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Northwest Atlantic Fisheries Organization

Serial No. N4561 **NAFO SCR Doc. 01/166**

SCIENTIFIC COUNCIL MEETING - SEPTEMBER 2001
(Deep-sea Fisheries Symposium – Oral)

Maturation and Occurrence of Atresia in Oocytes of Greenland Halibut (*Reinhardtius hippoglossoides*, Walbaum)

by

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Abstract

Measurements of oocyte diameters and histological examinations were conducted on gonads of Greenland halibut females (*Reinhardtius hippoglossoides*, Walbaum) from East-Greenland (1997 and 1998), Faeroe Islands (1999) and Hatton Bank (1999). Morphometric measures of the oocytes revealed that the diameter of the largest yolk vakuole and the thickness of *zona radiata* correlated well with oocyte diameter. Athretic oocytes were not found in immature gonads, but were observed in 70% of the gonads of sexually maturing and mature specimens. Atresia mostly affected the largest oocytes (500-800µ) in maturing specimens and the smallest vitellogenic oocytes (600-1200µ) in mature specimens. Atresia infrequently affected vitellogenic oocytes larger than 1200 µ. Intensity of atresia was negatively correlated with hepatosomatic index and with potential fecundity for the entire material, but no significant correlation was found within any area or year. There was also found no difference in oocyte morphometry or atresia between areas or years.

Key words: Greenland halibut, maturity, atresia

Introduction

Greenland halibut is a flatfish widely distributed in the north Atlantic, and it is observed from 400 m to depths of about 2000m (Boje and Hareide, 1993). Greenland halibut is described as a boreal-arctic species and is found mainly at temperatures between -1° and 4°C.

Greenland halibut in East Greenland, Icelandic and Faroese waters constitute one management unit (Figure 1). Little is known on the reproduction biology of this component. Greenland halibut off East Greenland, Iceland and the Faeroe Islands is so far considered one spawning unit and might have their spawning ground southwest of Iceland (Sigurdsson, 1977; Magnusson 1977). However, running females have also been found in East Greenland (A.C. Gundersen, unpublished data – presented to this meeting), Faroese waters (L. H. Ofstad, unpublished data) and Hatton Bank (Møreforsking unpublished data).

Recently, focus has been put on reproduction and recruitment processes for this species *e.g.* fecundity studies of Greenland halibut in East Greenland in 1997 (Gundersen *et al*. 2001), and distribution of young Greenland halibut around South Greenland in 1998 (Woll, 2000).

The fecundity study conducted in 1997 revealed that three types of oocytes were observed in the ovaries collected in late summer (Gundersen *et al*. 2001); non-vitellogenic oocytes, defined as recruitment oocytes (R), early vitellogenic oocytes (G2) with a diameter range of 490-1 050 µm after preservation (mean 730 µm), and fully vitellogenic oocytes (G1) that were easily distinguished early vitellogenic oocytes due to transparency, size and a small, distinct nucleus in the G2 oocytes. Diameter range of G1 was 900 – 1 650 µm after preservation (mean 1 250 µm). Potential fecundity of Greenland halibut in East Greenland waters was in the range 32 500-277 000 oocytes per female for individuals in the total length range 63cm – 108 cm. Mean gonadosomatic index (GSI) was in the range 1.0-4.9% indicating ovaries in a maturing state (Gundersen *et al*. 2001).

Degeneration and resorption (atresia) of developing oocytes is one of the mechanisms used to adjust fecundity. In most fish species atresia is rarely observed, but among Greenland halibut this seems to be commonly occurring (Fedorov 1968, 1971; Walsh & Bowering 1981; Junquera et al. 1999). Fedorov (1968) described the development of atretic oocytes from Greenland halibut from the Barents Sea and wrote " During histological analysis of Greenland halibut ovaries, ones attention is drawn to resorption of oocytes of the phases of vacuolation and primary yolk accumulation continously going on throughout the year". Walsh and Bowering (1981) also found that young (early maturing) greenland halibut females caught off nothern Labrador often had gonads with showed large-scale atresia. Junquera *et al*. 1999 in their study of Greenland halibut from east of Newfoundland found that females with high intensity of atresia had oocytes in the cortical alveoli stage or primary vitellogenesis. *Op. cit.* also showed that many females with more developed oocytes had atresia on a smaller scale (0-20% of the oocytes were affected).

The reason for atresia to occur in other fish species is mainly energy shortage due to lack of food supply or low HSI or other abiotic factors like temperature giving unfavorable conditions for spawning. Occurrence of atresia may result in overestimation of potential fecundity, depending on the timing of sampling versus onset of atresia.

