Time scale of ovarian maturation in Greenland halibut (*Reinhardtius hippoglossoides*, Walbaum)

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Evidence for a prolonged ovarian development phase in Greenland halibut is presented. The reproductive cycle in this species was originally described based on the assumption that this phase should last about one year. The results of the present study, which involves data series covering a long time period and different geographic areas, show instead that there is more than one year between the mean age of the females that are at the onset of ovarian development and the mean age of the females that are actually spawning. There are two possible interpretations for this observation. One is that the ovarian development phase (vitellogenesis) could last more than one year and thus as a consequence, individual spawning would not necessarily occur on an annual basis. The other would be the existence of a high proportion of non-spawning females every year for other reasons not related with the natural rhythm of oocyte development.

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Introduction

The cyclical change in the ovary during the development and maturation process is essentially similar in all oviparous teleosts. Eggs spawned in one year develop from a reservoir of resting or non-vitellogenic oocytes. During development they are supplied with yolk by the follicular cells during vitellogenesis. In most species, vitellogenesis ceases once oocytes reach their fully developed size and such oocytes eventually undergo maturation and ovulation after appropriate hormonal stimulation (Wallace and Selman, 1981). Oocyte maturation involves nuclear migration and hydration (Fulton, 1898). After maturation is complete, the oocyte is ovulated into the ovarian lumen and is ready to be spawned.

The time of spawning and the duration of the reproductive cycle is genetically controlled, although it has been shown experimentally that it can be modified by environmental variation. The majority of fish species outside the tropics show seasonal reproductive cycles that are maintained for as long as the fish is reproductively competent (Breder and Rosen, 1966; Woodhead, 1979), and most usually complete the reproductive cycle within one year.

Greenland halibut is considered to be a determinate, group synchronous spawning species, based on the existence of an hiatus separating the advance yolked oocytes, (Gundersen *et al.*, 1999; Junquera *et al.*, 1999). The fact that atresia rates show the highest values at the early vitellogenic stages (Junquera *et al.*, 1999; Tuene *et al.*, 2002) is also in support of this perception. This means that a single group of oocytes develops through vitellogenesis and matures to be spawned, without recruitment of any new group from the reservoir of non-vitellogenic oocytes. Up to now what was known about the Greenland halibut reproductive biology suggested the possibility that this species does not match the perception of a regular annual cycle of sexual maturity. Morgan and Bowering (1997) pointed out irregularities in the maturation process in the Northwest Atlantic Greenland halibut, possibly caused by variability in the distribution of adult fish, that hinders a precise estimation of maturity at age and size. Also, high variability in the main spawning seasons among areas and even within the same area is extensively documented. Besides the apparent lack of a clear seasonality in spawning, another outstanding characteristic in the Northwest Atlantic Greenland halibut population is that typically few mature (ripe) individuals are caught in

either research surveys or commercial catches. To explain this several hypotheses have been proposed, including: spawning migrations of mature fish to areas not sampled; and misinterpretation of the maturity stage of the ovaries (Walsh and Bowering, 1981); a maturation cycle of more than one year (Jorgensen and Boje, 1994); an unsuitable temperature regime that leads to a high frequency of gonad resorption (Jorgensen and Boje, 1994) and population asynchrony in the maturation process (Fedorov, 1968; Junquera, 1994).

In this study the hypothesis of a prolonged adolescent phase as a possible explanation of the peculiarities observed in the Greenland halibut sexual cycle is considered. The adolescent phase is the time span between the age a female changes from immature to adult and the age when a female actually spawns.

Material and methods

Spawning seasonality

Data used in this part of the study have been recorded by scientific observers on board commercial deep-water trawlers in the Northwest Atlantic (NAFO Divisions 3LMN) during the period 1990–2000 (Table 1A). The sampling method in the case of maturity data is a length-stratified sampling from the catch. Maturity stages are assigned after macroscopic examination, based on a simple four-point scale (Junquera and Zamarro, 1994). In females those stages are: (1) immature (juvenile); (2) Adult resting or developing the ovaries at any level; (3) Fully hydrated (imminent spawning); and (4) Recent postspawning. This scale is intended to avoid as much as possible, the use of stages based on structures not visible to the naked eye, to which assignment is highly subjective. However the differentiation between immature (stage 1) from the adult stages (2 onwards) proved to be frequently uncertain, especially in the case of the first maturing females. But the aim of this part of the study is just to separate the stage of final growth of the oocytes, the duration of which is presently unknown, from the hydrated stage that indicates imminent spawning. This stage is perfectly identifiable to the naked eye due to the large size attained by the ooctytes (4–5 mm). The proportions of the fully hydrated females over the total adult females, first per sample and then accumulated to the corresponding monthly proportions have been obtained. Though in principle the total number of adults per sample would be here the total of females in stage 2 onwards, due to the uncertainty associated to the distinction between juveniles and adults in certain cases, inherent to this method of diagnosis, it was considered as a more accurate alternative to establish a separation threshold at the length of 50% maturity. Based on previous microscopic estimates on female Greenland halibut length at 50% maturity (Junquera et al., 1999), females were considered as "adult" from a total length of 67 cm.

