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# Histological and Visual Observations on Oogenesis and Sexual Maturity in Greenland Halibut off Northern Labrador

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## Introduction

Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), spawn on the continental slopes from Newfoundland to West Greenland in the deep warm water where temperature conditions are favorable for ovarian and embryonic development (Templeman, 1973). Templeman noted that females with eggs close to spawning condition are very uncommon in the bays along eastern Newfoudland, where bottom temperatures are lower than a similar depths on the continental slope.

Greenland halibut is an important component of the marine resources exploited off eastern Canada in recent years, the average annual catch being 50,000 tons during 1975-78. Biological data accumulated for stock assessments indicate that the fishery is prosecuted almost entirely on immature fish (Bowering, MS 1979). The scarcity of mature fish in the catches led to consideration of the possibility that the maturity condition of the ovaries by visual examination was being misinterpreted, in that recovery from the spent condition may be so rapid that the ovaries of previously spawned fish were designated as immature. The accuracy of determining maturity stages of individuals by visual examination in the field is essential to understanding the maturation cycle and the onset of first spawning in Greenland halibut. Consequently, a study was undertaken to compare the results of maturity determinations for females by two methods: visual observation of stages based on descriptions developed for field studies, and analysis of histological sections of ovaries using the descriptions of Federov (1968).

## Materials and Methods

Ovaries were collected from 115 Greenland halibut taken by bottom otter trawl during a research vessel survey off northern Labrador (Divisions 2G and 2H) in August 1979. The specimens constituting the sample were taken from the catches of several sets at depths of 370-752 m with bottom temperatures from 0.7° to 4.1°C. Length measurements were recorded as fork length to the nearest cm, and the sample was stratified over a length range of 50-113 cm with a maximum of 11 specimens per 4-cm length group. The ovaries were assessed visually for maturity condition and then preserved in 10% buffered formalin (pH 7.0).

In the laboratory, pieces excised from the posterior portions of the ovaries were dehydrated through alcohol solutions and embedded in paraffin. Sections were cut at 6-8 microns thick and stained with Harris' Haematoxylin and Eosin and a variant of Mallory's Triple. A Zeiss Photomicroscope III was used for microphotography at a magnification of 78.75X. As a result of storage of ovaries in formalin for several days and subsequent storage in 70% alcohol, distortion of the shape of the larger oocytes was evident.

## Results

Histological sections of each ovary were examined under the microscope and a maturity stage assigned to the specimen, following Fedorov's (1968) descriptions of maturity stages for Greenland halibut in the Barents Sea (Table 1). More than one-half of the ovaries (54.8%) were classified as immature (stage II), 4.4% were maturing (stage III), 7.8% were spent, and 33.0% were recovering from the spent condition (stage VI ⟶ III) (Table 2). Because the sample was taken in August (several months after spawning), fish of maturity stage IV (yolk-filled eggs) and stage V (partly spent) were absent. Juveniles (stage I) were also absent, as there were no fish below 50 cm long in the sample. Examples of histological sections illustrating features of oocyte development and degeneration are given in Fig. 1.

Mass degeneration of oocytes at the beginning of vacuolation and primary yolk accumulation was evident in many immature fish, especially those in early Stage III (Fig. 1, C to F). Fedorov (1968, 1971) described this occurrence in Greenland halibut of the Barents Sea and concluded that oocyte absorption occurs mainly with the active participation of follicular cells. Immigration of freely wandering elements, possibly phagocytes, into the oocytes is evident during the

TABLE 1. Descriptive stages of maturity used for visual and histological analyses of Greenland halibut ovaries. [(Histological stages from Fedorov (1968).]

