Studies on the Formation of Fish Eggs
I. Annual Cycle in the Development of Ovarian Eggs in the Flounder, *Liopsetta obscura*[1)]

By
Kiichiro Yamamoto

(Akkeshi Marine Biological Station, Akkeshi, Hokkaido)

(With 2 Textfigures and 3 Plates)

The growing oocytes have provided, since the end of the last century, favourite and important material for analysis of many fundamental problems pertaining to localization and differentiation. The interest of earlier investigators was concentrated mainly on elucidation of the morphological changes taking place in the oocyte in the course of growth, a considerable amount of work having been produced as contributions to this field of study. Obviously, the mechanism of egg differentiation involves a number of complicated physical and chemical factors, and it is certainly impossible to obtain adequate understanding merely from the morphological researches. Thus, the trend in investigation has shifted in recent years from morphological observations to analyses of the conflicting data through microchemical study. Being attributed to technical difficulties, however, less progress has been made in this field of study and knowledge has remained insufficient. Hence, the need arises to carry out a cytochemical study of developing eggs as has been undertaken in the present investigation.

In a series of studies to be published hereafter, the subject will be treated and cosidered from the cytochemical standpoint. In the first place, however, the study has dealt with the morphological changes of the developing eggs in the course of growth, with particular concern for the annual cycle of ovarian eggs in the flounder, *Liopsetta obscura*.

It is the author's pleasant duty to acknowledge his sincere gratitude to Professor Tohru Uchida for his keen interest in the subject and his helpful suggestions. The writer is also very much obliged to Professor Sajiro Makino for his valuable advice and improvement of the manuscript for publication.

Material and method

The flounder, *Liopsetta obscura*, is a member of the family *Pleuronectidae*, common on the northern coasts of Japan, particularly in the coastal waters around Akkeshi. The fish on which the following observations were carried out were caught by a gill- or set-net, or

1) Contributions from the Akkeshi Marine Biological Station, No. 72.
Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 12, 1956.

a line during these three years ranging from January 1951 to June 1953. The body length, body weight and weight of ovaries were measured, and then the ovaries were fixed with Zenker's fluid, Bouin's mixture, Bouin-Allen's solution, Gilson's fluid, or formol-alcohol mixture. Bouin-Allen's solution was found best for the fixation of growing oocytes in early stages, while good results were obtained by the use of Zenker's fluid. For the eggs at the stage of the homogeneous yolk-mass formation, Gilosn's fluid proved most suitable. Serial sections, 10 μ in thickness, were prepared according to the usual paraffin method. Delafield's haematoxylin with counter-staining of eosin, and Heidenhain's iron-haematoxylin in combination with light green produced satisfactory results in staining. Mallory's triple stain also gave good results, particularly for the eggs in later stages.

Observations

1. Regarding the growing oocyte

For convenience' sake in description, the processes of differentiation of the oocyte may be divided into the following eleven stages.

(1) Chromatin-nucleolus stage : This term is used for the stage showing the oocytes of the smallest size with a thin accumulation of cytoplasm. This stage can be subdivided into the following three sub-stages according to the behaviour of the chromatin; (i) pre-synaptic, (ii) synaptic, and (iii) post-synaptic (Figs. 1 and 2). The oocytes at the pre-synaptic stage are characterized by an oval nucleus which contains several deeply staining chromatin-nucleoli lying on the meshwork of the chromatin-reticulum. The general features of the oocyte in synapsis are as follows: the large chromatin-nucleolus lies in one side of the nucleus and the chromatin-reticulum appears as a bunch of thick and deeply staining threads. After synapsis each spireme-thread undergoes splitting longitudinally. They vary considerably in length showing anastomosis with each other in a complicated way. This stage is denoted as "post-synaptic". Detailed descriptions of the oocytes of this stage will be reported in the following paper of this series (Yamamoto 1956 a).

(2) Early peri-nucleolus stage : Small oocytes showing deeply stained cytoplasm, measuring about 0.018 to 0.055 mm in diameter, are found in this stage. At the earliest period of this stage the oocyte has a large nucleus and a small amount of cytoplasm surrounded by a thin follicle layer. Each chromatin thread distributed dispersedly throughout the nucleus forms a loop following association at one end. In addition to the chromatin-nucleoli there are one or two true nucleoli which are strongly basophilic. With the growth of oocytes the cytoplasm increases much in relative volume and becomes more basophilic. The nucleus becomes enlarged, and in its central region are scattered the chromatin threads, loose in texture. At the same time the deeply stained basophilic nucleoli of different sizes arrange themselves gradually towards the periphery of the nucleus (Fig. 3).

(3) Late peri-nucleolus stage : Along with the growth of the oocyte, the cytoplasm gradually loses its basophilic nature and tends to stain faintly with haematoxylin. This is the oocyte at the late peri-nucleolus stage. The size of

the oocyte measured in the fixed material ranges from 0.060 to 0.10mm in diameter. The accumulated cytoplasm appears no longer to have good affinity to dye, showing vague outline. In the outer surface of the cytoplasm there is found the yolk nucleus of "Balbiani" in the form of a black dot. The nucleoli, spherical in form and variable in size, arrange themselves around the periphery of the round nucleus, where the chromosomes are no longer distinct (Figs. 4 and 5).