Studies on fish reproduction are fundamental for the implementation of the precautionary approach in fisheries management. It might give additional information for assessment but it is not fundamental for implementation of prec.app.
The fishery for Greenland halibut is a directed trawl fishery carried out since the 1960'ties. Landings peaked in the late 1980'ties with 61 000 t, and have since then declined to about 25 000 t in recent years.

Stock assessments suggest that the Greenland halibut stock biomass for the East Greenland, Icelandic and Faroe stock has been falling since the late 1980'ies. Fishing mortality has been substantially above the recommended level for a decade. The decline in biomass seems to have been halted since 1998, but biomass is still well below the level corresponding to maximum sustainable yield. A combination of unreliable maturity data and age readings from recent years is still the major barrier for carrying out any more precise assessment. More specific, the unreliable maturity data prevents an accurate estimate the spawning stock biomass (SSB) and impede any use of SSB as a reference point for management advice for the stock.

In recent years a new fishery for Greenland halibut in Hatton Bank west of the British Isles has developed.

The aim of this study was to obtain information on reproduction biology of the peripheral populations of the management unit stock in East Greenland, Iceland and Faroe Islands and particularly to study the onset, development and effect of atresia in oocytes during maturation.

Material and Methods

Fish catches

Ovaries were collected in East-Greenland waters during joint Norwegian-Greenland surveys July-August in 1997 and 1998. A total of 30 samples were taken from longline catches in 1997, and from trawl catches in 1998 (Table 1). Samples were stored at 3.6% buffered formaldehyde at sea and prepared for embedding in the laboratory.

Ovaries from Faroe Islands were collected duing the regular groundfish survey conducted by the Marine Laboratory of Faroe Islands in June 1998.

A total of 60 ovaries were collected. Ovaries from the waters around Faroe Islands were collected during a regular deepwater groundfish survey conducted by Faroese Fisheries Laboratory in June 1999. The trawl hauls (of duration 3-5 hours) were taken in the depth range 400-600 m on the Faroe Plateau where Greenland halibut comprised the majority of the catch. The Greenland halibut catches were usually dominated by small males (50-65 cm) wheras large females were seldom caught.

From Hatton Bank ovaries were collected from the longline catches of the commercial vessel "M/S Loran" in 1999. The Greenland halibut caught here were unusually large and 99% were females.

Measurements of oocyte diameter

At sea, samples from fresh fish were stored in 3.6% formaldehyde. In the lab, subsamples from the middle of the right gonad were taken for diameter analysis. The diameters were measured automatically using image analysis. The oocytes were first separated by pipette, using fast in/out movements through pipette tips of appropriate size. The oocytes were distributed in physiological saline on a petri-disc and measured. The oocytes were lighted to appear light on dark background. A Polaroid DMC 1a digital camera was mounted on a Wild MS5 microscope, and the pictures were analysed using the programme NIH-image on a Power Macintosh G3. For each individual, the first 80 oocytes were used in the data analysis.

Preparation of histological samples

Dehydration

Initially, tissue was sampled from the midsection of the right lobe of the ovary and stored in 70% ethanol for at least 24 hours. Then dehydration was continued successively by increasing the concentration of ethanol to 96% in 3 steps: 90% ethanol for 1 hour, repetition of this step and at last 96% ethanol for 1 hour. During these steps the ovary samples kept in bottles in motion.

Infiltration

Ovary tissue was then infiltrated by technovit. Infiltration consisted of 3 steps. First technovit was mixed with ethanol (1:1) and stored for 2 hours. Then 100% technovit was added in two steps, first with an incubation time of 36 and then 24 hours.

Polymerisation and mounting

Polymerisation was conducted by using newly activated technovit added activator making the fluid to polymerise. Blocks were kept at a cool place for 3-5 hours. Then blocks were mounted and prepared for sectioning.

Sectioning and staining

Blocks were sectioned using a mycrotome. Thickness of sections were 3µ. Then sectiones were stained in toluidine blue and prepared for microscopy.

Histological analyses

Sections from a total of 100 female gonads were analysed (Table 1). The numbers and diameters of atretic oocytes of each of four stages (Fig. 6) were registered for each section. Numbers and mean diameters of intact oocytes (G1 and G2) were also registered for each section. The total numbers of atretic oocytes and intact oocytes (potential fecundity) in the ovary were calculated from occurence in the section and from the relative sizes of atretic and intact oocytes. Potential fecundity numbers in the presented material were calculated from the sections, and not the standard volumetric method. For each individual, about five vitellogenic oocytes (where available) and five of the cortical alveoli stage which had been sectioned through the nucleus were measured as follows: (1): total diameter of oocyte (2) thickness of zona radiata (3) thickness of the cortical alveoli layer (non-vitellogenic oocytes only) (4) diameter of the largest yolk vakuole (vitellogenic oocytes only).

Data analyses

Microsoft Excel was used to manage the data and to make the graphs presenting oocyte diameters. Statistic tests and scatterplots were made using Statistica release 5.5.

