ON THE EFFECTS OF ENVIRONMENTAL FACTORS UPON THE REPRODUCTION OF FISHES

2. Effects of Short and Long Day-lengths on *Oryzias latipes* during Spawning Season**

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Introduction

It is generally accepted that light is an important environmental factor that exerts influence on the reproduction of fish (Hoover & Hubbard 1937, Bullough 1939, Medlen 1951, Hazard & Eddy 1951, Corson 1955, Burrows 1957, Combs et al. 1959, Harrington 1950, 1956, 1957, 1959 a, b and Shiraishi & Takeda 1961). From the standpoint of the manner of response to light fish may be classified into two types, long day type and short day type (Yoshioka, 1962).

When the females of *Oryzias latipes* belonging to the long day type were exposed to artificially shortened day-length during their spawning period, they all stopped laying within 20 days in spite of suitable water temperature for spawning (Yoshioka, 1962). In contrast to this, artificially prolonged day-length resulted in a delay in the spawning of *Salvelinus fontinalis* belonging to the short day type (Allison, 1951).

Up to the present time, however, it has not been clarified whether the decreasing or increasing change in illumination affects the reproduction of fish or whether length of illumination does. Moreover, few detailed analyses of the effects of day-lengths on the spawning process of fish have been carried out.

To make the above problem clear the present experiments were undertaken using the Medaka, *Oryzias latipes* during its spawning period as material.

Before going further the writer wish to record his heartful thanks to Prof. Kiichiro Yamamoto of Hokkaido University, for his valuable advice and helpful encouragement during the course of the present study and also for his kind correction of the present manuscript.

Materials and Methods

Materials used for the experiments were *Oryzias latipes* of a wild type, which had been born in a laboratory pond during the previous breeding season

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and reared in a large aquarium placed in the green house on the campus of the Faculty of Fisheries, Hokkaido University. At the beginning of the experiments they showed body length ranging from 26 mm to 29 mm and body weight from 250 mg to 310 mg. The conditions of the experiments were as follow.

(1) Pairs consisting of a female and a male were taken out at random from the stock colony.

(2) Each pair was kept in a glass aquarium 14.0 cm in length, 18.0 cm in width and 15.0 cm in depth (capacity of about 3 litres).

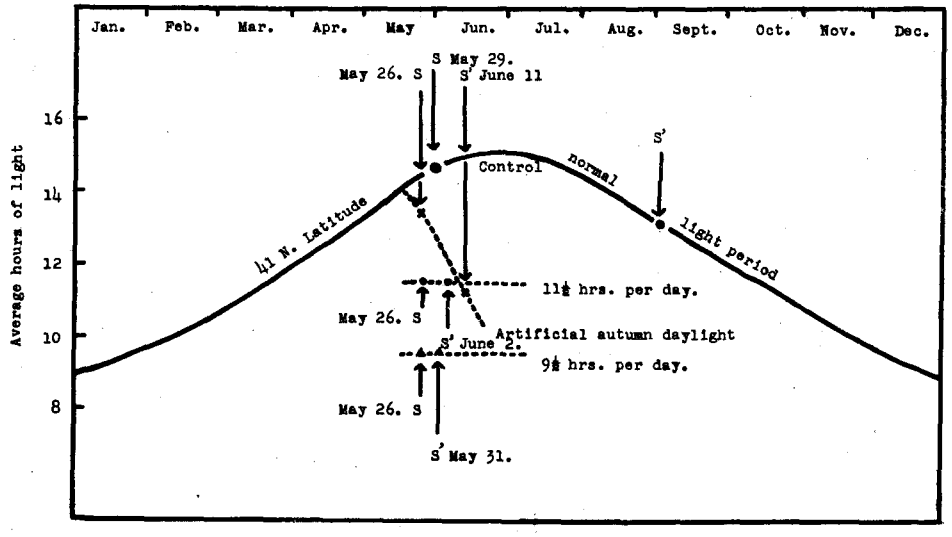
(3) The fish were fed only with living earth worms, which were given twice a day.