Table 1. Greenland halibut sampling description. (A) Northwest Atlantic (NAFO Divisions 3LMN) commercial sampling (scientific observers) from the Spanish deep-water bottom trawlers, analysed only macroscopically for maturity diagnosis. TF, total females sampled; AF, total adult females on the samples. (B) Number of females microscopically analysed in the NAFO area. A, number of females classified as adolescent; SP, number of females classified as spawning; C, commercial samples; R1, EU research survey in Flemish Cap (NAFO Division 3M); R2, EU selectivity research survey in NAFO Divisions 3LM; R3, Spanish long-line research survey in the same area; R4, Spanish research survey in the Grand Bank (NAFO Division 3N). (C) Number of females microscopically analysed in the Northeast Atlantic (ICES Subarea II). R5, autumn Spanish research survey. (D) Number of females macroscopically analysed from the autumn Canadian research survey (R6) in the NAFO area.

	TF	AF	Sampling
(A) Commercial Sampling in NAFO Divisions 3LMN			
1990	33581	7534	Monthly since May
1991	107227	13670	Monthly
1992	164818	15740	Monthly
1993	83340	4787	Monthly
1994	40136	6003	Monthly
1995	1459	133	Only January
1996	5497	853	Monthly
1997	3164	692	Monthly
1998	8242	1076	Monthly
1999	6889	973	Monthly
2000	11285	999	Monthly

	Total	A	SP	Source
(B) Sampling for microscopic analysis in NAFO Divisions 3LMN				
1991	150	82	7	C
1992	250	58	93	C
1993	130	28	57	C
1994	433	126	66	C-R1
1995	90	27	18	R2
1996	526	95	111	C-R3-R1
1999	286	119	10	C-R4
2000	1054	409	23	C-R4
(C) Sampling for microscopic analysis in ICES Subarea II				
1997	160	27	78	R5
1999	134	29	29	R5
2000	284	36	127	R5
(D) Research survey sampling in NAFO Subareas 0, 2 and Divisions 3KL				
1978	191	70	26	R6
1979	131	74	1	R6
1980	201	162	6	R6
1981	547	320	97	R6
1982	208	187	21	R6
1984	113	16	8	R6
1985	101	3	13	R6
1986	259	205	26	R6
1988	192	75	1	R6
1990	24	22	0	R6
1991	160	14	0	R6

Duration of the ovary development phase

The material used in this part of the study is listed in Table 1B, C and D. Data in Table 1B comes from the following sources: Spanish commercial deep-water trawlers in the Northwest Atlantic (NAFO Divisions 3LMN) (1991–1996, 1999–2000); Spanish annual research surveys on the Grand Bank of Newfoundland (NAFO Division 3N, 1999–2000); Spanish long-line research survey in the Northwest Atlantic (NAFO Divisions 3LM, 1996); European Union selectivity research survey in the same area (1995) and European Union annual research surveys on Flemish Cap (NAFO Division 3M, 1994 and 1996).

The method used is an indirect approach that consists of measuring the distance in time between the age frequency distributions of the females at the adolescent stage and females ready to spawn. A similar method has been applied by Everson (1994) to *Notothenia coriiceps*. For this component of the study, maturity stages were determined by microscopic examination of ovary sections prepared using conventional histological processing and H&E staining (Junquera *et al.*, 1999). The classification into maturity stages used here is an update of the one originally developed by Fedorov (1968), where Greenland halibut oogenesis is divided into oocyte growth (development), maturation and ovulation (Guraya, 1986). The presence/absence of oocytes in cortical alveoli, vitellogenesis, nuclear migration, hydration, previtellogenic and vitellogenic atresia, and postovulatory follicles has been recorded in each of the ovary sections, following the classification of Wallace and Selman (1981) and West (1990), and the photographic description of these stages in this species given by Fedorov (1968) and Walsh and Bowering (1981).