HSI (hepatosomatic index) = (weight of liver)/(total fish weight)
GSI (gonadosomatic index) = (weight of gonad)/(total fish weight)

Results

Oocyte diameters

The material from Greenland 1997 showed two distinct peaks, one around 700 and the other around 1250 u (Fig 2). The Greenland 1998 samples showed a single peak and a group recrouting to vitellogenesis (Fig. 3). The Faroe islands samples from 1999 looked similar to Greenland 1998, but had a separate small peak of larger oocytes (Fig.4). Hatton Bank 1999 samples showed two peaks, around 700 and around 1550 u (Fig 5).

Histology

A description of the development of atretic oocytes were made from the present material, and for convenience this development was subdivided into four stages (Fig. 6). The proportion of atretic oocytes in the gonads of the four stages was as 1:1:2.7:6.8 after correcting for the size differences between the stages.

The 100 gonads in this study ranged from immature with largest oocyte diameters of 200 u to maturing with largest oocyte diameters of 2200 u. There was not found oocytes in final maturation. The thickness of the layer with cortical alveoli had only a weak correlation with oocyte diameter. Diameter of the largest yolk globules increased with increasing oocyte diameter (Fig. 7). Thickness of *zona radiata* also increased with increasing oocyte diameter (Fig. 8).

Atresia was not found in gonads with oocyte diameters below 500µ (Fig. 9). Atresia was found in 70% of the individuals with leading oocyte cohorte larger than 500µ.

Atresia was observed in both cortical alveoli stage, early vitellogenesis and in later vitellogenesis.

When atresia was found in fish in early vitellogenic phase (oocytes<1000µ) the largest oocytes were the ones affected (Fig. 10). When atresia was found in fish in later vitellogenic phase (oocytes>1200µ) the smallest oocytes were the ones affected. In fish with oocytes between 800 and 1200µ atresia affected oocytes of the whole size range. There were large differences in oocyte development and atresia between the areas sampled (Fig 9), due to differences in fish size and sampling time. When all results were pooled, significant negative correlations were found between potential fecundity and atresia (Fig. 11) and between HSI and atresia (Fig. 12).

Discussion

Diameter

Size of yolk globules increased with increasing oocyte diameter, and this was used to assess the size / developmental stage the atretic oocytes had before they became atretic. The largest yolk globule observed here was 240 µ, and Fedorov (1968) registered sizes of up to 300 µ. Fedorov (1968) found thickness of zona radiata to be 45-50µ among fully developed oocytes, extrapolating results from this study indicated thickness of 45 µ. Four oocytes between 2000 and 2200 µ had *zona radiata* thickness of between 36 and 53 µ.

In this study there was found no atresia among oocytes below 500 µ, and this is in accordance with earlier works (Fedorov 1968, Walsh & Bowering 1981, Junquera *et al*. 1999). In the material presented here there was not found large-scale atresia in gonads with large oocytes. Two main types of atresia were seen, as in Fedorov (1968): One type affecting cortical alveoli stage or early vitellogenic stage that can seriously inflict or stop the maturation process. Other authors have also registered that this type of atresia can occur in large scale in gonads of fish in

puberty (Walsh & Bowering 1981 and Junquera *et al*. 1999). The other type of atresia attacs the smallest vitellogenic oocytes and results in a clear size separation between cohorts of oocytes. The material in the present work did not allow valid comparisons of atresia between areas, due to the small number of samples and the differences in fish size and developmental stage from the different areas.

A lot of atresia is often found in the gonads of fish in or approaching puberty. In Greenland halibut from the banks east of Newfoundland Junquera et al. 1999 found atresia in 80% of the gonads in cortical alveoli stage, and in 60% of the individuals more than 20% of the oocytes had been affected. In fish from Nothern Labrador caught in august Walsh & Bowering (1981) found that virtually every large fish in puberty had atresia among the oocytes in cortical alveoli and early vitellogenic stage. Furtermore, they found an abrupt decrease in atresia with increasing fish length.

Proportion of atretic oocytes in the gonads of the four stages used here (Figure 6) was as 1:1:2.7:6.8 after correcting for unequal size. The different stages can be assumed to have durations in the same proportion. That would imply that the first stage has a duration of about 10 % of the total duration of the atretic oocyte. The main resorption of the cytoplasma (stage 3) appears to occur quite fast, within 30 % of the total duration. The last stage (stage 4) with remnants of zona radiata and a thick capsule accounts to more than 50 % of the total duration.

