Northwest Atlantic Fisheries Organization

Serial No. N2525

NAFO SCR Doc. 95/18

SCIENTIFIC COUNCIL MEETING - JUNE 1995

Sexual Maturity of Greenland Halibut at West Greenland Based on Visual and Histological Observations

by

J. Gerup Nielsen and J. Boje

Greenland Fisheries Research Institute, Tagensvej 135
DK-2200 Copenhagen N, Denmark

Abstract

Gonads from female Greenland halibut (*Reinhardtius hippoglossoides* (Walbaum)) caught in West Greenland fjords at Uummannaq in August were examined in order to compare visual examination in field and histological analysis and to provide information on length at sexual maturity. When examined by means of histological slices, 20% of the fish were classified as juvenile/immature, compared to 33% of the fish when using visual examination. This degree of misclassification results in a discrepancy between the two estimated M_{50} values, being 58 cm and 65 cm for the visual and histological analyses, respectively. At this state, visual examinations in the field are therefore not sufficient in order to determine sexual maturity. A relation between gonadosomatic growth and maturity stage obtained by histological analysis, suggest that no enhancement in growth rate of gonads occurs when developing from immature to mature stage. For practical purposes, mature fish may be distinguished by means of gonadosomatic indices in the future. The general level of maturation, where no ripe, spawning or spent fish were recorded, are in accordance with previous investigations, suggesting that only insignificant spawning takes place in the West Greenland fjords.

Introduction

Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum) among other deep sea species, is receiving an increased interest as a commercial resource in the Northwest Atlantic. With the collapse of important cod stock components in the area, the fishery has now focused on the Greenland halibut resource. Along the continental slopes of Canada and Greenland, in the Davis Strait and in Baffin Bay annual catches of Greenland halibut has recently reached 90,000 tons. However, many biological parameters on the populations are still poorly known and the assessments of the resource suffer from this fact.

In the Northwest Atlantic Greenland halibut spawning has been observed on the continental slopes from Flemish Pass off Newfoundland (Junquera & Zamarro 1994) and are expected to extend northward to the Davis Strait off West Greenland south of the submarine ridge at 67°N latitude (Jensen 1935; Smidt 1969; Templeman 1973). Spawning has also been recorded in local areas in Gulf of St. Lawrence (Fairbairn 1981; Bowering 1982) and sporadically in the fjords of West Greenland north of 69°N latitude (Riget & Boje 1989). For Greenland halibut stocks around Iceland, development of the gonads are dependant on a

temperature range being about of 4-5°C (Sigurdsson 1977,1979). As bottom temperature conditions of about 3-4°C only exists south of the submarine ridge in the Davis Strait, distribution of the spawning grounds in this area are probably limited in accordance with this (Jensen 1935). The occurrence of adult fish in the fjords of West Greenland, Labrador and Newfoundland, and the contemporary lack of any observations of spawners in these areas are also assumed related to the low bottom temperatures of about 1-2 °C (Jensen 1935; Templeman 1973).

The probability to discover the ripe maturity stage by visual examination is small because this stage develop due to hydration of the oocytes and not due to somatic growth, and because after spawning the gonads very rapidly recovers to a stage similar to an early maturation stage (Fedorov 1968),

In order to confirm former assessments on sexual maturity of Greenland halibut in West Greenland which is based on visual examinations (Riget & Boje 1989), histological and visual examination of ovaries were carried out simultaneously in this study. The maturity ogives for Greenland halibut in the area are the first attempt to provide maturity data for future analytical assessments.

Materials and methods

Ovaries were collected from 117 Greenland halibut caught in Uummannaq fjord at West Greenland (in NAFO Division 1A, 70° 24'N latitude) at depth of 300-600 meters by longline and gillnet in August 1994. Bottom temperatures in the depth interval were recorded to 1° - 1.6° C, decreasing by depth. All fish were length measured (total length) to the centimetre below, and sampled stratified in the range 41 - 102 cm. Body weight and gonad weight were recorded. The maturity stage was assessed visually in the field using a description by Riget and Boje (1989) (Table 1). The ovaries were stored in 10% buffered formaldehyde for examination in the laboratory.

Gonadosomatic indices was estimated as gonad weight/length^3 , where length^3 is assumed a non-biased approximation for body weight.

