CORRELATION BETWEEN THE GROWTH OF THE OVIPOSITOR AND OVARIAN CONDITIONS IN THE BITTERLING, *RHODEUS OCELLATUS***

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Introduction

In spawning time, the female bitterling develops a plainly visible urogenital papilla, the ovipositor, with which she deposits her eggs in the gill-cavity of the fresh water mussel of the genera *Unio* and *Anodonta*.

Regarding the European bitterling, *Rhodeus amarus*, Bretschneider & De Wit (1947) have made sexual endocrinological studies and assumed that the ovary of the bitterling produces the progesterone-like hormone. The presence of numerous atretic oocytes in the ovary at the time of the growth of the ovipositor and further items of evidence obtained from experiments using hormonal substances led them to the conclusion that atretic oocytes produce that hormone, which is responsible for the development of the ovipositor. The same authors called these atretic oocytes "pre-ovulation corpora lutea" on the basis of the findings that though the atretic oocytes are derived from follicles before ovulation, they are analogous to the mammalian corpora lutea both in mode of formation and in structure.

In the viviparous fishes, *Lebistes reticulatus* and *Dermogenys pusillus*, Stolk (1951, 1957) studied the cycle of the corpus luteum and concluded that the corpora lutea of these fishes probably have to accomplish an important task during the gestation period. Gokhale (1957) in two *Gadus* species, and Beach (1959) in the gold fish, suggested the role of atretic oocytes in the production of hormone.

On the other hand, Hisaw & Hisaw (1959) from studies on some elasmobranchs came to the conclusion that the corpora lutea or corpora atretica may have not any endocrine function and that their formation does not come under the control of a pituitary luteinizing hormone. Pickford & Atz (1957) also examined the functions of the corpus luteum in fishes; they stated that there is no direct evidence to show that the so-called corpora lutea described by various investigators have a secretory function.

Recently, the effects of stress on the formation of atretic oocytes in the piscine

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ovary were discussed in the review of Ball (1960).

Therefore, it is of particular interest to analyse the correlation between the length of ovipositor and the conditions of the ovary, directing special attention to the significance of atretic oocytes. The present study is concerned with the subject, the Asiatic bitterling, *Rhodeus ocellatus* being used as material.

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Materials and Methods

Materials used in the present study were caught with seine nets from Lake Kasumi, Ibaraki Prefecture, on October 27, 1959, April 7, 1960 and on May 6, 1961.

They were brought to the Faculty of Fisheries, Hokkaido University and cultured in a large aquarium set in a green house by feeding with a boiled mixture of dried shrimp and wheat meal. The water temperature of the aquarium was kept at 9–16°C throughout the season. About 7–10 fresh water mussels, *Anodonta woodiana lauta* caught from Lake Onuma, Hokkaido, were put into the aquarium. Under these conditions, the fish spawned the first ova at the beginning of April and some fish continued their spawning up to the middle of September. During the two years, samples for the histological study were taken and preserved from the above stock colony at intervals of about two weeks.

In order to examine the effects of the absence of the fresh water mussel on ovarian conditions, a group of the fish from the stock colony was transferred to the aquarium without mussels, on May 11, 1961. Sixty days later, ten fish of this group were killed and 140 days later, the second sampling was made.

Experiments on the effects of high temperature on ovarian conditions were also carried out. Ten females and three males were reared in a large aquarium of 45×30×30 cm, water temperature of which was kept at 23–25°C during the period from July 23 to September 3. The second group of twelve females and three males were cultured under the conditions of excessively high temperature, ranging 30 to 33°C, during the period from September 28 to October 28. The ovaries of the fish kept under these experimental conditions were also examined by the histological method. At fixation, the body length, body weight, ovary weight and ovipositor length were measured and then ovary was fixed in Bouin's

and Allen-Bouin's solution or Gilson's fluid. Serial sections of the whole ovary, 10 micra in thickness, were made and stained in routine manner with Delafield's haematoxylin-eosin, Heidenhain's iron haematoxylin-light green and modified Mallory's azan stain. Index of ovipositor length, which is represented by ovipositor length in proportion to body length, was calculated for each fish.

Results

(*I*) *Morphological changes in the growing oocytes*

In order to know the stages of the developing oocyte exactly, cytological study was carried on. For convenience in description, ten stages were established as in the case of the flounder, *Liopsetta obscura* (Yamamoto, 1956 a).

(1) Chromatin-nucleolus stage

The youngest oocyte, 7–11 micra in diameter, has a spherical nucleus and thin layer of cytoplasm with an indistinct boundary. A single nucleolus is present in the central region of the nucleus. As the oocyte grows, it is characterized by a nucleus which has a net-like chromatin-element and several deeply stained chromatin-nucleoli of various sizes. In the oocyte of the synaptic stage, a large chromatin-nucleolus lies near the periphery of the nucleus and chromatin-elements appear as a thick intricate bunch of threads on the other side. After synapsis, the cytoplasm considerably increases in volume and one or two large chromatin-nucleoli are found in the nucleus with distinct outline. These oocytes have a diameter of 0.02 to 0.027 mm.

(2) Early peri-nucleolus stage

The diameter of the oocyte at this stage ranges from 0.03 to 0.12 mm. The oocyte at the earlier phase of this stage has a large nucleus and a small amount of cytoplasm. Chromatin threads are dispersed within the nucleus. In addition to many chromatin-nucleoli, there are found one or two large and basophilic true nucleoli in the nucleus. Along with the growth of the oocyte, the cytoplasm increases its volume and becomes more basophilic. At the later phase of this stage, the nucleus is much enlarged; deeply stained nucleoli, spherical in form and various in size, gradually arrange themselves on the periphery of the nucleus (Fig. 1).

(3) Late peri-nucleolus stage

As the oocyte grows, the cytoplasm gradually loses its affinity to dyes and comes to be stained faintly with haematoxylin. Nucleoli of spherical form and of various sizes are situated on the periphery of the nucleus as in the previous stage. The yolk nucleus is found in the outer part of the cytoplasm (Fig. 2). The size

of the oocyte at this stage ranges from 0.13 to 0.16 mm. A thin follicle layer surrounding the oocyte is recognized, but the zona radiata is not evident.