Atresia is generally thought to occur when the individual fish lacks the energy or fat reserves neccesary to develop all its oldest generation of oocytes into eggs. Kjesbu *et al*. (1991) showed that Atlantic cod (*Gadus morhua*) which received a reduced feed ration lost more oocytes through vitellogenesis than the full-fed fish. Among the Greenland halibut presented here there was found a significant negative correlation between atresia and HSI, but the heterogenous pooled material used can have given a false correlation. The negative correlation that was found between atresia and potential fecundity was probably due to two causes: (1) atresia occured less frequent among large females (2) atresia had reduced potential fecundity among the affected females.

It is neccesary to know the duration of the atretic oocytes in order to correctly evaluate the effect of atresia on potential fecundity. Hunter & Macewicz (1985) estimated alfa-atresia in nothern anchovy (*Engraulis mordax*) to last 8 days at 16 °C. Kjesbu et al. 1991 estimated mean duration in cod to be 13 days at 8 °C. The duration in Greenland halibut will be much longer because of tha large size and the low temperatures, controlled experiments in tanks will be neccesary to find the duration.

Atresia among the smallest oocytes of the leading cohort will seldom affect potential fecundity. There will be small or no errors in estimation of potential fecundity due to atresia in individuals that have mean size of leading cohort oocytes above 1000 µ. This is supported by the results from Junquera *et al* 1999. Potential fecundity may be overestimated due to atresia in individuals having leading cohorte oocytes under 1000 µ, and the possible error increases sharply with decreasing oocyte size.

Acknowledgements

The authors want to thank the Norwegian Research Council, The Nordic Ministry Council, the Greenland Institute of Natural resources and the Greenland Home Rule for funding the work and the Marine Laboratory in Faroe Islands for conducting the survey.

Further we would like to thank the crew on the vessels Loran, Møgstertrål, Fjellmøy and Magnus Heinasson for their help during sampling. Thank to A.H. Hellevik and J.E. Dyb Møre Research for their contributions to collect samples.

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Table 1. Catch data of the 100 Greenland halibut females analysed in the present work.

Area / year	*N*	*Depth*	*Gear*	*Month*
East Greenland 1996	*2*	*1200m*	*Longline*	*July*
East Greenland 1997	*19*	*1200m*	*Longline*	*July*
East Greenland 1998	*31*	*750m*	*Trawl*	*July*
Hatton Bank 1999	*20*	*1350m*	*Longline*	*Sept.*
Faroe Islands 1999	*28*	*450m*	*Trawl*	*June*
Total	*100*			

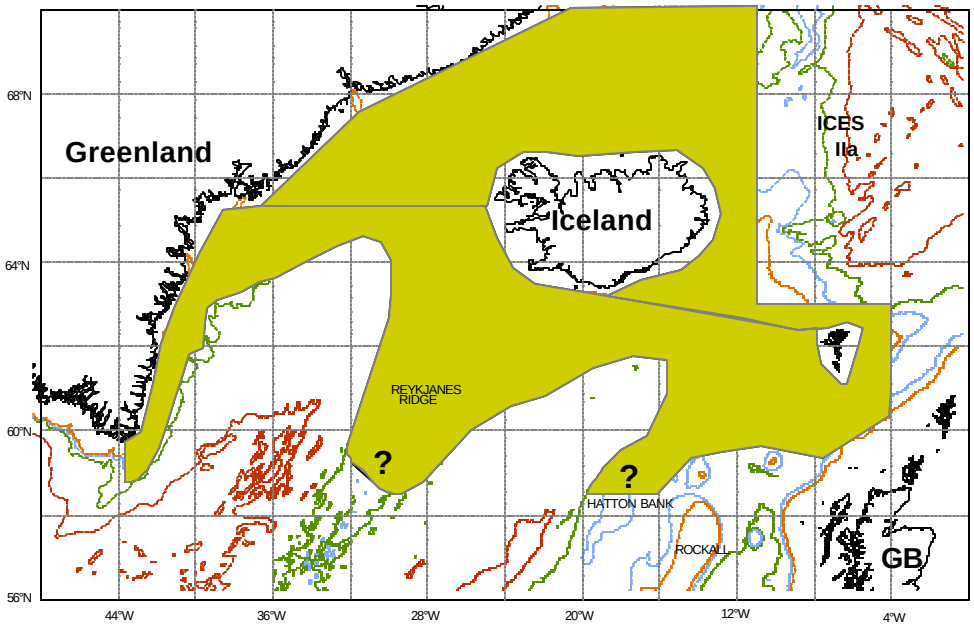

Fig. 1. Distribution of West-Nordic Greenland halibut.

