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CORRIGENDUM

SCIENTIFIC COUNCIL MEETING - JUNE 1995

Histological Assessment of Sexual Maturity in Greenland Halibut in Div. 3LM

by

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THE FOLLOWING NEW TABLE 2 REPLACES THE OLD TABLE 2.

Table 2. Percentage of spawning and number of adult females analyzed by month in 1992-94.

	1992		1993		1994			
					3LM		3N	
	N	%	N	%	N	%	N	%
January	2165	5	468	7	353	6	0	0
February	5020	2	737	4	834	8	144	0
March	6822	1	1308	2	1465	2	440	0
April	6733	1	1035	2	1031	0	608	0
May	7713	0	1010	1	1470	1	1410	0
June	6332	0	781	7	572	0	791	0
July	1558	5	463	26	288	0	500	0
August	2631	5	376	2	376	5	825	2
September	1508	3	292	3	473	7	829	11
October	1691	2	47	2	947	14	1101	2
November	1769	1	90	0	605	8	1708	1
December	312	2	130	30	118	18	572	1
TOTAL	45246		8730		8502		8928	

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Introduction

The Greenland halibut spawning in the northwestern Atlantic was believed to take place in the deeper waters south of the ridge between Greenland and Baffin Island, at about 67° N in the Davis Strait (Jensen, 1935, Smidt, 1969). However, as pointed out by Jorgensen and Boje (1994), the assumption on the location of the main spawning ground for the stock there was based on the observations of egg and larvae. No spawning and only very few fish in ripe or spent condition have been observed in the Davis Strait area. As they approach maturity, fish from the eastern Canadian shelf and western Greenland were believed to migrate northward to the spawning area in Davis Strait (Smidt, 1969, Templeman, 1973, Chumakov, 1975, Atkinson *et al.*., 1982, Chumakov and Serebryakov, 1982), and thus only immature fish remained in the southern areas (Zilanov *et al.*., 1976). Further investigations also showed the existence of spawning areas in the Gulf of St. Lawrence (Bowering, 1980), in the northern Flemish Pass (Junquera and Zamarro, 1994) and in West Greenland (Riget and Boje, 1989; Jorgensen and Boje, 1994).

The main spawning seasons also seem to differ from area to area: Spring (Jensen, 1935) and late winter-early spring (Templeman, 1973; Smidt, 1969) in Davis Strait; also in Subarea 0 in late winter - early spring (Chumakov and Serebryakov, 1982). Jorgensen and Boje (1994) and Riget and Boje (1989) in West Greenland (Subarea 1), indicate that the spawning occurs in off-shore areas in the first quarter of the year, while in the inner part of the fjords this 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 from 1991 to 1993, but a secondary peak occurred in December-January and a few fish in spawning condition were detected during all the year (Junquera and Zamarro, 1994, Junquera, 1994). In the Barents Sea, stock was reported to experience a reverse situation (Fedorov, 1968; Bulatov, 1983): main spawning was in winter with the secondary spawning in summer, and some spawning fish appeared sporadically throughout the year. In this same area, Andriayashev (1939) and Nikolsky (1954) (cit. by Fedorov, 1971) indicate that the spawning season lasted from October to June.

Apart of this apparent lack of a clear seasonality in the spawning season, it is a common feature in the Greenland halibut in the Northwest Atlantic that few mature individuals are found in either commercial catches or research surveys. Several arguments have been proposed to explain the escarcity of mature females: spawning migration of the mature fish to the northern 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); populational asynchrony in the maturation process (Fedorov, 1968; Junquera, 1994).

In order to clarify the seasonal maturation dynamics and the type of reproductive strategy in female Greenland halibut, this paper presents the results from the analysis of ovaries over a three year cycle using standard histological techniques.

Material and methods

During the years 1992 to 1994, 461 ovaries of Greenland halibut between 65 and 100 cm in total length were sampled on board the commercial deep-water trawlers in Div. 3LM and 3N and during the EC summer survey in Flemish Cap (Div. 3M) in 1994 (Table 1). The ovaries were fixed immediately after catch in 10 % buffered formalin (Hunter, 1985), and the length and weight of the fish recorded. For histological analysis, samples of the ovaries were dehydrated, embedded in paraffin and 6µm-thick sections were stained with Harris' haematoxylin and eosin.