(4) Yolk vesicle stage

Yolk vesicles appear from near the surface of the oocyte and gradually invade the inner region of the cytoplasm (Fig. 3). Finally, the ooplasm is filled with yolk vesicles excepting a narrow zone around the nucleus (Fig. 4). The nucleus which contains many deeply stained nucleoli with haematoxylin is situated in the central area of the oocyte. Yolk vesicles are small in size and spherical in form. They have weak affinity to dyes but are stained with eosin or light green. In the later phase of this stage, the zona radiata gains thickness and comes to show clear radial striation. The oocytes of this stage grow rapidly as the phase advances. They range from 0.2 to 0.58 mm in diameter.

(5) Primary yolk stage

The diameter of the oocyte in this stage is about 0.63 mm. Yolk globules appear as small bodies. They are stained moderately with haematoxylin. The formation of yolk globules proceeds centrally from the peripheral region along with the enlargement of their size. Simultaneously, there is increase in the affinity of the globules to the dyes. The outer half zone of the ooplasm is still found filled with yolk vesicles. The nucleus, spherical in form, lies in the central area of the oocyte and many nucleoli of various sizes are observed (Fig. 5).

(6) Secondary yolk stage

The oocytes become large, attaining a diameter of 0.80 to 0.87 mm. A great number of yolk globules are found in the ooplasm. They are of various sizes and are stained intensely with Heidenhain's haematoxylin (Fig. 6). The outer part of the oocyte is occupied with yolk vesicles of 5–6 rows. Between yolk vesicles, many minute globules stained intensely with haematoxylin are observable. They appear to grow into large yolk globules in time. The nucleus, spherical in form, is located in the central area of the oocyte. It contains many nucleoli of spherical form. The thick zona radiata showing clear radial striation is present.

(7) Tertiary yolk stage

The ooplasm is full of yolk globules except for the thin surface layer composed of 2–3 rows of yolk vesicles. Marked increase in the size of oocytes is recognized in this stage. They measure about 1.06 to 1.25 mm in diameter. The zona radiata becomes thinner than in the secondary yolk stage. The follicle layer also loses its thickness (Fig. 7).

(8) Migratory nucleus stage

In the earlier phase of this stage, the nucleus, spherical in form, begins to migrate to one pole of the oocyte. As the nucleus moves towards the pole, the

oocyte becomes oval in form (Fig. 8). When the migration has been completed, the nucleus assumes round form; it contains many spherical nucleoli of diverse sizes. A small number of large yolk globules are found around the nucleus. Between the yolk globules the hyaloplasm may be discernible. A micropyle is also detected at a point of the thin zona radiata. The size of the oocyte is about 1.33 to 1.40 mm.

(9) Pre-maturation stage

Oocytes become elliptical in form. The nuclear membrane becomes indistinct, the spherical nucleoli disappear from sight, and then there appear many minute twig-like nucleoli (Fig. 9). Hyaloplasm develops around the nucleus. The zona radiata is comparatively thin; the follicle layer at the animal pole is thick and distinct. The size of the oocyte in this stage is almost the same as that of the previous stage (Fig. 10).

(10) Ripe egg stage

The ripe egg extruded from the follicle is electric-bulb-like in shape, with a narrow neck-like end, yellowish in colour, and easily distinguishable from young oocytes. Many yolk globules of round form are distributed without fusion. A layer of hyaloplasm is detected in the neck-like end of the ovum; the micropyle is situated at a point of the same portion. The cortical alveoli are found embedded in the cortical layer. The long axis of a mature egg ranges from 2.4 to 2.6 mm, while the short axis ranges 1.1 to 1.2 mm (Fig. 11).

(II) Observations on the oocyte undergoing atresia and the empty follicle

Atretic phenomena occur mainly in the oocytes of advanced stages. Under natural conditions, disintegration has not been recognized in oocytes of the perinucleolus stage and yolk vesicle stage, but it has been frequently in the oocytes of the tertiary yolk stage and migratory nucleus stage. The process of atresia is somewhat different according to the amount of yolk in oocytes. In the oocytes of the secondary or tertiary yolk stage and migratory nucleus stage, the granulosa cells exhibit slight hypertrophy and lose their regularity at first. The nucleus of the oocyte also seems to be disintegrated in the early stage of atresia. A thick layer of colloidal substance appears in the outer part of the atretic oocyte (Fig. 12). Sometimes, spherical vacuole-like bodies which are variable in size, are recognized in this layer. Wrinkled zona radiata was embedded in the layer of colloidal substance. However, in atretic oocytes corresponding to the migratory nucleus stage, in which the zona radiata is very thin, remnants of the zona radiata frequently disappear in the early stage of atresia. In the inner part of the oocyte, intact yolk globules of spherical form are found.

The next step of atresia is characterized by the hypertrophy and elaborate

folding of granulosa cells (Fig. 13). The nucleus of the elongated granulosa cells is located in the basal part. The accumulation of the colloidal fluid in the lower part of the elongated granulosa cells and the inclusion of a few yolk globules within the granulosa cells suggest the digestive and phagocytic action of these cells (Fig. 14). A small amount of yolk materials still remains in the oocyte. Enlarged capillaries are found within the theca layer. Parallel to these changes, the granulosa cells increase in number and gradually penetrate towards the inner part of atretic oocytes. At the same time, a small number of cells, probably the lymphocytes, invade the atretic oocytes (Fig. 15). With the absorption of yolk materials, atretic oocytes collapse gradually and become irregular masses of cells. Sometimes, these solid masses of cells, together with connective tissue fibers, take a spherical form (Fig. 16). Later, granular yellow pigments are accumulated in the picnotic granulosa cells and then these cells migrate into the ovarian stroma. The final structure of atretic oocytes is a small heap of cells filled with yellowish granules, together with connective tissue fibers (Fig. 17). These bodies seem to persist for a long time in the ovarian stroma.

