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RHYTHM OF DEVELOPMENT IN THE OOCYTE OF THE GOLD-FISH, *CARASSIUS AURATUS*

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In order to understand the egg-production of fishes showing multiplicity of spawning, the first problem to be settled is to ascertain the developmental rhythm in the oocytes of the fishes. Studies along this line have already been performed by several workers using some kinds of pelagic fishes (Clark, 1934; Kambara *et al.* 1953; Andreu & Pinto, 1957, etc.). They investigated the problems principally by examining the seasonal changes in frequency curves of egg diameter. On account of the difficulty of direct observations on the spawning behaviour of those fishes, however, they could not make sure of the actual correlation between the rhythm of development of the oocytes obtained and spawning rhythm.

In the present study the writers have attacked the problem by using histological methods taking gold-fish as material. About the gold-fish, Beach (1959) has already published "Seasonal changes in the cytology of the ovary and of the pituitary gland of the goldfish". Because of the object of his study, he dealt rather briefly with the rhythm of development of oocytes; moreover his results appear not to reveal their natural rhythm because his materials were cultured in aquaria of constant temperature. The present results show that histological methods are more suitable for the study of the rhythm of development of gold-fish oocytes than biometrical methods based on egg size, and that the information obtained may reflect actually the rhythm of spawning.

Before proceeding further, the authors wish to offer their cordial thanks to Mr. Kazunori Takano for his kind help during the sampling of materials.

Material and Methods

The gold-fish, *Carassius auratus*, used in the present study was restricted to the variety, "Wakin". Fish hatched in the preceding season were purchased in early spring from a gold-fish dealer and cultured in a pond of 5.0 m length, 3.5 m width and 1.0 m depth, except during the winter when they were kept in an indoor aquarium whose water temperature showed about 10°C throughout the season. Sampling was performed monthly, six fish at each time, during the period from the month when the fish were gotten, to November of the following

year. During that period the fish grew in average from 4.6 to 13.5 cm in body length. The same sampling procedures were repeated three times during the five years, 1956 to 1960. Body weight, body length and gonad weight of the fish were recorded. Gonads were cut into small pieces, and fixed with several kinds of fixatives such as Bouin's, Bouin-Allen's, Zenker's, Champy's, Regaud's and Gilson's fluids. For the fixation of oocytes at early developmental stages, Regaud's and Bouin-Allen's fluids gave good results, while Gilson's and Champy's mixtures proved to be suitable for oocytes of advanced stages. For examination of gonadal structure, several gonads were carefully taken out of the coelomic cavity and fixed in total with Bouin's fluid. Sections were cut at 10 micron thickness by means of the usual paraffin method, and stained according to Delafield's haematoxylin-eosin method and Heidenhain's iron-haematoxylin-light green method. Mallory's triple stain and modified Mallory's azan stain were also employed. Modified Mallory's azan stain proved to be the best for the differentiation of egg components. PAS reaction was also used to test the presence of carbohydrate in oocytes.

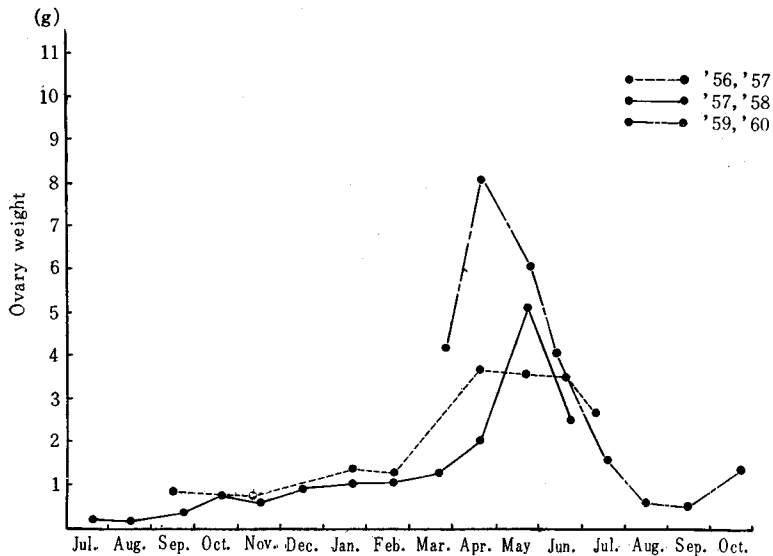
Results

1. *Ovarian changes in weight and external features*

The ovary of the gold-fish consists of two lobes of almost equal size, which are hung to the wall of the coelomic cavity by the short and thick mesovarium. The ovarian lumina are found in the upper part of each lobe. They are conjugated into one large lumen which is conducted backwards to the short oviduct which opens just behind the anus. Three layers may be distinguished in the wall of the ovarian lumen: the innermost layer composed of large and oblong cells with cilia, the middle layer of thick connective tissue, through which many blood vessels are running, and the outermost layer of compact and fibrous nature (Figures 14 and 15). The lower part of the lobes is occupied by ovarian stroma of lamelliform nature. Many ovigerous lamellae in which are embedded numerous oocytes of various sizes are found arranging themselves somewhat regularly.

The size of the ovary depends not only on its maturity, but also on the size of the fish. The greater the size of the fish, the larger is the size of the ovaries usually, if the oocytes present in the ovaries were the same in stage of development. So, ovary weight itself is not always the best indicator of ovarian maturity. In consideration of both weight and external features, however, the maturity of the ovary can be determined fairly properly.