Soon after the fish were killed, body length, body weight, and gonad weight were recorded. Gonads were fixed with Allen Bouin's solution. They were sectioned serially at 10 microns by the usual paraffin method, and stained with Delafield's haematoxylin and eosion.

Results

Experiment 1. Effects of decreasing and of constant short day-lengths on spawning.

The experiments were begun on May 22, 1962 and ended on June 30, 1962. During the experimental period the water temperature of the aquaria ranged from 18°C to 21°C. Three groups of fish, each composed of 10 pairs, were used



Text-fig. 1. Experimental conditions in short day-lengths. First and last spawning observed, S and S'

for the experiments. Another group composed of the same type of fish served as control.

The fish of the first group (Group A) were subjected to 9½ hours of light per day which corresponds to the shortest day-length of the year, the fish of the second group (Group B) to 11½ hours of light per day which usually causes them to stop laying, and the fish of the third group (Group C) to artificial autumn daylight which decreased from 13.0 hours to 11.0 hours at the rate of 5 minutes per day. The fish of the control group received natural summer daylight.

Dates of the first spawning and of the last are shown in Text-fig. 1. Number of females which laid eggs every day, days from the first spawning to the last and average oviposition frequency each day by all females of each group are shown in Table 1. Number of eggs laid every day is shown in Table 3.

On May 26, 4 days after the beginning of the experiment, females of each group began to lay eggs (Table 1). In the control group females continued to lay eggs throughout the period of the experiment. On the other hand, all females in the three experimental groups stopped laying until 20 days had elapsed since the experiment had started (Table 1).

On May 28, 6 days after the beginning of the experiment, the largest number of females in Group A laid eggs. Then, the spawning females decreased in number day by day and became zero after June 1. Although spawning females in Group B decreased gradually in number after May 30, some of them continued to spawn until June 2.

The females in Group C went on laying eggs until day-length had been reduced to 11½ hours of light per day, and they ceased laying completely when the amount of daylight was reduced further.

From the above facts, it becomes clear that the females respond to both kinds of short day-length, the decreasing one from 13.0 hours to 11.0 hours and the constant one of less than 11½ hours of light per day.

Experiment 2. Effects of increasing and constant long day-lengths on the spawning of artificially aborted females.

This experiment was carried out from July 7, 1962 to July 31, 1962, at 18°C–21°C. Fourty pairs of fish, which had been exposed to 11½ hours of light per day and had stopped laying completely two weeks before, were used for the experiment. At the beginning of the experiment, the ovaries of these fish were similar in appearance to those in the out-of-spawning season. They were divided into four groups. Each group was composed of 10 pairs of fish.

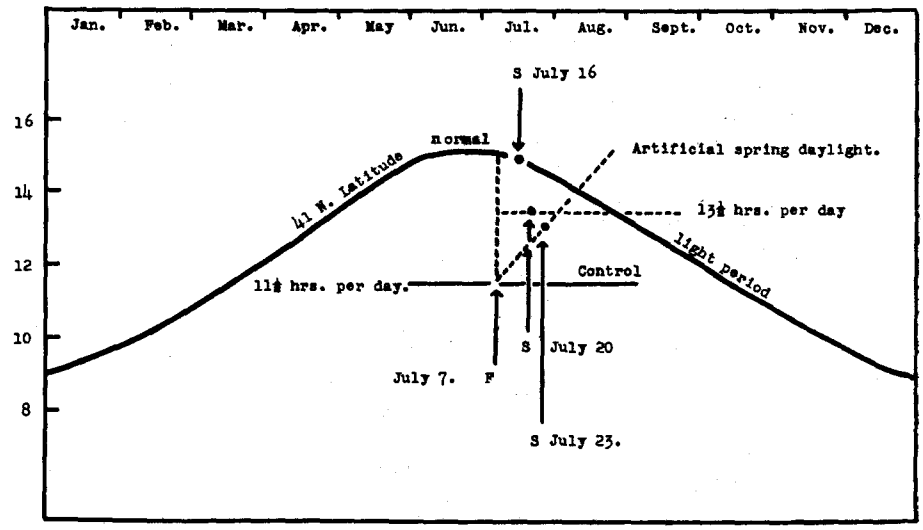
The first group (Group A) was subjected to artificial spring daylight which increased from 11½ hours to 13½ hours at the rate of 5 minutes per day, the

