RHYTHM OF DEVELOPMENT IN THE OOCYTE OF THE MEDAKA, *ORYZIAS LATIPES*[*)**)]

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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 clarify the developmental rhythm in the oocytes of the fishes. Yamamoto and Yamazaki (1961) studied the rhythm of development in the oocyte of the goldfish by means of histological methods and came to the conclusion that histological methods are more suitable for the study of the rhythm of development in goldfish oocytes than routine, biometrical methods based on egg size, and that the information obtained reflect actually the rhythm of spawning.

To accumulate knowledge in this field of study, the writers have studied the developmental rhythm of the oocyte of the Medaka, whose spawning season covers about three months from June to August and spawning times are counted usually some 30 times during that period.

Material and Methods

The fish used in the present study, *Oryzias latipes*, were obtained from a pond at Yunokawa Hot Spring in the suburbs of Hakodate City at various times of the years from 1958 to 1961. After being collected from their natural habitat, they were cultured in the pond located on the campus of the Fisheries School, Hokkaido University, until needed. For the study of seasonal change of ovaries sampling was made monthly, about ten fish at each time, through the period mentioned above. Materials for the study of ovarian change along with spawning cycle were mainly collected in July and August when the fish were at the height of spawning. Body weight, body length and gonad weight of the fish were recorded. The gonads were fixed with Regaud's solution containing five per cent of acetic acid or Bouin-Allen's and Champy's solution. For the routine histological study serial sections of the whole ovary were prepared by the usual paraffin method and stained with iron-haematoxylin or Delafield's haematoxylin followed by either light green or eosin. PAS reaction was employed on Bouin-Allen-fixed

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materials embedded in paraffin for the demonstration of carbohydrates and Sudan staining on Regaud's-fixed materials embedded in carbowax for the demonstration of lipids in the oocyte.

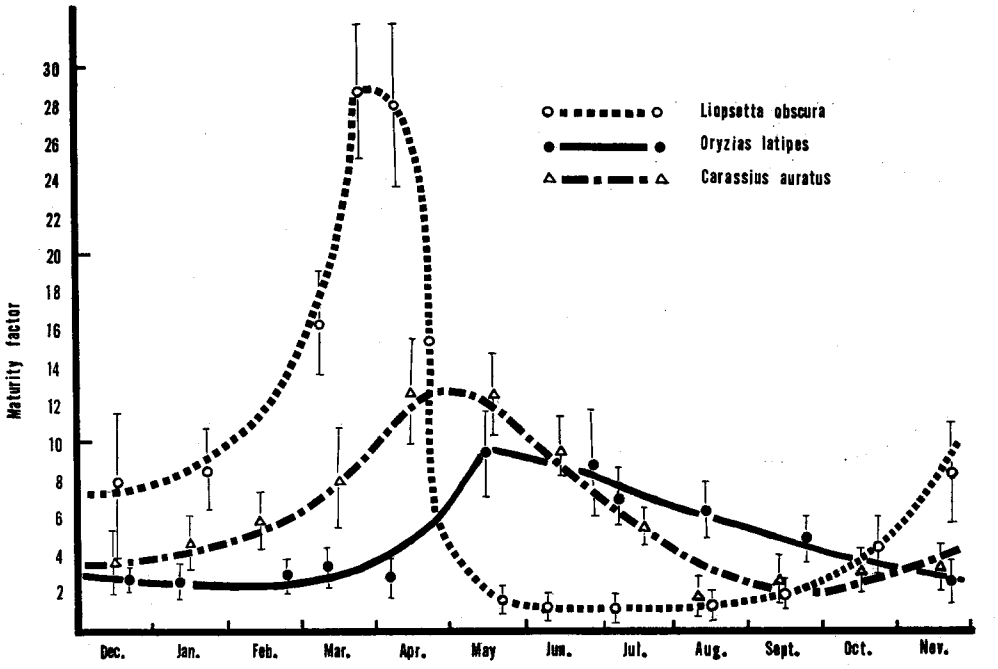
Results

I. *Changes in ovary weight*

The ovary of the Medaka is a median, unpaired sac-like organ, which is hung to the wall of the coelomic cavity by a mesovarium. It is covered with a thin peritoneal epithelium. Immediately within the epithelium lies a layer of mesenchymal connective tissues. From the ovarian wall many ovarian folds are projected into the ovarian interior. The folds consist of germ cells in varying stages of growth, lying in a loose connective tissue stroma. The ovarian lumen lies along the dorsal side of the ovary and is continuous with the oviduct which takes the form of a single straight tube about one mm in length. The wall of the ovarian lumen is composed of three components; epithelial lining, tunica albuginea and peritoneal epithelium.