(4) Yolk vesicle stage : This is the stage when yolk vesicles appear in the ooplasm. At the beginning of this stage the vesicles of minute granules appear as a thin layer situated beneath the surface of the oocyte. Simultaneously, a narrow pre-deposit of the zona radiata makes its appearance between the cytoplasm and the follicle layer. The germinal vesicle round in form lies in the central area of the oocyte, and many nucleoli are to be seen around the periphery of the nucleus (Fig. 6). As the formation of yolk vesicles proceeds, the oocyte becomes markedly enlarged; it is provided with a thick follicle layer and a distinct pre-deposition of the zona radiata. The yolk vesicles are now seen in the outer half area of the ooplasm exclusive of a narrow zone of peripheral hyaloplasm (Fig. 7). Many nucleoli scattering in the periphery of the germinal vesicle are now stained faintly with basic dyes; they are somewhat oval in form. The diameter of the oocytes at this stage ranges from 0.17 to 0.21 mm.

(5) Primary yolk stage : This stage of the oocyte is applicable to that in which the ooplasm is filled with yolk globules. It was found that the yolk globules of this stage are uniformly positive to the polysaccharides reaction of Hotchkiss (Yamamoto 1956b). The oocytes come to increase much in size and become somewhat oval, measuring about 0.25 to 0.30 mm in long axis. The ooplasm is thoroughly filled with yolk globules which are larger than the yolk vesicle, except the narrow zone of the egg-surface. The nucleoli lose their peripheral arrangement in the germinal vesicle, and become scattered at random throughout the latter. The follicle layer and pre-deposit of the zona radiata are more broad and more distinct than in the former stage (Fig. 8).

(6) Secondary yolk stage : This stage immediately follows the primary yolk stage. There occurs rapid thickening of the zona radiata which clearly shows radial striation. The yolk globules, much enlarged and stained intensely with Heidenhain's haematoxylin, are found numerously deposited in the ooplasm but not in the narrow zone of the egg surface. Among them three kinds of yolk globules are distinguished from the results of polysaccharide examination (Yamamoto 1956 b). The germinal vesicle assumes a round form with an irregular contour and contains faintly staining nucleoli of spherical form (Fig. 9). In the oocytes at the later part of this stage there is frequently found a narrow zone of homogeneous yolk surrounding the germinal vesicle. The diameter of the oocytes at this stage is about 0.35 to 0.45 mm.

(7) Tertiary yolk stage : The oocytes grow larger in size, measuring from 0.50 to 0.55 in diameter, and the zona radiata increases further in its thickness.

The large germinal vesicle, spherical in form and fairly smooth in contour, is found situated in the central area of the oocyte. Within the nucleus are distributed a few nucleoli being vacuolized in appearance. The ooplasm is filled with the yolk globules which appear as comparatively large spherical bodies (Fig. 10). All globules show a weak but positive Hotchkiss reaction, becoming yellow when subjected to staining with a combination of fuchsin sulfite and 2-4-dinitrophenylhydrazine after oxidation by periodic acid (Yamamoto 1956 b).

(8) Migratory nucleus stage : The germinal vesicle in the migratory stage moving towards the pole of the egg is found surrounded by a zone of apparently viscid substance. The nucleus contains many nucleoli stained fairly with haematoxylin. The ooplasm is surrounded by a marked zona radiata ; it contains yolk globules of large size. Between the yolk globules there is a network of hyaloplasm (Fig. 11). After the migration of the nucleus towards the pole has been completed, the germinal vesicle assumes a round form with a smooth contour. A large number of nucleoli variable in form are found within the nucleus. The distinct network of hyaloplasm lies in the space between the yolk globules. These globules become large in size, though few in number (Fig. 12). The size of the oocytes in this stage is almost the same as that in the previous one.

(9) Pre-maturation stage : A sudden disappearance of the nuclear membrane seems to take place following the former stage. The nucleus now appears as a clear body at an eccentric position in the egg. The nucleoli are found converted into convoluting thread-like bodies which stain deeply with haematoxylin (Fig. 13). Sooner or later they lose their affinity to the stain and finally fade away. On the other hand, the chromatin elements look like strings of beads; they are now distributed around the thread-like nucleoli. Along with the disappearance of the nucleoli they come to assume a distinct form as minute, rod-lik, chromosomal elements. At the same time the yolk globules come together and form several large bodies. The hyaloplasmic mass containing the diffused nuclear elements is found lying in one pole of the egg. Then the hyaloplasmic mass produces a clear network which covers the space between yolk globules on the one hand, and a cortical layer surrounding the whole egg on the other. Under the surface of the cortical layer the cortical alveoli are embedded uniformly. There is detected a micropyle almost completely formed at a point of the zona radiata just above the cytoplasmic mass. The measurement of the oocyte is about 0.55 to 0.60 mm in diameter in the fixed material (Fig. 14).