Table 1. Effects of short day-lengths on spawning

Light condition	Number of females which laid eggs (B) / Number of females examined (A)											Days from the first spawning to the last	Average oviposition frequency (B/A×100)
	May 26	27	28	29	30	31	June 1	2	3	4	5		
9½ hrs. per day (Group A)	1/10	3/10	3/10	2/10	2/10	1/10	0/10	0/10	0/10	0/10	0/10		
11½ hrs. per day (Group B)	1/10	3/10	4/10	4/10	2/10	2/10	1/10	1/10	0/10	0/10	0/10		
Artificial autumn daylight (Group C)	1/10	4/10	5/10	4/10	4/10	3/10	3/10	2/10	4/10	4/10	2/10		
Natural spring daylight (Control)	2/10	3/10	5/10	6/10	3/10	1/10	6/10	5/10	4/10	3/10	7/10		
	June 6	7	8	9	10	11	12	13	14	15	16		
9½ hrs. per day (Group A)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	6	5.4
11½ hrs. per day (Group B)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	8	8.1
Artificial autumn daylight (Group C)	3/10	2/10	3/10	1/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10	17	21.3
Natural spring daylight (Control)	2/10	3/10	2/10	2/10	3/10	3/10	3/10	6/10	5/10	4/10	2/10	—	36.3

Table 2. Effects of long day-lengths on the spawning of artificially aborted females

Light condition	Jun. 23 – Jul. 6	Light condition	Number of females which laid eggs (B) / Number of females examined (A)												Days from the beginning of the experiment to the first spawning	Total
			July 7	8	9	10	11	12	13	14	15	16	17	18	19	
11½ hrs. per day	0/140	Artificial spring daylight (Group A)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	
	0/140	13½ hrs. per day (Group B)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	
	0/140	Natural summer daylight (Group C)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	3/10	2/10	7/10	
	0/140	11½ hrs. per day (Control)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	
			July 20	21	22	23	24	25	26	27	28	29	30	31		
		Artificial spring daylight (Group A)	0/10	0/10	0/10	2/10	0/10	3/10	3/10	2/10	4/10	0/10	4/10	4/10	17	22/250
		13½ hrs. per day (Group B)	0/10	0/10	1/10	2/10	2/10	5/10	6/10	3/10	3/10	0/10	6/10	3/10	16	31/250
		Natural summer daylight (Group C)	1/10	3/10	6/10	0/10	5/10	2/10	4/10	5/10	5/10	2/10	6/10	3/10	10	55/250
		11½ hrs. per day (Control)	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	—	0/250



Text-fig. 2. Experimental conditions in long day-lengths. S, first spawning observed. F, first day of experiment

second group (Group B) to 13½ hours of light per day corresponding to the day-length of May, and the third group (Group C) to natural summer daylight. Another group remained in 11½ hours of light per day as the control group.

The conditions of experimental photoperiods and the results obtained are illustrated in Text-fig. 2 and Table 2. No females in the control group laid eggs at all throughout the period of the experiment, while females in the experimental groups begun to lay eggs from 10 to 17 days after the beginning of the experiment.

Seventeen days after the start of the experiment when the day-length reached $12\frac{11}{12}$ hours of light per day, spawning was observed first in the females of Group A. In Group B and Group C the fish began to lay eggs 16 days and 10 days after the start of the experiment respectively and they continued to spawn normally after that. It is noteworthy that in Group C day-length had been shortened from 15 hours to 14½ hours during the experiment.