Circumnuclear ring, cortical alveoli, vitellogenesis, fully yolked oocytes, hydrated oocytes, postovulatory follicles and atresic oocytes were identified in each section, 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 frequency of each one of those types of oocytes in the sections was recorded monthly for every year. In 1993, samples of ovaries were only available in August and December. In 1994, data from Div. 3LM and 3N were treated separately, as a one month difference was found in the peak of spawning between those areas (Table 2) which had not been observed in previous years (Junquera, 1994). During this same period, the maturity stage of 306,867 female Greenland halibut was determined by the observers on board the commercial ships, based on the macroscopic aspect of the ovaries using a four-point maturity scale. Table 3 shows this scale and its equivalent microscopic structures to analyse the accuracy of the macroscopic diagnosis of the stage of maturity. The macroscopic scale used is a simplification of those by Fedorov (1968), Walsh and Bowering (1981) and Riget and Boje (1989). This is to avoid as much as possible, the use of stages based on structures not visible to the nacked eye, whose assignment must be highly subjective. Furthermore, it is important to separate the stage of final maturation of the eggs (determined microscopically by the final vitellogenic and fully yolked stages), whose duration is at the moment uncertain, from the hydrated stage. Once the hydratation of the oocytes starts, the spawning takes place within the following few hours (Fulton, 1898; Hunter and Goldberg, 1980). So it is important to separate this stage clearly in order to determine the date of spawning.

Results

The accuracy of the observers in the diagnosis of the stage of maturity of female Greenland halibut using macroscopic criteria is assessed comparing their results with those from the histological examination of the same material (Table 4). The discrepancies are mainly related to spent individuals designated visually as resting or early maturing (26% incorrectly classified), and a further 10 % incorrectly classified as spawning (hydrated stage) which were, in fact, vitellogenic stages.

Primary growth stage

The first stage of the teleost primary oocyte growth is called the chromatin nucleolar stage. As the oocytes grow, the nucleus increases in size and multiple nucleoli appear at its periphery, which is called the perinucleolar stage. Both at the chromatin nucleolar stage and the perinucleolar stage are the only kind of oocytes which are present in ovaries from immature fish (macroscopic stage I). Besides, these can be found in ovaries at all the stages of maturity, throughout the year.

Cortical alveoli stage (CA)

This stage is characterized by the appearence of yolk vesicles in the cytoplasm. With Haematoxilyn-eosin preparation these structures appear as one or several rows of empty peripheral spheres. The presence of these oocytes indicate the onset of the maturation and spawning during the following breeding season. The frequency of AC in 1992 showed two main peaks (Table 5 and Fig.1), one appeared one between February-April and the other in November. A secondary increase was observed in July. For the rest of 1992, this stage was absent. In 1993, samples were only available for August and December; in both months CA were present, although their proportion was much higher in August. In 1994, in Div. 3LM one peak in CA appears in February and after, there is a period of high frequency of CA from May to September, with a maximum in July. In Div. 3N in 1994, all the ovaries sampled in April were in CA stage and a significant amount is found thereafter, increasing again in August. In general eight months elapsed between the peaks in CA stage and the peak of spawning every year (Tabla x).

Vitellogenic stages

The appearence of yolk proteins in fluid -filled spheres (yolk spheres, granules or globules) is characteristic of these stages. Three levels are considered: primary vitellogenesis (VIT1), where the granules are still very small, is one stage very close to the CA one. In the secondary vitellogenic stage (VIT2), the yolk spheres are increasingly greater, reaching maximum size in the fully yolked stage (VIT3), the final vitellogenic stage occurs when the yolk granules start to fuse. In every one of these stages, only one mode of vitellogenic oocytes appeared in the sections examined, with a few oocytes in previous stages of development which should better be considered as residual since their number does not justify a new group of maturing oocytes. The final stage of the oocyte maturation is indicated by the peripheral migration of the nucleus and dissolution of its membrane. This stage has never been found in the present study. In 1992, (Table 6, Fig. 1) VIT1 appeared