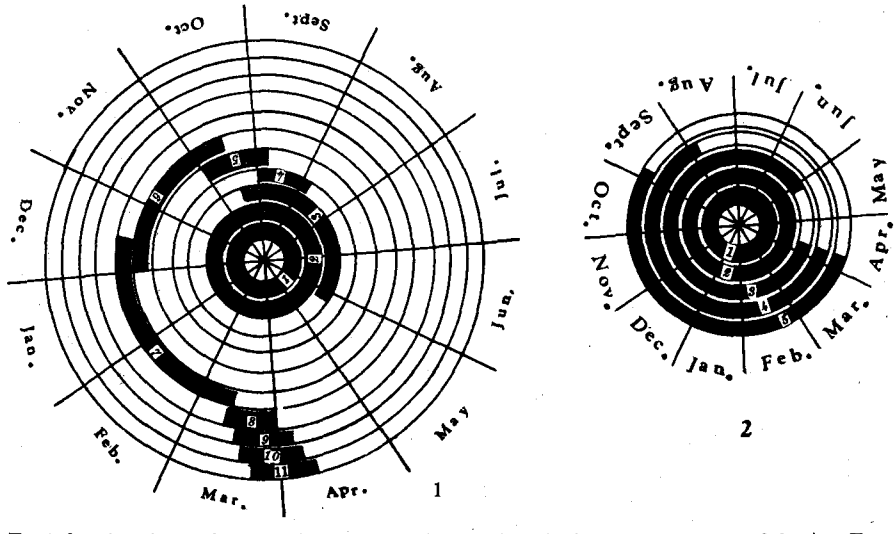
(10) Maturation stage : At the beginning of this stage the continuous yolk is to be found in the half globe of the egg, whereas the other half shows many yolk globules distributed in the hyaloplasm without fusion. The cortical alveoli embedded in the cortical layer become visible. The chromosomes, short rod-like in form, are found arranging themselves in an area beneath the periphery of the animal pole forming a metaphase spindle of the first maturation division (Fig. 15). The zona radiata lying under a thin follicle layer is moderately thick,

though its radial striation is rather indistinct. As the accumulation of hyaloplasm in the animal pole proceeds, the yolk mass is found covered by the hyaloplasm which is fairly thick at the animal pole and thin towards the opposite pole. Along with these changes the process of the maturation division proceeds and finally the extrusion of the first polar body is completed. A thin layer of the follicle is still retained enveloping the whole egg (Fig. 16). Then the eggs are extruded from the follicle, and come to lie the lumen of the ovary. The oocytes of this stage measure about 0.55 to 0.63 mm in diameter according to observation of the fixed material.

(11) Ripe egg stage: The ripe eggs are demersal and slightly adhesive in nature in living condition. They are spherical in form, measure some 0.70 mm in diameter, transparent, pale blue in colour, and covered with a compaıatively thin egg membrane which shows no particular adhesive apparatus. Careful observations made clear the cortical alveoli embedded in the cortical protoplasm and the micropyle in the animal pole (Fig. 17).

2. Regarding the annual cycle of oocytes

A) Mature fish: In Text-figure 1 is shown a schema of the annual cycle in development of oocytes of this species. Referring to this figure it is clear that the oocytes of the chromatin-nucleolus stage (1) and also of the early peri-nucleolus stage (2) can be found in the ovaries of mature fish throughout the



Text-fig. 1. Annual cycle of the oocyte in the descriptive stage (mature fishes). For explanations see text.

Text-fig. 2. Annual cycle of the oocyte in the descriptive stage (immature fishes). For explanations see text.

year. However, the oocytes of advanced stages are observable in ovaries for different lengths of time in various seasons, some stages being found for a few months, and others for a few weeks only. The oocytes corresponding to the late peri-nucleolus stage (3) were observed in ovaries during a period from late May to the middle of October. In autumn the oocytes at the yolk vesicle stage (4), and the primary yolk stage (5) appeared in ovaries. The former were detected in ovaries from late August to the end of September, while the latter appeared in ovaries from late September to the end of October. The oocytes of the secondary yolk stage (6) were found in ovaries for a comparatively long duration extending from the middle of October to the end of December. Then, the oocytes of the tertiary yolk stage (7) were detected in ovaries covering the period from middle December to the middle of March. The oocytes of the advanced stages, such as the migratory nucleus stage (8), the pre-maturation stage (9), the maturation stage (10), and the ripe egg stage (11), appeared successively in the ovaries taken in early spring from middle March to the middle of April. They appeared for only a few weeks in the months from March to April.

The annual cycle in development of oocytes in the present species differs considarably from that observed in *Fundulus*; according to Matthews (1938) the ovaries of *Fundulus* close to the spawning season include oocytes in various stages of development.

B) Immature fish: As Hickling (1930) has already noted in the hake, the ovaries of an immature flounder of comparatively large size show the oocytes of pre-spawning maturation stage. Many oocytes are found at the yolk vesicle stage, a few advancing to the primary yolk stage. But no oocyte beyond the primary yolk stage has been found in these ovaries. The annual cycle of the developing oocyte is shown in Text-figure 2. The oocytes of the chromatin-nucleolus stage (1) and the early peri-nucleolus stage (2) were found in the ovaries of immature fish all the year round, as occurred also in mature fish. The oocyte of late peri-nucleolus stage (3) continued to appear for a long period; they first appeared in the ovaries in late May and their occurrence was continuous up to early April of the following year. The oocytes of the yolk vesicle stage (4) were included in some ovaries obtained from late August to early April. Further, ovaries containing oocytes of the primary yolk stage (5) were found in the fish collected in late September or in early April.