Considering the determination of the length of first maturity, it is of major importance to record the most apparent oocyte stages in the ovary. As it is too time consuming to examine all oocytes in an ovary, only few slice sections from each ovary are typically examined. In order to determine the effect of choice of different slice sections in the ovary on the assessment of maturity, one ovary from a fish of 65 cm was sliced at three sections dividing the ovary in approximately four equal parts. As a rough measure of maturity stage was used diameters of oocytes. All measurable oocyte diameters were recorded in the three sections and differences between the sections was tested by means of a non-parametric test (Kruskal-Wallis test, 95% significance level) as the data was non-normally distributed. However, the test was not significant, *viz.* there were no difference between oocyte diameters in the three sections.

In the laboratory, samples were taken from the middle part of the ovaries and placed in a phosphate buffer for 24 hours. Subsequently, the samples were cooled for 2 minutes in an ethanol/ice mixture at about -70 °C. Sections of 20 μm thickness were sliced at -20° C using a Microm HM500 OM and stained with Heamatoxylin and Eosin (1% aqueous solution). Then slices were dehydrated 1 minute in each of the following series; 70% ethanol, 99% ethanol, 99% ethanol, toluene and toluene. Finally, slices were mounted with Entellan-Neu. Apart from these slices, additionally 4 ovaries were sliced embedded in paraffin. This method allows to cut at 10 μm and subsequently more details are visible. The method is however time consuming and the slices were only made in order to compare with the freeze-cut method to ensure correct assessment.

Ovaries were classified using a description by Fedorov (1968) (Table 1.).

Probit transformed values of maturity proportions by length (5-cm groups) were used to

generate M_{50} by means of linear regression. The line formula obtained by regression was used to recalculate values, which were retransformed in order create maturity ogives. Standard deviation of the M_{50} estimate was calculated by an approximation using the length interval corresponding to [probit(maturity prop.)=0] -[probit (maturity prop.)=1].

Gonadosomatic values (GI) were logit transformed in order to be normal distributed. Trends in mean values of the different maturity stages assessed histologically were tested by variance analyses after the model: overall mean logit(GI)=length+maturity stage+residuals.

Results

Greenland halibut in the length range 41-102 cm were sampled for the analysis. Based on the histological assessment 20% of the total material consisted of juvenile and mature fish (stages I and II), while the remaining were all maturing (stages IIIa and IIIb). Gonads in ripe, spent or recovering stages were observed by any of the methods (Table 1).

In general the histological analyses were in accordance with the visual assessment for fish with length less than about 60 cm. For longer fish a larger part were determined as being maturing (stages IIIa and b) by histological assessment than by visual assessment. As a total this results in a misclassification of 13% between the two categories immature (stages I and II) and maturing (stages IIIa and IIIb). In Table 3 is given the percentage maturation by length from visual and histological assessments and in Fig. 1 given the corresponding maturity ogives deriving from probit analysis. Range and slope of the two curves are very similar, but length at 50% maturation (M_{50}) is very different. This reparation between the two methods results in a M_{50} of 65.4 cm ± 0.88 cm (95% confidence levels) for the visual method compared to 58.1 cm ± 1.06 cm using histological analysis. Thus, the visual examinations seem to underestimate sexual maturity substantially.

There were only observed degenerating cells in 13% of the gonads, which were classified as maturing by histological examinations. Degenerating cells were observed within fish length ranges of 70-90 cm, most abundant in the range 80-84 cm.

In order to compare the histological examinations with somatic growth of the gonad, a gonadosomatic index (GI) have been established and is given in Fig. 2. The gonadosomatic index express the gonad weight as a proportion of whole body weight. From the figure it is obvious that the major part of the gonads weight less than 1% of the body weight. Few fish accelerate somatic growth when reaching lengths of about 75 cm. Mean values for each maturity stage are connected by a dotted line and also given in Table 4. Fedorov (1968) has previously provided ranges of gonadosomatic indices representative for the different maturity stages. The present material is compared to these figures in Table 4. Because of deviation in the two scales of determination of stage II, a comparison is not easy carried out. The stage II determined for present data is comparable with the lower range of Fedorov's stage III. From the comparison it is obvious that values deriving from present study are lower than the ones obtained by Fedorov except for stage I where values are similar. Highest value in present study for stage III is 3.1 compared to 10 in Fedorov's material. Smaller fish, determined to be in stage I in present study reach longer sizes than do corresponding stage in Fedorov's study.