only in March and July and in a very low proportion, which can be explained because it is not always easy to distinguish this stage from the CA one. VIT2 showed in 1992 two peaks - one in June and the other in October - and VIT3 in August and December. These two peaks correspond to the modes of CA detected in the previous February and July. In 1993, a significant number of vitellogenic stages were found in August (VIT1) and December (VIT3). In 1994, in 3LM there are two modes in VIT1, one in March-April and the other in August-September; VIT2 occuring from April to July and VIT3 varying from 100 % in January then decreasing and increasing again in June to September. It can be identified a running mode through the vitellogenic stages starting in March (VIT1) and ending in September (VIT3), which corresponds to the one in CA detected in the previous February and with the long spawning season in late 1994 (Table 2) and early 1995 (Cárdenas per. comm.). In 3NO 1994, no such clear pattern is detectable as VIT1 appeared in all the months sampled except in April, where 100 % of the observed oocytes were in CA stage; VIT2 appeared between May and June, and VIT3 from May to August which is related to the peak spawning observed in September in this area (Table 2).

Hydrated stage

During the hydration, the oocytes enlarge due to a massive intake of water, and the ovaries fill the body capacity. During final maturation and also at the late vitellogenic stage in this species it is characteristic for the mature oocytes to be arranged in the inner part of the ovary, close to the lumen, while oocytes in earlier stages of development are grouped in the outer portion, proximal to the ovary wall.

Hydration is a very rapid process and ovulation occurs within the few hours following its onset. Then the oocytes drop the lumen into the ovary and, very frequently, they are lost during the tissue preparation procedure. For this reason, it is very difficult to observe this event in the samples obtained.

Postovullatory follicles

The presence of POF indicates that this fish has already spawned. POF are present in the ovaries, in spent resting and maturating females. It is possible to identify POF even in the early vitellogenic stage. The months with a lower proportion of POF occur when a high proportion of females are in vitellogenesis phase. So in the first semester of 1992, very few ovaries had POF, but the proportion of these structures increased from July onwards, with the onset of spawning (Table 2). In 1994, when two wide spawning periods occurred, POF were present throughout the year, though the lower proportion was between April to July when there was no spawning activity (Table 2). 12 % of the ovaries with POF analyzed during the whole period had hydrated oocytes or oocytes in the late vitellogenic stage.

Atresic oocytes

It is possible to observe atretic oocytes in all mature females, though the abundance of those oocytes in the ovaries is highly variable. Most of the atresia process was present in postspawning females affecting residual oocytes which were not extruded. During the vitellogenic phase it was

possible to detect atresia, but in most cases only very few oocytes were in such stage. Only 4 % of mature females showed signs of mass degeneration of the oocytes.

Discussion

The accuracy of staging ovarian maturity can be enhanced by histological analysis. However the results obtained demonstrate that macroscopic staging can provide satisfactory results if a suitable scale is used. As Walsh and Bowering (1981) pointed out, in this species the main source of error lies in distinguishing fish maturing for the first time or starting maturation from fish recovering from a previous spawning, since the most characteristic feature of the post-spawning fish, (the presence of empty follicles), is not visible by eye. The confusion rate on ripe females, however, was very low (10 %).

The Greenland halibut oogenesis follows a similar pattern to other demersal fishes of Newfoundland waters such as cod (Zamarro *et al.*, 1993), American Plaice (Zamarro, 1992) and redfish (Saborido-Rey, 1994). Chromatin nuclear and perinucleolar stages are present in all females sampled, but only in immature ones does their frequency reach 100 %. Mature females are identified by the presence of cortical alveoli, vitellogenic or hydrated oocytes or because the ovary show postovullatory follicles.