Based on these findings it is reasonably considered that the adolescent phase reflects the spawning rhythm in mature fish, without resulting in spawning. Now the history of the growth of these abortive oocytes will be traced.

In early or late autumn there are in the ovaries many eggs showing the peri-nucleolus stage (Fig. 18). They exhibit two divided zones of cytoplasm; the inner zone surrounding the nucleus is more intensely stained than the outer zone which is granular in nature. The oocyte always contains a visibly healthy nucleus characterized by well defined nuclear membrane, homogeneously colourless nucleo-

plasm, thread-like chromatin elements, and basophilic nucleoli. The abortive oocyte of the yolk vesicle stage possesses the yolk vesicle lying in the inner zone of cytoplasm. In an early phase of this stage the outer zone of the ooplasm shows the granular condition stained faintly with haematoxylin (Fig. 19), and in the later phase that zone exhibits the appearance of viscid substance, being faintly stained with eosin (Fig. 20). The abortive oocytes in the primary yolk stage show also the zoning of cytoplasm (Fig. 21). The yolk globules are distributed in the inner zone only and seem to come together giving up their regular appearance. The inner zone is surrounded by the outer zone which is stained faintly with eosin. These abortive oocytes also have visibly healthy nuclei.

The abortive oocyte found in the winter material also exhibits the ooplasm divided into two zones between which there occurs a narrow cavity. In some oocytes probably at the late peri-nucleolus stage the outer zone appears as a densely granular layer, while the inner zone shows a fine structure, being deeply stained with haematoxylin (Fig. 22). The inner zone of the oocytes of a somewhat advanced stage is broad, rough and alveolar in texture. The large germinal vesicle occurring in the oocyte is circular in shape and vague in ontline. The nucleoli are distributed in the periphery of the nucleus : they show weak affinity to stains (Fig. 23).

The much advanced oocytes which were found in the ovaries of immature fish collected in late March show the granulated outer zone surrounding the deeply stained inner zone. The large germinal vesicle enclosed by the inner zone contains a few faintly stained nucleoli and highly granular nucleoplasm (Fig. 24). In addition to these abortive oocytes there are found many oocytes, such as those represented by Fig. 25. The oocyte lying just under the investing follicle shows the outer zone in the form of a narrow layer, and the deeply stained inner zone which contains a nucleus having peripheral basophilic nucleoli. Fig. 26 indicates an oocyte preserved on the 30th, April 1951. It shows the very narraw outer zone and the inner zone which is both deeply stained and sharply defined. Between the two zones there is still present a cavity, comparatively narrow in appearance. The nucleus seems healthy in nature. The sections of the ovaries preserved in early July show the ovigerous lamellae containing a number of young oocytes at the chromtin-nucleolus stage and the early peri-nucleolus stage. Though there are some oocytes which show a slight zoning of cytoplasm, the majority show healthy condition as seen in Fig. 3.

The observations described above suggest the possibility that the zoning of cytoplasm taking place in the abortive oocyte may be a preliminary process of the partial reabsorption of the oocyte, and further that through this phenomenon the abortive oocyte reabsorbs some amount of certain substances which have been accumulated during the period of growth. Probably this leads to the restoration of the old oocytes into young ones. As a result, the rejuvenescence of old oocytes is to be completed; they renew their activity in the next season.

Discussion

As shown in Text-figure 1, the sections of the mature ovaries of the flounder taken at the early stage of recovery show developing oocytes which are in two different stages, namely, (a) the youngest oocytes at the chromatin-nucleolus stage, and (b) the "reserve fund" eggs corresponding to the early peri-nucleolus stage. It is most probable that at the time of recovery and of preparation for spawning, the "reserve fund" eggs form the current season's crop with renewed activity. In addition to them, there are active eggs which originated from the youngest oocytes. Calderwood (1892) in the dab, Franz (1910) in the flounder, Hickling (1930) in the hake, have offered similar evidence stating that the "reserve fund" eggs grow into the current season's crop of active eggs.

As to the origin of the yearly crop of the youngest oocytes the present writer does not yet fully understand. Many workers have called attention to signs of mitotic figures in spent ovaries in order to make clear the origin of the new crop of young oocytes, but the evidence seems inadequate. Brock (1878) and Kolessnikow (1878) stated that the ovum is produced from a single epitherial cell of the ovary. In addition to this, Calderwood (1892) described a process of nuclear coalescence by which ten to twelve nuclei of the epithelium fuse. Later the nuclei or a collection of deeply staining bodies were found to occupy the position of nuclei in the ovum. Franz (1910) supported the view of Calderwood (1892). On the contrary, Wheeler (1924), based on his observations of the dab, has suggested that the "reserve fund" eggs originate from certain of the follicle cells which are sister cells of the oocyte. The results of the present study provide data which agree with the view of Wheeler, showing that a good number of the youngest oocytes, not the "reserve fund" eggs, are yearly regenerated from certain of the follicle cells. The empty follicles contain some nuclei which are large in size and intensely stained, together with the elongated follicle cell nuclei. In some follicles these cells with sharply staining nuclei are regarded as the youngest oocytes (Fig. 1). This interpretation is supported by the facts that empty follicles disappear with extreme rapidity, and that many of the youngest oocytes are found deposited in the re-formed lamellae.