In order to know the maturity of the ovary, changes of ovary weight were examined at first. Ovary weight is represented by the maturity factor, the percentage of ovary weight to body weight (Text-figure 1). Generally speaking, mean values of the maturity factor are changed very little during the period from November to early April. They ranged from 2.3 to 3.2. Then the values tend to increase slowly but steadily. At the end of April the ovaries gained weight promptly and maturity factor attained to 9.6 in average by the middle of May. As shown in Text-figure 1, 95% confidence limit in May was conspicuously large; this means that there is a large fluctuation in the weight of the individual ovary due probably to the rapid growth of ovaries during that period. The maturity factor of June was about the same as in May, being 8.7 in average. The confidence limit was also large as in the last month, and the smallest value in 95% confidence limit was 4.5. As almost all fish are laying in June, the large fluctuation of the values in this month may be due to the difference in the spawning phase of the sampled fish; some fish in the way of spawning and others just immediately after spawning. From July to September the maturity factor decreased gradually, and the mean values of the maturity factor in July, August and September were 6.9, 6.2 and 4.8 respectively. At the same time the fluctuation in values became small by and by.

This seasonal change in the maturity factor of the Medaka is very characteristic in comparison with those seen in fishes which spawn once a year. As shown in Text-figure 1, the annual curve of the maturity factor in *Liopsetta obscura*



Text-figure 1. Annual curves of the maturity factor in the Medaka, goldfish and flounder. Mean values in each month are represented by the marks, ○, △, ● and 95% confidence limits of the values by black bars.

which spawns once in a season, falls abruptly immediately after the beginning of spawning; the value, being 28 at the highest, is reduced to 1.5 in an elapse of only one month. The same annual change in maturity factor is recognized in other species showing the same spawning behavior, such as *Limanda yokohamae* (Hatanaka and Iwahashi, 1952), *Hippoglossoides platessoides* (Bagenal, 1957).

On the other hand, the goldfish which spawns two or three times in a season, gives another kind of annual curve in maturity factor (Text-figure 1). The descending of the curve after the beginning of spawning is comparatively slow in rate and continues to keep a high value for about two months. An annual curve of maturity index similar to that of the goldfish has been given by Tateishi *et al.* (1957) in *Scomber japonicus* which is surmised to spawn two or three times in a season. From the above findings it is reasonable to surmise that the annual curve in maturity factor reflects fairly well the spawning behavior of fishes.

II. *Morphological changes of the maturing oocytes*

Descriptions on the morphological change of the maturing oocyte in the Medaka have already been made by Aketa (1954) and T. S. Yamamoto (1955). The results obtained in the present study generally agree with their's, but there are some

points which should be added to their descriptions.

1. Chromatin-nucleolus stage (Figs. 1–3)

Throughout the year small oocytes of more or less 20 micra were found distributed sparsely in ovaries. They are in the stage on the way of synapsis and immediately after synapsis. They have a large nucleus surrounded with a thin layer of the cytoplasm. The nucleoli and chromatin threads in the nucleus are changed in form and number during that period, the same as in other teleost fishes (K. Yamamoto, 1956; Bara, 1960; Chouinard, 1963): chromatin-threads in network appearance become long and thin threads intermingled with one another, and then the threads show a pronounced polarization with their ends turned towards the pole of the nucleus near which one large nucleolus lies. At the next step the chromatin threads become thicker and shorter with scattering at random throughout the nucleus. In place of disappearance of the large nucleolus, several small nucleoli appear in the nucleus.

2. Peri-nucleolus stage (Figs. 4–5)

Oocytes grown up to about 50 micra in diameter have a large nucleus with many nucleoli of peripheral arrangement, which are stained deeply with haematoxylin. Chromatin threads become loose in both texture and outline, and they give rise to the so-called "lamp-brush" chromosomes. The cytoplasm surrounding the nucleus increases in relative volume and becomes stained more deeply with haematoxylin in comparison with the previous stage. Around the oocyte a very thin layer of the follicle may be revealed.

When oocytes have grown up to about 100 micra in diameter, threads and villi which are the adhesive organs of the eggs are found clearly between the follicle layer and the oocyte. The follicle layer is thick and distinct, but the zona radiata which surrounds the oocyte is very thin and indistinct. The cytoplasm has decreased its affinity to haematoxylin and is stained faintly, except the perinucleus region which is deeply stained. No notable yolky substance is demonstrated in the cytoplasm. The yolk nucleus of "Balbiani" is found deposited in the rather outer region of the cytoplasm as a black dot. The nucleoli showing a peripheral arrangement become large in size and number. The "lamp-brush" chromosomes in the nucleus gradually become obscure.

3. Yolk vesicle stage (Figs. 6–10)

The first yolky substance was demonstrated in the oocyte of about 150 micra in diameter. It comes in sight as small vesicles deposited in the outer region of the cytoplasm. The vesicles gave a positive reaction to PAS test as already reported by Aketa (1954) and T. S. Yamamoto (1955). Soon after the beginning of vesicle formation, minute fatty droplets appear in the cytoplasm. They are ac-

cumulated mainly in the peri-nucleus region (Fig. 6).

Then the vesicles gradually grow in size and occupy the greater part of the outer cytoplasm. Along with this change the accumulation of the fatty droplets has taken place mostly in the peri-nucleus region and in champy-unstained sections they form a black annulus surrounding the nucleus. The filaments and villi have increased in size and number during this period. They are found embedded between the follicle layer and the zona radiata which is still thin and indistinct. The nucleus has lost its spherical shape and smooth contour and it becomes elliptical in shape and rough in contour. The nucleoli have lost the affinity to haematoxylin and are stained faintly, but they still retain their peripheral arrangement (Figs. 7 and 8). At the later phase of this stage the oocytes show the diameter of about 400 micra.