Though Stenger (1959), Bara (1960) and Polder (1961) have insisted that any structures corresponding to the lutein cells and corpus luteum of mammals were not found in their material fishes, small tissues with yellowish pigments found in the present material resemble closely the corpus luteum of mammals. Therefore, it may be justifiable to conclude that the atretic oocytes which resulted from the above processes are identical to the so-called pre-ovulation corpora lutea described by Bretschneider & De Wit.

As for the empty follicles, Bullough (1942) stated that masses of empty follicles are homologous with the corpora lutea of mammals. The empty follicles found in *Rhodeus ocellatus*, shrink immediately after ovulation and show complicated folds. Then, the granulosa cells of the follicles undergo picnotic degeneration and become vacuolar. Empty follicles thus formed are similar to atretic oocytes of later stages. But they seem to disappear in a little while and do not make a heap of cells with yellow granules. Therefore, it is impossible to compare the empty follicles with corpora lutea as has already been pointed out by Mendoza (1943).

(*III*) *Correlation between ovipositor length and ovarian conditions*

As Yokote (1958) pointed out, morphological changes in the ovary coincide generally with the fluctuations of ovipositor growth.

In order to examine this correlation, the relation between ovipositor length and stage composition in oocytes of each ovary was observed. The results are summarized in Tables 1 and 2.

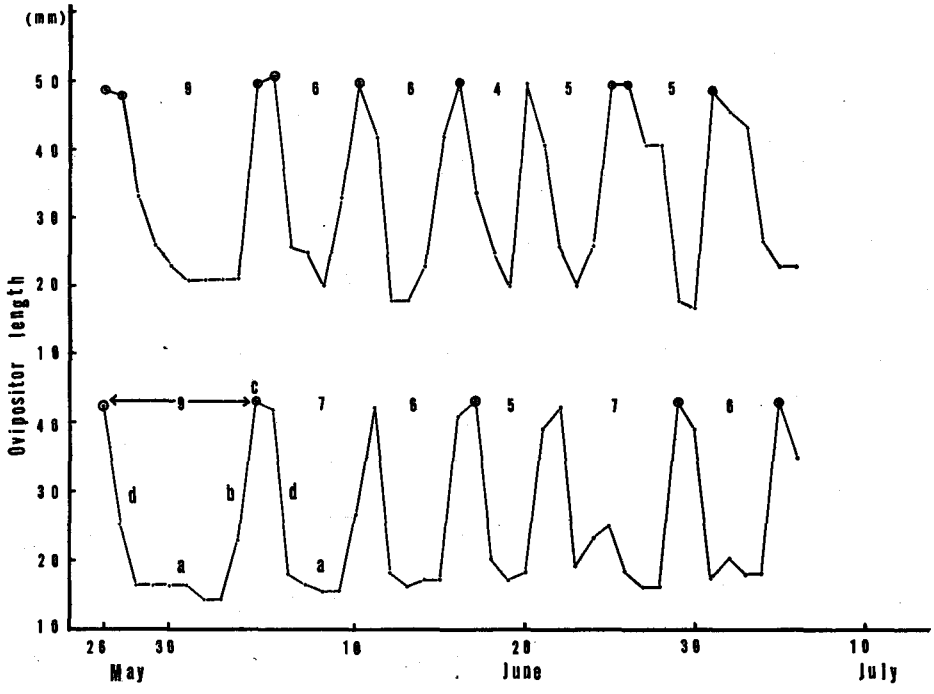
As shown in Table 1, fish out of spawning season have small ovipositors which

Table 1. Correlation between ovipositor length and stage composition in oocytes

Date	Ovipositor length (mm)	Body length (mm)	Total number of examined eggs	Percentage of oocytes at each stage									
				Peri-nucleolus stage		Yolk vesicle stage	Yolk stage			Migratory nucleus stage	Pre-maturation stage	Ripe egg stage	Atretic ocytes
				Early	Late		Primary	Secondary	Tertiary				
Nov. 6	1	26	1073	61.6	13.8	24.6							
Nov. 13	1	35	2183	68.7	10.6	20.6							
Nov. 24	1	29	816	60.0	9.7	30.7							
Nov. 18	6	42	1147	57.6	11.6	30.6							0.1
Dec. 17	1	36	755	66.1	9.5	24.2							0.1
Dec. 17	1	37	750	58.1	10.5	31.2							0·1
Dec. 17	2	42	854	61.0	10.3	28.7							
Feb. 19	2	46	1087	56.9	12.4	30.5							0.2
Mar. 21	9	41	1130	58.4	10.0	31.6							
Mar. 11	10	53	896	49.1	9.6	41.3							
Mar. 11	8	45	1272	57.7	10.7	29.2	2.4						
Mar. 19	9	40	853	56.4	10.2	28.3	5.0						0.2
Oct. 31	15	43	227	38.3	9.3	38.3	2.2	3.5	1.8				6.6
Oct. 31	16	42	327	43.7	8.9	40.1	0.3	0.9	0.6	1.8			3.7
Nov. 6	6	40	910	45.4	15.9	38.1	0.1						0.3
Nov. 18	9	46	718	36.3	15.1	39.6	1.9	3.3	0.8	0.4			2.2

range from 2 to 10 mm in length with 0.04 to 0.29 in index value. The ovaries of these fish contain only younger oocytes than the primary yolk stage. Up to February, more or less than 70 per cent of the oocytes in the ovaries belong to the peri-nucleolus stage and about 30 per cent to the yolk vesicle stage. No oocytes in the primary yolk stage are to be found. In March, 3 to 5 per cent of primary yolk stage oocytes were detected in the ovaries, besides those in the peri-nucleolus stage and yolk vesicle stage. Ovipositor length was more or less 10 mm at that time. A fish killed on March 15 had an ovipositor of 15 mm length and 0.38 index value. The largest oocytes in her ovary had arrived at the tertiary yolk stage. In contrast with the observations of Dutch workers, however, the so-called pre-ovulation corpora lutea or atretic oocytes were found scarcely in the ovaries obtained during these months.