Females were classified as "adolescent" when they were at either the cortical alveoli stage or any level of vitellogenesis, without signs of previous spawning, such as postovulatory follicles or vitellogenic atresia. Females were classified as "spawning" when they were either in the nuclear migration or hydration stages. All females analysed in this part of the study have been aged directly using their otoliths.

To examine whether the observed maturity patterns are just local from this particular area of the Northwest Atlantic or could be generalized as a species characteristic, the geographic scope has been enlarged by analysing samples collected during the annual autumn Spanish research surveys in the Northeast Arctic (ICES Subarea II) in 1997, 1999 and 2000 (Table 1C). These samples were examined histologically as described earlier.

In order to have a longer time perspective, data from the Canadian research surveys in NAFO Subareas 0, 2, and 3 during the 1978–1991 period have been included (Table 1D). These surveys were usually conducted annually in the NAFO Division 2J3KL area, and occasionally in the other Divisions. In 1987, 1989 and since 1991, no spawning females were found in these surveys. Only data from years in which females with hydrated eggs were observed are

included. In this case, females were classified into maturity stages by the macroscopic approach. The maturity scale in this data set includes many stages, but among them only one (defined as "no evidence of previous spawning, new opaque eggs for spawning in the next year visible to the naked eye") has been selected as a proxy of the adolescent stage as histologically described. It is assumed that this method of determining adolescent fish will not be as accurate as the histological method since it is more difficult to detect evidence of previous spawning macroscopically. As a spawning stage proxy, three macroscopic stages have been chosen, defined as follows: (i) "Opaque and clear eggs present with less than 50% of the volume being clear eggs; maturing to spawn in the present year" (ii) "50% or more of the volume are clear eggs; this stage also includes the ripe condition where the ovarian content is almost liquid with clear eggs to spawn or spawning in the present year" and (iii) "Partly spent, ovary not full as in the previous stage; some eggs extruded but many clear eggs remaining". Females from this data set were also aged individually using otoliths.

Results

In the Northwest Atlantic the Greenland halibut spawning activity is irregularly distributed over the year (Figure 1). The low monthly proportions of spawning females, only occasionally exceeding 25% (in numbers) of the sampled adult females, is also striking. In the Northeast Atlantic (Table 1C) the proportion of spawning females in the single month sampled (October of 1997, 1999, 2000) is substantially higher (63% of the adult females in 1997, 34% in 1999 and 68% in 2000), however, no inference can be made on the seasonal pattern of occurrence due to the lack of year round sampling in this area.

Frequency distributions at age of the maturity stages for the sample examined by histology show that adolescent (A) females appear for the first time at age 7 whereas spawning (SP) ones start to appear at age 10 in the Northwest Atlantic (Figure 2A). In the data set in which only the macroscopic approach has been used (Figure 2B), it is not possible to distinguish unambiguously the adolescent stage, but the spawning (hydrated) one can be determined easily by visual diagnosis. The stage used as a proxy of the adolescent one in this case appears first at age 5, while hydrated females first occur from age 8. However, in this data set the frequency distribution at age of both stages largely overlaps. In the Northeast Atlantic (Figure 2C) the adolescent stage appears first at age 6 and the spawning one at age 8.