In text-figure 1 is shown seasonal changes of ovary weight. From the figure it is clear that in one year gold-fish the ovary grows very slowly during the



Text-Fig. 1. Seasonal changes in ovary weight

summer. They weighed less than 0.5 g. Throughout the season they were almost colourless and transparent. In autumn, the growth of ovaries becomes rather fast. They attained to some 1.0 g in weight by the end of that season. Their colour changed into gray, and the large oocytes of the interior were visible to the naked eyes. As will be shown below, oocytes begin to be filled with yolk globules during this season. Throughout the winter the ovary continues to grow steadily and becomes 1.5 g in weight by the middle of March. The colour of the ovary is light brown and interior oocytes of large size are detected readily from outside. In spring ovaries grow quickly. Their weight attains to above 3 g by the middle of May. The ovaries filled with large eggs are opaque and bluish gray. The gold-fish cultured in the present writers' laboratory usually begin to spawn at the end of May and are accustomed to spawn twice or thrice in the same season as do also the fish of commercial culturists. The ovary near spawning was translucent, and gained weight conspicuously, being more than 5.0 g. Spent ovaries found in August were muddy, and contained a lot of fluid. They had lost weight, but still were about 1.0 g. After that, the recovery of the ovaries was rather fast. In late October some ovaries had already attained a weight of more than 2.0 g.

The ratio of ovary weight to body weight was also examined. The values were almost similar in tendency as those of total body weight, but the relative weight showed larger variations in individuals than the total weight.

Gokhale (1957) classified the gonadal maturity of the whiting and Norway pout into seven stages, viz., (1) immature virgin, (2) recovery spent or developing virgin, (3) maturing, (4) maturing, (5) full or ripe, (6) running or spawning and (7) spent. According to this classification, the ovarian maturity of the gold-fish was changed seasonally as follows. All ovaries obtained from one year fish in summer corresponded to the "immature virgin" stage. Some ovaries preserved in autumn were equivalent to the "developing virgin" stage and others to the "primary maturing" stage. In winter many ovaries showed the features characteristic of the "primary maturing" stage, though some ovaries were ranked with the "secondary maturing" stage. Ovaries corresponding to the "full" stage and the "running" stage were found during late May to July. Besides the ovaries of these stages, those belonging to the "secondary maturing" stage were recognized almost throughout the spawning season. The ovaries of the "spent" stage were found in late August and those of the "recovery spent" were obtained extending from September to early October.

It is noteworthy that in the gold-fish several kinds of ovaries in maturity such as the secondary maturing, full and running stages, were found at the same time during spawning season.

2. *Morphological changes of the maturing oocytes*

Concerning the histological study on the maturing oocyte of the gold-fish information has already been published by Beach (1959). However, his descriptions are rather brief and are concerned mainly with early phase of vitellogenesis. Therefore, the present writers would offer more detailed descriptions about the morphological changes of maturing oocytes in conformity with the senior author's paper on the flounder (Yamamoto, 1956 a).

1) Chromatin-nucleolus stage (Figure 1)

All through the year, very minute oocytes below 0.02 mm in diameter are found embedded in the ovigerous lamellae. They have a very thin sheath of cytoplasm and a large nucleus. These oocytes correspond to the chromatin-nucleolus stage. This stage may be subdivided into three sub-stages according to the features of the nucleus, viz., pre-synaptic, synaptic and post-synaptic stages.

2) Early peri-nucleolus stage (Figure 2)

The oocytes of this stage show sizes ranging from 0.02 to 0.15 mm in diameter. They have the cytoplasm which stains deeply with haematoxylin. The nucleus is relatively large in size. Within the nucleus there are found many chromatin threads distributed throughout it and many nucleoli arranged themselves on the periphery of the nucleus. The yolk nucleus may be found in the cytoplasm, though it is not so clear as in the following stage. In the early phase

of this stage the yolk nucleus lies close to the nuclear membrane and then moves to the periphery of the cytoplasm with the growth of oocytes. The oocytes are surrounded with a very thin follicular layer.

3) Late peri-nucleolus stage (Figures 3 and 4)

The diameter of oocytes in this stage ranges mostly from 0.11 to 0.16 mm. The cytoplasm loses its good affinity to haematoxylin and tends to be stained only faintly therewith. The yolk nucleus of dot form and of 10~15 micron diameter is often found situated in the outer part of the cytoplasm. Some oocytes demonstrate the phenomenon of zoned cytoplasm, showing a faintly stained outer layer of smooth structure and a deeply stained inner layer of granular structure. Such a zoning phenomenon has already been reported by Eimer (1872), Scharff (1887), Calderwood (1892), Wallace (1904), Franz (1910), Wheeler (1924) and Yamamoto (1954) in various kinds of fish oocytes. The follicular layer around the oocyte comes to be clear. Many nucleoli of spherical, hemispherical or elliptical shape and of various sizes are situated on the periphery of the nucleus. Black spots of nucleolar size are frequently found in the cytoplasm close to the nucleus. The nucleoplasm contains indistinct chromosomes and minute granules evenly distributed within it.

4) Yolk vesicle stage (Figures 5 and 6)

This is the stage when yolk vesicles are formed in the ooplasm. Most of the oocytes range from 0.15 to 0.35 mm in diameter. The yolk vesicles come into sight as minute bodies situated in the peripheral region of the ooplasm. Then, the vesicles are accumulated centripetally with accompanying enlargement in size. The vesicles at this stage were stained black with Delafield's haematoxylin, if they were fixed with fixatives containing potassium bichromate such as Zenker's, Regaud's and Champy's solutions. The zona radiata is also recognizable between the cytoplasm and the follicular layer. At first the zona radiata is a very narrow, compact and homogeneous layer, and then becomes thick with the growth of oocytes. At the beginning of this stage the nucleus is spherical in form and contains many nucleoli situated on its periphery. In a late phase of this stage it takes a somewhat oval form and becomes irregular in outline. The nucleoli become elliptical, bar-shaped or amoeboidal in form. The yolk vesicles exhibit a strong positive PAS reaction even after treatment with saliva. Therefore, the vesicles of this species contain mucopolysaccharides as has already been established in other fish oocytes (Konopacka, 1935, 1937; Yamamoto, 1955, 1956 c; Arndt, 1956, 1960, etc.).

