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Time scale of ovarian maturation in Greenland halibut

by

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Abstract

In this paper possible evidence of a prolonged ovarian development phase in Greenland halibut is presented. The reproductive cycle in this species has been originally described based on the assumption that this phase should last about one year. The results of several years of analysis showed that there is more than one year between the mean age of females at the onset of the ovarian development and the mean age of actually spawning females. Two possible interpretations of this fact are discussed: the ovarian development phase (vitellogenesis) could last more than one year, and individual spawning does not necessarily occur on an annual basis as a consequence, or the incidence of non-spawning females every year could be very high. Both possibilities have important implications for the species' reproductive potential and stock dynamics of this valuable deep water resource.

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 (now egg) 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 its been shown experimentally that it can be modified by environmental variation. The majority of fish species outside the tropics shows 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 a determinate, group synchronous spawning species (Junquera et al., 1999). 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. What is known up to now dealing with the Greenland halibut reproductive biology indicates a series of discrepancies suggesting that possibly 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, which led to difficulties to precisely estimating maturity at age and size. Also discrepancies on the main spawning seasons among areas and even within the same areas are extensively documented: spring or late winter - early spring in both Davis Strait (Jensen, 1935; Smidt, 1969; Templeman, 1973; Chumakov and Serebryakov, 1982) and in off-shore areas of West Greenland (Riget and Boje, 1989; Jorgensen and

Boje 1994). In the inner part of the West Greenland fjords, spawning occurs sporadically and apparently in a different manner from year to year. In northern Flemish Pass and Div. 3NO, peak spawning has been observed in summer, with a secondary peak in winter (Junquera and Zamarro, 1994, Junquera, 1994). Besides, some spawning fish everywhere throughout the year. In the Barents Sea stock (Fedorov, 1971) indicated that the spawning season lasted as long as October to June with a secondary peak sometimes in summer.

Apart from an apparent lack of a clear seasonality in the spawning season, it is a common feature in the Northwest Atlantic Greenland halibut population that few mature (ripe) individuals are found in either commercial catches or research surveys. Several arguments have been proposed to explain this scarcity of mature females: spawning migrations of the mature fish to other areas; a possible misinterpretation of the maturity condition of the ovaries (Walsh and Bowering, 1981); possible maturation cycle of more than one year (Jorgensen and Boje, 1994); inadequate thermic regime leading to a high frequency of resorption of the gonads (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 analyzed. We call the 'adolescent phase' the time span between the age a female changes from immature to adult and the age when a female is actually or about to spawn.

Material and Methods

• SPAWNING SEASONALITY.

Data used in this part of the study have been collected by scientific observers on board the commercial deep-water trawlers in the Northwest Atlantic (NAFO Divisions 3LMN) during the period 1990-2000 (Table 1A). The sampling procedure in the case of maturity data is a length-stratified sampling from the catch of the hauls selected. Maturity stages are assigned after macroscopic examination, based on a simple four-point scale. In females those stages are: 1- immature (juvenile); 2- Adult resting or developing the ovaries at any level; 3- Fully hydrated (imminent spawning); 4- Recent postspawning. This scale is intended to avoid as much as possible, the use of stages based on structures not visible to the necked eye, whose assignment is highly subjective. Also the objective is to separate the stage of final growth of the eggs, whose duration is at the moment uncertain, from the hydrated stage, which means imminent spawning. For the purpose of this part of the study the proportions of the fully hydrated stage over the total adult females per sample have been accumulated to obtain the corresponding monthly proportions. According to previous results (Junquera et al. 1999), females were here considered as 'adult' from a length of 67 cm.

• DURATION OF THE OVARY DEVELOPMENT PHASE.

The method used is an indirect approach that consists in measuring the time distance between the age frequency distributions of the females at the 'adolescent' stage and the one of the females actually ready to spawn. This method has been applied by Everson (1994) in *Notothenia coriiceps.* Those two maturity stages have been determined by microscopic examination of ovary sections prepared using a standard histological method (Junquera et al. 1999). The classification into stages that has been used is an update of the one originally made by Fedorov (1968). 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 have 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 given by Fedorov (1968) and Walsh and Bowering (1981). The stages   description is summarised in annex 1. For the purpose of this part of the study, females were classified as 'adolescent' when they were at either cortical alveoli or any level of vitellogenesis and 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 the females classified in those two categories have been directly aged using their otoliths.