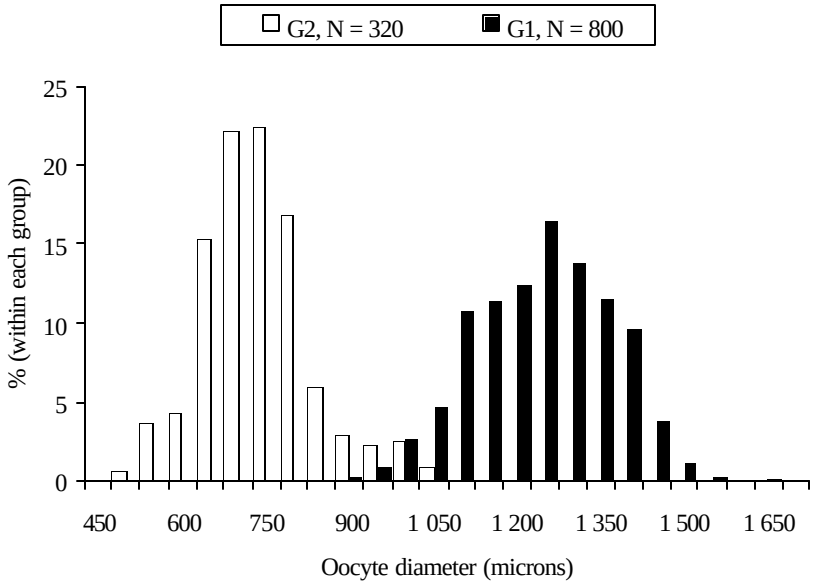

Fig. 2. Diameter of Greenland halibut oocytes sampled in East Greenland in July 1997.

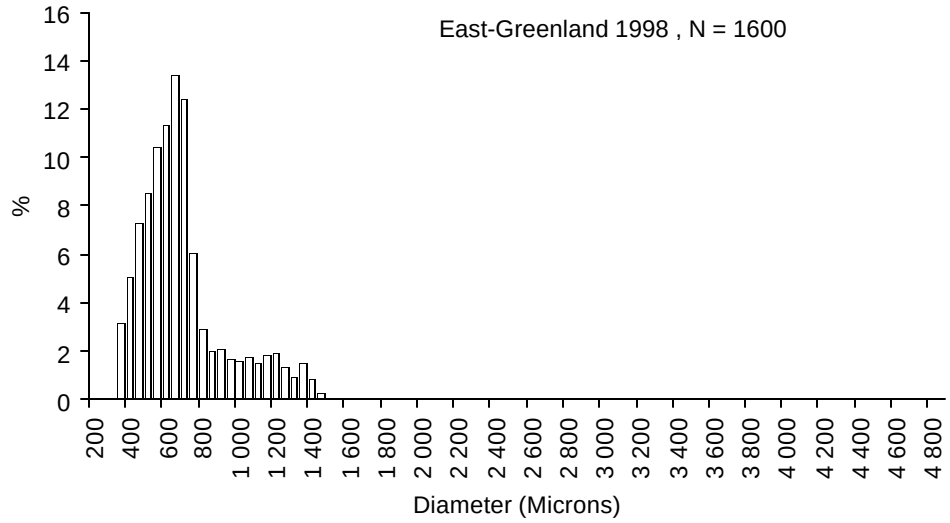

Fig. 3. Diameter of Greenland halibut oocytes sampled in East Greenland in July 1998. Stage 1-4 are present in the material.

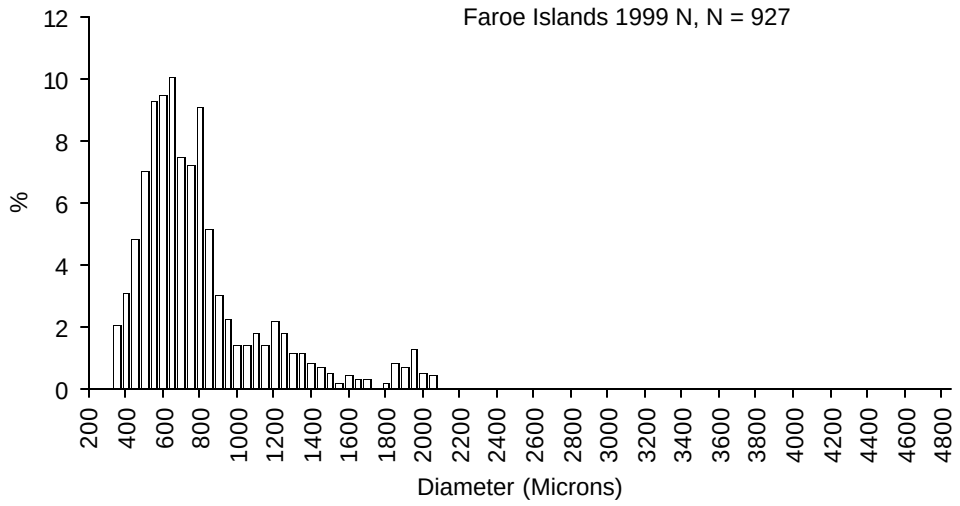

Fig. 4. Diameter of Greenland halibut oocytes sampled in the waters around Faroe islands in June 1999