From the results obtained in this experiment, it is clear that spawning can be induced in artificially aborted females by increasing day-length to 13½ hours, decreasing normal summer daylight from 15 hours to 14½ hours and keeping constant 13½ hours of light. Therefore, it may be concluded that one indispensable factor for the inducement of spawning in the present species is to keep the illumination time at a certain length.

Table 3. Effects of short day-lengths on egg number

Light condition	Number of eggs																						Total number of eggs laid
	May						June																
	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
9½ hrs. per day (Group A)	6	22	30	15	14	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	102
11½ hrs. per day (Group B)	12	31	38	34	21	15	7	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	179
Artificial autumn daylight (Group C)	2	52	41	28	32	25	30	19	21	9	11	12	21	31	10	9	5	0	0	0	0	0	358
Natural spring daylight (Control)	19	14	31	65	22	7	61	60	28	43	69	20	33	11	15	15	12	30	51	48	45	11	710

Experiment 3. Effects of short day-lengths on feeding activity.

It is thought that feeding activity may have an influence on egg formation. Therefore, this problem should be considered first.

The experiment for this purpose was carried out simultaneously with Experiment 1. Light and temperature conditions were the same as those of Experiment 1. Observations on feeding activity had been made with four pairs of fish in each group from May 26 to June 2. The food used for the experiment was living earth worms.

Every day at 10 a.m. minced fresh earth worms of about 100 mg kept in a box 5 cm×5 cm×3 cm were given to the fish. One hour later the bait in the box was weighed. Food-intake of the fish was calculated by the following formula. W=I.W.−R.W. (W, estimated weight of food taken by one pair of fish; I.W., initial weight of food given to one pair of fish; and R.W., weight of foods remaining in the box).

In each group, food weight given to one pair and food-intake by one pair every day and average food-intake by one pair per day were recorded in Table 4. Almost all fish of both control and experimental groups took a fair amount of food. Explaining it in detail, all pairs of the control group took more than 80 mg of earth worms. Three pairs in Group A, 3 pairs in Group B and 2 pairs in Group C consumed the same amount of food as the control. One pair in both Group B and Group C took less than 70 mg of food. As they had shown low feeding activity even in the pre-experimental period, it scarcely seems probable that low feeding activity of these fish during the observations was responsible for artificially shortened photoperiods.

The above observations revealed no conspicuous differences in feeding activity between the fish of the control group and of the experimental groups. Thus it may be concluded that the feeding activity of *Oryzias latipes* is not affected by artificially shortened photoperiods.

Experiment 4. Events in the spawning process affected by short day-lengths.

From the above experiments, it is clear that under daylight of less than 11½ hours the females completely stop laying for 9–20 days. Generally the spawning process of the females consists of three main events. viz., egg formation, ovulation and oviposition. The present author has already detected that large oocytes in the ovaries tended to degenerate in the fish exposed to artificial autumn day-length (Yoshioka, 1962). Yet the problem of whether or not the other events are also affected under the above conditions remained an open question.

(*1*) *Effects on ovulation and oviposition*

Egami (1959) reported that under natural daylight conditions, females of the

Table 4. Effects of short day-lengths on feeding activity of fish

Light condition	No. of pairs	Weight of food taken by one pair per day (mg) / Weight of food given by one pair per day (mg)								Average weight of food taken by one pair per day (mg)
		May 26	27	28	29	30	31	1	2	
9½ hrs. per day (Group A)	1	64/100	85/100	86/105	90/100	90/96	65/107	90/97	100/100	84
	2	92/100	60/100	40/100	90/100	85/100	63/99	90/93	75/100	74
	3	70/100	40/100	48/102	70/108	81/104	80/99	90/98	66/100	68
	4	80/100	76/100	81/98	80/104	80/98	80/101	90/95	90/97	82
11½ hrs. per day (Group B)	1	85/101	80/100	78/106	90/100	90/100	90/106	90/98	80/103	85
	2	80/100	40/100	77/105	98/98	90/100	80/103	90/99	80/95	80
	3	75/100	85/100	82/108	80/98	80/100	90/98	90/99	100/100	85
	4	65/100	36/100	76/96	77/100	71/100	56/102	90/103	25/96	62
Artificial autumn daylight (Group C)	1	100/100	80/100	65/104	50/102	80/100	90/100	90/100	90/100	81
	2	100/100	37/101	90/100	82/100	90/100	80/100	90/100	80/100	81
	3	75/105	30/100	78/105	90/96	80/94	90/95	86/105	65/100	74
	4	70/100	26/100	69/95	47/97	90/102	90/99	90/101	70/105	70
Natural spring daylight (Control)	1	85/100	87/100	90/100	68/98	90/101	90/105	90/101	90/100	86
	2	80/100	82/102	90/100	90/105	80/101	90/102	90/97	50/100	81
	3	82/100	80/100	88/100	77/101	90/99	90/105	77/97	70/96	82
	4	80/95	92/100	80/100	76/100	99/101	90/102	90/106	80/100	86