5) Primary yolk stage (Figure 7)

When yolk vesicles have occupied the outer half zone of the cytoplasm, yolk

globules begin to appear between the vesicles. The formation of the globules proceeds by and by to the inner part of the cytoplasm. The globules are minute and spherical, and stained black with Heidenhain's haematoxylin. Yolk vesicles also increase in size and number during this phase, and occupy the outer two-thirds of the cytoplasm. They come now to show weak affinity to haematoxylin. The follicular layer increases in thickness, especially granulosa cells grow large in size. The zona radiata through which the radial striations are running becomes thick, measuring about 10 micra in thickness. The nucleus has lost its round shape and takes polyhedral form. Nucleoli are distributed at random in the nucleus. The size of the oocytes in this stage ranges from 0.35 to 0.55 mm in diameter.

6) Secondary yolk stage (Figure 8)

Oocytes in this stage measure from 0.45 to 0.75 mm in diameter. Yolk globules seem to be accumulated very rapidly in the inner part of the ooplasm; this accumulation results in the rapid growth of oocytes. Simultaneously with this change, yolk vesicles are gradually shifted outwards to take arrangement in a few rows in the periphery of the ooplasm. The nucleus recovers somewhat its round shape. Nucleoli are almost the same in form, size and distribution as in the previous stage. Sometimes the micropyle can be found with one large micropylar cell.

7) Tertiary yolk stage (Figure 9)

As yolk globules increase further in number and size, oocytes become large, measuring 0.6 to 0.9 mm in diameter. The yolk vesicles distributed in the peripheral region of the oocyte make one or two rows. They seem almost the same in size and number as in the previous stage. The nucleus shows a spherical form and fairly smooth contour. A few nucleoli showing spherical form are located in the interior of the nucleus free from its membrane. The micropyle having a large micropylar cell, is detected at one pole where yolk vesicles are scattered loosely or have disappeared completely.

8) Migratory nucleus stage (Figure 10)

The oocytes in this stage are the same size as those in the previous stage. The nucleus is moving towards the animal pole of the egg; it is nearly circular with smooth contour. Around the nucleus there is found frequently viscid substance stained blue with modified Mallory's azan stains as already seen in flounder's eggs (Yamamoto, 1956 b). Nucleoli are few in number and round in shape. They are situated in the inner part of the nucleus or close to the nuclear membrane. The yolk globules increase further in size, large ones being 26 micra in diameter. The zona radiata of about 7-8 micra in thickness has clear radial striation.

9) Pre-maturation stage (Figure 11)

After the nucleus has arrived at the animal pole, the nuclear membrane disappears suddenly and no boundary can be seen between the nucleoplasm and the cytoplasm. Nucleoli take complicated form and then lose their affinity to the stain and finally fade away. Chromatin elements become thick and round, and are found distributed in the cyto-nucleoplasmic mass of the animal pole. At the animal pole one can find the micropyle nearly completed. The micropylar cell of large size is still found plugging the micropyle canal, but the nucleus of the cell has come to be obscure as already reported by Eigenmann (1890) (Figure 13).

10) Ripe egg stage (Figure 12)

Ripe eggs are demersal and adhesive in nature in living condition. The adhesive nature of the egg appears to be attributed to the very thin porous membrane lying on the outermost part of the egg. The eggs are spherical in form, white in colour and translucent. In fixed condition they measure about 0.9 mm in diameter. The yolk globules of large size are enclosed by cortical cytoplasm which is somewhat thick at the animal pole but becomes thin towards the opposite pole. The cortical alveoli are found embedded in the cortical cytoplasm. Between the yolk globules a network of cytoplasm connected with the cortical layer may be discernible. The zona radiata is 13 micra in thickness. At the animal pole the micropyle may be seen.

The oocyte of the gold-fish as well as that of the flounder has two kinds of yolk substances, viz., yolk vesicle and yolk globule. No fatty droplets have been detected at any time in vitellogenesis. In both species yolk vesicles are accumulated from the peripheral region to the inner part of the cytoplasm. The yolk globules begin to appear in the peripheral region when the vesicles have made two or three rows, and then invade the extra-vesicle cytoplasm and finally fill up the inner cytoplasm between the nucleus and the vesicle zone. After that, the formation of the yolk globule becomes more and more active in the inner area, while the formation of the yolk vesicle ceases in time. Thus the most part of the ooplasm is filled with yolk globules; the vesicles change their position to further peripheral region, where they are found forming one or two rows and finally give rise to the cortical alveoli as already ascertained in a variety of fishes (cf. Yamamoto, 1958). In the late phase of vitellogenesis in the flounder the yolk globules come together and make a continuous mass of yolk, while those in the gold-fish do not make a continuous mass of yolk; they remain in the state of large globules. On account of this difference in vitellogenesis, the exact stage corresponding to the maturation stage established in the flounder can not be found in the gold-fish.

Konopacka (1935) made a detailed study of vitellogenesis in the oocyte of the carp. The vitellogenesis in the gold-fish resembles well that observed in that related species, excepting one point that in the carp the yolk vesicles situated in the inner part break down during the late phase of vitellogenesis and contribute to the formation of yolk globules.