Some mention, in connection with the partial reabsorption, should be made regarding the abortive egg in the adolescent and the "reserve fund" egg in the adult fish. The partial reabsorption is a subject to which earlier authors gave little attention, although it is one of the important phenomena in connection with the egg fomration in vertebrates. As early as 1872, Eimer described the circular division of the cytoplasm in the young oocyte. Then Scharff (1887) and Cunningham (1894) reported the division of the plasm into two zones, an external light zone and an inner deeply stained zone. Calderwood (1892) also found in the "reserve fund" egg of the dab a circular division of the cytoplasm into two zones, the inner one being granular and more deeply stained than the outer one. He further revealed that the light plasm was separated from the dark plasm, resulting in the formation of a cavity between them. Then the light protoplasm appeared to be absorbed until only a narrow layer of plasm was left inside of the investing follicle. Franz (1910) has reported that the protoplasmic ring—"growth ring" according to him—appears in the plasm of the oocyte at the "third period", further, that the growth of oocytes alternates between a rapidly growing period and a resting period which is marked by the appearance of a plasmatic ring. Wheeler (1924) studying the development of oocytes in the dab, pointed

out that the oocytes of some ovarian pieces produced the cytoplasmic zoning, whereas those of other pieces from the same ovary fixed with a different fixative showed no trace of zoning. Based on these findings he asserted that the zoning is an artificial product caused by fixation.

So far as the author's observations are concerned, the zoning of the cytoplasm was constantly detected in both the abortive eggs from the immature fish and the "reserve fund" egg from the mature fish. Observations with the abortive egg indicated that the zoning is not an artifact due to fixation. Franz (1910) expressed the view that the ring was marked by the sudden plasmatic growth. The author is not in agreement with that opinion, because the light zone ultimately diminishes as shown by Calderwood (1892). In spite of this peculiar evidence, Calderwood was not concerned with the significance of this phenomenon. The present investigation revealed that the zoning of the cytoplasm is a preliminary process to the partial reabsorption of the egg. Through the partial reabsorption the abortive eggs are rejuvenated into young healthy oocytes, smaller in size than before. Under this condition they are ready for the renewal of activity for the following season. The ovary of the mature fish contains many small oocytes in addition to the current season's crop of active eggs. As the ovary maturates, the zoning of the cytoplasm becomes visible in the small oocytes. The latter seem also to be renewed into active young oocytes due to the partial reabsorption as in the case with the abortive eggs in immature fish.

Summary

The present study deals with the annual cycle of the oocyte in the flounder, *Liopsetta obscura*, with special reference to the morphological changes of the oocytes during the growing period. The results obtained are summarized as follows:

1. The course of differentiation of oocytes was described as occurring in eleven stages.

2. The annual cycle in development of oocytes is shown in Text-figures I and II. An ovary from the mature fish at a considerably advanced stage contains oocytes at several different stages. The ovaries of the adolescent female show also a tentative pre-spawning maturation which corresponds to the spawning rhythm as observed in the mature fish.

3. abortive eggs found in the adolescent female always show two cytoplasmic zones divided into the inner deeply stained zone surrounding the nucleus, and the outer light zone lying in close contact with the follicle. It seems probable that the zoning of the cytoplasm is a process proceeding the partial reabsorption of the oocyte, and that through this process some amount of the substance accumulated during the growth period is to be reabsorbed from the abortive eggs, so as to serve to renew the activity of oocytes in the following season.

4. The ovary of mature fish contains small oocytes at very early developing

stage in addition to the current season's crop consisting of active eggs. After the discharge of the ripe eggs, the "reserve fund" eggs start their growth to form the current season's crop, and the youngest oocytes grow into the "reserve fund" eggs for the following season. The findings of the present study strongly support the view that certain numbers of the youngest oocytes are yearly supplied through the transformation ot certain follicle cells. A process of partial reabsorption also takes place in the "reserve fund" eggs.

Literature

Brock, J 1878. Beiträge zur Anatomie und Histologie der Geschlechtsorgane der Knochenfische. Morph. Jahrb., Bd. 4, s. 505.

Calderwood, W. L. 1892. Contribution to the knowledge of the ovary and intra-ovarian egg in Teleosteans. J. Mar. Biol. Assoc., vol. 2, p. 298.

Cunningham, J. T. 1894. The ovaries of fishes. J. Mar. Biol. Assoc., vol. 3, p. 151.

Eimer, Th. 1872. Untersuchung über die Ei der Reptilien und Fische. Arch, f. Mikro. Anat., Bd. 8, s. 216.