The material for this analysis come from the following sources: Spanish commercial deep-water trawlers in the Northwest Atlantic (NAFO Divisions 3LMN) (1991-1996, 1999-2000); Spanish spring survey in NAFO Division 3NO (1999-2000); Spanish longline survey in NAFO Div. 3LM (1996); EU selectivity survey in NAFO Div. 3LM (1995) and EU summer surveys in NAFO Div. 3M (1994 and 1996). A description of the samples is presented in table 1(B).

In order to determine weather the observed patterns are just local from this particular area of the Northwest Atlantic or can be generalized as an species characteristic, the geographic scope have been increased by adding samples collected during the autumn Spanish surveys in the Arctic (ICES Subarea II) in 1997, 1999 and 2000 (table 1(C)). Those samples have been treated in the same way as explained above. Also to increase the time scale of the study, data obtained during the autumn Canadian surveys in NAFO Suareas 0, 2, 3 (1967-1981) have been included. Those data (Table 1D) consist in females classified into maturity stages using the macroscopic approach. The maturity scale in this survey series includes a lot of stages, but among them only one have selected as a proxy of the 'adolescent' stage. A margin of error in this approximation is assumed. The definition of this stage is: 'no evidence of previous spawning, new opaque eggs for spawning in the next year visible to the necked eye'. As 'spawning' stage, two stages have been chosen in this scale, defined as follows: (i) '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 (ii) '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

The monthly proportions of spawning females (hydrated stage) during the period 1990-2000, based on commercial sampling in the Northwest Atlantic (NAFO Divisions 3LMN) are presented in Fig. 1. In 1995 there is information only from January, but for the rest of the period what is outstanding is the irregular distribution of spawning over the time and the low proportions of spawning females, that only occasionally exceed 25% (in numbers) of the sampled adult females. In the Northeast Atlantic (table 1) the proportion of spawning females found in the single month sampled (October 1997-2000) was substantially higher (63% of the adult females in 1997, 50% in 1999 and 72% 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 show that 'adolescent' females appear for the first time at age 7 whereas 'spawning' ones start to appear at age 11 in the Northwest Atlantic (Fig. 2 A). According to the macroscopic approach used in the Canadian data set it is not possible to distinguish exactly the 'adolescent' stage, but still the spawning (hydrated) one can be easily determined by visual diagnosis. In this case (Fig. 2 B) the stage used as a proxy of the 'adolescent' stage first appears at age 5 while hydrated females first occur at age 8. However in this data set the frequency distribution at age of both stages largely overlap. In the Arctic data set the 'adolescent' stage appears first at age 6 and the 'spawning' one at age 8.

The linear trends of the cumu lative frequency distribution of the maturity stages at age are used to obtain an average distance in time between the adolescent and spawning stages (Fig. 3). In both the Northwest and Arctic Greenland halibut a distance of four years is observed, although, in the later area both the age to became adult and to achieve spawning are younger. Mean age at the 'adolescent' stage is 11 ($\pm$ 1.7) and that of the 'spawning' stage 16 ($\pm$ 2.1) in the Northwest Atlantic Greenland halibut using the microscopic approach. When the macroscopic one is used, those ages are 12 ($\pm$ 2.5) and 13 ($\pm$2.2) respectively. In the Arctic Greenland halibut mean age at the 'adolescent' stage is 10 ($\pm$ 1.4) and that of the 'spawning' stage 14 ($\pm$ 2.8). Converted into lengths, this would be 65 cm ($\pm$ 6.2) and 83 cm ($\pm$ 15.4) respectively in the Northwest Atlantic with the microscopic approach; 79 cm ($\pm$ 11.8) and 89 cm ($\pm$ 9.7) with the macroscopic one and 59 cm ($\pm$ 5.1) and 77 cm ($\pm$ 10.7) respectively in the Arctic Greenland halibut.

Discussion

Based on the results we can conclude that there is an interval of about four years between when female Greenland halibut became adult and when they are actually ready for spawning. Since a similar pattern is observed in both sides of the Atlantic and across a fairly large time series, it might be considered as characteristic of the species. Apparently the data set where the macroscopic approach for ovary staging has been used would not give much support to this conclusion, as 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 unambiguous and clearly distinguishable by eye, the 'adolescent' one is impossible to define precisely without histological examination, as it is necessary to distinguish the adult females that never spawned before from the adult repeat spawner resting females, and also from the immature ones. In addition, this data set covers a long time period and any changes in

ageing criteria over time would affect the results. However even in this case a three-year difference between the starting point of the two stages is observed, and this consistent with the results produced by the microscopic approach.