4. Primary yolk stage (Figs. 11–13)

At the next step small granules faintly stained with haematoxylin appear in the cytoplasm between the vesicles (Fig. 11). Then they seem to come together and increase in size. And they become fused platelets locating around the yolk vesicles. They are found crowded rather in the outer part of the cytoplasm, but not limited strictly in the part and are spreaded to almost all parts of the cytoplasm (Figs. 12 and 13). During this period the yolk vesicle and fatty droplets remain almost unchanged in size, number and distribution. The nucleus takes fairly haematoxylin and is coloured almost black. The grown filaments and villi are found located between the follicle layer and thickened zona radiata. The dimension of the oocytes ranges from 390 to 450 micra.

5. Secondary yolk stage (Figs. 14–16)

The migration of the nucleus takes place at the next step. The yolk platelets seem to be accumulated more vigorously at the antipolar side and make large masses of yolk. These masses of yolk are fused and enlarged into a large yolk mass situated in the central part of the oocyte. Thus the nucleus seems to be passively driven to one pole by and by. At the same time yolk vesicles and fatty droplets are also pushed towards the outer region of the cytoplasm. The dimension of the oocytes becomes large and measures 425 to 633 micra. It is noteworthy that before the beginning of the migration of the nucleus, polarity has already been recognized in the follicle layer, that is, the antipolar side is thicker than the pole side.

6. Tertiary yolk stage (Figs. 17–19)

Thereafter, the yolk mass increases more and more in size along with the enlargement of oocytes, the diameter being measured 616 to 683 micra. The yolk vesicles are pushed further to the peripheral region and take the form or a nar-

row annulus enclosing the yolk mass. The fatty droplets seem to fuse into large ones and are found located near the boundary between the yolk mass and yolk vesicle zone. The nucleus has lost its spherical form and shows a rough contour, but it contains distinct nucleoli and obscure "lamp-brush" chromosomes. In the vicinity of the nucleus, the follicle layer shows a small expansion in which is located the micropyle on the way to development.

7. Maturation stage (Figs. 20–21)

The yolk mass grows further and occupies almost all interior of the oocyte. As already clarified by Aketa (1954), the yolk vesicles are found embedded in the cortical layer as cortical alveoli. The fatty drops are usually found near the place between the cortical layer and the yolk mass. The nucleus which has lost its boundary from the surrounding cytoplasm is found situated in the thickened cortical cytoplasm at the animal pole. The nucleoli decrease their affinity to the stain and commence to fade away. The chromosomes are thickned and shortened, and stained intensely, and they are on the way to conversion into metaphase chromosomes. The follicle layer becomes thin, but still remains to envelope the oocytes. The oocytes show almost the same size as the ripe eggs, being 833 to 866 micra in diameter.

8. Ripe egg stage (Fig. 22)

Ripe eggs in the ovarian lumen are spherical in form and have no covering follicle layer. In fixed conditions the eggs have the dimension of about 0.9 mm in diameter, but in living condition they measure about 1.3 mm. The membrane enclosing the egg proper is comparatively thick. Many villi are found distributed on the whole surface and a bundle of filaments are situated on its vegetal pole. The cortical layer is thin and embedded with many, rather large cortical alveoli. A large yolk mass is enclosed by the cortical layer and many oil drops are seen located between the cortical layer and the yolk mass.

The oocytes of the Medaka have three kinds of yolky substances as already reported by previous investigators (Aketa, 1954; T. S. Yamamoto, 1955): yolk vesicle, yolk platelet and fatty droplet. As for the formation of yolk platelets, T. S. Yamamoto (1955) reported that the yolk platelets are accumulated at first in the peri-nucleus region and make a continuous mass of yolk with the accumulation of yolk outwards. This finding, however, corresponds to the events found in the oocyte of the secondary and tertiary yolk stage. As mentioned above, the first appearance of the yolk platelet is discerned as small granules distributed in the outer part of cytoplasm and then the platelets are found accumulated around the yolk vesicle due probably to the fusion of the granules. This mode of yolk formation is fairly in agreement with those known in many fishes (c. f. K. Yamamoto, 1958).

The characteristic feature in egg formation of the Medaka, however, must be the early appearance of the formation of yolk mass. In all fishes except the Medaka the formation of a continuous yolk mass starts after the formation of yolk globules has been almost completed, while in the Medaka the formation of yolk mass proceeds simultaneously with the accumulation of yolk platelets. And this seemingly results in the occurrence of the migration of the nucleus to one pole in very early stage of oogenesis.

On account of this characteristic feature in vitellogenesis, the exact stages corresponding to the migratory nucleus and pre-maturation stage established in the flounder (K. Yamamoto, 1956) cannot be detected in the Medaka.

III. *Stage composition in oocytes*

a. Seasonal changes

Investigations on stage composition in oocytes have been taken place with a view to knowing the developmental rhythm of ovarian eggs.