In order to compare the respective 'A' and 'SP' age distributions in the three data set analysed, a non-parametric two-sample Kolmogorov–Smirnov test has been applied. It showed that the differences between 'A' and 'SP' distributions are significant in all the three cases (KS = 0.50; n = 16; $p < 0.05$ in (A); KS = 0.56; n = 16; $p < 0.05$ in (B) and KS = 0.31; n = 16; $p < 0.001$ in (C)).
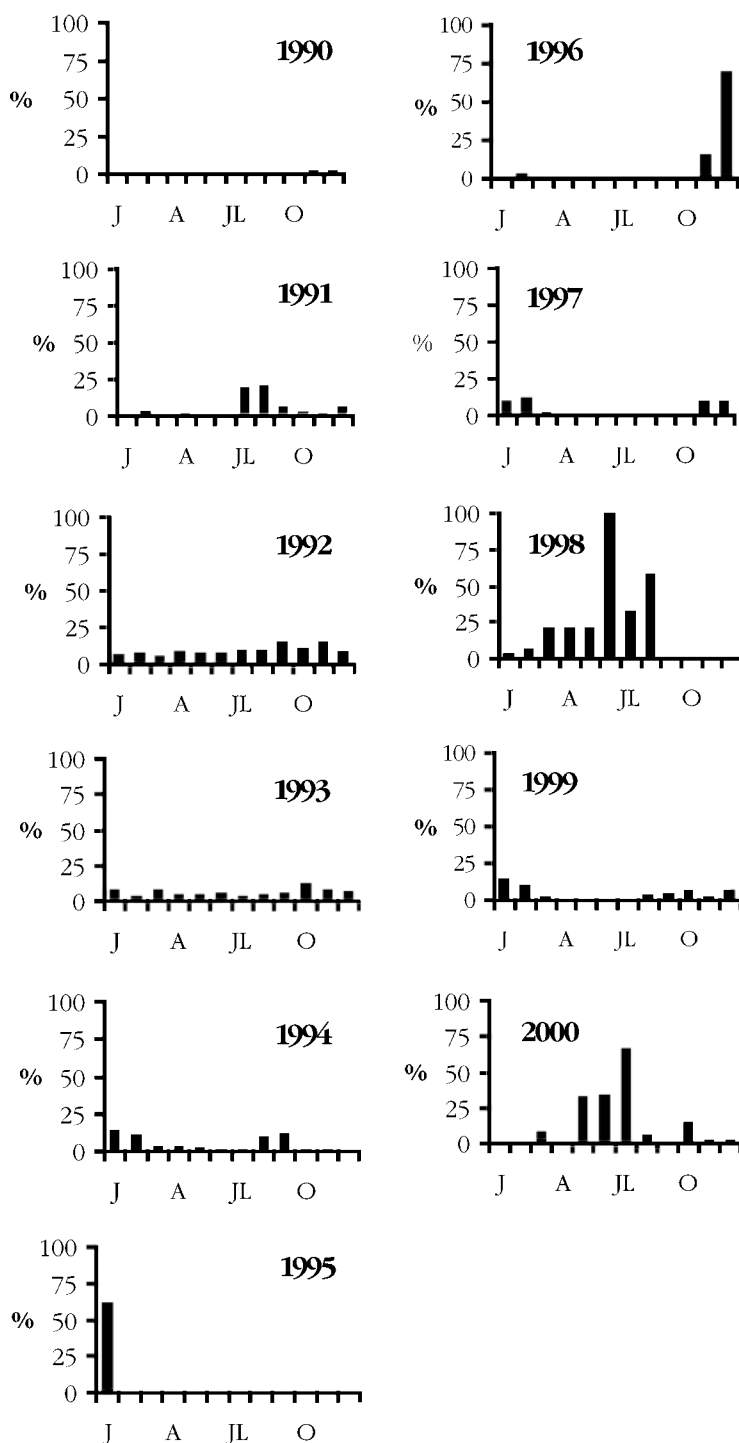
Figure 1. Monthly proportions of Greenland halibut spawning females (hydrated stage) during the period 1990–2000, based on commercial sampling (Table 1A) in the Northwest Atlantic (NAFO Divisions 3LMN). In 1995 only data from January are available.

(A) Northwest Atlantic - microscopic classification
(n= 2919)

(B) Northwest Atlantic - macroscopic classification
(n = 2127)