The situations of non-annual spawning can be divided in two categories: one is when spawning is skipped due to some adverse reason, which prevent the final development of the gametes. In this situation, massive resorption of sexual products occurs. In the second category are the species whose natural sexual cycle extends for several years. Fedorov (1971) pointed out that this category included species which were usually long lived, with irregular spawning, inhabiting areas with extreme climatic conditions. However examples of species with lengthy sexual cycles are not uncommon: two years to complete vitellogenesis in south Atlantic grenadier (Alekseeva and Alekseev 1984), four years for Nototheniids and other Antarctic fishes (Everson 1994, Shandikov and Faleeva 1992) and high 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. This was accompanied by a continuous process of degeneration of oocytes entering the secondary growth phase that is the ovary development was halted in its initial stage. In our perspective, based on previous analysis (Junquera et al. 1999), atresia rate in the Northwest Atlantic Greenland halibut effectively peaks at the initial vitellogenesis (secondary growth phase) and became absent in fully yolked oocytes prior spawning, but it should not be regarded as a response to adverse conditions or a mechanism for spawning cancellation but as a mechanism of fecundity regulation. Besides, it has been demonstrated that those large previtellogenic and early vitellogenic atretic oocytes are later used to produce interstitial gland cells, thus constituting a feed back element in the normal cycle of the ovary development (Saidapur 1978, Guraya 1972). Although in experimental conditions massive prespawning atresia is a common response to environmental stress, based on our experience this is an uncommon event in physiologically normal females in natural conditions, thus playing little, if any, role in the normal ovarian dynamic during the breeding season, as has also been pointed by Wallace and Selman (1981).

If it is accepted that the process of vitellogenesis lasts 4 years, the immediate question arising would be whether individual females do spawn only once every 4 years or if they are able to spawn at shorter time intervals after their first spawning, for instance annually. In the first instance, it would mean that only about 25 % per year of the adult females would actually spawn. This is quite in agreement with what we observe in Northwest Atlantic in most of the time series analysed, as the proportion of spawning females rarely exceeded 25 %. However the proportion of spawning females found in the Northeast Atlantic is much larger than this. A possible explanation could be that sampling from that population was conducted in an spawning area, in a season close to the peak spawning as reported by Albert et al. (1998), where probably only the reproductively active adults were present. In support of it is the fact that during this survey the sex ratio was 4 males:1female, which is a very unusual situation out of the breeding season. Also Bowering and Nedreas (2000) reported the existence of seasonal migrations of the mature fraction in this stock to the spawning areas. So the stock structure detected in one survey, in that particular season and area would not be a realistic figure of what the whole stock structure would be, as a part of the adult females (the inactive ones) would be absent.

The prolonged vitellogenesis process could also explain the irregularity or lack of a clear-cut seasonality in the spawning season in this species in the Northwest Atlantic. 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 case specific endogenous stimuli. Individuals would be arrested at the vitellogenic stage for as long as appropriate conditions are met (Bone et al. 1995). Opposite to what is observed in the Northwest Atlantic, in the Northeast Atlantic Greenland halibut, spawning is more synchronized in time, since the peak always occurs in the last quarter of the year (Albert el al. 1998). As pointed out by Bowering and Nedreas (2000), in the Northwest Atlantic spawning is performed at considerably deeper waters (i.e. beyond 1000 m) than in the Northeast (between 500 – 800 m). It could be that in those shallower depths photoperiod could act as cue factor in switching the final maturation. Under constant photoperiod as in the case of spawning in deeper water, other unknown, and not necessarily environmental, factors must act as cue. Also the factors operating as cues may be different for each phase of the reproductive cycle. Bye (1989) indicated that endogenous circannual reproductive cycles are probably universal and

are synchronized with the seasons by response to environmental conditions. Though there is the overwhelming perception that even in the constant environment of the deep seas fish species have circannual cycles in reproductive activity, the Northwest Greenland halibut case seems not to support this view.

