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The Process of Oogenesis in Masked Greenling, *Hexagrammos octogrammus**

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Abstract

A total of 184 female masked greenling, *Hexagrammos octogrammus*, were collected from the shores of Usujiri, Hokkaido, from January 1984-October 1985, and their ovaries were examined by histological and histochemical methods. The process of oogenesis was divided into twelve stages. Two distinct features of oogenesis of masked greenling were observed. The first feature was that oil droplets appeared after yolk vesicles, and thereafter yolk globules accumulated. Second, the coalescence of yolk globules began before the nuclear migration, and yolk globules continued to accumulate during the nuclear migration toward the animal pole. Ripe eggs were 1.8-2.1 mm in diameter and transparent blue in color. The ovarian cavity was located on the ventral side of the ovary, and ripe eggs existed along with gelatinous material. Some oocytes developing beyond the migratory nucleus stage were atretic and remained in the ovary for over one year. Therefore, post-spawners could be identified by the presence of these atretic oocytes.

Introduction

Masked greenling *Hexagrammos octogrammus* and other hexagrammids are distributed along the shores of the North Pacific and adjacent regions. Masked greenling have the widest distribution among hexagrammids ; they are found in the seaweed beds of the Aleutian islands, the Okhotsk Sea and northern Japan (Rutenberg, 1962 ; Quast, 1964). It is known that male masked greenling parents protect egg masses, and the larvae and juveniles drift near the sea surface, as do other hexagrammids (Wilby, 1937 ; Yamamoto and Nishioka, 1948 ; Gorbunova, 1962 ; Jewell, 1968 ; Kanamoto, 1976 ; Phillips and Barraclough, 1977 ; Low and Beamish, 1978 ; DeMartini and Anderson, 1980 ; DeMartini, 1986). There is, however, no published data concerning the maturation of hexagrammids. We have examined the process of oogenesis, mode of ovarian development, and the reproductive cycle of masked greenling using specimens captured near Usujiri, Hokkaido. The present study deals with the process of oogenesis.

Materials and Methods

Masked greenling were collected from the shores of Usujiri, southern Hokkaido

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from January 1984-January 1985, and from July-October 1985 on every months by trammel net. Ovaries of 184 females were fixed in Bouin's fluid, 10% formalin, or 20% formalin, depending upon the intended analytical procedure. Serial paraffin sections of the specimens were sectioned to 8-10 μm and stained with Derafield's haematoxylin and eosin. Some of the sections were treated with periodic acid Schiff (PAS) reagent for detection of yolk vesicles. In order to detect oil droplets, some of the ovaries were embedded in carbowax after fixation in 20% formalin, sectioned to 3-4 μm, and stained with Sudan III.

Results

General anatomy of the ovary

The paired ovaries of masked greenling were found nearly equal in size and located post-dorsally within the abdominal cavity. The ovarian cavity is located on the ventral side of the ovary and is connected to a short and thick oviduct which exits behind the anus (Fig. 1A).