Discussion

Walsh and Bowering (1981) also conducted histological and visual examinations of gonads. Their study showed that maturity ogives obtained by the two methods were different so that higher maturity proportions at length were obtained by visual than by histological assessment. This is in contradiction to the present study where highest proportions at length were obtained by histological assessment. Moreover, the difference in M_{50} between the two methods are even higher in present study, 7.3 cm compared to approximately 2 cm in the study by Walsh and Bowering (1981). Bowering (1983) later concluded that for practical purposes visual observations may be adequate to determine the onset of sexual maturity. In the present study the difference between the two methods is outstanding and further research

in the area are to be conducted before visual examinations can be used to determine onset of sexual maturity as well as a maturity ogive.

Missing observations of ripe, spent or recovering fish in present study are in accordance with previous investigations in Greenland waters. Observations of ripe or ripening female Greenland halibut in coastal or fjord areas of West Greenland are very sparse. Smidt (1969) summarises observations from the period 1908-1960, in which 5 specimens with ripe eggs and 26 spent females were observed (March and August). Later, Riget and Boje (1989) reported a total of 9 females in ripe or running condition among 3,600 females examined in 1987 and 1988, all in March. More present observations of fully mature Greenland halibut are within the same order of magnitude as previous records (personal data, not published). Due to the sampling time in August, which is supposed to be several month after spawning, no gonads in ripe or spent stages are expected to be present. It is, however, surprising that no recovered fish were found, as there previously have been observed females in ripe and spent condition in February and March (Riget and Boje, 1989).

Expressing gonadosomatic indices by means of histological assessed maturity stages seem to give a better understanding of the somatic growth of the gonads along with the maturation process. Mean values of GI for the maturity stages as given in Table 4 are all significant different at the 95% level when treated as logit transformed values. However, some overlap is observed between the stages. Thus, fish having gonads weights of more than 0.5 % of their body weight can roughly be classified as maturing according to the present data. On the other hand, some gonads having weights less than 0.5% of the body weight, are classified mature, and therefore the limit at 0.5% should not be taken conclusive. Although more data have to be compiled in order to make such a distinction, this method may be straightforward and objective in order to be carried out in the field.

When comparing gonadosomatic indices by maturity stage from present study some disagreements with Fedorov's (1968) values are obvious (Table 4.). Only for stage I gonadosomatic index (GI) values are within the same interval. For stages II and III, GI values in present study is much lower than for the corresponding values in Fedorov's study. There is no immediate explanation for this fact, other than area effects causing a difference in condition factor. Moreover, immature fish in present study reach longer sizes than corresponding fish in Fedorov's study, suggesting that some fish in the West Greenland fjord never reach sexual maturity in their life span.

Conclusions

A comparison of two methods, histological and visual, to evaluate the maturity ogive for Greenland halibut in the West Greenland fjords, displayed substantial differences in both length at first maturity as well as in length at 50% maturation probability. Therefore, the previously used method, visual examination in the field, must be considered insufficient in order to determine a maturity ogive. A relation between somatic growth of the gonad and the maturity stage suggest that the major increase in growthrate occurs between stage IIIa and IIIb, and thus not between the juvenile/immature stages and the maturing stages.

The present study confirms previous investigations on the Greenland halibut fjord components in West Greenland, that no comprehensive maturation towards spawning occurs for any ages, but rather an insignificant maturation.

References

Bowering,W.R. (1982). Greenland halibut in Gulf of St. Lawrence. *J. Northwest Atl. Fish. Sci.*, Vol. 3: 141-147.

Bowering,W.R. (1983). Age, growth, and sexual maturity of Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), in the Canadian Northwest Atlantic. *Fisheries Bulletin*: Vol. 81, No. 3: 599-611.

Fairbairn, D.J. (1981). A biochemical genetic analysis of population differentiation in Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), from the Northwest Atlantic, Gulf of St. Lawrence and the Bering Sea. *Can. J. Fish. Aquat. Sci.*, **38**: 782-794.

Fedorov, K.Y. (1968). Ovogenez i polovoi tsiki chernogo paltusa. Tr Polyarn. Nauchno-Issled. Proektn. Inst. Morsk. Rybn. Khoz. Okeanogr., **23**: 425-450. [Oogenesis and sexual maturity of the Greenland halibut. *Can. Fish. Aquat. Sci. Transl.* No. 4559. 1979.]

Jensen, Ad.S. (1935). The Greenland halibut (*Reinhardtius hippoglossoides* (Walb.)) its development and migrations . *K. Danske Vidensk. Selsk. Skr.*, 9RK., **6**(4): 1-32.