Table 5. Effects of short day-lengths on ovulation and oviposition

Light condition	Group	Time at checking	Number of pairs examined (A)	Number of females with ovulated eggs at 4 a.m. (B)	Number of females which laid eggs at 8 a.m. (C)	Ovulation frequency (B/A×100)	Oviposition frequency (C/A×100)
May 27. 9½ hrs. per da (Group A)	A	8 a.m.	5	4	—	80	—
	A	8 a.m.	5	—	4	—	80
11½ hrs. per day (Group B)	B	8 a.m.	5	5	—	100	—
	B	8 a.m.	5	—	4	—	80
Natural daylight (Group C)	C	8 a.m.	5	4	—	80	—
	C	8 a.m.	5	—	5	—	100
June 3. 9½ hrs. per day (Group A)	A	8 a.m.	5	4	—	80	—
	A	8 a.m.	5	—	4	—	80
11½ hrs. per day (Group B)	B	8 a.m.	5	3	—	60	—
	B	8 a.m.	5	—	5	—	100
Natural daylight (Group C)	C	8 a.m.	5	3	—	60	—
	C	8 a.m.	5	—	4	—	80
June 10. 9½ hrs. per day (Group A)	A	8 a.m.	5	4	—	80	—
	A	8 a.m.	5	—	4	—	80
11½ hrs. per day (Group B)	B	8 a.m.	5	4	—	80	—
	B	8 a.m.	5	—	4	—	80
Natural daylight (Group C)	C	8 a.m.	5	4	—	80	—
	C	8 a.m.	5	—	3	—	60

red variety of *Oryzias latipes* lay eggs at dawn almost every day, and that ovulation takes place at about 2 a.m., between 1 and 4 a.m. and oviposition at about 5 a.m., between 4 and 7 a.m.

However most of the present specimens lay eggs at an interval of 2 or 3 days and only a few of the females lay eggs every day (Yoshioka, 1962). Therefore, of the fish which had laid eggs every day, 80 pairs were selected and used for this experiment. The chosen fish were transferred to the experimental boxes at 8 a.m. The fish of 11½ hour day-length were sealed from daylight with light-proof cloths at about 4 p.m. In the case of 9½ hour day-length, the same treatment was given at about 2 p.m. The fish of the control group remained in natural day-length.

Five pairs of fish were provided for each test. To examine ovulation, the females were killed at 4 a.m. the following morning and their ovaries were examined. Oviposition was checked at 8 a.m. the following morning. The above tests were repeated three times, on May 27, June 3, and June 10. The results of the tests were presented in Table 5. At all tests, ovulation or oviposition had taken place in more than 80 percent of the females of each group regardless of light-length conditions. That is, in the control group ovulation frequencies ranged from 60 to 80 and in the group subjected to 11½ hours of light per day the values ranged from 60 to 100 and in another group subjected to 9½ hours of light per day all the values were 80. Oviposition frequencies in the control group ranged from 60 to 100 and in the two experimental groups from 80 to 100.