3. Seasonal changes in stage composition

In order to find out about the developmental rhythm of ovarian eggs, stage composition in the oocytes of ovaries preserved monthly has been determined by counting the oocytes in each stage under the microscope. The oocytes at the chromatin-nucleolus stage were excluded from counting, because their number in this stage is difficult to decide exactly. The obtained results of counting are

Table 1. Seasonal changes of stage composition in oocytes

No.	Date	Ovary weight (g)	Total number of examined eggs	Percentage of oocytes at each stage						Migratory nucleolus stage	Pre-maturation stage	Remarks
				Peri-nucleolus stage		Yolk vesicle stage	Yolk stage					
				Early	Late		Primary	Secondary	Tertiary			
1	Jun. 18	0.1	206	60.7	36.9	2.4						
2	Jul. 20	0.2	241	61.0	29.9	9.1						
3	Aug. 22	0.2	1231	65.1	31.0	3.8						
4	Sep. 21	0.5	179	60.9	30.7	8.4						
5	Oct. 18	0.7	417	46.4	29.3	19.2	2.2					
6	Nov. 10	0.7	437	65.1	24.6	9.3	1.0					
7	Dec. 10	0.7	634	67.4	13.8	17.1	0.6	1.1				
8	Jan. 22	1.5	199	54.8	27.1	8.0	5.5	4.5				
9	Feb. 22	1.2	605	55.7	15.0	14.4	12.7	2.1				
10	Mar. 17	3.0	486	45.9	9.1	23.3	7.3	3.9	10.5			
11	Apr. 16	11.2	461	39.5	17.6	14.2	5.7	4.6	14.6	3.8		
12	May 23	5.5	133	49.1	14.3	14.3	4.4	4.4	10.5	3.0		
13	May 26	8.4	376	42.8	14.6	12.0	7.7	3.5	4.5	9.6	5.3	
14	Jun. 18	2.2	257	46.7	26.6	12.0	6.9	5.4	2.3			Just after spawning
15	Jun. 22	2.5	310	64.2	18.1	7.1	1.9	2.6	6.1			
16	Jun. 30	3.0	689	51.8	17.6	15.8	7.3	5.5	2.0			Just after spawning
17	Jul. 9	3.5	484	51.4	26.1	5.8	2.9	3.6	10.1			
18	Jul. 18	1.2	840	46.9	14.6	16.2	1.1		0.2			21% Atretic oocytes
19	Aug. 19	1.0	821	76.1	19.0	4.8			0.09			
20	Sep. 15	0.8	509	69.2	27.9	2.9						
21	Oct. 18	3.0	758	47.1	23.7	21.8	5.4	2.0				

summarized in Table 1.

During the period from June to September, only oocytes younger than the yolk vesicle stage were found in ovaries corresponding to Gokhale's immature virgin stage. As far as the present results are concerned, no marked differences in stage composition are recognized among ovaries in this season. The oocytes of the early peri-nucleolus stage supply about 60 per cent of all oocytes counted, those of the late peri-nucleolus stage some 30 per cent and of the yolk vesicle stage more or less than 10 per cent. In October, oocytes of the primary yolk stage appeared in the ovary. The frequency of the oocyte at each stage was as follows: The oocytes of the early peri-nucleolus stage were 49.4 per cent, those of the late peri-nucleolus stage 29.3 per cent, and those of the yolk vesicle and of the primary yolk stages were 19.2 and 2.2 per cent respectively. An ovary preserved in November showed almost the same stage composition in oocytes as that in October. These ovaries were in a stage equivalent to Gokhale's developing virgin stage.

In December oocytes of the secondary yolk stage were detected in the ovary. It is noteworthy that the oocytes at the late peri-nucleolus stage decreased to 13.8 per cent, while those laden with yolk totaled up to 18.8 per cent. The same tendency was found in ovaries fixed in January and February. For the oocytes at the late peri-nucleolus stage, values in both months were found at 27.1 and 15.0 per cent respectively. The oocytes at the early peri-nucleolus stage have also decreased somewhat in number. On the contrary, the number of those laden with yolk, especially those of the primary yolk stage and of the secondary yolk stage, tends to increase progressively. The values in January were ascertained to be 5.5 per cent for the primary yolk stage and 4.5 per cent for the secondary yolk stage; those in February were 12.7 and 2.1 per cent respectively. These ovaries were classed in the primary maturing stage of ovarian maturity.

In March the oocytes of the tertiary yolk stage appeared in an ovary at Gokhale's secondary maturing stage. The percentage of oocytes at this stage was counted as 10.5. Oocytes more advanced than the yolk vesicle stage have increased considerably in number over those in the preceding stage. They summed up to 45.0 per cent, while oocytes at the early peri-nucleolus stage and late peri-nucleolus stage decreased markedly in number, the former being 45.9 per cent and the latter 9.1 per cent. The reverse correlation in number between oocytes laden with yolk and those without yolk leads to the supposition that the resource of the current season's crop may depend mainly on the oocytes at the late peri-nucleolus stage and partly on the oocytes at the early peri-nucleolus stage, which have arrived at these stages more than about one year before

spawning.

In April the oocytes of more advanced stage, the migratory nucleus stage, were discernible. Their percentage was 3.8. The oocytes at the tertiary yolk stage indicated a comparatively high percentage as in the preceding month. One ovary preserved in late May contained oocytes showing the same composition of oocyte stages as above. A few days later an ovary corresponding to Gokhale's full stage was collected from a fish which was exhibiting distinct courtship behaviour. Such a fish should spawn within a few days if she was kept in an aquarium with some males. The stage composition of oocytes was characterized by high percentage of oocytes at the migratory nucleus and pre-maturation stages. The values were 9.6 for the former and 5.3 for the latter. Two fish killed on June 18 and 30 just after spawning possessed ovaries apparently in Gokhale's secondary maturing stage. No oocytes corresponding to the migratory nucleus stage or the pre-maturation stage could be found in the ovaries. These ovaries could be distinguished easily from those of May ranked of the same ovarian maturity due to the presence of empty follicles and low percentage of oocytes at the tertiary yolk stage (Figure 16). From the above observations it is most reasonable to suppose that oocytes advanced beyond the migratory nucleus stage develop quickly into ripe eggs and that they should be laid at one time. A datum obtained shows that the presumptive eggs laid in the primary spawning amount to more or less than 15 per cent of the total oocytes counted.