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Table 1.- Greenland halibut sampling description. **(A)** Commercial sampling (observers) in the Spanish bottom trawlers. This data was set analysed only macroscopically. TF = total females sampled; AF= total adult females on the samples (> 67 cm). **(B)** Number of female Greenland halibut analyzad microscopically in the NAFO area. Nº A = number of females classified as 'adolescent'; Nº SP = number of females classified as 'spawning'; C= commercial samples; R1 = EU summer survey in Div. 3M; R2 = EU selectivity survey in Div. 3LM; R3= Spanish longline survey in Div. 3LM; R4 = Spanish spring survey in Div. 3NO. **(C)** Number of female Greenland halibut analysed microscopically in the ICES Subarea II. R5 = autumn Spanish survey in ICES SA II. **(D)** Total of females analysed macroscopically in the autumn Canadian survey (R6) in NAFO Subareas 0,2,3.

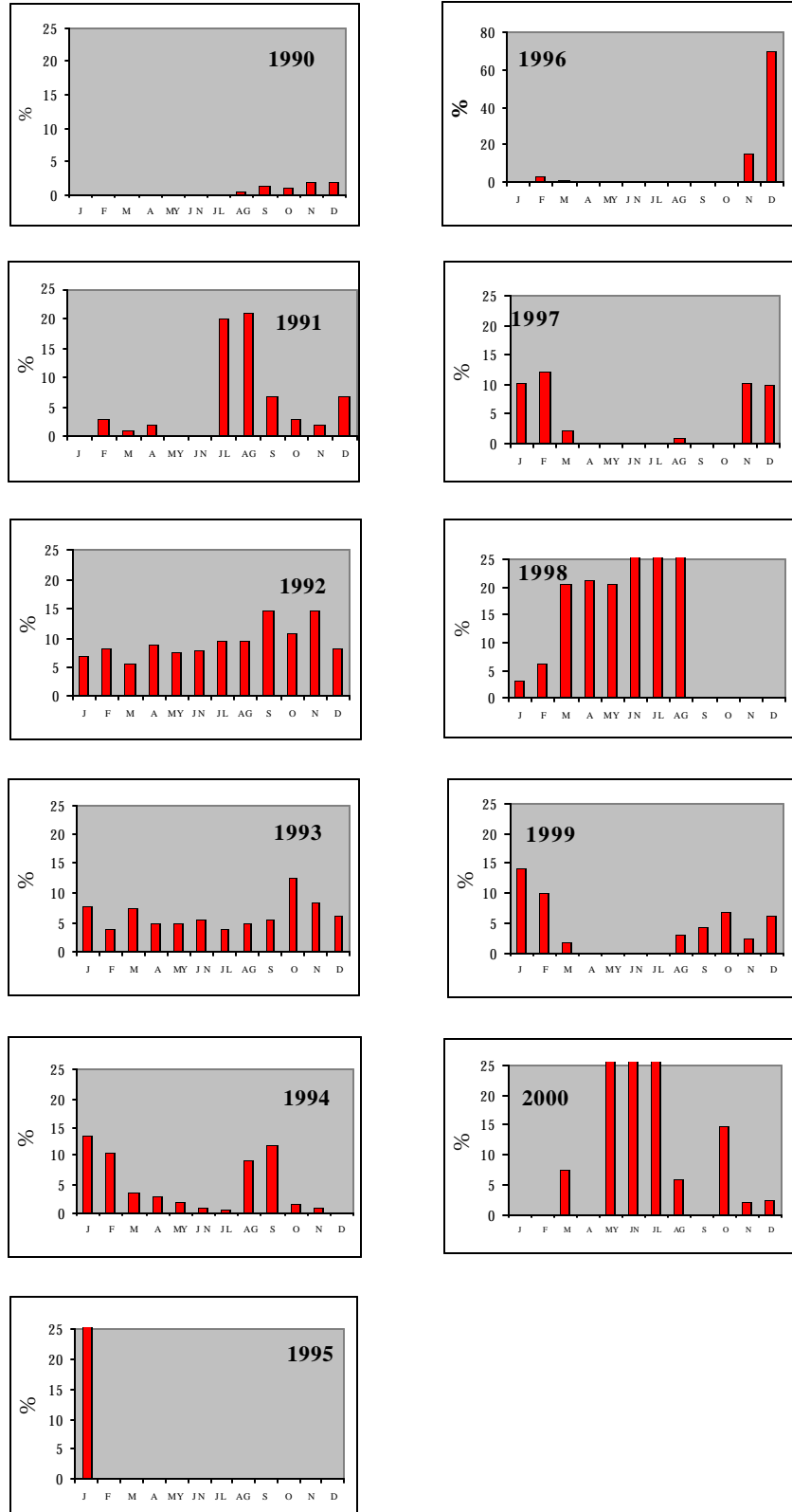| (A) Commercial Sampling in NAFO Div. 3LMNO			
	TF	AF	sampling
1990	33581	7534	Monthly since May
1991	107227	13670	Monthly
1992	164818	15740	Monthly
1993	83340	4787	Monthly
1994	40136	6003	Monthly
1995	1459	133	Only January
1996	5497	853	Monthly
1997	3164	692	Monthly
1998	8242	1076	Monthly
1999	6889	973	Monthly
2000	11285	999	Monthly
(B) Sampling for microscopic analysis in NAFO Div. 3LMNO			
	Total	Nº A	Nº SP	Source
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			
	Total	Nº A	Nº SP	Source
1997	160	27	78	R5
1999	134	29	29	R5
2000	284	36	127	R5
(D) Research survey sampling in NAFO Subareas 0 + 2 + 3			
	Total	Nº A	Nº SP	Source
1967	1		1	R6
1977	1		1	R6
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