Histology of the maturing oocytes

The morphological changes of the maturing oocytes are described following the classification of Yamamoto (1954) and Yamamoto et al. (1965). Oogonia and oocytes of the chromatin-nucleolus stage exist in a cluster at the surface of the ovigerous lamella throughout the year. The resting oogonia, 4-7 μm in diameter, had a large nucleus and very thin cytoplasm. The nucleus contained one or several chromatin-nucleoli. In the chromatin-nucleolus stage, oocytes and nuclei were 8-30 μm and 6-20 μm in diameter, respectively. The cytoplasm was unstainable. This stage was subdivided into three sub-stages according to the features of the nucleus, viz., pre-synaptic, synaptic and post-synaptic stages (chromatin-nucleolus stage, Fig. 2A). At the next stage the cytoplasm became gradually stainable with basic dye. Oocytes and nuclei were 30-110 μm and 20-70 μm in diameter, respectively (early peri-nucleolus stage, Fig. 2B). The cytoplasm had increased in volume with oocyte growth, but the cytoplasm gradually became less stainable with haematoxylin. Oocytes and nuclei were 100-170 μm and 60-80 μm in diameter, respectively (late peri-nucleolus stage, Fig. 2C). At an oocyte diameter of about 170 μm, yolk vesicles occurred on the periphery of the cytoplasm. Yolk vesicles could not be stained with Delafield's haematoxylin and eosin, but showed a strong positive PAS reaction. The zora radiata was distinctly present between the follicle layer and the cytoplasm of the oocyte (yolk vesicle stage, Fig. 2D, E). When oocytes reached about 230 μm in diameter oil droplets appeared in the vicinity of the nucleus and the oil droplet zone was formed in this region. Oil droplets stained red with Sudan III (oil droplet stage, Fig. 2F, G). At an oocyte diameter of about 350 μm, yolk globules appeared in the cytoplasm between the yolk vesicles and the oil droplet zone as small granules stained with haematoxylin. They gradually accumulated and formed the yolk globule zone. Three zonal structures, i.e., inner-most oil droplet zone, middle yolk globules and outer-most yolk vesicle zone, were clearly found at this stage (early yolk globule stage, Fig. 2H). Yolk globules began fusing when they occupied about two thirds of the cytoplasm. Oil droplets simultaneously began moving toward the outer region of the cytoplasm. Oocytes at this stage were

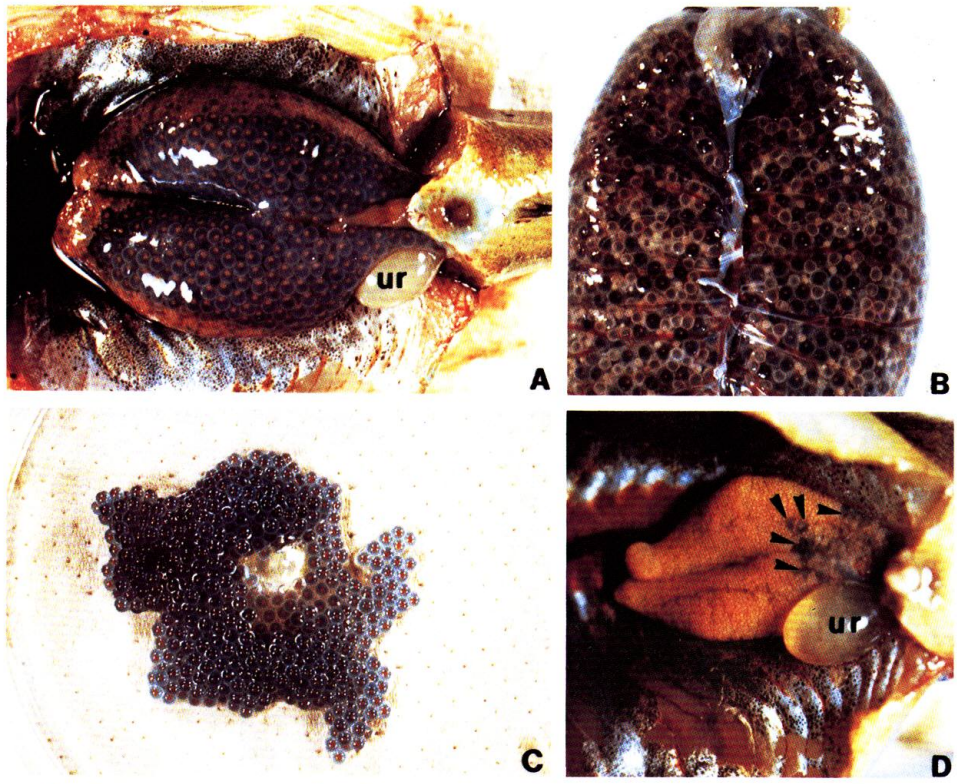

Fig. 1. Ovary and oocytes of masked greenling. A-mature ovary, *ur*, *urinary bladder* ; B-blue translucent oocytes developing beyond the migratory nucleus stage ; C-ripe eggs and gelatinous material in ovarian cavity ; D-residual eggs in the ovary, arrows indicate atretic eggs.