The fish preserved on June 22 and July 9 showed stage composition in oocytes the same as in those just spawned. Empty follicles, though they had now become small in size due perhaps to absorption, were also found within the ovaries. It is certain that in these fish at the time of sacrificing a pretty long time had elapsed since spawning. Stage composition in oocytes of these ovaries was striking in high percentage of oocytes at the tertiary yolk stage in comparison with those in ovaries just spawned. As some fish in the same group spawned after the time when the above fish were killed, it is reasonable to suppose that these fish might have spawned once more if they had been cultured longer.

A fish killed on July 18 yielded an ovary showing an interesting feature. At first sight the preparations from this ovary resembled those from ovaries just spawned. However, careful observations revealed a marked difference between the two. In this ovary, only a small percentage of oocytes laden with yolk were healthy and most of them were beginning to show degeneration. The degenerating oocytes had characteristic deformed shapes, hypertrophied granulosa cells, deformed yolk globules and the zona radiata broken into fragments. Oocytes from such an ovary seem to correspond to the primary, secondary and tertiary

yolk stages, and also to the yolk vesicle stage, though the correct stage of each oocyte could not be detected. They amounted to 21 per cent of the total oocytes counted. A spent ovary preserved on August 19, when the spawning season had completely ended, contained a lot of atretic oocytes as above. Degeneration in these oocytes was so striking, that it is now impossible to decide the number of these degenerating oocytes (Figure 17). The findings on the processes of degeneration agree well with the detailed observations of Bretschneider and De Wit (1947) and of Beach (1959). Throughout this season there could not be found a notable number of yolk-less oocytes showing the degenerating figures, though many of them showed the cytoplasm zonning phenomenon.

In September, only traces of atretic oocytes were detected in the recovering ovary (Figure 18). The characters of oocyte stage in this ovary resembled well those of immature virgin ovaries. The ovary contained a majority of yolk-less oocytes and a small number of oocytes with yolk vesicles. A two year fish killed in October yielded an ovary at the primary maturing stage. In the ovary, oocytes at the primary yolk stage and even at the secondary yolk stage were detected. This suggests that the oocytes develop faster in a two year fish than in a one year fish.

From the findings obtained from the spent and recovering ovaries it is most reasonable to surmise that near the end of the spawning season many of the oocytes with yolk fail in maturation and they remain in the spent ovary for a while, but all of them were absorbed during the phase of recovery, while most of yolk-less oocytes found in spent ovaries, which were denominated by Hickling (1930) as "reserve fund eggs", and by Vladykov (1956) as "recruitment stock" eggs, remain unabsorbed in the ovary and give rise to mature eggs during the following season.

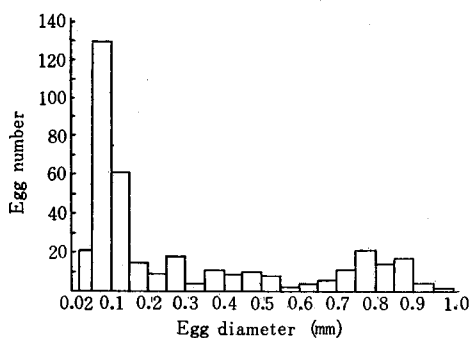
Discussion

So far, the rhythm of development of animal oocytes may be classified into three main types, that is, total synchronism, group synchronism and asynchronism (Marza, 1938).

In the species belonging to the type of total synchronism, all oocytes in an ovary develop synchronously. In the classes of Cyclostomata and Piscis, only two species, *Petromyzon planeri* (Champy *et* Gley, 1923) and *Oncorhynchus masou* (Yamamoto *et al.* 1959) have so far been ascertained to come under this type. These species show characteristic reproductive behaviour with spawning once in a life followed by death. The type of group synchronism is most common in the fish kingdom. Near the spawning season, two groups of oocytes are recognized

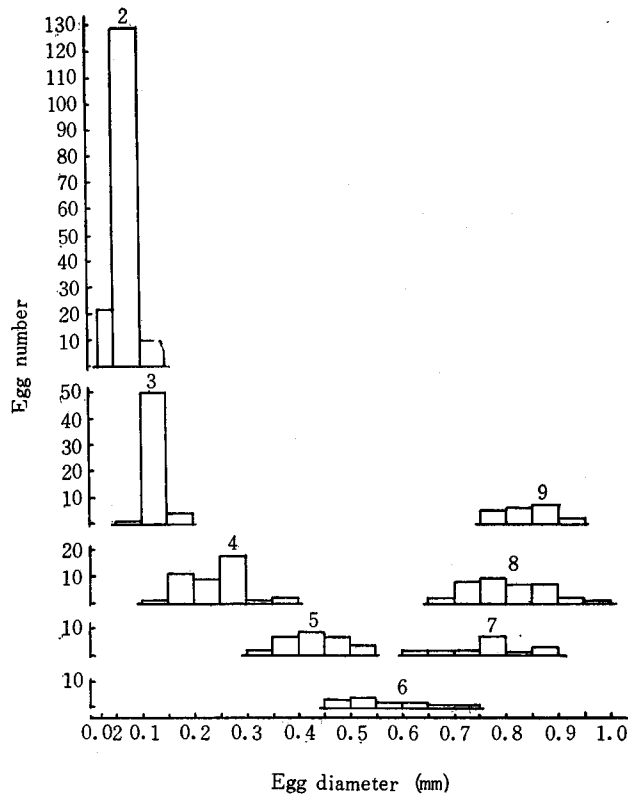
in an ovary, which are distinguished clearly from each other by their developmental stage. One group is composed of large and maturing oocytes. They are destined to become the current season's crop. The other consists of very small and yolk-less oocytes. They may be the recruitment stock for the following year. Herrings (Hickling & Rutenberg, 1936; Yamamoto *et al.* 1959), speckled trout (Vladykov, 1956) and flounders (Yamamoto, 1956 a) are the typical species belonging to this type. The fish of this type usually spawn once in a year and will do so several times in a lifetime. Their spawning season is ordinarily short and definite. The fish belonging to the type of asynchronism are also numerous. They have an ovary containing various kinds of developing oocytes. Sardines (Clark, 1934; Andreu & Pinto, 1957; Ishida *et al.* 1959), and Atka mackerel (Kambara *et al.* 1953) belong to this type. They have a comparatively long spawning season and ordinarily spawn several times within a season. As stated above, the gold-fish belongs naturally to this type.