Fig 1.   Percentages of adult female Greenland halibut at the spawning stage in NAFO Div. 3LMN (Northwest Atlantic) in commercial samp les (Table 1A).
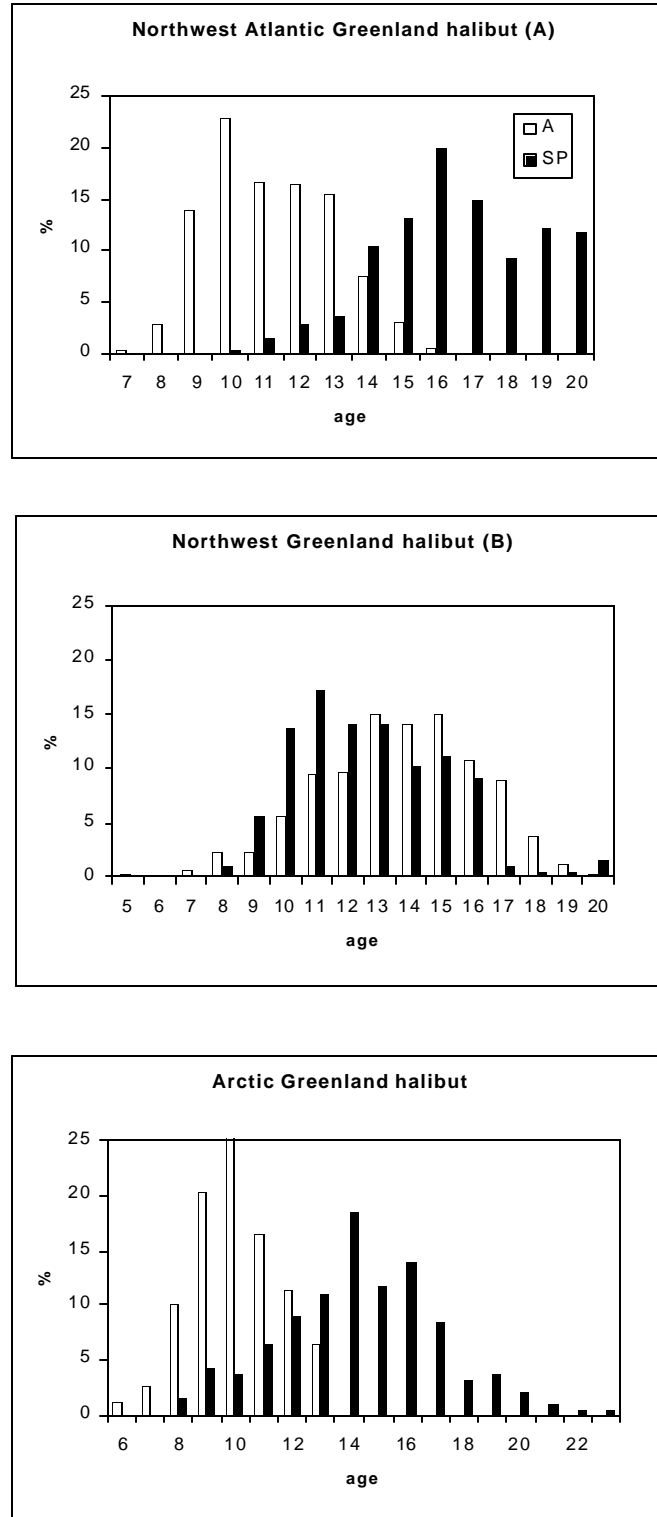
9