Franz, V. 1910. Die Eiproduktion der Scholle. Wiss. Meeresunter., Abt. Helgoland, Bd. 9, s. 63.

Hickling, C. F. 1930. The natural history of the hake. III. Seasonal changes in the condition of the hake. Fish. Invest:, Ser. 11, vol. 12, no. 1.

Kolessnikow, N. 1878. Ueber die Eientwickelung bei Batrachien und Knochenfischen. Arch. Mikro. Anat., Bd. 15, s. 382.

Matthews, S. A. 1938. The seasonal cycle in the gonad of *Fundulus*. Biol. Bull., vol. 73, p. 66.

Scharff, R. 1887. On the intra-ovarian egg of some osseous fishes. Quart. J. Micro. Sci., vol. 28, p. 53.

Wheeler, J. F. G. 1924. The growth of the egg in the dab. Quart. J. Micro. Sci., vol. 68, p. 641.

Yamamoto, K. 1954a. Formation of the cytoplasmic circular zone in the oocytes of the flounder, *Liopsetta obscura* (in Japanese). Zool. Mag., vol. 63, p. 47.

———— 1954b. Studies on the maturity of marine fishes. II. Maturity of the female fish in the flounder, *Liopsetta obscura* (in Japanese). Bull. Hokkaido Fish. Resea. Laboratory. no. 11, p. 68.

———— 1956a. Studies on the formation of fish eggs. II. Nuclear changes in the oocyte of *Liopsetta obscura*, with special reference to the activity of the nucleous. In press.

———— 1956b. Studies on the formation of fish eggs. III. Localization of polysaccharides in oocytes of *Liopsetta obscura*. In press.

Explanation of Plate X to XII

All figures in the Plate X to XII are photomicrographs taken from the sections of ovaries, with the exception of Fig. 17.

Plate X

Fig. 1. Portion of an empty follicle. Bouin-Allen and iron-haematoxylin. ca. ×1200.

Fig. 2. Oocytes in synapsis. The same preparation as above. ca. ×1200.

Fig. 3. The "reserve fund" eggs corresponding to the early peri-nucleolus stage. The same preparation as above. ca. ×410.

Fig. 4–5. Oocytes of the late peri-nucleolus stage. Bouin-Allen and iron-haematoxylin. y. n. Yolk nucleus. ca. ×410.

Fig. 6–7. Oocytes at the yolk vesicle stage. The similar preparation as above. ca. ×410, ×260.

Fig. 8. Oocyte corresponding to the primary yolk stage. Bouin and Delafield. ca. ×260.

Plate XI

Fig. 9. Oocyte at the secondary yolk stage. Bouin-Allen and iron-heamatoxylin. ca. ×66.

Fig. 10. Oocytes corresponding to the tertiary yolk stage. Alcohol-formol and Mallory's tri-colour. ca. ×66.

Fig. 11–12 Oocytes at the migratory nucleus stage. Bouin-Allen and iron-haematoxylin. ca. ×66.

Fig. 13–14. Oocytes at the pre-maturation stage. Fig. 13. Bouin and Delafield. Fig. 14. Zenker and Mallory. ca. ×66.

Fig. 15–16. Oocytes at the maturation stage. Fig. 15. Bouin-Allen and Mallory. Fig. 16. Bouin-Allen and Delafield. ca. ×66.

Fig. 17. Ripe egg in living state. ca. ×66.

Plate XII

Fig. 18. Abortive oocyte at the late peri-nucleolus stage. Bouin and iron-haematoxylin preparation. From a fish, 18 cm in body length and caught on September 5, 1951. ca. ×410.

Fig. 19. Abortive oocytes at the early part of the yolk vesicle stage. The same section as above. ca. ×410.

Fig. 20. Abortive oocyte at the later part of the yolk vesicle stage. Bouin and Delafield. From a fish, 19 cm in body length and caught on September 13, 1951. ca. ×260.