From the view point of fishery biology, it is most urgent to clear up the frequency of spawning and the number of eggs laid each time in fishes showing multiplicity of spawning. Studies along this line have been made by Clark (1934) about the California sardine, by Kambara *et al.* (1953) about Atka mackerel and by Andreu and Pinto (1957) about the European sardine. They determined the frequency of spawning and the number of eggs laid by these fishes by examining seasonal changes in the frequency curves of egg diameter. However, this method seems not enough for the investigations on *Carassius auratus*. As shown in Text-figure 2, the frequency curve of egg diameter from an ovary



Text-Fig. 2. Size composition of the oocytes from an ovary at the full stage

of the full stage preserved in May represents at least three distinct groups of oocytes, i. e., large oocytes measuring from 0.65 to 1.0 mm in diameter, medium oocytes from 0.35 to 0.55 mm and small oocytes from 0.02 to 0.2 mm. This



Text-Fig. 3. Size composition of the oocytes in each stage, from the same ovary as in Text-Fig. 2. For explanation see text

observation creates the impression that each of these groups consists of oocytes which are destined to be developed and laid at one time. However, if the curve is examined by analyzing it into the graphs of oocytes of every maturity stage (Text-figure 3), it can be readily understood that each group of oocytes recognized in Text-figure 2 consists of oocytes of several stages. For instance, the group of large oocytes contains individuals at the tertiary yolk stage, migratory nucleus stage and pre-maturation stage. Examinations of the materials just after spawning showed that in the gold-fish the oocytes advanced beyond the migratory nucleus stage are spawned at one time while those of the tertiary yolk stage remain in the ovary as the resource of the next spawning. This proves that the groups of oocytes judged from egg diameter do not coincide exactly with the groups spawned actually.

As stated above, there is little doubt that oocytes advanced beyond the

migratory nucleus stage should be spawned at one time. Thus, there is need here to consider whether all of the corresponding oocytes found in sections should be laid or not.

Clark (1934) has already detected the fact that in the sardine some of the ripe eggs remain unspawned in the ovaries. The same phenomenon was ascertained in various kinds of fishes (Kambara *et al.* 1953; Vladykov, 1956; Suzuki *et al.* 1957). In the gold-fish this might also be the case, but under normal conditions these unspawned eggs are so few as to be negligible. Therefore, the number of eggs spawned in one time may be determined fairly exactly by counting the oocytes advanced beyond the migratory nucleus stage in the ripe ovary; such value was found in the present study to be some 15 per cent of all oocytes counted.

Next, some mention should be made regarding the possibility whether the secondary group of oocytes spawned simultaneously can be detected by means of histological investigations like the present one.

As the oocytes at the primary, secondary and tertiary yolk stages were rarely found in spent ovaries, it is fairly reasonable to surmise that most of these oocytes found in the early phase of spawning season may probably be ripened and spawned during the same spawning season.

This supposition may be supported by the following items of evidence: (1) The total number of these oocytes is almost the same in all ovaries preserved during any one spawning season. The number of these oocytes totals about 15 per cent, which agrees well with the number of eggs laid in the primary spawning. (2) Among the oocytes of the 3 above-noted stages those at the tertiary yolk stage increase in number with the elapse of time after spawning; for instance, the ovaries preserved on June 18 just after the primary spawning (no. 14) contain 2.3 per cent of the corresponding oocytes, while the ovary examined a rather long time after the primary spawning (no. 17) contains 10.1 per cent of the oocytes. Therefore, the oocytes at the primary, secondary and tertiary yolk stages found in such ovaries as above may be regarded as the secondary group of eggs spawned. During the maturation of oocytes laden with yolk, the reduction in number of eggs due to atresia was ascertained in the plaice (Cunningham, 1898) and in the speckled trout (Vladykov, 1956). In the gold-fish, the same phenomenon was also observed in the present study, though such atretic oocytes were not many in number. Thus, the number of eggs spawned in the secondary time may be reduced somewhat from the presumptive eggs of the secondary group counted in the present study.

In connection with this, there is a noteworthy fact to be considered here.

As stated above, near the end of the spawning season, degeneration and absorption of oocytes laden with yolk were usually found. No marked difference was found in stage composition of the oocytes between the atretic group and the spawning group present in the ovary soon after spawning. Therefore, in the late phase of the spawning season it is very difficult to decide whether the group of oocytes laden with yolk will proceed to spawning or to degeneration. The presence of many atretic oocytes in the spent ovary has already been ascertained in fishes mostly showing the multiplicity of spawning, such as the hake (Hickling, 1930), minnow (Matthews, 1938), bitterling (Bretschneider & De Wit, 1947), sardine (Andreu & Pinto, 1957), and also mackerels (Tateishi *et al.* 1957), while the flounder (Yamamoto, 1956 a) and herring held only a few of such atretic eggs in spent ovaries. This suggests that the presence of many atretic eggs in the spent ovary is a characteristic feature found in the fish of the type of asynchronism.

As already emphasized by Hickling (1930) in the hake, Yamamoto (1956 a) in the flounder and by Vladykov (1956) in the speckled trout, it may also be true in the gold-fish that the oocytes at the early and late peri-nucleolus stages found in both developing virgin ovaries and in recovering ovaries are the source of eggs spawned in the current season, whilst those found in full and running ovaries remain unabsorbed and give rise to the recruitment stock for the following year's spawning.