In the spawning season, the ovipositor undergoes periodical fluctuations and changes from about 10 to 60 mm (Text-fig. 1 and Fig. 18). The processes of cyclic changes in the ovipositor have been divided into four phases and denoted as ovula-



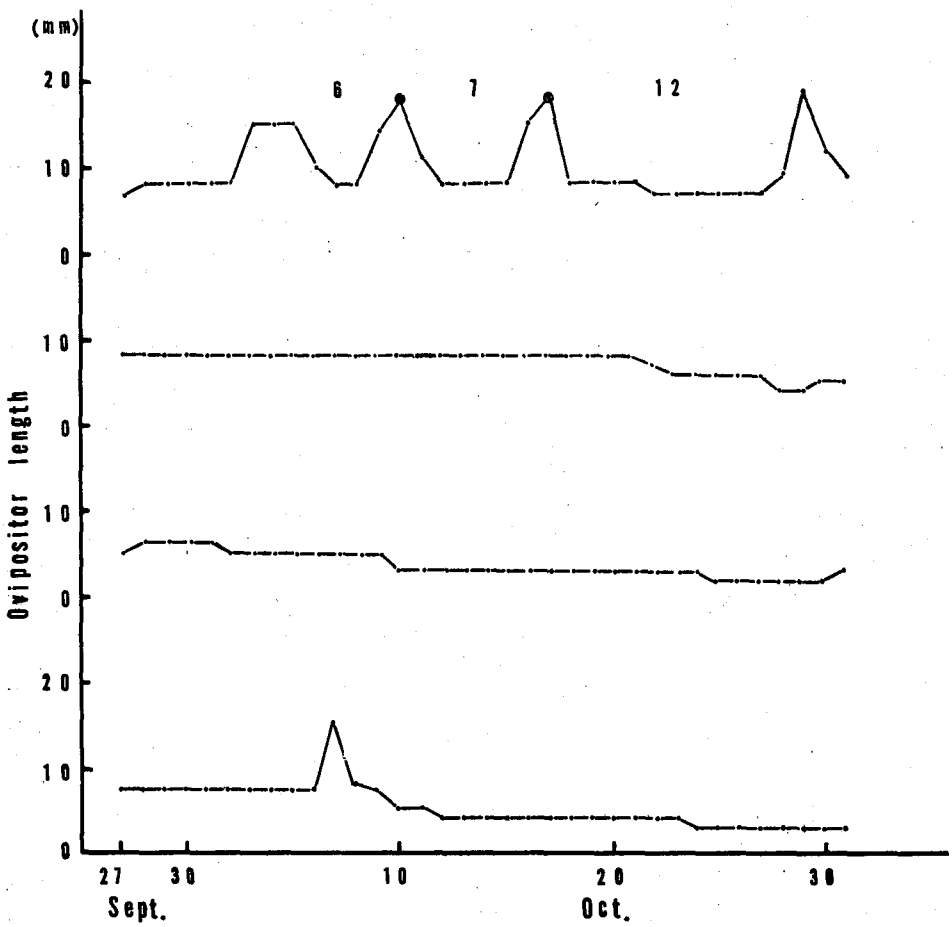
Text-fig. 1. Periodical fluctuations in ovipositor growth shown by the two females in the spawning season
Ovipositions observed are marked with a double circle.
a: resting phase; b: ovulation phase;
c: oviposition phase; d: post-oviposition phase.

Table 2. Correlation between ovipositor length and stage composition in oocytes in the spawning season

Date	Ovipositor length (mm)	Phase	Body length (mm)	Total number of examined eggs	Percentage of oocytes at each stage									
					Peri-nucleolus stage		Yolk vesicle stage	Yolk stage			Migratory nucleus stage	Pre-maturation stage	Ripe egg stage	Atretic oocytes
					Early	Late		Primary	Secondary	Tertiary				
Sept. 30	11	a	37	888	38.8	10.0	37.7	3.4	5.4	0.2	4.5			0.1
Apr. 22	13	a	42	575	65.3	12.5	16.5	1.0	1.0	0.7	2.8			0.5
Sept. 30	14	a	40	592	38.6	13.0	32.9	4.7	5.6	0.8	4.2			
Sept. 30	14	a	44	331	49.8	6.7	31.1	3.6	3.0	0.3	5.4			
Mar. 6	25	b	40	755	47.3	9.4	34.8	1.9	2.0	1.1	2.8	0.3		0.3
Sept. 30	26	b	44	765	29.4	13.7	44.1	4.1	2.5		5.5	0.7		
Sept. 30	28	b	43	622	38.2	20.5	30.5	2.3	2.7	2.9			2.7	
Jul. 17	32	c	38	218	47.2	8.7	20.6	1.8	4.6	2.3	4.1		8.7	1.8
Jul. 13	40	c	42	254	42.9	11.0	33.5	2.4	1.2	2.8	0.4		5.9	
Jul. 3	41	c	35	650	58.0	12.4	24.0	1.6	1.2	0.2	2.3		0.2	
Jul. 13	47	c	43	164	9.1	10.9	39.0	10.3	6.7	11.5	8.5		3.0	0.6
Nov. 18	63	c	44	346	29.5	17.1	35.3	3.2	4.0	6.9			4.0	
Feb. 23	20	d	39	362	51.1	11.3	31.5	1.9	0.6	1.9	1.7			
Sept. 30	23	d	38	493	63.6	7.7	20.5	1.2	2.6	2.2	1.8			0.2
Nov. 18	27	d	40	819	55.1	13.4	27.2	1.1	0.5	0.2	2.3			
Sept. 30	27	d	41	410	46.6	15.8	31.2	3.2	0.5	0.2	2.4			

a: resting phase; b: ovulation phase; c: oviposition phase; d: post-oviposition phase.

tion, oviposition, post-oviposition and resting phase. At the time when the ovipositor reaches its maximum length, spawning occurs. This phase is denoted as oviposition phase. The maximum length of ovipositor was different somewhat by individuals and ranged from 32 to 63 mm. After spawning, ovipositors reduce their length rapidly and attain to 20–27 mm in about a day. This reducing phase is called the post-oviposition phase. The resting phase follows after the post-oviposition phase. At this phase ovipositors become shorter and reduce to 11–14 mm. They remain in that state for three or four days. Then, ovipositors lengthen rapidly, attaining to more or less 25 mm in a day. This is the ovulation phase. The changes in length of ovipositor are repeated cyclically at intervals of about 6–9 days.