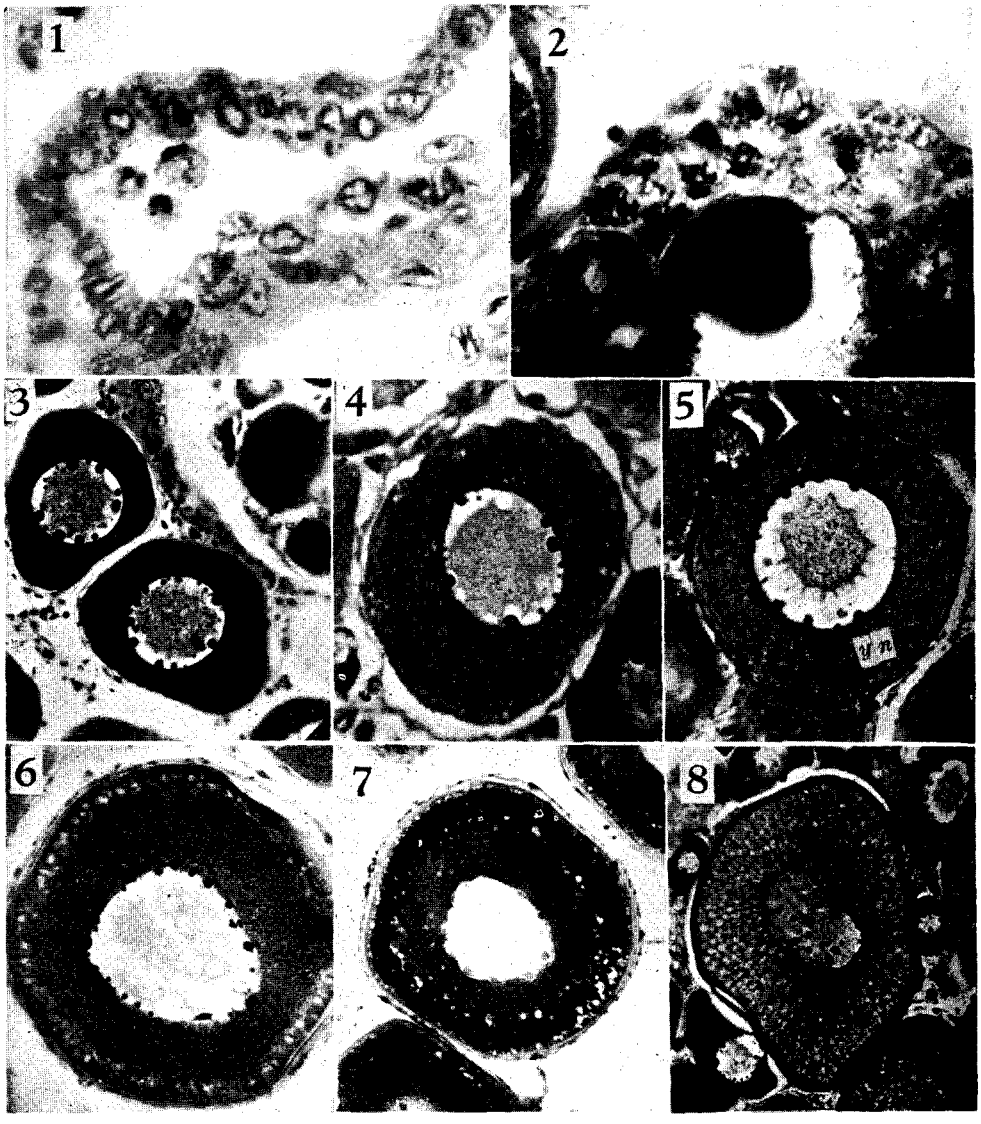
Fig. 21. Abortive oocyte at the primary yolk stage. Bouin and Delafield. From a fish, 18 cm in body length and caught on April 5, 1951. ca. ×260.

Fig. 22. Abortive egg at more advanced stage than Fig. 18. Zenker and iron-haematoxylin. From a fish, 20 cm in body length and caught on November 24, 1952. ca. ×410.

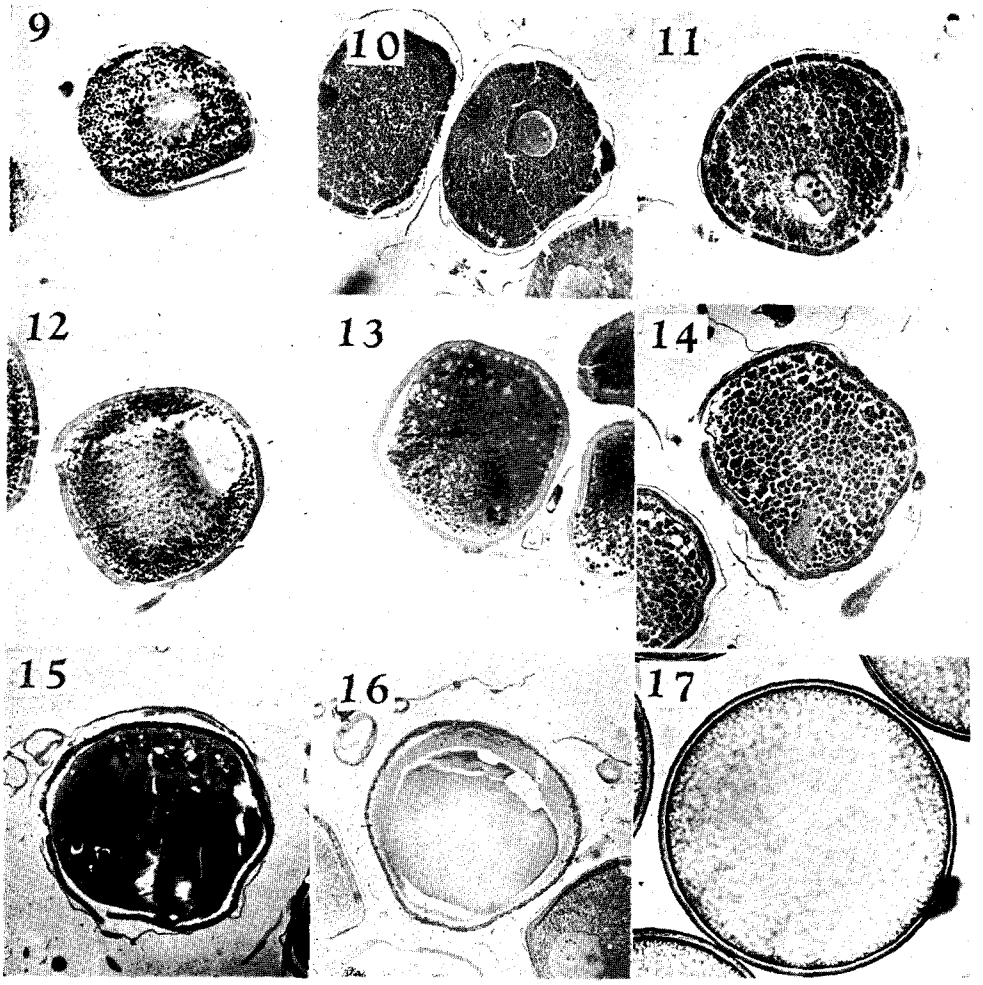
Fig. 23. Abortive egg showing the cytoplasmic zoning. Bouin-Allen and iron-haematoxylin. From a fish, 21.5 cm in body length and caught on November 20, 1952. ca. ×410.