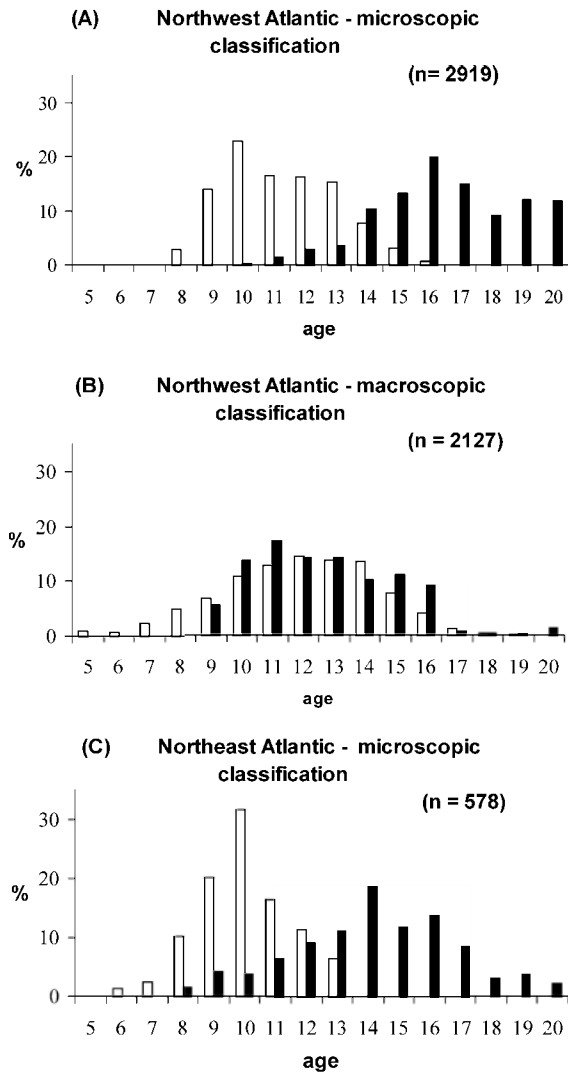**(C) Northeast Atlantic - microscopic classification**
(n = 578)

Figure 2. Percentages at age of Greenland halibut females in adolescent (□) and spawning (■) stages. Northwest Atlantic (A) and Northeast Atlantic (C) panels have been obtained by microscopic examination of the ovaries. Northwest Atlantic (B) is based on a macroscopic classification.

The linear trends of the cumulative frequency distribution of the maturity stages at age were produced to obtain an average difference in time between the adolescent and spawning stages (Figure 3). In both the Northwest and Northeast Atlantic Greenland halibut, a distance of four years is observed, though in the latter area both the age to become adult and to achieve spawning are younger.

Discussion

The results show an interval of about 4 years between when female Greenland halibut became adult and when they are
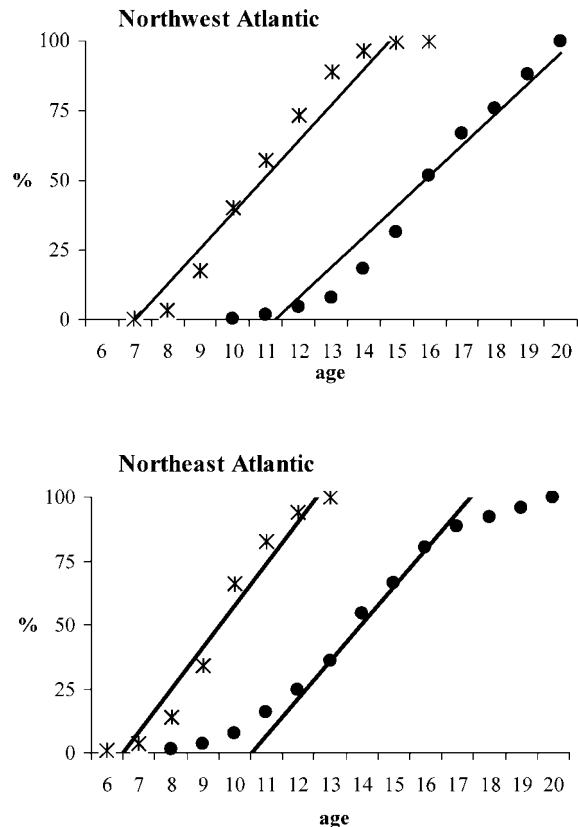

Northwest Atlantic

Northeast Atlantic

Figure 3. Cumulative proportions at age of Greenland halibut females at the adolescent and spawning stages, and respective linear trends (only results from microscopic analysis are included). ✗——adolescent; ●——spawning.

actually ready for spawning. Since a similar pattern is observed on both sides of the Atlantic and across a fairly long time period, it might be considered as a characteristic of the species. The data set where the macroscopic approach for ovary staging was used gives only limited support to this conclusion, since the frequency distributions at age of the "proxy-adolescent" and spawning stages show substantial overlap. However it must be noted that while this later stage is clearly distinguishable by eye, the adolescent stage is impossible to define precisely without histological examination, since it requires the ability to distinguish adult females that never spawned before from adult repeat spawner resting females, and also from the immature. Nevertheless even in this case, a three year difference between the starting point of the two stages is observed, consistent with the results produced by the microscopic approach.

