes or POFs present in their ovaries were similar to the LC of fish in January; this supports the hypothesis that the oocytes of VOC2 are used for the following year’s spawning. The reason for the absence of CAO or VO in a spent individual may be because that individual will skip the following breeding season (Fedorov 1971). Fedorov (1968) suggested that the CAO are eventually reabsorbed or may be used for a second spawning season in the summer in addition to the main one in winter. Again, from the oocyte development rate of VOC2, which is about 7.5 times lower than that of VOC1, this appears to be unlikely. From the hiatus present in the oocyte distribution between the VOC1 and VOC2 in both the present study and Cooper et al. (2007), we support the current consideration that Greenland halibut is a determinate spawner (Junquera and Saborido-Rey 1995; Junquera et al. 1999). Potential fecundity refers to the standing stock of vitellogenic oocytes, while the definition of determinate fecundity is that the standing stock of advanced vitellogenic oocytes before spawning is equivalent to the total potential fecundity for the year (Hunter et al. 1989). For Greenland halibut, this is not strictly true, as the ovary contains two sets of vitellogenic oocytes, only one of which contributes to the annual fecundity. However, no oocytes appear to move from VOC2 to VOC1 after the two groups split; this therefore sets a point in ovary development where the annual fecundity can no longer be increased. Hence, the maximum fecundity is determined before the onset of spawning, which is the main characteristic of determinate fecundity.

It appears that this study and one previous study on wolffish (*A. lupus*) (Beese and Kändler 1969) are the only documented examples of CAO being present throughout the ovary development in a determinate spawner inhabiting high latitude, cold waters. In Greenland halibut, this is likely to be a result of a combination of (i) the low development rate of the smaller cohort of oocytes (CAO and VOC2), which results in an extended period of recruitment of CAO to VOC2 and (ii) that as the fish approaches spawning, the next cohort oocytes begin to develop, leading to the recruitment of more CAO from PVO. This results in the CAO being present throughout the entire development.

The results showed that full maturation and hydration occurs when the average oocyte diameter reaches approximately 2300–2500 μm and that individual oocytes can reach up to 3000 μm before hydration. One fish, which was in the process of hydration, had unhydrated oocytes with an average diameter of 2300 μm. It is unclear whether this represents variation in investment in oocytes between individuals or if the hydration occurs in larger oocytes before smaller oocytes. The number of samples with such advanced oocytes were small, so further studies with a greater number of samples are needed to accurately assess the variation in the maximum size oocytes attain before hydration.

**Annual cycle**

It was seen that there were two distinct groups of sexually mature females that differed in their pattern of oocyte devel-
opment throughout the year: one with the LC increasing in diameter from January to December and another where the LC remained relatively constant through the year. The fish in the group that showed a constant LC through the year (group 2) were generally shorter than fish from the group with an increasing LC (group 1). This leads us to believe that the fish from group 2 may never have spawned before and had begun ovary maturation for the first time in that year. A previous analysis of Greenland halibut survey data taken between October 1997 and May 1998 using log-transformed gonadosomatic (GSI) values also showed two distinct groups of “maturing” females: one with less-developed gonads and another close to spawning (Albert et al. 2001). The GSI of the smaller gonads began to increase in May, and it was concluded that this group consisted of first-time spawners that would go on to develop their ovaries for spawning in the next spawning season. The GSI data from Albert et al. (2001) is taken from the same population as the data in the present study; however, no oocyte growth data were presented, which is clearly needed to get a better insight of the underlying reproductive strategy (Kainge et al. 2007).

Combination of the results from Albert et al. (2001) and the present study leads us to conclude that the development of the ovaries for the first spawning event will take more than 1 year, as the LC in January is between 700 and 1000 μm and reaches a maximum in December–January. Therefore, to reach 700 μm the fish must have started oocyte development in the previous year; we considered it impossible for the fish to develop their oocytes from 180 to 700 μm in less than 1 month. If these were to grow at a rate similar to the VOC1 oocytes, which grew about 140 μm per month, then it would take close to 4 months. Because of the low number of sampled fish in group 2, it is difficult to correctly assess the growth rate of oocytes from previtellogenesis to vitellogenesis around 700–1000 μm. It is also difficult to discern the point of initiation of development because of spread of the LC diameter in the group 2 fish. Group 1 fish also had a large spread in LC diameter, and this may be due to a large spread in the timing of the initiation of development. However, this large spread may also indicate that ovary development may take up to 3 years, as there are indications of a third group of fish, with group 2 having an LC between 500 and 1000 μm between June and December and group 3 having an LC between 250 and 500 μm. However, owing to the low number of fish in group 2, it is difficult to be sure which is correct. Previous evidence for such a prolonged phase of ovarian development has been seen indirectly for Greenland halibut in the Northwest Atlantic, where there is an interval of 4 years between the age of the females that are at the onset of ovarian development and the age of the females that are actually spawning (Junquera et al. 2003).

Junquera et al. (2003) suggested that as a consequence of ovarian development taking more than 1 year, individual spawning would not necessarily occur on an annual basis. From our results, it appears that spawning could occur on an annual basis because of the simultaneous development of the current and following year’s oocytes. This seems to be a unique strategy, i.e., to our knowledge not shown previously for any teleost. The reason for such a unique strategy is unknown, but because of the large diameter of the oocytes and the low temperatures experienced by this species, they may be physiologically unable to complete the entire maturation process within 1 year; ovary development rate decreases with decreasing temperatures (Kjesbu 1994). If this is the case, then to avoid the need for biennial spawning as seen in white sturgeon (Acipenser transmontanus) (Doroshov et al. 1997), which is also known to have large eggs (Webb et al. 1999), then the fish must carry out this simultaneous development of the current and following year’s oocytes. The only known boreal species that have eggs of a comparable size is the Atlantic halibut (Hippoglossus hippoglossus), with a maximum unhydrated oocyte of 2050 μm (Haug and Gulliksen 1988), but they have an annual ovary cycle. However, assuming the oocytes are a sphere, this equates to an oocyte volume of 40% less than that of Greenland halibut, assuming maximum oocyte size is 2500 μm, which can account for the difference between the development times of the ovary of the two species.