The enumeration of all oocytes has been made in each stage except the smallest ones at the chromatin-nucleolus stage, using the serial section of the whole ovary from the samples obtained monthly from September to May. The results obtained are summarized in Table 1.

In September when the spawning season of this species had been just finished, three kinds of oocytes were found in the ovaries, *i. e.*, the oocytes in the peri-nucleous, yolk vesicle and primary yolk stage. They were counted to be 82.6, 15.0 and 2.4 per cent respectively. The most remarkable feature of these ovaries lies in the containing of many, degenerating oocytes. The degenerating oocytes are characteristic of deformed shapes, hypertrophied granulosa cells, deformed yolk globules and broken zona radiata as already reported by many authors, recently, by Yamamoto and Yamazaki (1961) on goldfish, Shirai (1962) on rose bitterling and by Barr (1963) on plaice. Such degenerating oocytes seem to correspond to the stages of the primary, secondary and tertiary yolk, and sometimes to the yolk vesicle stage, though the correct stage of each oocyte could not be determined in general (Fig. 24).

By October all oocytes at the primary yolk stage had disappeared from sight and during the period from October to February only young oocytes corresponding to the peri-nucleolus and yolk vesicle stages were found in the ovaries. From Table 1 it is clear that the number of the oocytes at the yolk vesicle stage is the smallest in October, being 4.8 per cent in average, and that it increases, thereafter, from month to month up to 10.5 per cent on the average by February. The degenerating oocytes mentioned above were also found throughout this period, but they were few in number. Besides these degenerating oocytes there were

Table 1. Seasonal changes of stage composition in oocytes

Months	No.	Total number of examined oocytes	Number and percentage of oocytes at each stage														Degenerating oocytes		Remarks
			Perinucleolus stage		Yolk vesicle stage		Yolk stage						Maturation stage		Ripe stage				
							Primary		Secondary		Tertiary								
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	mean	
Sept.	1	1013	879	86.9	114	11.3	18	1.8									34		
	2	1212	957	79.0	217	17.9	38	3.1									16		
	3	2056	1698	82.6	312	15.2	46	2.2									55		
	Total	4279	3534	82.6	643	15.0	102	2.4									105	35.0	
Oct.	1	1959	1923	98.2	36	1.8											0		Many tissue masses*
	2	2406	2330	96.8	76	3.2											6		Small tissue masses
	3	3330	3075	92.3	255	7.7											0		Many tissue masses
	Total	7695	7328	95.2	367	4.8											6	2.0	
Nov.	1	1325	1239	93.5	86	6.5											1		Many tissue masses
	2	3034	2801	92.3	233	7.7											8		Small tissue masses
	3	2883	2637	91.5	246	8.5											1		Small tissue masses
	Total	7242	6677	92.2	565	7.8											10	3.3	
Dec.	1	2611	2417	92.6	194	7.4											2		
	2	1510	1335	88.4	175	11.6											1		
	3	2243	2006	89.4	237	10.6											6		
	Total	6364	5758	90.5	606	9.5											9	3.0	
Jan.	1	2526	2357	93.4	169	6.6											0		
	2	2409	2199	91.3	210	8.7											2		
	3	1766	1607	91.0	159	9.0											8		
	Total	6701	6163	92.0	538	8.0											10	3.3	
Feb.	1	3066	2730	89.0	336	11.0											1		
	2	2043	1835	89.8	208	10.2											1		
	3	2788	2499	89.6	289	10.4											8		
	Total	7897	7064	89.5	833	10.5											10	3.3	
Mar.	1	1206	1096	90.9	108	8.9	2	0.2									1		
	2	1087	932	85.7	112	10.3	43	4.0									8		
	3	1316	1108	84.2	136	10.3	68	5.2	4	0.3							5		
	Total	3609	3136	86.9	356	9.9	113	3.1	4	0.1							14	4.7	
Apr.	1	1753	1375	78.4	335	19.1	43	2.5									8		
	2	1416	1231	86.9	149	10.5	34	2.5	2	0.1							11		
	3	1870	1586	84.8	244	13.1	40	2.1									2		
	Total	5039	4192	83.2	728	14.4	117	2.3	2	0.04							21	7.0	
May	1	2119	1559	73.6	312	14.7	221	10.4	27	1.3							21		
	2	2542	1990	78.3	402	15.8	136	5.3	14	0.6							15		
	3	1999	1534	76.7	310	15.5	147	7.3	7	0.4	1	0.05					17		
	Total	6660	5083	76.3	1024	15.4	504	7.6	48	0.7	1	0.02					53	17.6	

* Tissue masses are composed of atretic oocytes.

frequently found tissue masses composed of degenerated oocytes in the ovaries obtained in October and November.

In addition to oocytes younger than the yolk vesicle stage, the oocytes at the primary yolk stage were found in all ovaries examined in March. They were still

few in number. The frequency of the oocytes at that stage was 3.1 per cent on the average, while those of the peri-nucleolus and yolk vesicle stage were 86.9 and 9.9 per cent respectively. Of three ovaries only one yielded the oocytes at the secondary yolk stage, the number was very few and summed up to 0.3 per cent.