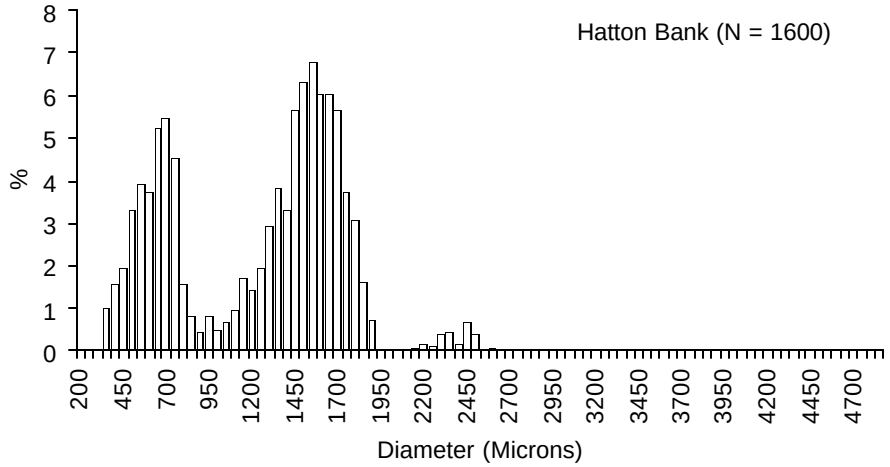

Fig. 5. Diameter of Greenland halibut oocytes sampled at Hatton Bank, west of the British Isles in September 1999.

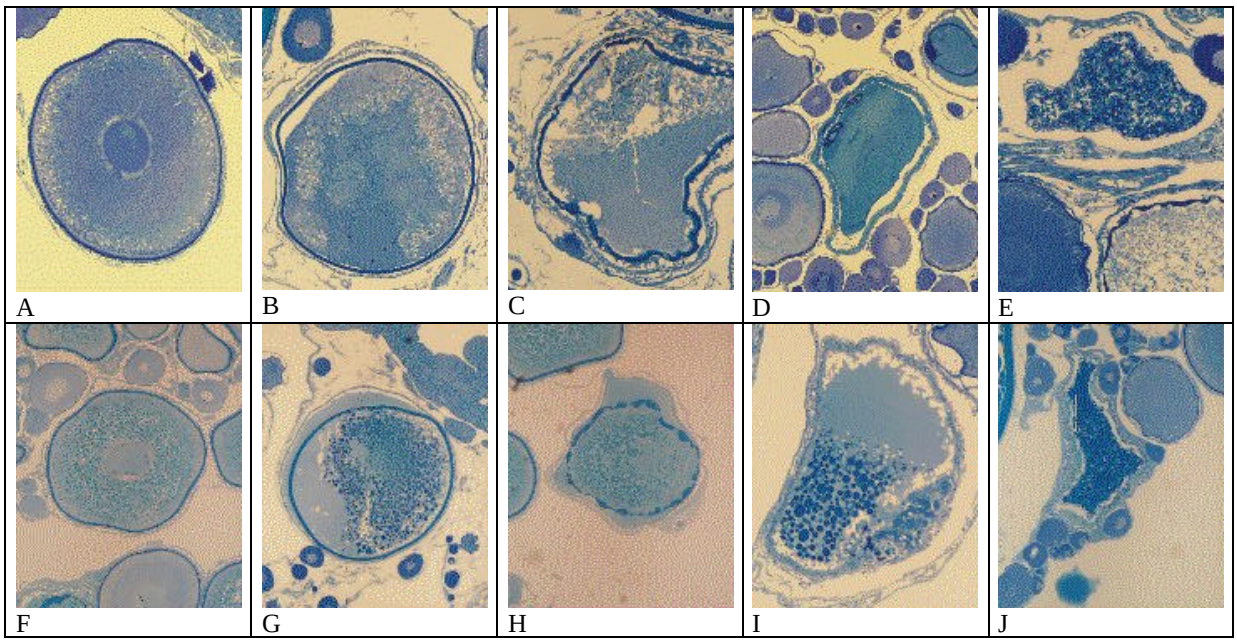

Fig. 6. Progression of atresia in oocytes that just have started vitellogenesis (A to E) and more developed oocytes (F to J). Intact oocytes (A and F). Recently started atresia (B and G) where the symmetrical orientation of the organelles is lost, ovoplasma is miscolored but the internal *zona radiata* is not broken down. Atretic capsule is forming (C and H) where zona radiata still remains more or less continuos. Resorption occurring fast (D and I) with thick capsule and a reduced size of the oocyte. Finished major resorption (E and J) with remains of zona radiata and only small remains of cytoplasma.