| Visual | | Histological | |
|---|---|---|---|
| I(A) | Juvenile stage; ovary very small, no signs of egg development. | 1. | Oocytes in protoplasmic growth stage, nests of oogonia and oocytes present, ovarian wall thin (juvenile stage). |
| I(B) | Immature; ovary small and pinkish, eggs not visible to naked eye, ovarian wall thin and transparent. | 2. | Monolayer follicle phase in primary oocyte development with stage 1 present. |
| II. | Maturing A: eggs becoming visible to the naked eye, all eggs opaque, ovarian wall thin. | 3A. | Early stage 3, oocyte entering trophoplasmic growth, with beginning vacuolation and primary yolk accumulation, chorion (zona radiata) visible. |
| III. | Maturing B: mixture of opaque and clear eggs with less than half of eggs clear. | 3B. | Late stage 3 oocytes enter intensive trophoplasmic growth and heavy yolk deposition, mixture of early and late stage 3 and some stage 1 and 2. |
| IV. | Maturing C: mixture of opaque and clear eggs with more than half of eggs clear; this category includes the ripe condition where contents are almost liquid with translucent eggs. | 4. | 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. | 5. | 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 reddish purple in appearance, wall is thick and tough, some residual clear or opaque eggs are seen; this stage passes gradually into Maturing A conditon. | 6. | Ovary contains oocytes of new generation in the phase of vacuolation and primary yolk accumulation (stage 3) and the entire complex of sexual cells of stage 2 are present, ovarian wall is thick, large number of ruptured follicles and unreleased oocytes are undergoing resorption (stages 5-3); the latter part of this stage is sometimes referred as the "resting stage". |

TABLE 2. Comparison of visual (V) and histological (H) analysis of maturity stages of Greenland halibut by fish length. (Fish over 97 cm in length are combined into a single group.)

| Length | No. of | Immature (stage II) | | Maturing (stages II-III) | | Spent (stage VI) | | Recovering (stage VI→III) | |
|---|---|---|---|---|---|---|---|---|---|
| (cm) | fish | V | H | V | H | V | H | V | H |
| 50-53 | 5 | 5 | 5 | — | — | — | — | — | — |
| 54-57 | 9 | 9 | 9 | — | — | — | — | — | — |
| 58-61 | 11 | 11 | 10 | — | 1 | — | — | — | — |
| 62-65 | 9 | 8 | 8 | 1 | 1 | — | — | — | — |
| 66-69 | 8 | 7 | 8 | 1 | — | — | — | — | — |
| 70-73 | 10 | 8 | 9 | — | 1 | — | — | 2 | — |
| 74-77 | 10 | 4 | 7 | — | — | 3 | 1 | 3 | 2 |
| 78-81 | 9 | 1 | 3 | 2 | 1 | 2 | 1 | 4 | 4 |
| 82-85 | 10 | 1 | 2 | 2 | 1 | 2 | 1 | 5 | 6 |
| 86-89 | 10 | — | 1 | 2 | — | 1 | 1 | 7 | 8 |
| 90-93 | 9 | 1 | 1 | 1 | — | — | 2 | 7 | 6 |
| 94-97 | 7 | — | — | 1 | — | — | 1 | 6 | 6 |
| 98-113 | 8 | — | — | 1 | — | 1 | 2 | 6 | 6 |
| Total | 115 | 55 | 63 | 11 | 5 | 9 | 9 | 40 | 38 |
| % | | 47.8 | 54.8 | 9.6 | 4.4 | 7.8 | 7.8 | 34.8 | 33.0 |

beginning of degeneration (Fig. 1C). These degenerating oocytes stained heavily with haematoxylin, whereas normal oocytes stained with eosin.

Degeneration of oocytes was evident in immature fish of 54-93 cm. The percentage of immature fish showing degeneration of oocytes was very high over the length range of 58-77 cm but decreased rapidly to zero in ovaries of fish larger than 93 cm (Fig. 2). It is interesting that the length at which 50% degeneration of oocytes occurred coincides almost exactly with the length at which 50% of the specimens were sexually