Text-fig. 2. Fluctuations in ovipositor growth shown by the four females in the post-spawning season

Data on the stage composition in oocytes of the fish at each phase are summarized in Table 2. The fish showing the maximum length of ovipositor always have ovaries with ripe eggs extruded into the ovarian lumen. Besides mature eggs, many oocytes of all stages younger than the migratory nucleus stage and empty follicles were found in the ovaries. Atretic oocytes were very few, and of five females examined only two contained 0.6 and 1.8 per cent of atretic oocytes respectively. In the post-oviposition phase, the most advanced oocytes found in ovaries were those at the migratory nucleus stage. Atretic oocytes were also very few. The ovaries of fish in the resting phase were filled with oocytes of the migratory nucleus stage, the tertiary yolk stage and of many younger stages. During the ovulation phase, maturation and ovulation take place. The ovaries obtained in this phase included a few ripe eggs mingling with oocytes at the pre-maturation stage and many younger oocytes. Only one ovary out of three held a small number of atretic oocytes.

At the end of September, the length of ovipositors gradually becomes shorter until it reaches less than 10 mm at the middle of October, though there were found some exceptions (Text-fig. 2). Some fish killed in October and November showed the characteristic conditions of the ovary with many oocytes of various atretic stages (Table 1 and Fig. 19).

From above observations, it is clear that the ovipositor length is intimately correlated to egg maturity, but not the presence of atretic oocytes.

(IV) Ovarian conditions of the fish reared under unsuitable conditions

(A) Effects of high temperature on the ovary

Under high temperature condition, ranging from 23 to 25°C, the fluctuations of ovipositor growth in spawning season were not cyclic; histological studies revealed that the gonadal condition was not always in accordance with the ovipositor growth. Some oocytes at the pre-maturation stage and many oocytes of later phase atresia were observed in the ovary of a fish whose evipositor length was 15 mm, index value 0.37. In spite of the short length of ovipositor, a fish showing the ovipositor length of 5 mm contained many atretic oocytes corresponding to the secondary or tertiary yolk stage. The most remarkable regression of the oocytes was recognized in the individual of 15 mm ovipositor. Almost all oocytes laden with yolk seemed to be in the course of degeneration (Fig. 20).

In the second group of fish reared for 30 days at 30 to 33°C, the ovipositor length of all fish was reduced to 1–2 mm by the end of experiment. In the ovaries of these fish, all oocytes advanced beyond early phase of yolk vesicle stage were atretic. Many atretic oocytes corresponding to the corpora lutea of beta, gamma and delta phases described by Dutch workers were also detected (Figs. 21 and 22).

It is highly probable, therefore, that atretic oocytes of any stages are not responsible for the growth of the ovipositor.

(*B*) *Effects of the absence of the mussel on the ovary*

All the fish kept without mussels indicated no cyclic changes of ovipositor in spawning season and always had short ovipositors. Ten fish killed after 60 days, had ovipositors ranging from 6 to 13 mm. In the ovaries of these fish, many oocytes corresponding to the tertiary yolk stage and most of oocytes at the migratory nucleus stage were in the course of atresia, though younger oocytes at the secondary, the primary yolk stage and yolk vesicle stage were intact (Fig. 23).

From these findings, it is certain that in the condition of rearing without mussels, development from the tertiary yolk stage to the migratory nucleus stage can be induced in some oocytes, but maturation and ovulation are inhibited completely, because neither any oocytes at the pre-maturation stage nor mature eggs were present in the ovaries.

Ten fish autopsied on the 140th day also showed short length of ovipositor as quiescent season. Oocytes at the yolk vesicle stage are the most advanced and small masses of cells, which seem to be derived from atretic oocytes and connective tissue fibers, filled the ovarian stroma (Fig. 24).

Discussion

The role of pre-ovulation corpora lutea as only a secretory gland in the piscine ovary was emphasized by Bretschneider & De Wit (1947), De Groot & De Wit (1949 a, 1949 b, 1949 c, 1949 d), Van der Veen & De Wit (1951 a, 1951 b), Stolk (1951, 1957), Hoar (1955) and Ball (1960).

According to the opinion of Bretschneider & De Wit, a luteinizing hormone which transforms some eggs into pre-ovulation corpora lutea is secreted from the anterior hypophyseal lobe. During March and April prior to the spawning season of the European bitterling, the eggs seemingly corresponding to the yolk vesicle stage or primary yolk stage rapidly decrease in number along with the formation of pre-ovulation corpora lutea. In the spawning season, these bodies become the highest in number at the time when the ovipositor shows its maximum length.

In the present study, atretic oocytes at the yolk vesicle stage, primary, secondary and tertiary yolk stage have been rarely found in the bitterling sampled in March and April when the growth of the ovipositor was going on steadily. In the spawning season, this is also true. Three fish which were killed just after oviposition had no atretic oocytes although the ovipositors were of full length. In two fish sampled at ovulation phase, atretic oocytes were not found. Thus, the

growth of the ovipositor seems not to be caused by the presence of atretic oocytes.

On the other hand, the appearance of atretic oocytes was not always accompanied by the growth of the ovipositor. For instance, the fish cultured under unsuitable conditions provided the ovaries which contained atretic oocytes of various stages but the ovipositor showed short length.