Although the origin and development of these yolk-less oocytes are important and urgent problems to be settled, this study could not cover them.

Summary

The rhythm of development in the oocyte of the gold-fish, *Carassius auratus* has been studied mainly by histological methods using one year and two year old fish as materials. The results obtained are summarized as follows:

1. Macroscopically, seasonal changes in the ovary are characterized by the occurrence of ovaries in several stages of maturity during a spawning season.
2. The morphological changes in maturing oocytes were described in ten stages. The oocyte has two kinds of yolky substance, yolk vesicle and yolk globule. No fatty droplets have been detected at any time during vitellogenesis. The yolk globules do not make a continuous mass of yolk but remain in the state of large globules.
3. The stage composition in oocytes shows characteristic features of the type of asynchronism, that is, the ovary in the full stage contains oocytes of all degrees of maturity.
4. Three groups of oocytes are recognized in the ovary of the full stage, the

first group is composed of oocytes more advanced than the migratory nucleus stage, the second group is composed of oocytes at the primary, secondary and tertiary yolk stages, and the third group comprises small, yolk-less oocytes.

5. It seems probable that the oocytes advanced beyond the migratory nucleus stage should be spawned simultaneously as one group.

6. There are some items of evidence favorable to the supposition that the oocytes of the second group found in the early phase of the spawning season may be ripened and spawned during the same spawning season as the secondary group of eggs.

7. The third group of oocytes corresponding to the early and late peri-nucleolus stages appear to remain in the ovary and give rise to the recruitment stock for the following year's spawning.

8. Degeneration and absorption in the oocytes laden with yolk were usually found in ovaries near the end of the spawning season.

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EXPLANATION OF PLATES

Plate I

All figures are photomicrographs of sections from the oocytes of the gold-fish.

Fig. 1. Oocytes in the chromatin-nucleolus stage. Zenker's solution and Heidenhain's haematoxylin preparation. $\times 1400$

Fig. 2. Oocytes in the early peri-nucleolus stage. Zenker's solution and Heidenhain's haematoxylin preparation. $\times 550$

Fig. 3. Oocyte in the late peri-nucleolus stage. Zenker's solution and Delafield's haematoxylin preparation. $\times 270$

Fig. 4. Oocyte showing phenomenon of cytoplasm zoning, the same stage as above. Zenker's solution and Heidenhain's haematoxylin preparation. *yn* yolk nucleus. $\times 220$

Fig. 5. Oocyte in the early phase of yolk vesicle stage. Bouin's solution and PAS preparation. *yv* yolk vesicle. $\times 120$

Fig. 6. Oocyte in the late phase of yolk vesicle stage. Zenker's solution and Delafield's haematoxylin preparation. $\times 120$

Fig. 7. Oocyte in the primary yolk stage. Bouin's solution and Heidenhain's haematoxylin preparation. $\times 105$

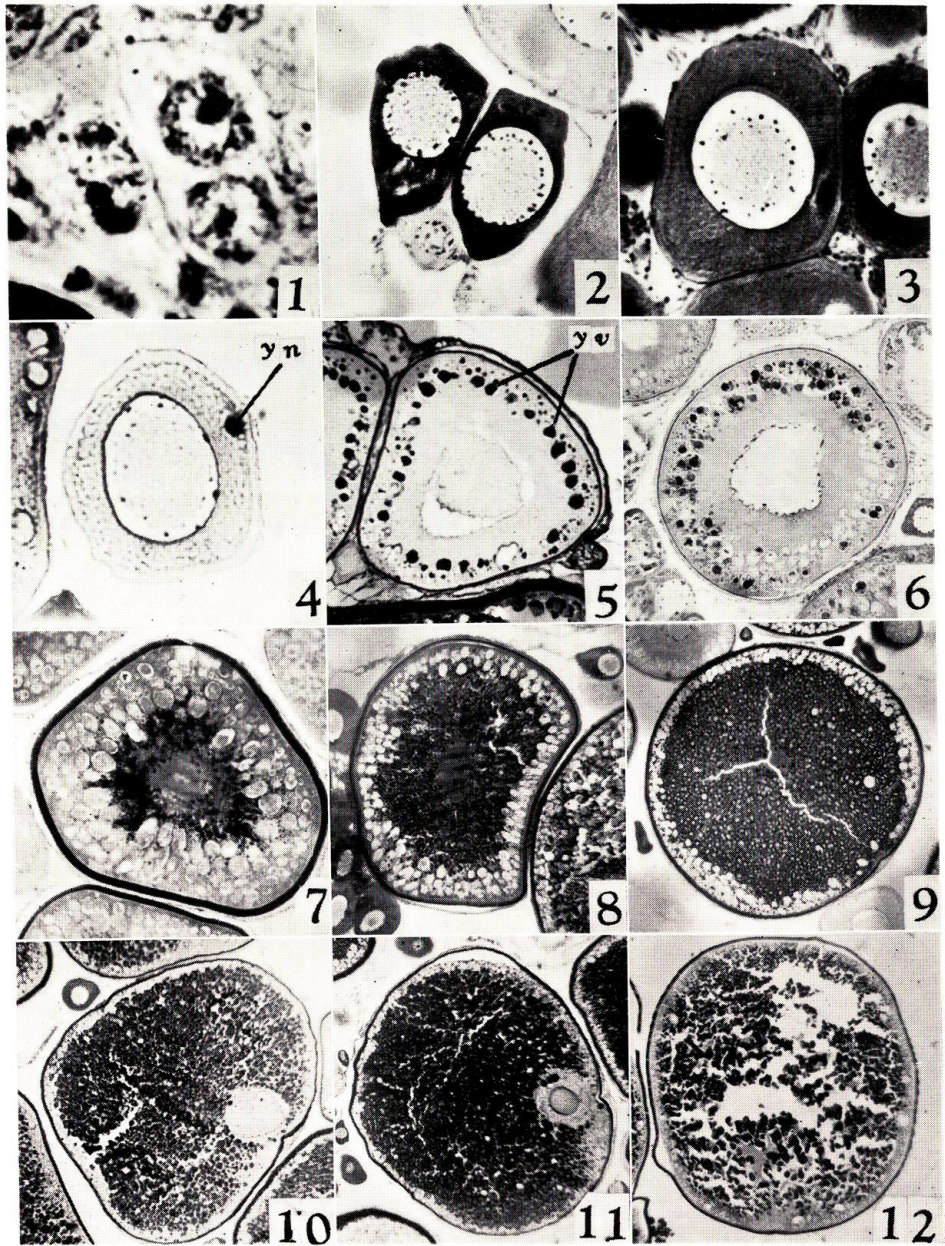
Fig. 8. Oocyte at the secondary yolk stage. Bouin's solution and Delafield's haematoxylin preparation. $\times 60$