Examples of species with lengthy sexual cycles are not uncommon. Two years to complete vitellogenesis are reported in south Atlantic grenadier (Alekseeva and Alekseev, 1984) and four years in Nototheniids and other

Antarctic fishes (Shandikov and Faleeva, 1992; Everson 1994). Large proportions of non-reproductive adults in orange roughy have been documented (Bell *et al.*, 1992). Also in Greenland halibut the idea of a lengthy reproductive cycle is not new. Fedorov (1971) concluded from visual and histological analysis of gonads that some sexually mature females in the Barents Sea Greenland halibut do not spawn annually and that the pause in reproduction could last for at least two years, with ovarian development halted in the initial stage.

The situations of non-annual spawning can be classified as two types: (a) spawning is abnormally skipped due to some adverse condition which prevents the final development of the gametes. In this situation, massive resorption of sexual products occurs, and (b) species whose natural sexual cycle extends for several years. According to Fedorov (1971) this category usually includes long-lived species, with irregular spawning, inhabiting areas with extreme climatic conditions. Based on previous results (Junquera *et al.*, 1999), atresia rates in the Northwest Atlantic Greenland halibut peak at initial vitellogenesis, as also pointed out Fedorov (1971), and become virtually absent in fully yolked oocytes prior to spawning. This should not be regarded always as a response to adverse conditions or a mechanism for spawning cancellation, but as a mechanism of fecundity regulation. Although in experimental conditions massive prespawning atresia is a common response to environmental stress, this is an unlikely event in physiologically normal females under natural conditions (Wallace and Selman, 1981). Oocytes resorbed at early development stages can be used later to produce interstitial gland cells (Guraya, 1972; Saidapur, 1978), thus constituting a feed back element in the normal cycle of the ovary development. But on the contrary, for determinate spawners it is a waste of energy to fully develop a significant number of oocytes that will not be spawned.

If the process of vitellogenesis lasts up to 4 years, the question arises as to whether individual females spawn only once every 4 years or if they are able to spawn at shorter time intervals after their first spawning. If spawning normally occurs only once every 4 years, this would mean that only about 25% of the adult females would actually spawn per year, which is in agreement with the results from the Northwest Atlantic for most of the time series analysed. However, the proportion of spawning females found in the Northeast Atlantic is much larger than this. Sampling in this case was conducted in a known spawning area of the stock, and in a season close to the peak spawning, (Albert *et al.*, 1998), where probably only the reproductively active adults would be present. The ratio of males/females in this survey area was 4 : 1, which is a very unusual one out of the breeding season, and supports the idea that the sampling took place in a spawning area, during the spawning season. In addition, Bowering and Nedreaas (2000) reported the existence of seasonal migrations of the mature fraction in this stock to the spawning areas. This could mean that the observed proportion of spawning females in this area was not reflective of the whole stock but rather that it was inflated by the absence of non-spawning females in the area.

The prolonged vitellogenesis process also contributes to explaining the irregularity or lack of a clear-cut seasonality in the Northwest Atlantic Greenland halibut spawning. The timing of reproduction in female teleosts may be viewed as the product of numerous biotic and abiotic stimuli which exert both long-term effects on ovarian growth and short-term effects on final maturation and ovulation of the oocytes (Stacey, 1989). It is known experimentally that the oocyte maturation switch in Pleuronectiform species is not a simple consequence of oocyte development (vitellogenesis) being completed. Maturation and subsequent spawning would not be undertaken in the absence of specific endogenous stimuli. Individuals would be arrested at the vitellogenic stage for as long as appropriate conditions are met (Bone *et al.*, 1995). In contrast to the Northwest Atlantic, spawning of Greenland halibut in the Northeast Atlantic is more synchronized in time, since the peak always occurs in the last quarter of the year (Albert *et al.*, 1998). According to Bowering and Nedreaas (2000) in the Northwest Atlantic spawning occurs at considerably greater depths (beyond 1000 m) than in the Northeast Atlantic (between 500 and 800 m). It is possible therefore that in those shallower depths a variable photoperiod could act as a cue in switching the final maturation, and leading to a clear seasonality in spawning. Under a constant photoperiod, as is the case of spawning in deeper waters, the lack of seasonal cues may lead to less synchronous spawning within the population. Though there is the overwhelming perception that even in the constant environment of the deep seas fish species maintain circannual cycles in reproductive activity, the Northwest Greenland halibut case seems not to support this view.

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