Junquera,S and J. Zamarro. (1994). Sexual maturity and spawning of Greenland halibut (*Reinhardtius hippoglossoides*) from Flemish Pass area. *NAFO Sci. Coun Studies* 20: 47-52.

Riget,F and J.Boje (1989). Fishery and some biological aspects of Greenland halibut (Reinhardtius hippoglossoides) in West Greenland waters. *NAFO Sci. Con. Studies*. 13: 41-52

Sigurdsson, A. (1977). On the spawning grounds of Greenland halibut in Icelandic waters. ICES C.M. 1977/F:28, 10 pp.

Sigurdsson, A. (1979). The Greenland halibut, *Reinhardtius hippoglossoides* (Walb.) at Iceland. *Hafrannsoknir*, **16**, 31 p.

Smidt, E. (1969). The Greenland halibut, *Reinhardtius hippoglossoides* (Walb.) biology and exploitation in Greenland waters. *Medd. Danm. Fisk.- og Havunders* , N.S., **5**: 79-148.

Templeman,W. (1973). Distribution and abundance of the Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), in the Northwest Atlantic. *ICNAF Res. Bull.*, No. 10: 63-98.

Walsh,S.J. and W.R.Bowering (1981). Histological and visual observations on oogenesis and sexual maturity in Greenland halibut off northern Labrador. *NAFO Sci. Coun Studies*, No 1: 71-75.

Table 1. Descriptive stages of maturity used for visual and histological assessments of Greenland halibut (Visual stages after Riget and Boje (1989), Histological stages after Fedorov (1968)).

Visual		Histological	
I	Juvenile or immature: ovary very small, eggs not visible to naked eye	I	Oocytes in protoplasmic growth stage, nests of oogonia and oocytes present, ovarian wall thin (juvenile stage)
		II	Monolayer follicle phase in primary oocyte development with stage 1 present.
II	Maturing (A): eggs becoming visible to the naked eye	IIIa	Early stage III, oocyte entering trophoplasmic growth, with beginning vacuolation and primary yolk accumulation, chorion (zona radiata) visible
III	Maturing (B): eggs 1-2 mm in diameter	IIIb	Late stage III oocytes enter intensive trophoplasmic growth and heavy yolk deposition, mixture of early and late stage III and some stage I and II
IV	Maturing (C): eggs 2-4 mm in diameter. The category includes the ripe condition where contents are almost liquid with translucent eggs	IV	Most developed oocytes are filled with yolk or are completing growth, the next generation of oocytes are in vacuolation and primary yolk accumulation, radial striations appear in the chorion (zona radiata)
V	Running stage (partly spent): some eggs extruded but several thousand clear eggs remaining	V	Eggs have hydrated and the appearance of flowing sexual products is noted, commencement of spawning is ready to begin, older generation oocytes are entering periods of maturation.
VI	Spent stage: ovary appears very reddish purple, wall is thick and tough, some residual clear or opaque eggs are seen	VI	Ovary contains oocytes of new generation in the phase of vacuolation and primary yolk accumulation (stage III) and the entire complex of sexual cells of stage II are present, ovarian wall is thick, large number of ruptured follicles and unreleased oocytes are undergoing resorption (stages V-III); the latter part of this stage is sometimes referred as the 'resting stage'.

Table 2. Comparison of visual (V) and histological (H) assessments of maturity stages of Greenland halibut by fish length.

Length (cm)	No of fish	Juvenile/Immature		Maturing	
		V	H	V	H
40-44	1	1	1	0	0
45-49	2	2	2	0	0
50-54	6	6	5	0	1
55-59	10	10	9	0	1
60-64	13	10	5	3	8
65-69	9	5	1	4	8
70-74	8	3	0	5	8
75-79	19	0	0	19	19
80-84	23	0	0	23	23
85-89	17	1	0	16	17
90-94	7	0	0	7	7
95-99	1	0	0	1	1
100-104	1	0	0	1	1
Total	117	38	23	79	94
%		32.5	19.7	67.5	80.3

Table 3. Percentage maturation from visual and histological assessments by length group for Greenland halibut.

Length (cm)	No. of fish	Visual		Histological	
		No.	%	No.	%
40-44	1	0	0	0	0
45-49	2	0	0	0	0
50-54	6	0	0	0	0
55-59	10	0	0	0	0
60-64	13	3	23	0	0
65-69	9	4	44	5	56
70-74	8	5	63	3	38
75-79	19	19	100	14	74
80-84	23	23	100	18	78
85-89	17	16	94	11	65
90-94	7	6	86	6	86
95-99	1	1	100	1	100
100-104	1	1	100	1	100

Table 4. Gonadosomatic indices (GI), (mean value and range), and corresponding fish lengths, from present study using a maturity scale by Riget and Boje (1989) compared to values given by Fedorov (1968). The dashed line indicate boundary between immature and mature stages.