450-830 μm in diameter (late yolk globule stage, Fig. 2I). After the oil droplets had changed position, the nucleus also began moving toward the animal pole. Oocytes were at this stage about 1050 μm in diameter (migratory nucleus stage, Fig. 2J, K). Oocytes in this stage were distinguishable from that of the previous stage with the naked eye, because the oocyte color had changed to transparent blue (Fig. 1B). After the nucleus had arrived at the animal pole, the nuclear membrane disappeared and no boundary was found between the nucleoplasm and the cytoplasm. Yolk globules became a yolk mass. The oocytes were 1050-1530 μm in diameter (pre-mature stage, Fig. 2L). Oil droplets, which had occurred at the periphery of the yolk mass, transferred to the animal pole. The oocytes were 1450-1650 μm in diameter (mature stage, Fig. 2M). Mature oocytes were ovulated into the ovarian cavity on the ventral side of ovary. Ripe eggs were the same in appearance as at the mature stage except for the lack of an enclosing follicle (ripe egg, Fig. 2N). Freshly ovulated eggs were 1.8-2.1 mm in diameter. Ripe eggs were enclosed with transparent gelatinous material in the ovarian cavity (Fig. 1C).

Some of the developing oocytes became atretic, and were gradually absorbed by the granulosa cells. The first step of atretic changes of oocytes at the oil droplet,