As stated above, atretic oocytes of some stages certainly correspond to the so-called pre-ovulation corpora lutea of Bretschneider & De Wit morphologically. Therefore, it is reasonable to conclude that the pre-ovulation corpora lutea possess no secretory function indispensable to ovipositor growth.

Thus; there arises a question: What factors participate in the growth of the ovipositor? The present study clearly shows that ovipositor length is intimately correlated to ovarian maturity.

In spawning season, the ovipositor lengthens rapidly at ovulation phase when maturation and ovulation occur. In the condition of rearing without musssel, however, ovipositors always keep the short length. Maturation and ovulation are inhibited under the same condition. It would seem, therefore, that factors participating in maturation and ovulation are related to the rapid growth of the ovipositor at the same time. Hormonal control of pituitary is surmised to be one of the factors. And also, it is reasonable to suppose that physical stimulus caused by the extrusion of mature eggs into the lumen brings ovipositor growth under nervous control. Detailed study on this problem is now being carried out.

As for the cause of atresia in oocytes, the present writer agrees with Ball (1960). Summarizing the results of Stanworth (1953) and of Rasquin & Atz (1952), Ball arrived at the opinion that gonadotrophs, as well as thyrotrophs, are affected by stress conditions and this results in a transitory disturbance of gonadotrophin output in association with the thyroid and adrenocortical changes, all of which would be expected to inhibit oocyte growth. The conditions such as the absence of mussel and high temperature may be considered as stress conditions exerting effects on gonadotrophs and thyrotrophs. As the results of these effects, actual atresia in oocytes, through the pathway mentioned by Ball, may be expected to occur. Hypertrophy and phagocytic action of the granulosa cells are the usual changes along with the ceasing of growth in oocyte.

Summary

Histological studies of the ovary in the bitterling, *Rhodeus ocellatus* were undertaken, for the purpose of clarifying the correlation between the growth of the ovipositor and the ovarian conditions.

1. Ovarian conditions are described on the basis of oocyte maturity which progresses through ten stages.
2. The atretic oocytes are identical to the so-called pre-ovulation corpora lutea, morphologically speaking.
3. Under natural conditions, the process of maturation of ovaries is generally correlated to the fluctuations of the ovipositor length.
4. The fish cultured in high temperature exhibited regression of the oocytes. The same phenomenon was recognized in the fish of post-spawning season.
5. The absence of the fresh water mussel exerts an influence which prevents maturation and ovulation of oocytes in the present experimental fish.
6. It would seem that the so-called pre-ovulation corpora lutea possess no secretory function necessary for the growth of the ovipositor.

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Explanation of Plates

PLATE I

All figures are photomicrographs obtained from sections of the ovaries of the bitterling except Fig. 18. Fixed in Bouin's fluid and stained with Delafield's haematoxylin-eosin.

Fig. 1. Oocytes at the early peri-nucleolus stage. ×350

Fig. 2. An oocyte at the late peri-nucleolus stage. yn yolk nucleus. ×315

Fig. 3. Oocytes at the early phase of yolk vesicle stage. ×88

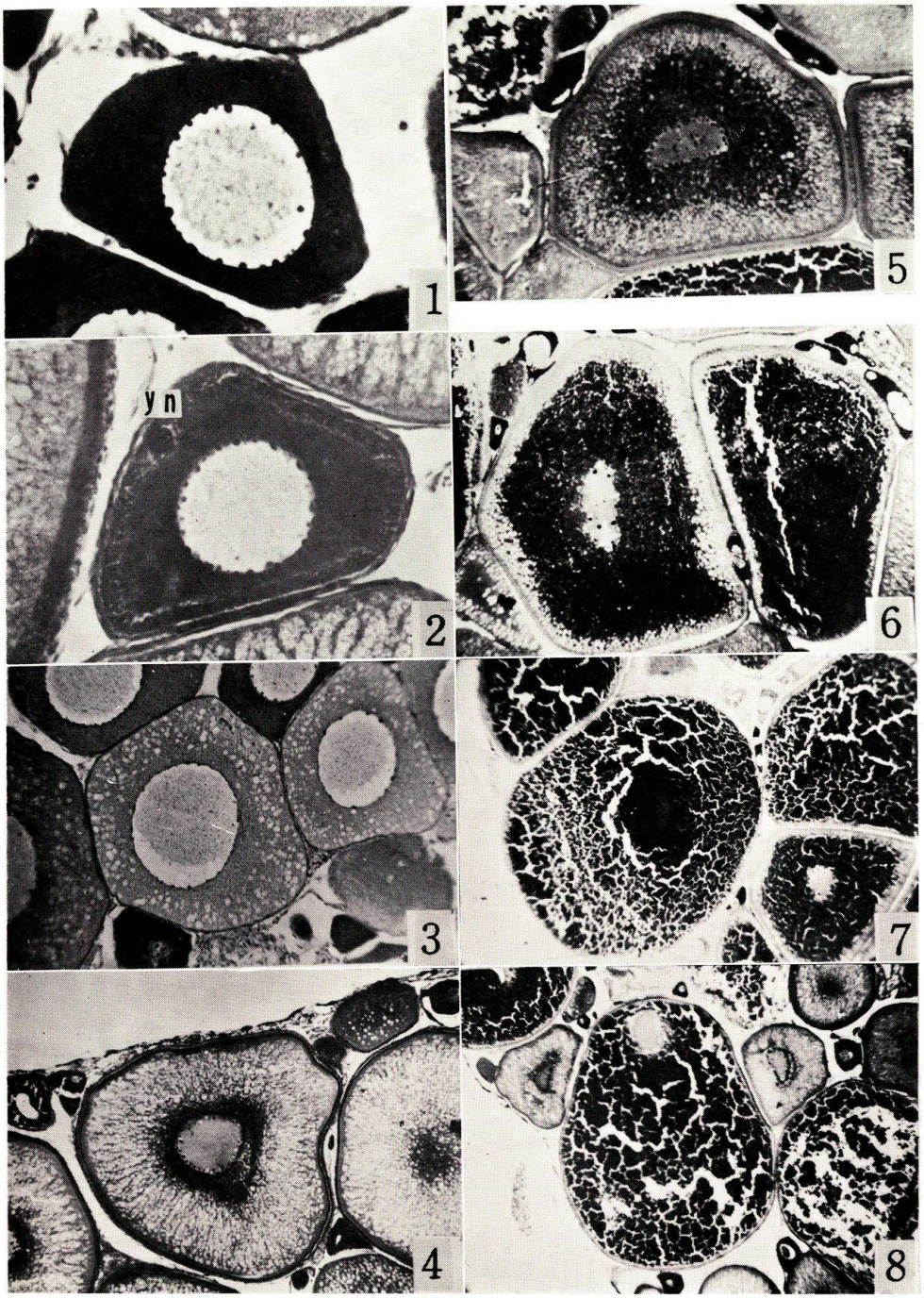
Fig. 4. Oocytes at the late phase of yolk vesicle stage. ×70