CA oocytes in Greenland halibut decline rapidly in number as yolk accumulation is initiated. These are not observed again in the ovaries until after spawning and the beginning of the reorganization of ovary. This peculiarity is used to distinguish multiple spawners from single spawners (Moser, 1967; Bowers, 1992), i. e., asynchronous from synchronous ooocyte development. Our results suggest that Greenland halibut in Newfoundland is a single spawner although eggs may be laid in batches in contrast with data reported by Feodorov (1968), who indicate more than one spawning season and eggs spawned in a single batch. In all mature ovaries analyzed, only a modal group of developing oocytes was detected. The final maturation oocytes never coexist with oocytes in CA or in earlier vitellogenic stages. Only the modal group corresponding to chromatin nucleolar or perinucleolar stages were present at the same time with maturing oocytes. This indicates that Greenland halibut have ovaries of the 'synchronous group' type of development, as described by Wallace and Selman (1981).

The presence of recent POF in ovaries with oocytes at the final maturation show that Greenland halibut is a fractional spawner, as described by DeVlaming (1983). The term 'fractional spawning' is frequently confused with 'multiple spawner'. While multiple spawning generally refers to more than one spawning in a season, fractional spawning is used for species that spawn part of an ovulated clutch and purely to describe the release of eggs (West, 1990). The hydrated eggs are released in batches over a moderately long period until all the eggs are shed. In reared cod, 17-19 batches were released over 50-60 days (Kjesbu, 1989) and in haddock observed in aquarium, an average of about 16 batches of eggs were shed over 32 days (Hislop *et al.*, 1978).

The temporal variability in the occurrence of CA oocytes does not follow any fixed apparent seasonal pattern. This is related with the fact that the Greenland halibut population has a protracted spawning season with two or more peaks during the year. A protracted breeding season in itself

does not necessarily imply multiple spawnings for each female although it may simply reflect a lack of population synchrony in gonad development (DeJong, 1940; DeVlaming, 1983).

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Table1.- Number of females analyzed by year and division.

Year	1992		1993		1994	
Division	3LM	3N	3LM	3N	3LM	3N
Size range	36-104	-	58-93	68-96	40-104	60-106
Number	99	0	45	45	207	121

Tabla 2.- Percentage of spawning females by months in 1992-1994.

	1992	1993	1994	
			3LM	3N
January	5	7	6	0
February	2	4	8	0
March	1	2	2	0
April	1	2	0	0
May	0	1	1	0
June	0	7	0	0
July	5	26	0	0
August	5	2	5	2
September	3	3	7	11
October	2	2	14	2
November	1	0	8	1
December	2	30	18	1

Table 3.- Summary of female reproductive 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
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.

Tabla 4.- Accuracy of macroscopic diagnosis compared with microscopic examination (numbers in percentage of accuracy)

Micro./macro.	1	2	3	4
1	98	4		
2	2	91	1	26
3		2	85	
4		3	5	74

Tabla 5.- Percentage of Cortical alveoli oocytes by month, 1992-94.

	Jan	Feb	Mar	Apr	May	June	July	August	Sept.	Octob	Nove	Decem
1992	-	50	25	33	0	0	24	8	0	0	57	0
1993	-	-	-	-	-	-	-	63	-	-	-	16
1994	0	55	9	29	43	53	58	52	55	-	-	-

Tabla 6.- Percentage of vitellogenic oocytes by month, 1992-94

			1	2	3	4	5	6	7	8	9	10	11	12
	1992		-	0	17	0	0	0	10	0	0	0	0	0
Vit1	1993		-	-	-	-	-	-	-	25	-	-	-	5
	1994	3LM	0	0	36	40	13	14	4	17	20	-	-	-
		3N	-	-	-	0	25	12	16	15	-	-	-	-
	1992		-	0	17	0	0	100	24	8	10	25	0	0
Vit2	1993		-	-	-	-	-	-	-	13	-	-	-	11
	1994	3LM	0	0	9	30	13	14	12	0	5	-	-	-
		3N	-	-	-	0	13	6	19	0	-	-	-	-
	1992		-	6	0	0	0	0	24	46	60	25	21	100
Vit3	1993		-	-	-	-	-	-	-	0	-	-	-	37
	1994	3LM	100	11	9	10	19	29	4	28	10	-	-	-
		3N	-	-	-	0	10	3	16	23	-	-	-	-