Fig. 2. Percentages of Greenland halibut females at the 'adolescent' (A) and 'spawning' (SP) at age. Northwest Atlantic (A) and Arctic Greenland halibut are obtained by microscopic examination of the ovaries. Northwest Atlantic (B) is based on a macroscopic classification.
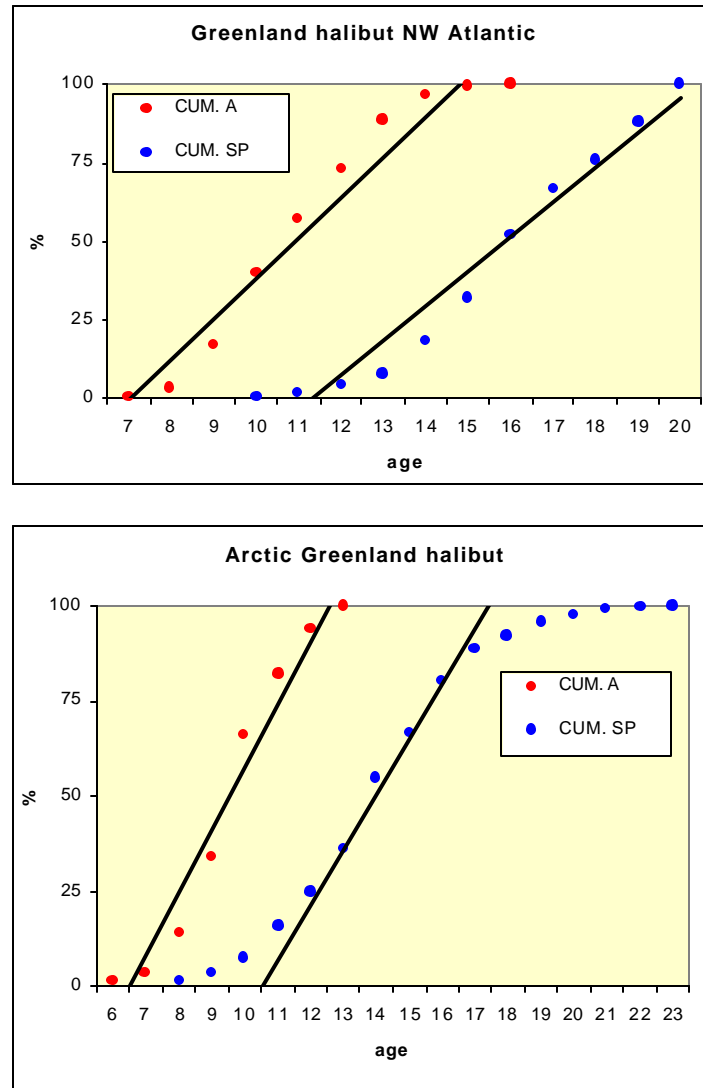
Fig. 3.- Cumulative proportions of Greenland halibut females at the 'adolescent' and 'spawning' stages at age and
respective linear trends (only results from microscopic analysis are included).

ANNEX 1

Description of the maturity stages:

	MACROSCOPIC	MICROSCOPIC
Immature (I)	Ovary small, translucent restricted to posterior part of the body cavity	All the oocytes in circumnuclear ring stage. No sign of previous spawning, such as postovulatory follicles neither oocytes starting the phase of maturation (cortical alveoli). Ovary wall thin.
Maturing and resting (II)	Ovary starts to enlarge. At first eggs may not be visible, then becoming visible although opaque, giving finally having a granular appearance. Ovary wall thin.	Oocytes starting the growth phase: Cortical alveoli, vitellogenesis and finally fully yolked oocytes. Ovary wall thin.
Spawning (III)	Ovary with eggs partially or totally hydrated, with hyaline aspect. Running stage.	Some or all the oocytes in the ovary are hydrated. Nuclear migratory oocytes are included in this stage.
Spent (IV)	Ovary wall fairly thick and tough reddish purple appearance, sometimes with residual eggs.	Ovary wall very thick, a high number of postovullatory follicles and blood vessels. Unreleased oocytes at different stages of development and at the onset of reabsorption remain very dispersed within the ovary.