The ovaries obtained in April were almost the same in stage composition of oocytes as in March. Two ovaries out of three were composed of oocytes of three stages, *i. e.*, the peri-nucleolus, yolk vesicle and primary yolk stage: one ovary was made of four kinds of oocytes including those of the secondary yolk stage. The average percentages of oocytes of four stages were 83.2, 14.4, 2.3 and 0.04 respectively.

In May not only the occurrence of oocytes at the secondary yolk stage was common, but also the development of the tertiary yolk stage oocyte was discerned. The figures in Table 1 show that the average percentage of oocytes in each stage is 76.3 in the peri-nucleolus stage, 15.4 in the yolk vesicle stage, 7.6 in the primary yolk stage, 0.7 in the secondary yolk stage and 0.02 in the tertiary yolk stage. Throughout the period when the yolk globule develops in oocytes, degenerating oocytes were always observed in ovaries, though not so many in number.

It seems worthy to note that the oocytes at the yolk vesicle stage do not decrease in number due to the development of the oocytes at the primary and secondary yolk stages, but rather increase steadily in number during that period. This shows that the development of oocytes from the peri-nucleolus to the yolk vesicle is proceeding in parallel with those from the yolk vesicle to the primary yolk and from the primary yolk to the secondary yolk.

Although the percentage of oocytes at the peri-nucleolus stage clearly continues to decrease during the period when yolk formation is proceeding, it seems likely that the development of oocytes from the chromatin-nucleolus to the peri-nucleolus is also going forward at the same time, because the total number of the oocyte in the peri-nucleolus stage is not always diminished.

b. Changes in the cycle of spawning

At the next step, changes in stage composition along with the cycle of spawning have been investigated. As already reported by the junior author (1962), the fish used in this study usually spawns at intervals of two or three days. Thus, the fish in the height of spawning were sacrificed every morning during the period extending from the last spawning to the next. After the preparation of serial sections of the whole ovary from the specimens, the enumeration of oocytes in each stage was made in the same ways as above.

Table 2 represents the results obtained. Oocytes of all stages below the

Table 2. Changes of stage composition in oocytes during a spawning cycle

Phases in spawning cycle	No.	Total number of examined oocytes	Number and percentage of oocytes at each stage														Empty follicles		Degenerating oocytes	
			Peri-nucleous stage		Yolk vesicle stage		Yolk stage						Maturation stage		Ripe stage					
							Primary		Secondary		Tertiary									
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
The day just after spawning	1	1597	1294	81.0	215	13.5	76	4.7	8	0.5	4	0.3					26		41	
	2	893	773	86.6	91	10.2	12	1.3	16	1.8	1	0.1					28		29	
	3	1125	910	80.9	161	14.0	40	3.8	12	1.1	2	0.2					21		52	
	Total	3615	2977	82.3	467	13.0	128	3.5	36	1.0	7	0.2					75	25.0	122	40.6
One day after spawning	1	2910	2195	75.4	521	17.9	154	5.3	29	1.0	11	0.4					13		42	
	2	1353	1187	87.7	103	7.6	45	3.3	11	0.8	7	0.6					12		38	
	Total	4263	3382	79.3	624	14.6	199	4.7	40	0.9	18	0.5					25	12.5	80	40.0
Two days after spawning	1	2245	1808	80.5	246	11.0	121	5.4	45	2.0	25	1.1					15		26	
	2	1691	1385	82.0	169	10.0	126	7.4	7	0.4	4	0.2					6		22	
	3	1183	922	77.4	158	13.7	69	6.0	20	1.7	14	1.2					7		31	
	Total	5119	4115	80.3	573	11.1	316	6.4	72	1.4	43	0.8					28	9.3	79	26.3
Three days after spawning	1	1610	1337	83.1	168	10.4	67	4.1	18	1.1	17	1.1	1	0.1	2	0.1	0		30	
	2	1057	835	79.0	133	12.6	54	5.1	9	0.8	18	1.7	6	0.6	2	0.2	4		32	
	Total	2667	2172	81.6	301	11.2	121	4.5	27	1.0	35	1.3	7	0.3	4	0.1	4	2.0	62	31.0

tertiary yolk stage were included in the ovaries obtained on the day just after spawning: the ratios of oocytes in each stage were 82.3:13.0:3.5:1.0:0.2 in order of stage from the peri-nucleolus to the tertiary yolk.

The characteristic feature of the spent ovaries lies in the presence of empty follicles, which appeared as shrunken bags composed mainly of theca cells (Fig. 23). The number of empty follicles amounted to 25 on the average in one ovary. This figure is larger than the number of eggs spawned at one time, mean value of the latter in August being 5.3 (Yoshioka, 1962). The presence of many degenerating oocytes is also one of the striking features of the spent ovaries. The atretic oocytes range in number from 29 to 52, being 40.6 on the average. They showed the similar morphological changes as seen in the post-spawning season.

The ovaries obtained on one day after spawning were composed of the similar stage oocytes as above. The percentage of oocytes in each stage, however, differed somewhat from that seen on the day spawned: the increase in number of oocytes at the secondary and tertiary yolk stage was noticeable. The average value obtained was 0.9 per cent in the secondary yolk stage and 0.5 per cent in the tertiary yolk stage. Empty follicles decreased clearly in number, but atretic oocytes remained unchanged.