Fig. 24–25. Abortive eggs showing the cytoplasmic zoning. Bouin and iron-haematoxylin. From a fish, 18.5 cm in body length and caught on April 5, 1951. ca. ×410.

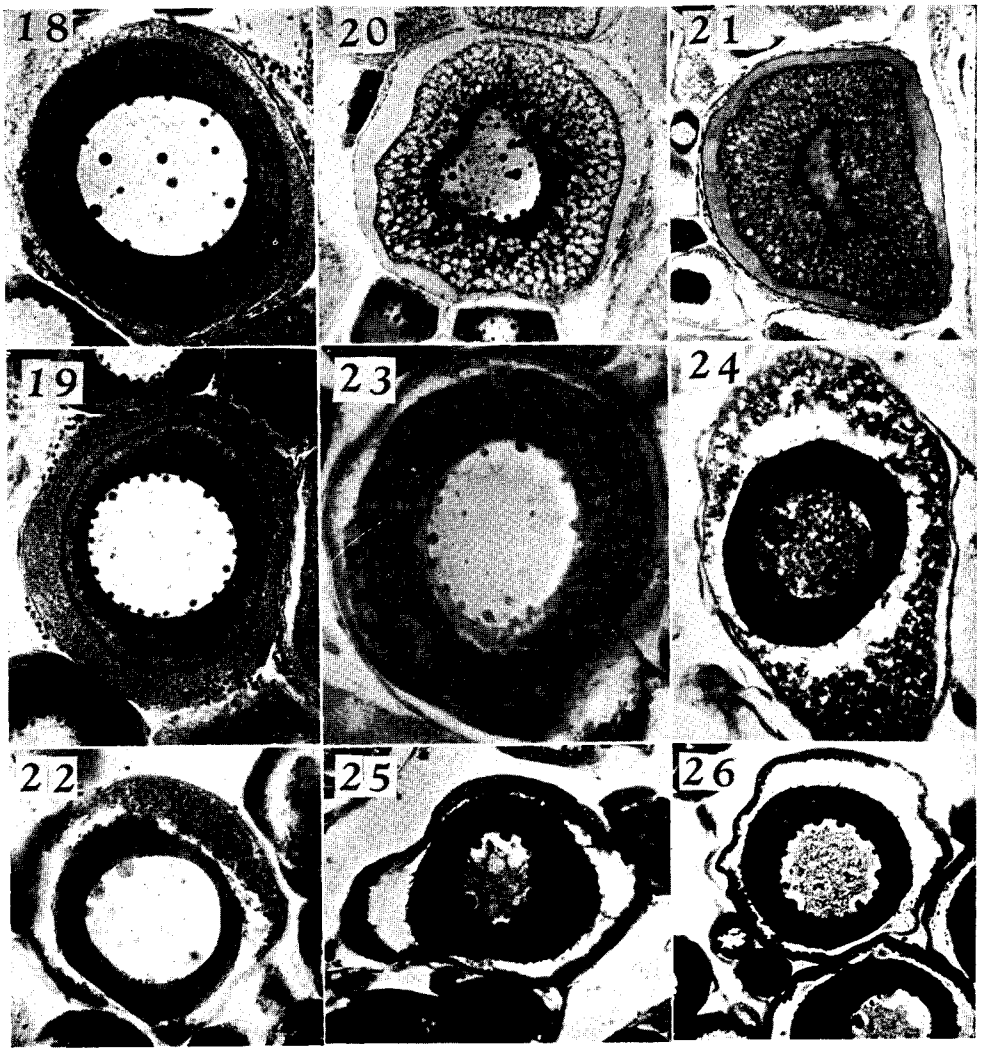
Fig. 26. Abortive eggs at recovering period. Bouin-Allen and iron-haematoxylin. From a fish, 19 cm in body length and caught on May 30, 1951. ca. ×410.



K. Yamamoto : Studies on the Formation of Fish Eggs, I



K. Yamamoto: *Studies on the Formation of Fish Eggs, I*



K. Yamamoto: Studies on the Formation of Fish Eggs, I