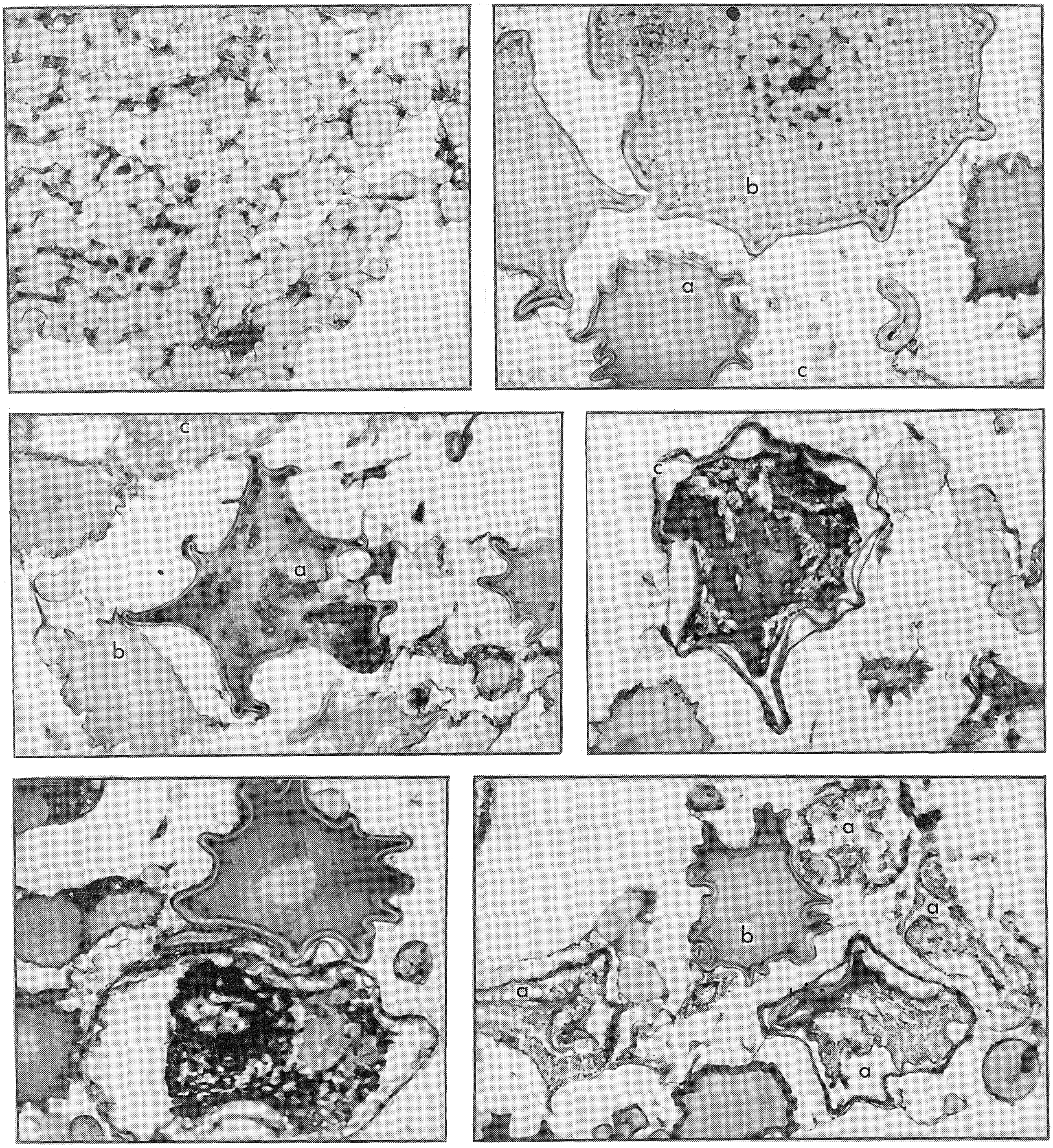


Fig. 1. Histological sections of Greenland halibut ovaries stained with Haematoxylin and Eosin. **A.** Stage II, immature, showing oogonia and oocytes in monolayer follicular growth; fish length 51 cm. **B.** Stage VI ⟶ III, spent and recovering; (a) oocyte beginning vacuolation and primary yolk accumulation, (b) oocyte in intensive yolk accumulation, (c) remains of absorbed follicles from previous spawning; fish length 90 cm. **C.** Early stage III; (a) oocyte undergoing degeneration by an influx of elements, possibly phagocytes, (b) stage II oocyte, (c) remains of degenerated oocyte; fish length 72 cm. **D.** Oocyte at mid-point of degeneration with chorion (c) or zona radiata still intact; fish length 72 cm. **E.** Further degeneration of stage III oocyte; fish length 80 cm. **F.** Oocytes close to complete degeneration (a), and oocyte at the beginning of stage III development; fish length 68 cm.