Two days after spawning, only the oocytes younger than the tertiary yolk stage were found in the ovaries examined. But the number of oocytes at the secondary and tertiary yolk stage had become greater; the former was 1.4 per cent on the average and the later 0.8 per cent. Empty follicles decreased in number and added up to 9.3 in average. Degenerating oocytes found in the ovaries became fewer in number than those on the last day, but the decrease in the oocyte number seems insignificant.

Three days after spawning two fish were killed at about 4 A. M. before dawn. Oocytes in the maturation and ripe stage could be found in the ovaries of the fish. The number of the maturation stage oocytes was one or six and that of the ripe stage was two in each. Total number of eggs of these stages agrees well with egg number laid in each spawning. Thus, it is reasonable to surmise that the oocytes developed more than maturation stage will be extruded as one group. Thanks to the development of these ripe eggs, however, there is found no discernible decrease in number of oocytes younger than the tertiary yolk stage. And the percentage of oocytes was 81.6, 11.2, 4.5, 1.0, 1.3, 0.3 and 0.1 in order from the peri-nucleolus stage to the ripe stage.

From the data obtained it is clear that the oocytes at the tertiary yolk stage can develop into the ripe stage through the maturation stage during about half a day.

Discussion

From the results mentioned above, it is clear that the Medaka as well as sardines (Andreu and Pinto, 1957; Ishida et al. 1959), goldfish (Yamamoto and Yamazaki, 1961), Red Sea Bream (Mio, 1962) and Anchovy (Usami, 1963), has also the oocytes displaying asynchronous development. The types of development such as total synchronism, partial synchronism, and asynchronism, result in the difference of spawning habits, *i. e.*, spawning once in a life or in a year, or multiplicity of spawning in a season.

So far, these types of spawning have been judged roughly from the frequency curves of egg diameter (Usami & Sugiyama, 1962; Nakai, 1962) and more exactly from stage composition in maturing oocytes (Champy and Gley, 1923; Yamamoto & Yamazaki, 1961; Mio, 1962). As shown in Text-figure 1, however, the annual curves in the maturity factor represent fairly well the difference in the type of spawning. The fishes showing the multiplicity of spawning in a season give an annual curve which descends very gradually after the beginning of spawning, while those which spawn once in a year give a curve which drops abruptly immediately after the beginning of spawning. Therefore, these two types of fishes may be distinguished roughly from each other by the examination of the annual curve of maturity factor in conformity with its confidence limit. The fishes which spawn once in a life, however, give a similar annual curve in maturity factor to those which spawn once in a season (Shiraishi, 1961). The only accurate method for the identification of these two types of fish, at present, is the histological method.

Next, considerations should be extended to the frequency of spawning and the number of eggs laid at each time in fish showing multiplicity of spawning. There is little doubt that in the Medaka the oocytes advanced more than maturation stage are laid at one time. As already stated above, however, the development of oocytes from the tertiary yolk stage to the ripe is taken place during about half a day. Therefore, this group of eggs cannot be determined until half a day before spawning. In the goldfish an egg group to be laid in the following spawning may be roughly determined on the basis of seasonal changes in stage composition in oocytes, whereas in the Medaka the determination of this egg group seems very difficult. Throughout spawning season, the oocytes of the Medaka develop in order from one stage to the following older stage unceasingly. Thus, the stage composition in oocytes is always kept in the same aspect, *i. e.*, younger oocytes always hold a higher percentage than the older ones and not any egg stages distinct from others are discernible. This seems to be a characteristic feature of fishes which spawn many times at intervals of short length.

The presence of empty follicles in ovaries is a certain evidence to prove that

the fish have already experienced spawning. Therefore, if the empty follicle remained unabsorbed in the ovaries for a long time, it might be possible to determine the experienced spawning times and number of laid eggs of the fish based on the conditions and number of empty follicles. However, this is not a true case in the Medaka. As ascertained in the present study, empty follicles disappeared very quickly and in three days after spawning only a few empty follicles were hardly discernible. Therefore, the determination of the experienced spawning time and number of laid eggs based on the conditions and number of empty follicles is impossible, except in the fish soon after spawning. This result agrees with the supposition given by Wheeler (1924) on a dab and K. Yamamoto (1956) on a flounder.

Summary

The rhythm of development in the oocyte of the Medaka, *Oryzias latipes* has been studied mainly by histological methods. The results obtained are summarized as follows:

1. An annual curve of maturity factor is characterized by very slow descending after the beginning of spawning.

2. A characteristic feature in vitellogenesis lies in the early formation of a large yolk mass, though the yolk globules appear at first in the outer region of the cytoplasm as seen in many other fishes. This seemingly results in the occurrence of nucleus migration to one pole in a very early stage of oogenesis.

3. The oocytes show the development of asynchronism. Even in spawning season, the oocytes develop in order from one stage to the following older stage unceasingly.

4. It seems certain that the oocytes advanced more than maturation stage are laid at one time and the development of oocytes from tertiary yolk stage to the ripe stage is taken place during about half a day.