Therefore, no significant differences in the frequencies of ovulation and oviposition were revealed between the control fish and the experimental fish. Thus, it is evident that the ovulation and the subsequent oviposition had taken place normally under artificially shortened day-lengths.

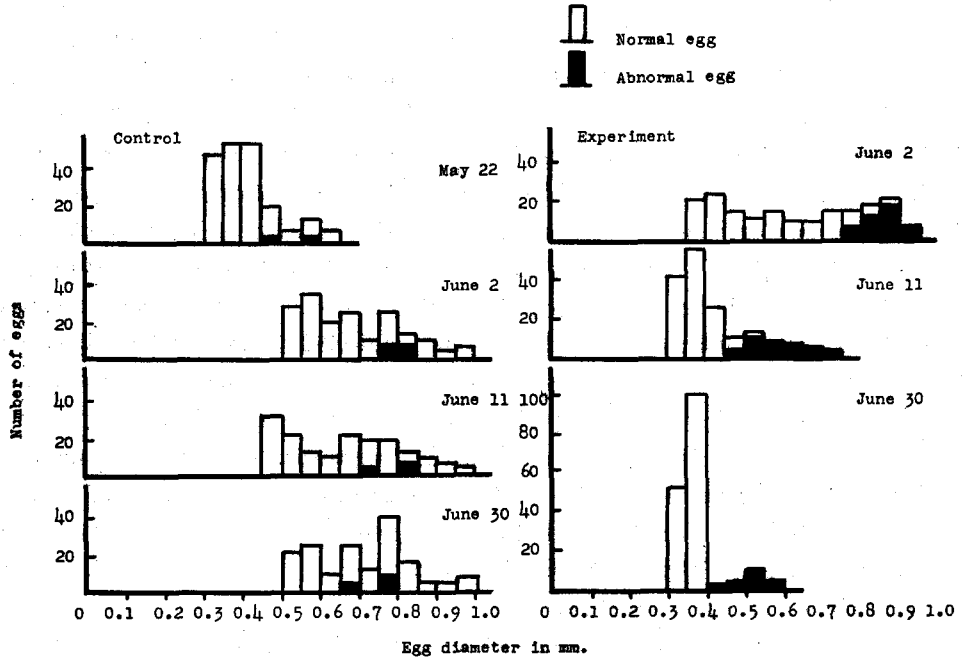
(2) *Effects on egg formations*

The present experiment was carried out from May 22 to June 30, at 18°C–21°C. Two groups, each composed of 10 pairs of fish, were used for this study. One was kept under 11½ hours of light per day and the other under natural day-length.

During the course of the experiment, some females were sampled from each group on May 22, June 2, June 11, and June 30, and oocytes composition in each ovary was examined. The largest about 60 oocytes in each ovary were chosen for measuring of diameter. The diameters of all oocytes were measured by a micrometer under microscopes. The frequency distribution of the diameter is shown in Text-fig. 3.

In the ovaries of the females sampled on May 22, there were found a large

number of oocytes less than 0.65 mm in diameter. Observations on ovaries from the control group preserved on June 2 revealed the presence of many oocytes at different growth stages. At that time females were laying eggs at the interval of 2 or 3 days. The largest oocytes attained 1.0 mm in diameter. These large oocytes were undoubtedly ones to be laid at the next oviposition time. Except for a few regressing oocytes, almost all oocytes were intact. The composition of oocytes in the ovaries obtained from the control group on June 11 and June 30 was similar to that of June 2. In those ovaries a lot of intact oocytes less than 1.0 mm in diameter were found. The diameters of oocytes found in their ovaries varied from 0.017 to 1.0 mm.