It can be seen that there was a large range in LC within each month. The oocyte development pattern of the fish with the largest oocytes from February, March, May, and August demonstrates a pattern that is consistent with fish spawning in August–September. This was supported by the capture of fish with hydrated oocytes in August. The large range of oocyte size within each month supports an extended spawning season, with the fish with the smallest oocytes in August–October probably not having fully matured oocytes until February; additional evidence of this extended season is the capture of a fish in May that contained hydrated oocytes, as well as POFs being present in the gonads many months of the year. However, the presence of POFs alone can be misleading, as it is unknown how long POFs persist in the ovaries of Greenland halibut. The present study largely agrees with the findings of Fedorov (1968), who found a peak spawning event between November and January, but also found females in spawning condition throughout the year. Albert et al. (2001) document this summer spawning component and ask where these fish came from, as they state these were not present in the offshore grounds in winter (based upon analysis of GSI). How-

### Table 3. Proposed maturity scale for Greenland halibut deduced from measurement of oocyte distribution.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immature. Only previtellogenic oocytes present.</td>
</tr>
<tr>
<td>2</td>
<td>Cortical alveoli. LC &lt; 500 μm.</td>
</tr>
<tr>
<td>3</td>
<td>Vitellogenesis 1. LC &gt; 500 μm but there is no hiatus present within the cohort of vitellogenic oocytes.</td>
</tr>
<tr>
<td>4</td>
<td>Vitellogenesis 2. LC &gt; 1300 μm and a hiatus is present in the cohort of vitellogenic oocytes.</td>
</tr>
<tr>
<td>5</td>
<td>Spawning. Hydrated oocytes are present in the ovary.</td>
</tr>
<tr>
<td>6</td>
<td>Spent. Oocytes have been released. Ovary may be red.</td>
</tr>
</tbody>
</table>
ever, we hypothesize that they were there and that these were not detected because of the inaccuracies in using GSI as an indicator of maturity; GSI is affected, in addition to development stage, by the size of the fish and also by fecundity. Similar observations of spawning Greenland halibut in many months of the year have been documented in other areas; in East Greenland a large number of spawning Greenland halibut have been caught in August (A.C. Gundersen, J. Kennedy, A. Woll, I. Fossen, and J. Boje, unpublished data), and individuals with advanced ovary development are also caught in March (Kennedy et al. 2009). In the western Atlantic, spawning Greenland halibut have been caught in 10 months of the year (Junquera and Zamarro 1994).

Maturity scale

The results from this study confirm earlier statements that macroscopic identification of maturity stages does not provide accurate results (Nielsen and Boje 1995), and it is clear that a revision of the maturity scale for Greenland halibut is needed. The scale used during research surveys and sampling of commercial catches has differed between the opposite sides of the Atlantic and also has been modified over time (Riget and Boje 1989; Fotland et al. 2000; Albert et al. 2001). Previous to 1998, a five-point scale (immature, maturing, spawning, spent, and uncertain) was used by the Institute of Marine Research in Norway (Fotland et al. 2000). The criterion for “maturing” is that oocytes are visible to the naked eye, larger than 1 mm, and up to 4 mm in diameter (stages 3 and 4). This is subjective and will differ between personnel because of the quality of their eyesight and what they deem “visible to the naked eye”. Fish are considered mature after the initiation of true vitellogenesis with the assumption that the fish will spawn in the coming spawning season. However, the results from the present study showed that this is not necessarily the case for Greenland halibut; therefore, only fish that are in stages 4 to 6 of the macroscopic maturity scale should be considered mature.

The use of the current macroscopic maturity scale would lead to an underestimation of SSB. This is due the tendency for wrongly identified ovaries to be classified as earlier rather than later stages. Ovaries in stage 6 appeared to be the most problematic (Albert et al. 2001), with these ovaries being assigned to many different stages. Albert et al. (2001) attempted to address the problem of misidentification by fitting normal components to the distribution of log-GSI histograms and showed that “maturity groups” could, in theory, be identified. However, this is only feasible in periods when individuals in different stages of the maturation cycle are separated along the GSI axis, which, in the present study, only occurred between September and December (results not shown); this severely limits the application of this method. This method also has several uninvestigated problems, such that GSI is, in addition to development stage, affected by both size (Packard and Boardman 1999) and fecundity of the fish. A recommended methodological improvement for the macroscopic identification could be the development of a photographic guide for the identification of stages such as is available for herring (Bucholtz et al. 2008).

Our results show that the most accurate method to assess the maturity status of Greenland halibut is to measure the size of the oocytes. Today, this can be done easily and quickly using an appropriate image analysis system, similar to that described in Thorsen and Kjesbu (2001). From the oocyte diameter distribution, the appearance of a hiatus within the VO would then indicate if the fish is likely to spawn in the coming spawning season. We thus propose an alternative maturity scale for consideration that could be used if such a method was adopted (Table 3). Only fish in stages 4 and 5 should be considered to be capable of spawning in the coming spawning season, with the inclusion of stage 6, which will have recently spawned in the current spawning season. This is, however, not ideal in all situations, as the samples need to be stored in formalin, which is generally forbidden on commercial fishing vessels, and taking samples also increases costs. Therefore, if macroscopic identification is used it is important to understand and take into account the problems associated with it.

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