Fig. 9. Oocyte at the tertiary yolk stage. Bouin's solution and Mallory's stain preparation. $\times 42$

Fig. 10. Oocyte at the migratory nucleus stage. Bouin's solution and Mallory's stain preparation. $\times 42$

Fig. 11. Oocyte at the pre-maturation stage. Bouin's solution and Mallory's stain preparation. $\times 42$

Fig. 12. Egg at the ripe egg stage. Bouin's solution and Mallory's stain preparation. $\times 40$



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Plate II

Fig. 13. Micropyle of an oocyte at the pre-maturation stage. Bouin's solution and Mallory's stain preparation. *mpc* micropylar cell. $\times 330$

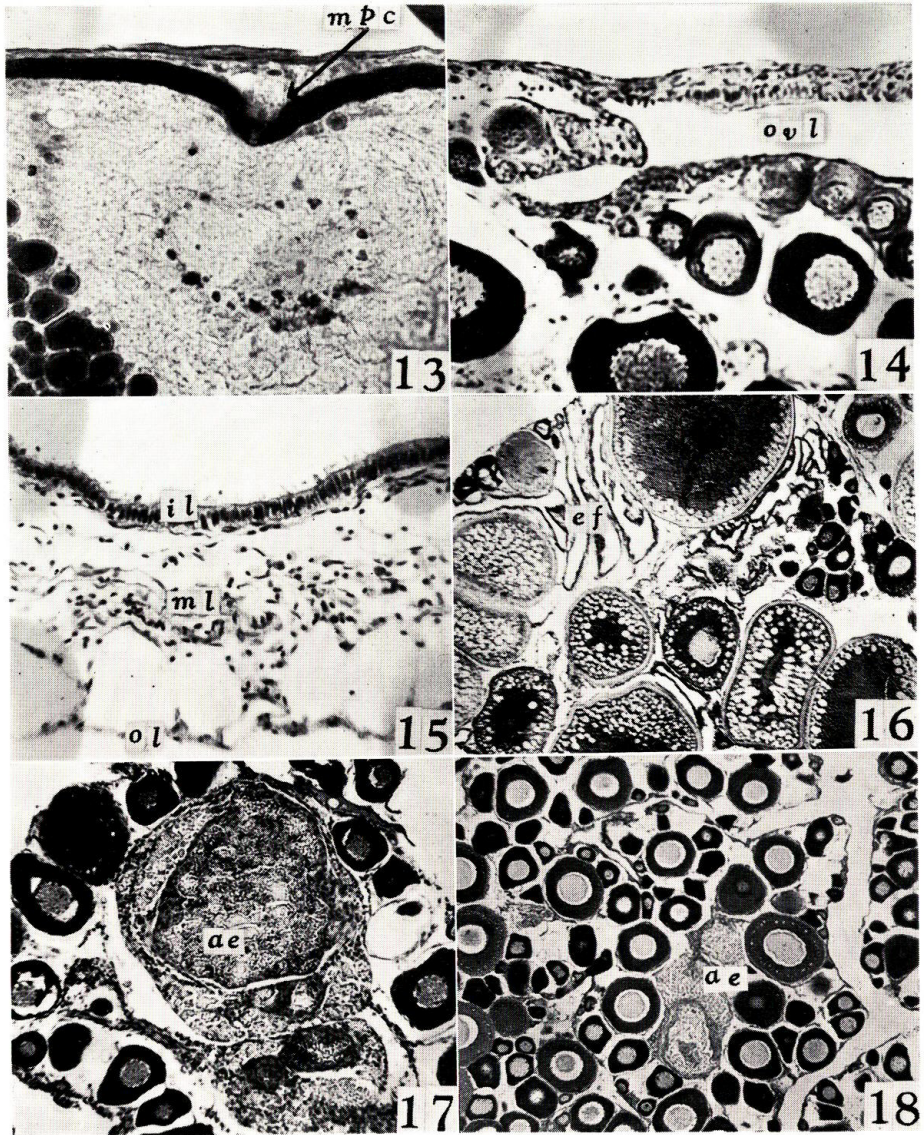
Fig. 14. Portion of an ovary, showing the ovarian lumen. Bouin's solution and Delafield's haematoxylin preparation. *ovl* ovarian lumen. $\times 230$

Fig. 15. Wall of ovarian lumen. Bouin's solution and Delafield's haematoxylin preparation. *il* inner layer: *ml* middle layer: *ol* outer layer. $\times 270$

Fig. 16. Portion of the ovary from a fish killed just after spawning. Bouin's solution and Delafield's haematoxylin preparation. *ef* empty follicle. $\times 43$

Fig. 17. Portion of the ovary from a fish killed on August 15. Bouin's solution and Delafield's haematoxylin preparation. *ae* atretic egg. $\times 90$

Fig. 18. Portion of an ovary from a fish killed on September 13. *ae* atretic egg. $\times 50$



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