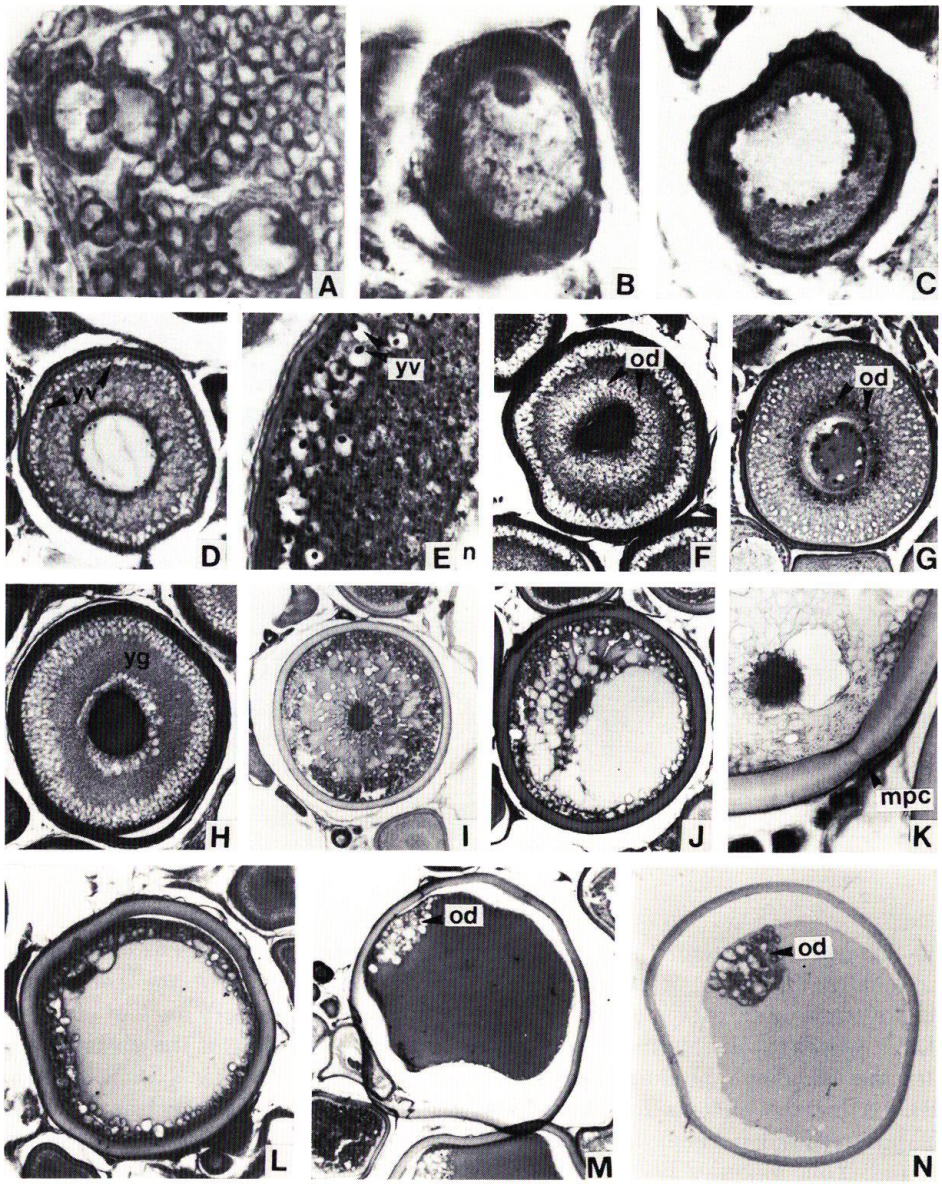

Fig. 2. Oogenesis of masked greenling. All figures are photomicrographs of sections from the ovaries of masked greenling. All samples were fixed in Bouin's fluid and stained with Delafield's haematoxylin-eosin except for E, G and M. A-oocytes at the chromatin-nucleolus stage (×800); B-oocyte at the early peri-nucleolus stage (×500); C-oocyte at the late peri-nucleolus stage (×250); D-oocyte at the yolk vesicle stage, *yv*, *yolk vesicles* (×150); E-yolk vesicle, Bouin's fluid and PAS preparation, *n*, *nucleus* (×600); F-oocyte at the oil droplet stage, *od*, *oil droplets* (×120); G-oocyte at the oil droplet stage embedded in carbowax and stained with Sudan III (×130); H-oocyte at the early yolk globule stage, *yg*, *yolk globules* (×85); I-oocyte at the late yolk globule stage (×45); J-oocyte at the migratory nucleus stage (×37); K-migration of nucleus to micropyle, *mpc*, *micropyle* (×300); L-oocyte at the pre-mature stage (×32); M-oocyte at the mature stage (×30); N-the ripe egg embedded in carbowax and stained with Sudan III (×30).