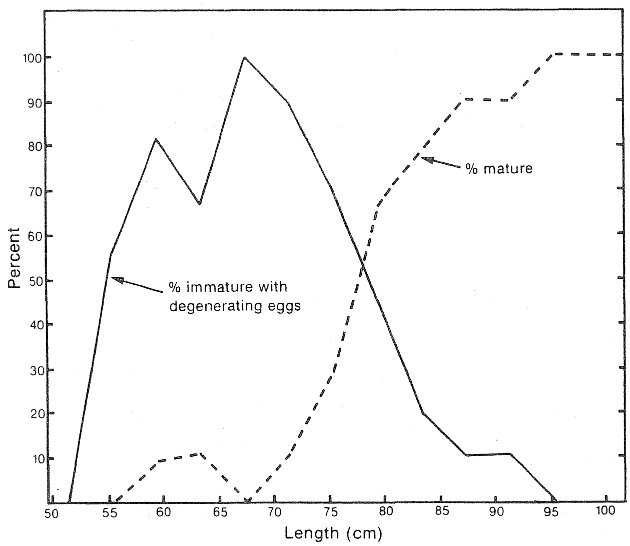


Fig. 2. Length distribution of Greenland halibut females, in which the ovaries exhibited mass degeneration of oocytes entering early stage III, and maturity-at-length distribution from histological analysis.

mature. No evidence of sterility or degeneration of oocytes was noted in sexually mature females, although Fedorov (1971) reported that degeneration of sexually mature oocytes occurs in Greenland halibut of the Barents Sea.

The accuracy of observers in appraising the stages of maturity of female Greenland halibut was assessed by comparing the visual observations with those from the histological analysis (Table 3). The inaccuracies were almost all associated with specimens designated as maturing, spent, and recovering from spawning by visual examination, in that 9 of the 60 specimens were actually immature, and 8 of the 11 fish, noted as maturing for the first time, were actually recovering from a previous spawning. In the first case, because mass degeneration of oocytes cannot be detected visually, the observers were lead to mistake these immature fish as fish which were maturing, spent or recovering, whereas, in the second case, recovery from spawning was

TABLE 4. Percentage maturation from visual and histological analyses and percentages showing degeneration of stage III oocytes by length group for Greenland halibut.

| Length | No. of | Visual | | Histological | | Stage III egg degeneration | |
|---|---|---|---|---|---|---|---|
| (cm) | fish | No. | % | No. | % | No. | % |
| 50–53 | 5 | — | — | — | — | — | — |
| 54–57 | 9 | — | — | — | — | 5 | 55.6 |
| 58–61 | 11 | — | — | 1 | 9.1 | 9 | 81.8 |
| 62–65 | 9 | 1 | 11.1 | 1 | 11.1 | 6 | 66.7 |
| 66–69 | 8 | 1 | 12.5 | — | — | 8 | 100.0 |
| 70–73 | 10 | 2 | 20.0 | 1 | 10.0 | 9 | 90.0 |
| 74–77 | 10 | 6 | 60.0 | 3 | 30.0 | 7 | 70.0 |
| 78–81 | 9 | 8 | 88.9 | 6 | 66.7 | 4 | 44.4 |
| 82–85 | 10 | 9 | 90.0 | 8 | 80.0 | 2 | 20.0 |
| 86–89 | 10 | 10 | 100.0 | 9 | 90.0 | 1 | 10.0 |
| 90–93 | 9 | 8 | 88.9 | 8 | 88.9 | 1 | 11.1 |
| 94–97 | 7 | 7 | 100.0 | 7 | 100.0 | — | — |
| 98–113 | 8 | 8 | 100.0 | 8 | 100.0 | — | — |

sufficiently well advanced from stage VI to stage III that they were mistaken for fish which were maturing from the immature condition. The inaccuracies have the effect of shifting the maturity-at-length ogive from visual observations about 3 cm to the left of that derived from histological analysis, with a corresponding reduction in the mean length at 50% maturity (Table 4, Fig. 3).