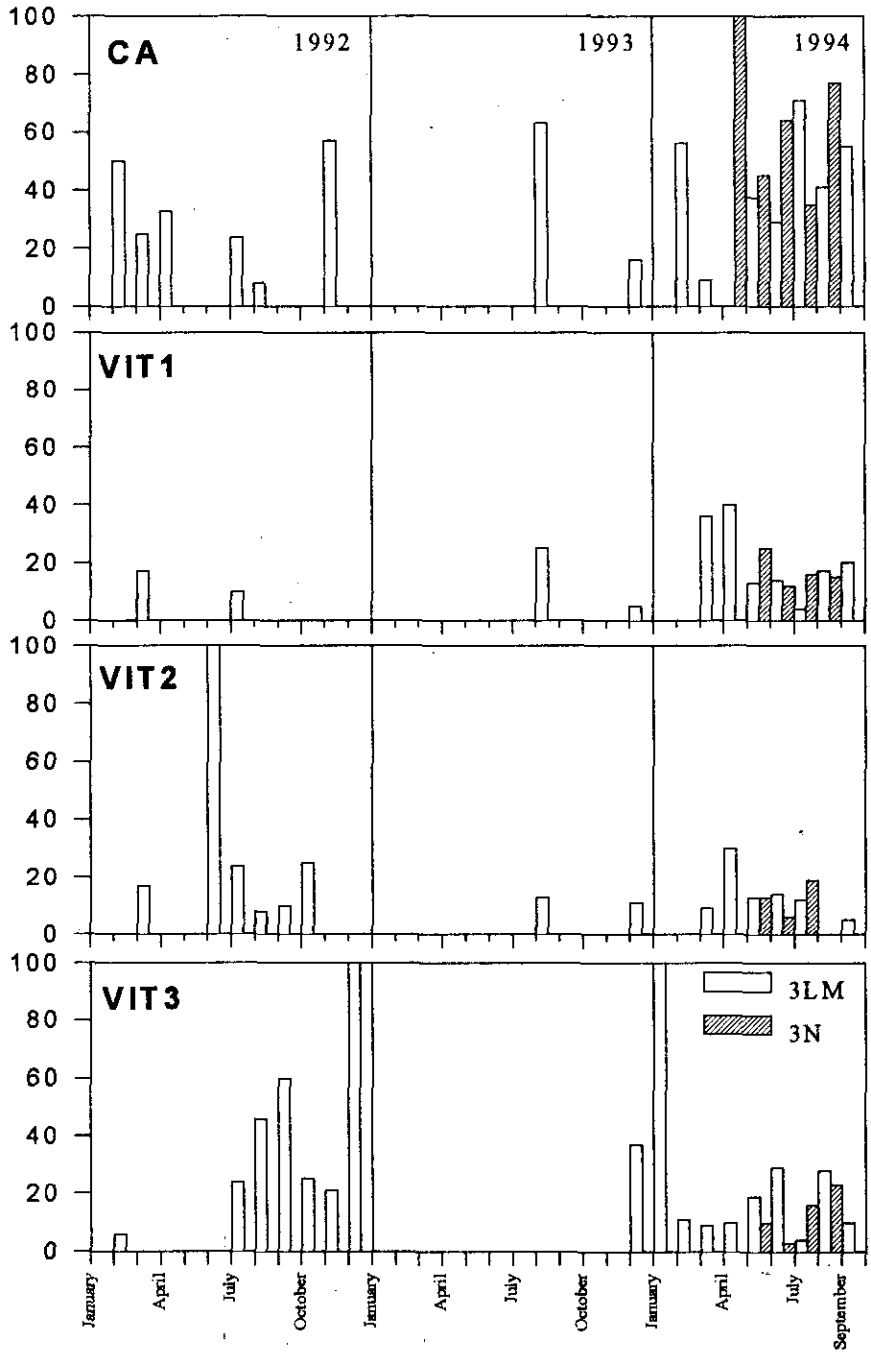

Figure 1.- Frequency of Cortical alveoli and Vitellogenic satges of Greendland halibut in 1992-94. 1994 data separated in Div. 3LM and 3N.