5. Empty follicles disappear in about three days. Thus, it is impossible to determine the experienced spawning time and number of laid eggs based on the conditions and number of empty follicles present in the ovary, except in the fish soon after spawning.

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Explanation of Plates

PLATE I

Figs. 1, 2 and 3. Oocytes in the chromatin-nucleolus stage. Regaud's solution and Heidenhain's kaematoxylin-light green preparations. ×860.

Figs. 4 and 5. Oocytes in the peri-nucleolus stage. The preparations were made by a similar way as above. ×170.

Fig. 6. Oocyte in the early phase of yolk vesicle stage. Champy's solution and Heidenhain's haematoxylin-light green preparation. ×170.

Figs. 7 and 8. Oocytes in the middle phase of yolk vesicle stage. Champy's solution and unstained preparations. *fd* fatty drop. ×170.

Fig. 9. Oocyte in the early phase of yolk vesicle stage. Regaud's solution and P.A.S. preparation. *yv* yolk vesicle. ×190.

Fig. 10. Oocyte in the late phase of yolk vesicle stage. Regaud's solution and Delafield's haematoxylin-eosin preparation. ×90.

Figs. 11, 12 and 13. Oocytes in the primary yolk stage. Regaud's solution and Heidenhain's haematoxylin-light green preparations. *yp* yolk platelet. ×90.

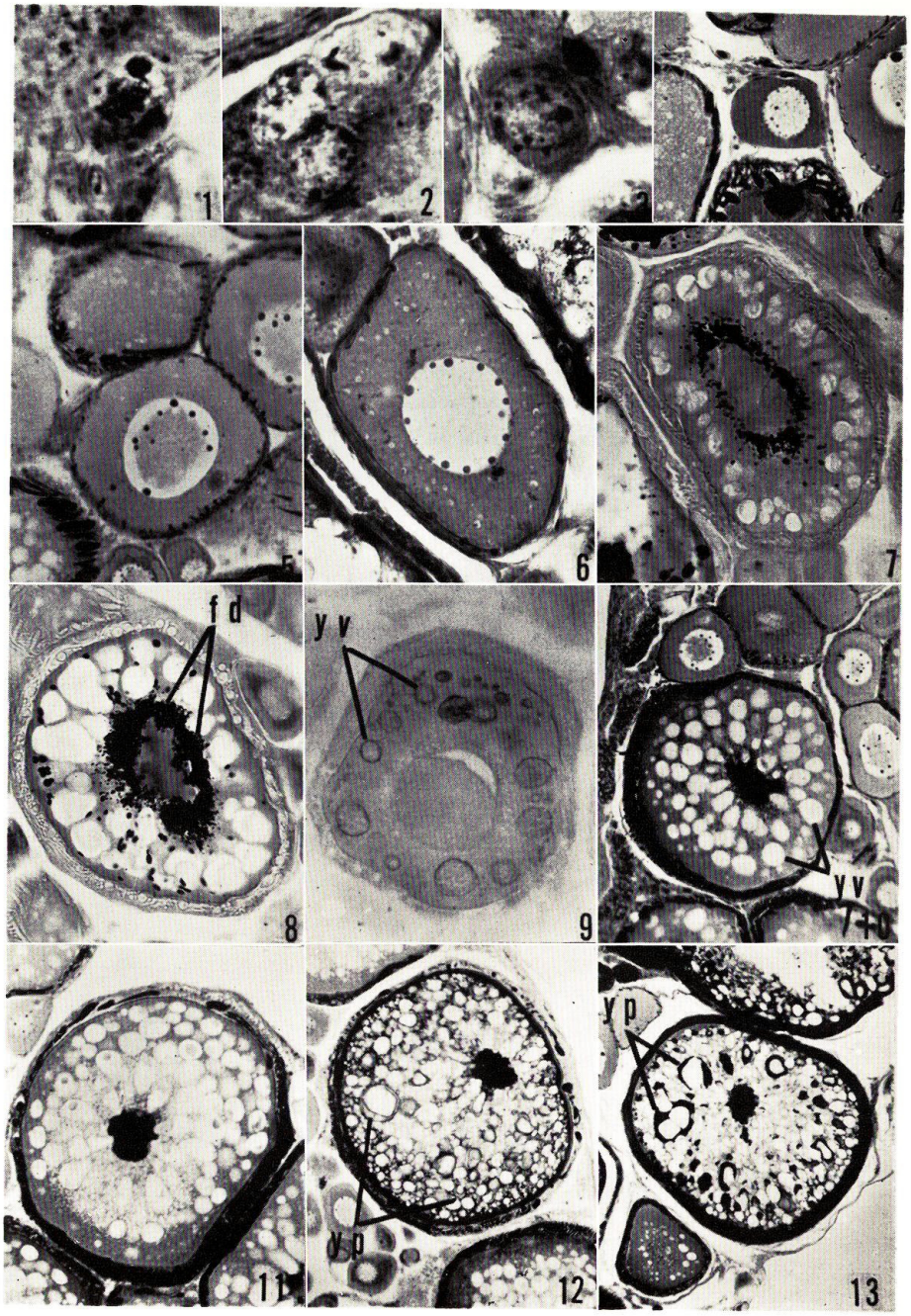

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PLATE II

Figs. 14, 15 and 16. Oocytes in the secondary yolk stage. Regaud's solution and Heidenhain's haematoxylin-light green preparations. ×45.