## Discussion and Conclusions

Templeman (1973) noted that the presence of Greenland halibut off northern Labrador is often related to spawning on the continental slope and to migrations to and from the spawning grounds. Zilanov *et al.* (MS 1976) noted that Greenland halibut upon reaching maturity migrate to the deep areas along the continental slope, whereas immature fish are found on the continental shelf and in the deep bays of eastern Newfoundland. Maturity stages of specimens collected from the shelf and slope areas of Div. 2G and 2H in August, based on both visual and histological analyses of ovaries, indicated that the sample consisted of immature fish and fish that had spawned earlier in the year.

TABLE 3. Errors in estimation of maturity in Greenland halibut females by visual observation and corrections by histological analysis.

| Maturity condition | Visual analysis | Corrections by histological analysis |
|---|---|---|
| Immature (stage II) | 55 | 54 observations correct; 1 ovary was maturing (stage III). |
| Maturing (first time) (stages III–IV) | 11 | 1 observation correct; 2 ovaries were immature; 8 ovaries were spent and maturing again. |
| Spent (stage VI) | 9 | 3 observations correct; 4 ovaries were immature; 1 ovary was maturing (stage III); 1 ovary was spent and maturing again. |
| Spent-recovering (stage VI ⟶ III) | 40 | 29 observations correct; 3 ovaries were immature; 2 ovaries were maturing (stage III); 6 ovaries were in spent condition. |

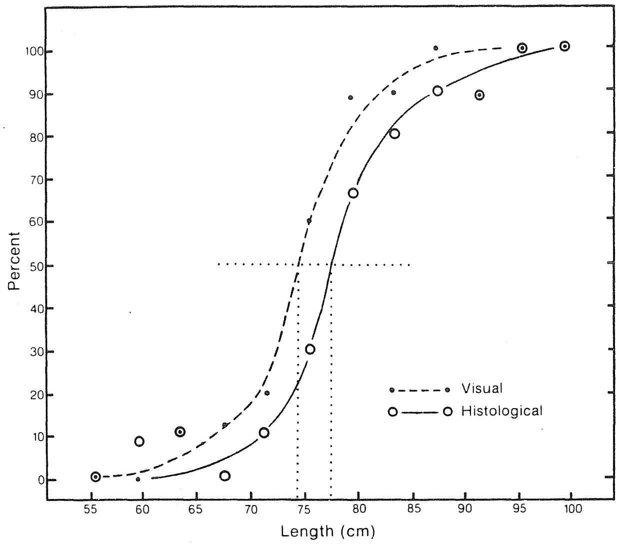


Fig. 3. Maturity ogives for visual and histological stages of maturity in Greenland halibut.

Fedorov (1968, 1971), from observations on Greenland halibut of the Barents Sea, noted that the occurrence, in many immature fish, of mass degeneration of oocytes just beginning vacuolation and primary yolk accumulation inhibits the subsequent course of oogenesis and disrupts the reproductive rhythm. This phenomenon was also common in Greenland halibut taken off northern Labrador, with the result that the process of maturation occurs over a length range of about 80-90 cm with the average size at 50% maturity being about 75-80 cm, which is substantially higher than the average sizes of fish caught commercially off eastern Newfoundland and Labrador (Bowering, MS 1977). It is not surprising, therefore, that samples from the commercial fishery consist mostly of immature fish.

The accuracy of visual observations on different stages of ovarian maturity can be enhanced by histological analysis. The main sources of error in the sample related to the problem of distinguishing fish maturing for the first time and fish recovering from a previous spawning. Although the maturity ogives from visual and histological observations are not greatly different, further sampling is needed to give a more accurate picture of oogenesis and the sexual cycle in Greenland halibut.

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