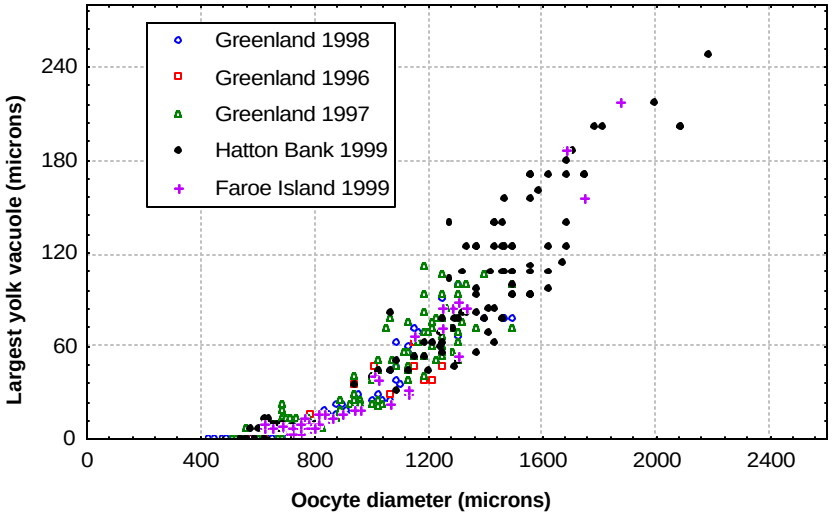

Fig. 7. Diameter of largest yolk vakuole increased with increasing oocyte diameter.

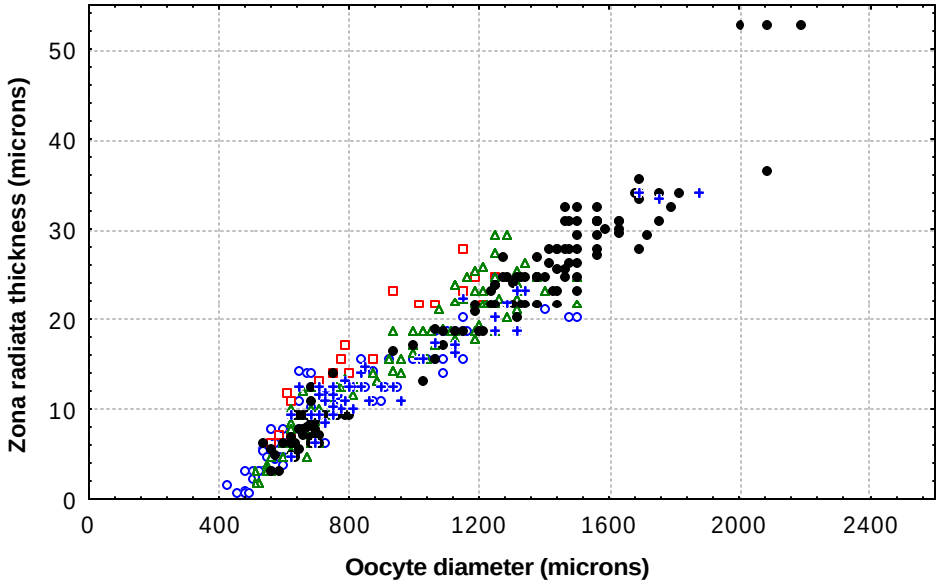

Fig. 8. Thickness of *zona radiata* increased with increasing oocyte diameter.

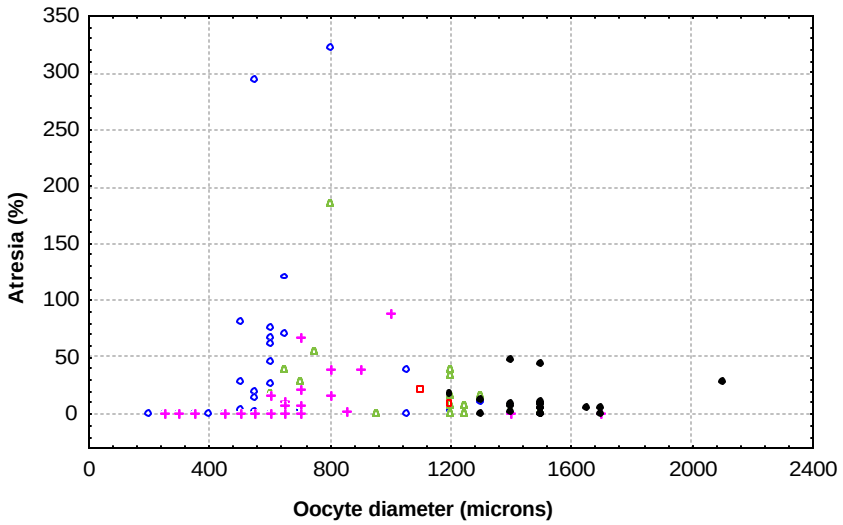

Fig. 9. Atresia varied between sampling stations, reflecting variations in fish size and maturity stage.