Figs. 17 and 18. Oocytes in the tertiary yolk stage. Preparations were made by a similar way as above. ×45.

Fig. 19. Micropyle on the way of development. Regaud's solution and Delafield's haematoxylin-eosin preparation. *m* micropyle; *n* nucleus. ×860.

Fig. 20. Oocyte in the maturation stage, from the preparation made by a similar way as above. ×40.

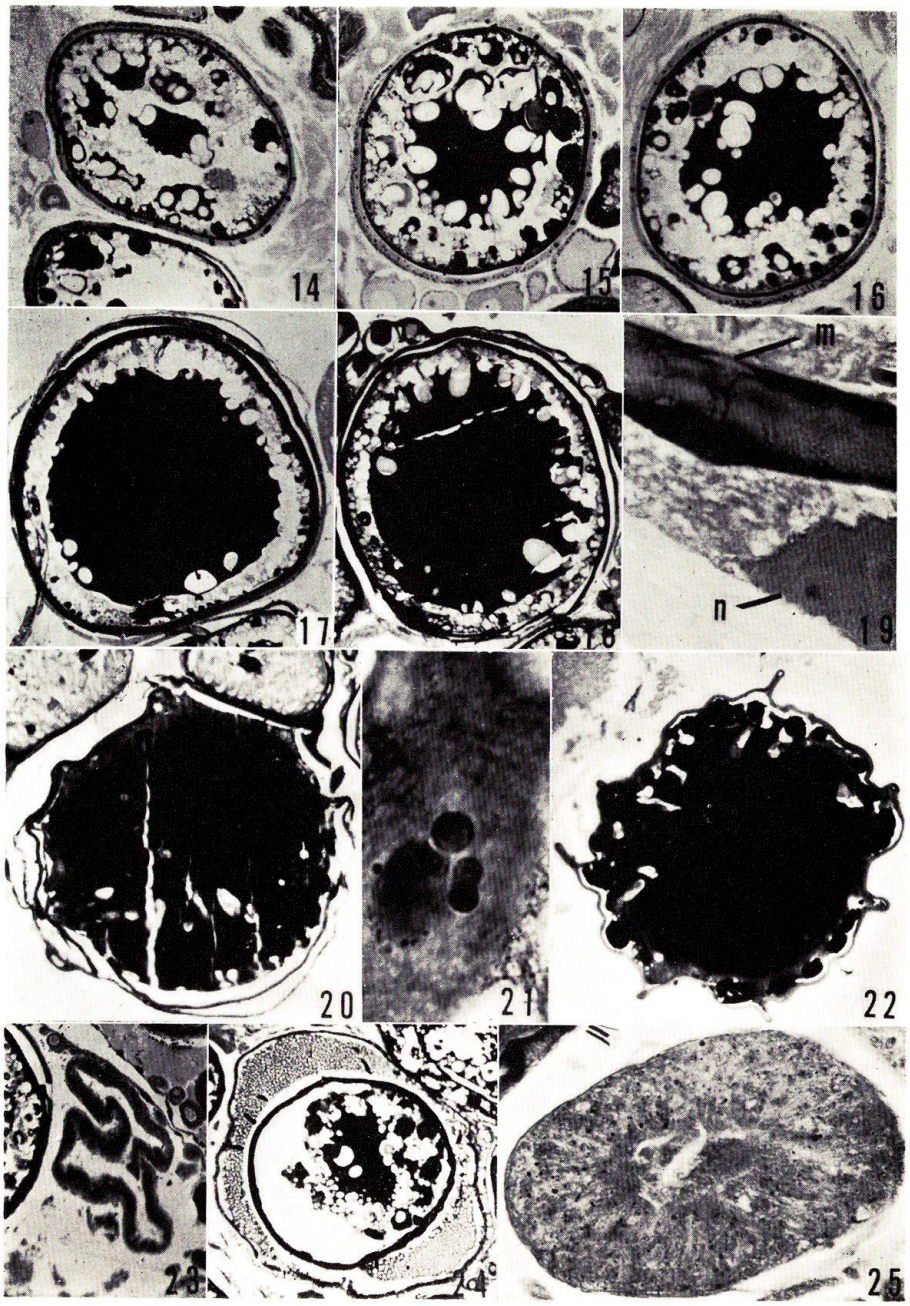
Fig. 21. Nucleus in a maturation stage oocyte, from the same preparation as above. ×860.

Fig. 22. Ripe egg. Champy's solution and Delafield's haematoxylin-eosin preparation. ×40.

Fig. 23. Empty follicle in spent ovaries. Regaud's solution and Heidenhain's haematoxylin-light green preparation. ×45.

Fig. 24. Degenerating oocyte in spent ovaries. Regaud's solution and Heidenhain's haematoxylin-light green preparation. ×45.

Fig. 25. Corpus atretica in spent ovaries. Regaud's solution and Heidenhain's haematoxylin-light green preparation. ×170.



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