Fig. 5. An oocyte at the primary yolk stage. ×70

Fig. 6. Oocytes at the secondary yolk stage. ×70

Fig. 7. An oocyte at the tertiary yolk stage. ×36

Fig. 8. An oocyte at the migratory nucleus stage. ×32



Shirai: Ovarian Conditions in the Bitterling

PLATE II

Fig. 9. Nucleus of an oocyte at the pre-maturation stage. Minute twig-lik uncleoli are shown. ×350

Fig. 10. An oocyte at the pre-maturation stage. ×32

Fig. 11. An egg at the ripe egg stage. ×20

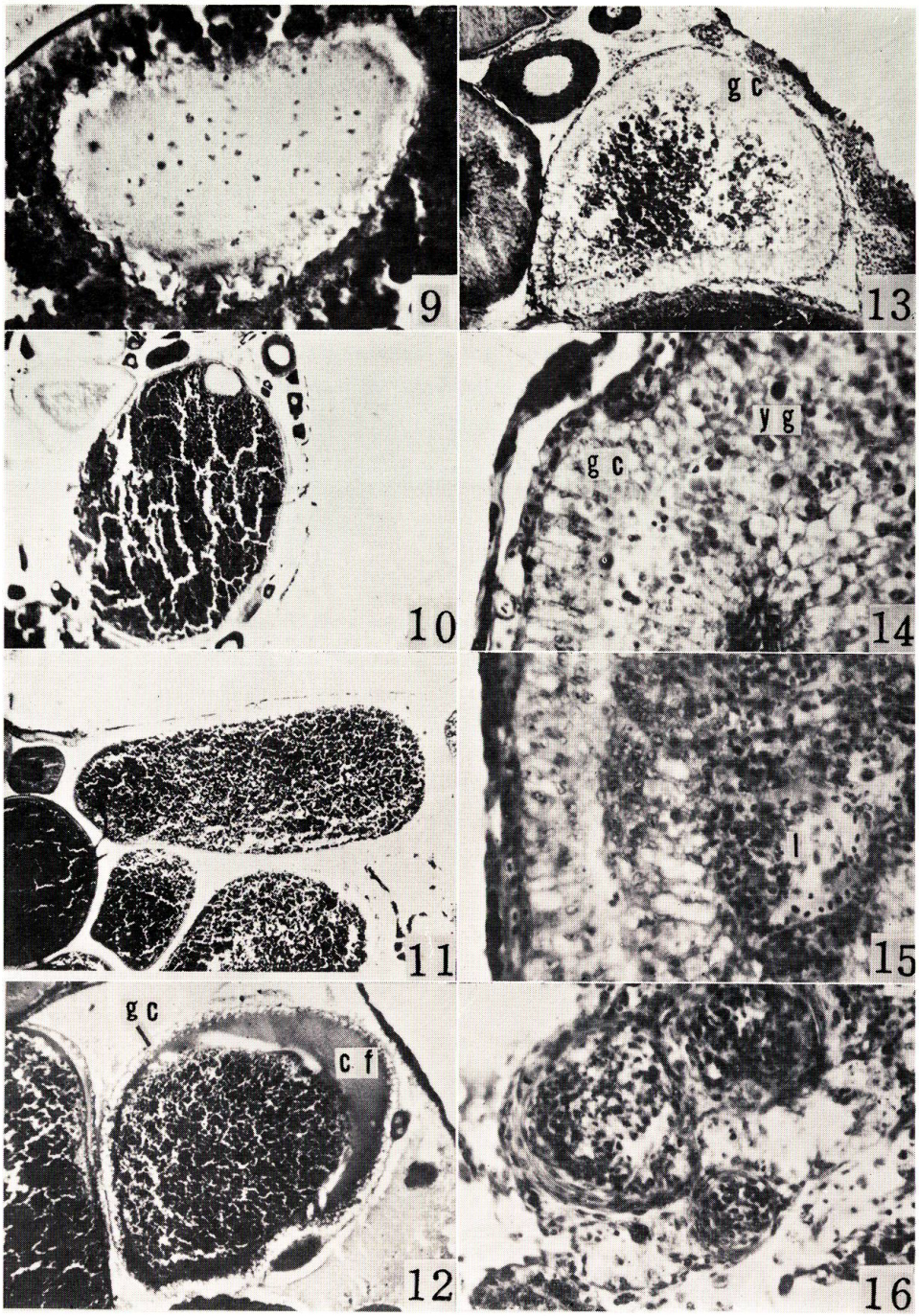
Fig. 12. An atretic oocyte in the early phase of degeneration. cf colloidal fluid. gc granulosa cells. ×70

Fig. 13. An atretic oocyte showing further hypertrophy of granulosa cells. ×70

Fig. 14. Magnified portion of an atretic oocyte showing the digestive and phagocytic action of the elongated granulosa cells. yg yolk globule. ×350

Fig. 15. Magnified portion of an atretic oocyte showing the granulosa cells and lymphocytes which have penetrated inward. l lymphocyte. ×350

Fig. 16. Spherical masses derived from atretic oocyte and connective tissue fibers. ×350



Shirai: Ovarian Conditions in the Bitterling

PLATE III

Fig. 17. Final appearance of an atretic oocyte filled with yellow pigment granules. ypg yellow pigment granules. ×350

Fig. 18. The ovipositor length of the fish at various phases.
a, in the resting phase. b, in the ovulation phase.
c, in the oviposition phase. d, in the post-oviposition phase.