Text-fig. 3. Frequency distribution of the largest 60 eggs diameter in ovaries of 21 females kept under different day-length conditions

On June 2, 12 days after the fish had been exposed to an artificially shortened photoperiod, the ovaries obtained differed from those of the controls. Marked differences in the size of oocytes were not found between the experimental group and control group. But most of large oocytes in the experimental group, 0.76–0.95 mm in diameter, were regressive. In the ovaries of females sampled on June 11, the oocytes decreased conspicuously in size and were less than 0.80 mm in diameter. Large oocytes of 0.51–0.80 mm were regressive, but small ones of 0.017–0.60 mm were intact. Moreover in the ovaries of 3 females sampled on June

30, involved oocytes were clearly distinguished into 3 groups from their size. One group consisted of larger oocytes, 0.41 mm–0.60 mm in diameter, all of which were yolk-laden and regressive. Another group consisted of smaller oocytes, less than 0.40 mm in diameter, all of which were yolkless and intact.

The above observations show that if laying females are subjected to 11½ hours of light per day at 18°C–21°C, oocytes larger than 0.41 mm are completely degenerated 40 days later and no intact oocytes above that size can be found in the ovaries. From these results it seems reasonable to assume that the laying was stopped under artificially shortened photoperiod because of an interruption in the growth of the oocytes.

Discussion

The writer has already reported that in *Oryzias latipes* the females in breeding season under an artificial autumn photoperiod which decreases from 13.0 hours to 11.0 hours daily, stopped laying eggs completely within 20 days at 18°C–21°C (Yoshioka, 1962). The present study makes it clear that not only the decreasing autumn day-length causes the fish to stop laying eggs, but also constant short day-lengths of less than 11½ hours of light cause it. Therefore it is justifiable to suppose that such an inhibition of spawning in females is not related to "the decreasing change" in illumination time, but to "the lower level" in the duration of the illumination time.

Moreover, the fact obtained from the experiments about the effects of long day-lengths on the spawning of artificially aborted fish gives powerful evidence to support the above opinion. In the experiments all groups of fish were able to recover their spawning function and lay eggs from 10 to 17 days after they were subjected to long day-length conditions, regardless of the different conditions of illumination time, i.e., increasing, decreasing and constant. Thus, the present author tends to draw the conclusion that a certain length of illumination time is indispensable for the spawning of fishes, though its critical value may be different for different species. This agrees with Burger's findings (1940) in birds that development of gonads is not concerned with the daily changes of day-length, but with a certain amount of daylight.

Ovulation and oviposition seem to have no relation to the duration of illumination time. The tests performed between May 27 and June 10 showed that in two experimental groups subjected to both 11½ hours and 9½ hours of light per day no difference in ovulation and oviposition was observed. In addition to the evidence given by the present study, Egami (1954) has already reported that the same result was obtained in the same species kept under day-length of less than

12 hours.

As has already been shown in a previous paper (Yoshioka, 1962) and demonstrated again by the present study, the oocytes of above 420 μ in diameter are markedly affected by short day-length, but smaller ones remain intact. This is the reason the fish exposed to short day-length ceased laying eggs.

According to Yamazaki (1961), in goldfish all oocytes with yolk were degenerated within 8 weeks after the hypophysectomy, while yolk-less oocytes remained almost intact, and failure of growth of oocytes may be a result of the lack of pituitary hormones. Therefore, it may be reasonable to suppose that short day-length of less than 11½ hours weaken the activity of the pituitary gland and then exert the same effect on egg formation as those of hypophysectomy.

Summary

The pairs of *Oryzias latipes* were kept under artificial photoperiods during the breeding season at 18°C–21°C. The following conclusions may be drawn from the present experiments.

(1) Inhibition or acceleration of spawning in the fish kept under artificial photoperiods is not related to "the decreasing or increasing change" in illumination time, but to "lower or higher level in amount" of illumination time.

(2) The critical length of illumination time to maintain the spawning in this species is estimated to lie in 13.0 hours –12.0 hours of light per day.

(3) The females kept under day-lengths of less than critical values stop laying eggs within 20 days. If they were returned to the condition of day-length higher than the value, they begin to lay eggs within 10–17 days.

(4) Feeding activity, ovulation and oviposition take place normally under artificially shortened day-lengths.

(5) But the growth of oocytes markedly delayed; especially the oocytes above 420 μ in diameter are inhibited in growth by artificially shortened day-lengths.

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