Present data					Fedorov (1968)		
stage	mean GI	range GI	mean length (cm)	length range (cm)	stage	range GI	length range (cm)
I	0.26	0.1-0.6	54.3	41-87	I	0.2-0.4	30-35
					II	<2	30-60
II	0.46	0.1-1.1	76.0	59-92			
III	1.40	0.7-3.1	85.2	75-102	III	2-10	
IV					IV	6-15	
V					V	15-18	
VI					VI	2.3-5	

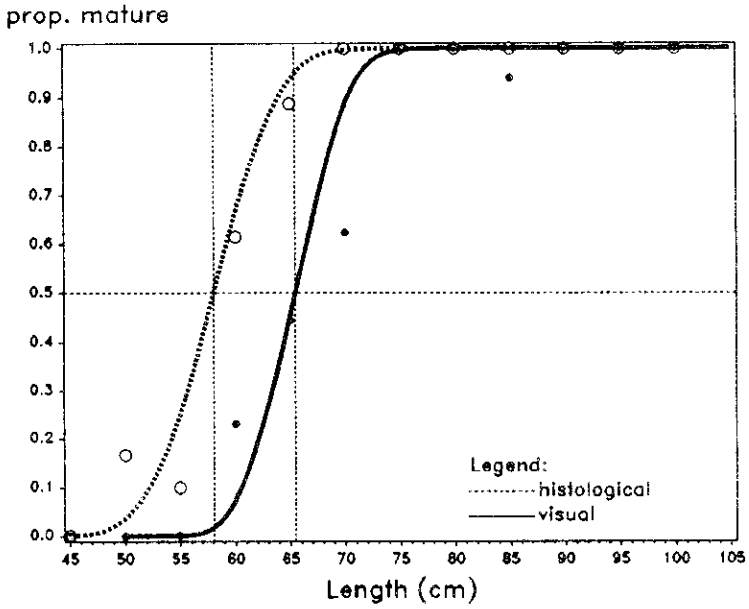

Fig. 1. Maturity ogive for Greenland halibut based on histological (circles and dotted curve) and visual (dots and solid curve) analyses.

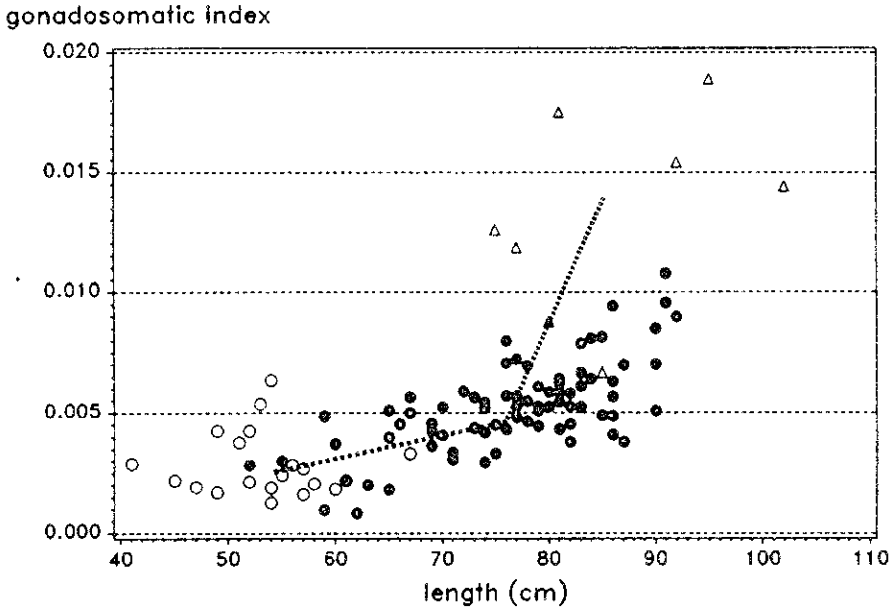

Fig. 2. Gonadosomatic indices. Empty circles represents histological stage II, filled circles represents stage IIIa and triangles represents stage IIIb. Mean values for each stage are connected by a dotted line.