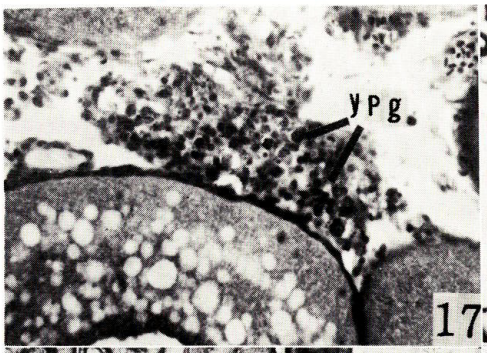
Fig. 19. Portion of the ovary from a fish killed on November 18. ×27

Fig. 20. Portion of the ovary from a fish reared under high temperature condition ranging from 23 to 25°C for 40 days. ×27

Figs. 21 and 22. Portions of ovaries from fish reared under high temperature condition ranging from 30 to 33°C for 30 days. ×27

Fig. 23. Portion of the ovary from a fish cultured without presence of mussels for 60 days. ×27

Fig. 24. Portion of the ovary from a fish cultured without presence of mussels for 140 days. ×27



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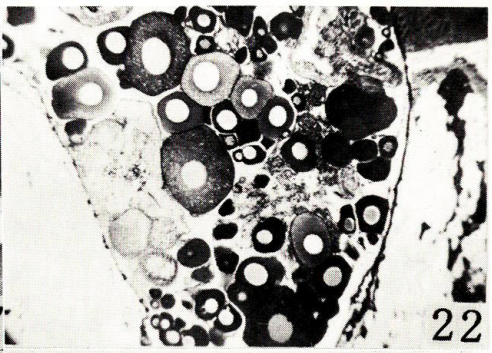

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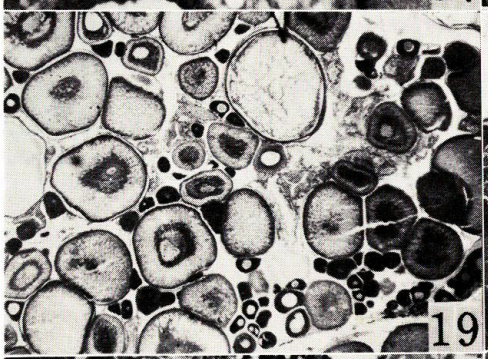

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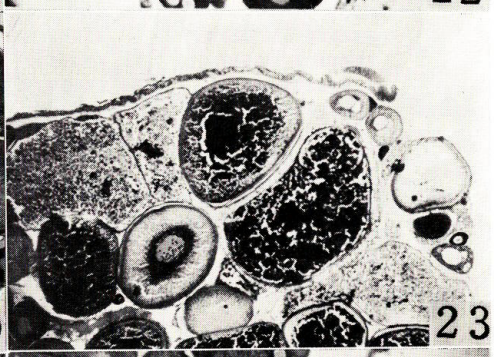

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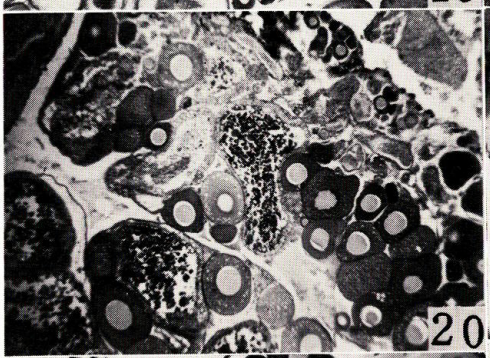

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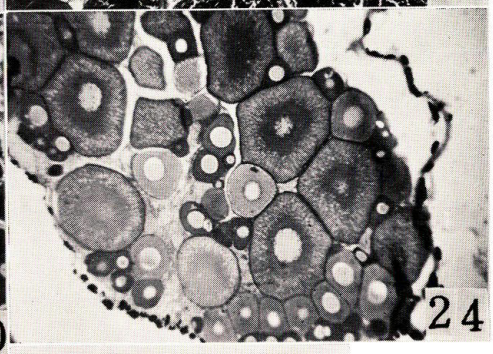

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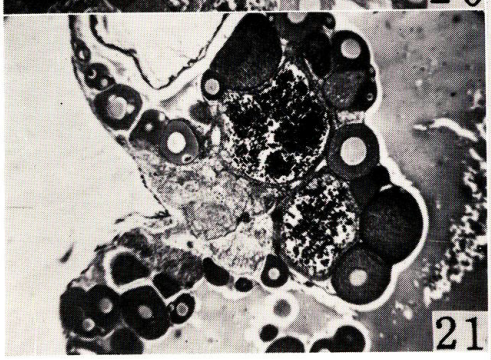

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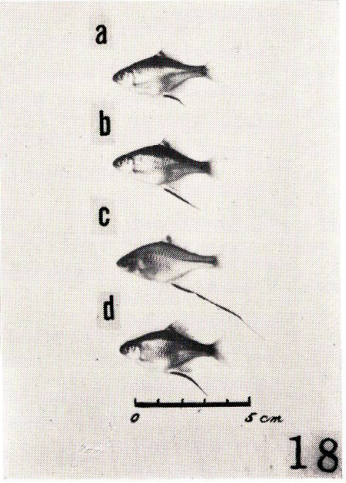

18

Shirai: Ovarian Conditions in the Bitterling