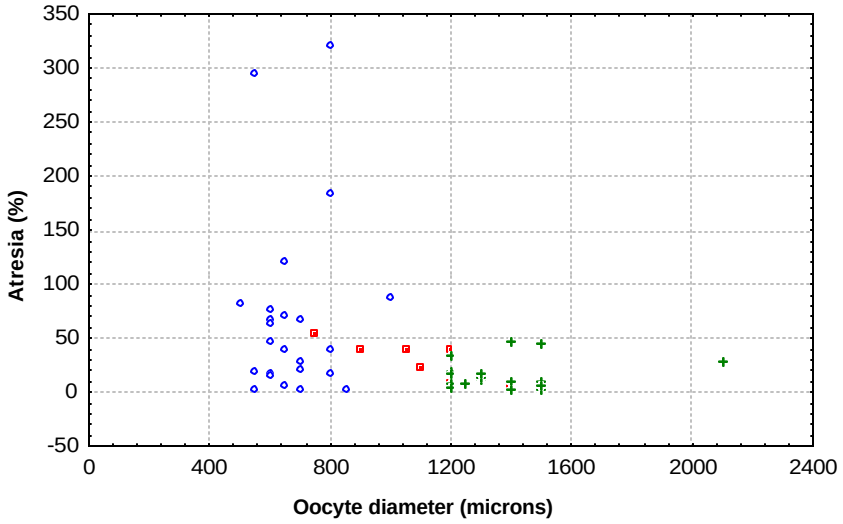

Fig. 10. In gonads with early maturing oocytes (mean diameter<1000 µ) atresia had affected the largest of those oocytes (circles). Atresia had affected the smallest of the oocytes in gonads where mean diameter was above 1200 µ (crosses). Oocytes of average size were affected in gonads with intermediate oocyte diameters (squares).

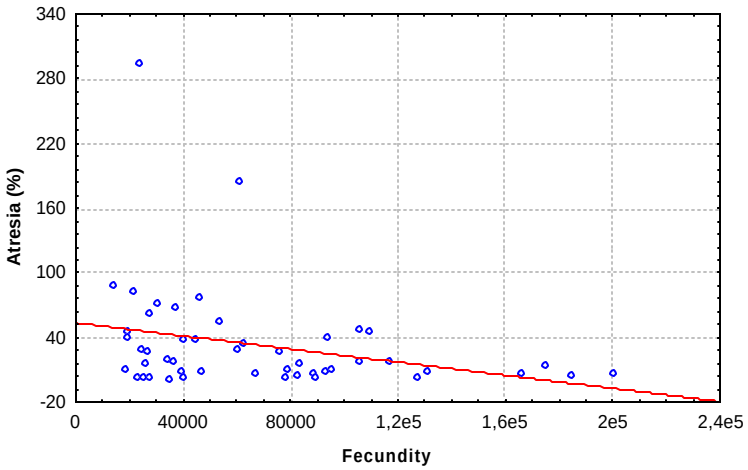

Fig. 11. Potential fecundity against atresia (%). There was found a significant negative correlation between amount of atresia and potential fecundity (N=55, R^2=0.08, p=0.047). Only individuals with atresia > 0 were used in this analysis.

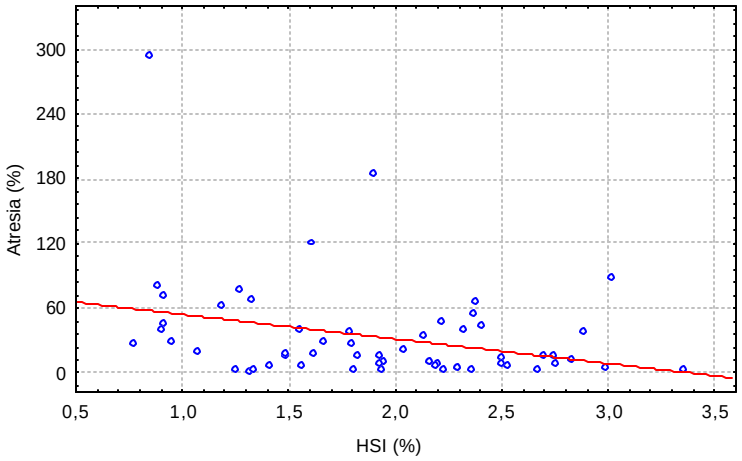

Fig. 12. HSI against atresia (%). There was found a significant negative correlation between amount of atresia and HSI (N=58, R^2=0.09, p=0.021). Only individuals with atresia > 0 were used in this analysis.