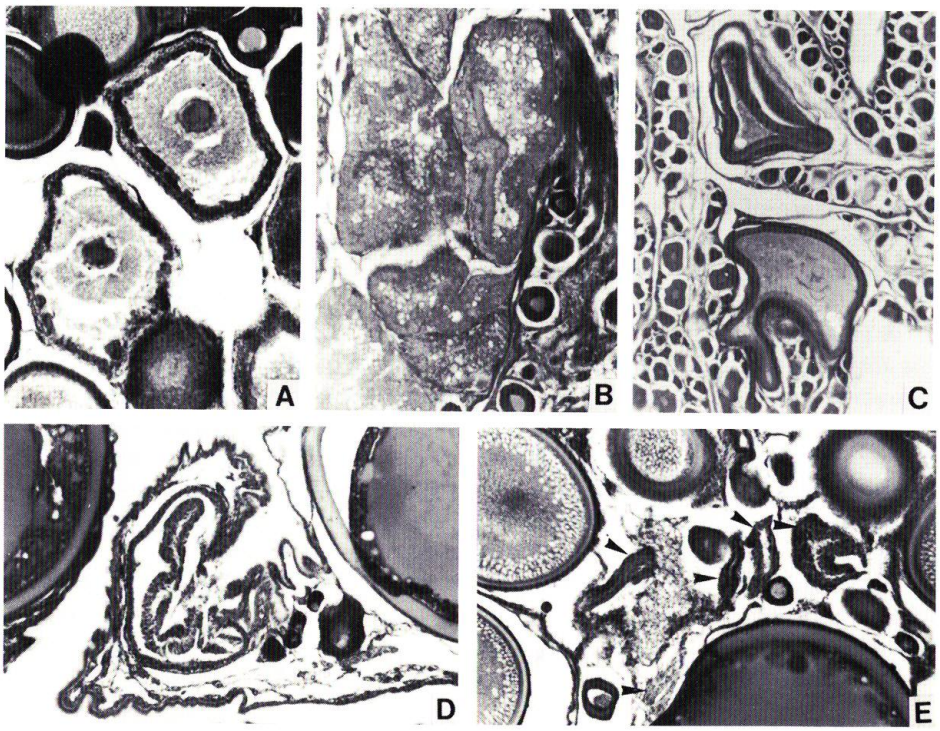

Fig. 3. Degeneration of atretic oocytes and empty follicles of masked greenling. All were fixed in Bouin's fluid and stained with the Delafield's haematoxylin-eosin. A-atretic oocytes at the yolk formation, the fragmented zona radiata (×50); B-atretic oocytes at the yolk formation, the yolk absorbed by granulosa cells (×50); C-atretic oocytes at the mature stage (×50); D-empty follicle in the ovary after ovulation (×60); E-the degenerated empty follicles, arrows indicate empty follicles (×50).

early yolk globule, and late yolk globule stage, was the breaking down of the zona radiata (Fig. 3A). The granulosa cells gradually thickened and yolk globules were absorbed through the fragmented zona radiata (Fig. 3B). After the yolk had been absorbed, the granulosa cells and the fragmented zona radiata remained in the ovigerous lamella for about four months as an atretic follicle. Oocytes developing beyond the migratory nucleus stage were also degenerated and absorbed through the same process but they remained in the ovary for over one year (Fig. 3C). They changed into a brown or black color, and thereby were easily recognizable with the naked eye (Fig. 1D). On the other hand, empty follicles were sac-like just after ovulation and both thecal and granulosa cells were clearly present (Fig. 3D). When the degeneration advanced, empty follicles could be seen as a tissue mass which stained with eosin (Fig. 3E), and they were fully absorbed in about four months.

Discussion

The process of oogenesis in fishes has been distinguished into different types according to whether and when oil droplets are accumulated (Yamamoto, 1958;

Ishida, 1980). In masked greenling oil droplets appears after yolk vesicles, and then yolk globules are accumulated. This type of oogenesis is similar to that found in salmonids (Ishida et al., 1961; Yamamoto et al., 1965) and Pacific pomfret *Brama japonica* (Yoon and Shimazaki, 1981).

The process of final maturation differs during the time of nuclear migration and the coalescence of yolk globules among species (Goetz, 1983). In masked greenling the coalescence of yolk globules begins before the nuclear migration, and the yolk continues to accumulate after the nucleus begins to migrate to the animal pole. These characteristics also occur in medaka *Oryzias latipes* (Yamamoto and Yoshioka, 1964).

A gelatinous material was found to exist with ripe eggs in the ovarian cavity. This material might function to glue eggs to each other at the time of spawning (Yamamoto, 1951), and/or play a role in the process of fertilization.

Oocytes developing beyond the migratory nucleus stage remained in the ovary for over one year. A similar protracted presence of atretic eggs is known in fatty greenling *Hexagrammos otakii* (Matsunaga et al., 1974) and Japan Sea greenling *Pleurogrammus azonus* (Kanbara, 1957). In these hexagrammids, post-spawners can be identified by the presence of atretic eggs.

This study has elucidated the process of oogenesis in masked greenling. In these observations, the nature and origin of the gelatinous material present in the ovarian cavity with ripe eggs remains unknown. Data on the role of the gelatinous material could help interpret the spawning mode of masked greenling.

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