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A Daily Rhythm in the Photosensitive Development of the Ovary in the Bitterling, *Rhodeus ocellatus ocellatus*

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Abstract

Five groups of adult females of the bitterling, *Rhodeus ocellatus ocellatus*, were subjected, during a 24-hour cycle, to the main light period of 6 hours plus an additional 2 hours of light given during the subsequent dark period beginning at the 8th, 10th, 12th, 14th, 16th or 18th hour, respectively, counting the onset of the main photophase as O hour, under constant temperature at 21°C. A control group was maintained under the short photoperiod of 8-hour light and 16-hour darkness. The experiment was carried out in the out-of-breeding season, lasting for 8 weeks from late October to late December.

Ovarian maturation, or vitellogenesis in particular, was evidently induced to occur in the fish exposed to an additional 2 hours of light beginning at the 12th, 14th and 16th hours. The gonad-stimulating effect was maximum when the additional light period was set around the 16th hour of the 24-hour cycle. The above results suggest the presence of a daily rhythm of sensitivity to light in the photosexual response in the bitterling. The significance of the rhythm in the photosensitivity as a basis for photoperiodism in fishes was discussed.

It is well established that the photoperiod influences the reproductive cycle of many species of teleost fishes[1)-3)]. Recently, the presence of a photosensitive circadian rhythm has been demonstrated for photosexual responses in some fishes, as indicated first by Baggerman[4)5)] in the stickleback, *Gasterosteus aculeatus*. In Indian catfish, *Heteropneustes fossilis*, Sundararaj and Vasal[6)] revealed a possible implication of a circadian rhythm in the occurrence of photosensitive ovarian recrudescence. Also, Chan[7)] reported a significant relation of a photosensitive daily rhythm to the ovarian maturation of the medaka, *Oryzias latipes*.

The bitterling, *Rhodeus ocellatus ocellatus*, spawns during the months from April to September. The fish appear to be classified as the so-called long-day type, for a long photoperiod accompanied with a warm temperature can induce ovarian maturation in the out-of-breeding season[8)]. Thus it is interesting to examine whether or not a rhythm of the photosensitivity may play an important role in the day-length measurement regarding the photosexual response of the bitterling as well as other photoperiodic species of teleosts. The present work was carried out in order to ascertain the presence of such a rhythm as a basis for sexual photoperiodism in *Rhodeus ocellatus ocellatus*.

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Material and Methods

The specimens of the bitterling, *Rhodeus ocellatus ocellatus*, used in the present study were obtained from the stock cultured under natural conditions in running water of outdoor ponds set in the campus of the Faculty of Fisheries, Hokkaido University. They were maintained in an air-conditioned room where room temperature was regulated at 21°C and light condition to 8 hours of light (8L) and 16 hours of darkness (16L) in mid October for about 1 week prior to the beginning of the experiment.

Adult fish ranging from 3.4 to 4.8 cm in body length were used. Each group of fish was kept in a 60-liter glass aquarium with males, floating vegetation and freshwater mussels, and they were fed daily on a commercial fish pellet. Illumination was provided by a 20-W fluorescent lamp, and the light intensity was conditioned at about 2000 lux at the level of water surface.

The experiment lasted for 8 weeks from late October to late December 1977. At the start of the experiment, 6 females and 6 males were sacrificed and served as initial controls. Experimental photoperiodic regimens and the number of the fish used are summarized in Table 1. Control fish were exposed to a short photoperiod (8L–16D) and a warm temperature (21°C). Fish of 5 experimental groups were daily exposed first to continuous 6 hours of light and then to additional 2 hours of light beginning at the 10th, 12th, 14th, 16th and 18th hour during the dark period, respectively, counting the onset of the main light period as 0 hour. The temperature condition was kept constant at 21°±1°C for all these groups.

To examine the ovarian condition fish were sacrificed at the end of the experiment. At autopsy, the body length and the body weight of the fish were recorded. After killing the fish by decapitation, gonads were excised, weighed and fixed in Bouin's fluid for histological observation. The specimens were sectioned at 10 μ in thickness by the routine paraffin method, and the sections were stained with Delafield's hematoxylin and eosin. For the evaluation of gonadal condition the gonosomatic index (gonad weight/body weight $\times 100$) was determined for each fish. To examine a possible relationship between the ovarian condition and the ovipositor length, the ovipositor index (ovipositor length/body length) was calculated. For statistical analysis, the "t" test was used.

Table 1. *Experimental procedures.*

Group	Light condition	Temperature condition	No. of	
			female	male
Initial control	—	—	6	6
Control	8L+16D	21°±1°C	5	5
I	6L+4D+2L+12D	21°±1°C	6	6
II	6L+6D+2L+10D	21°±1°C	7	6
III	6L+8D+2L+8D	21°±1°C	6	6
IV	6L+10D+2L+6D	21°±1°C	8	6
V	6L+12D+2L+4D	21°±1°C	5	6

Results

Changes in gonosomatic indices

Changes in mean gonosomatic indices (GSI) of female fish in control and experimental groups are indicated in Table 2. The initial control group showed a low level of GSI. In the fish serving as controls, GSI was on a similar level with that of the initial controls after a 8-week exposure to the short photoperiod (8L–16D) and the warm temperature (21°±1°C). In the experimental group I, the mean GSI showed a slight decrease, but the change was statistically insignificant. The experimental groups II, III and IV showed notable increases in GSI values. The values were significantly greater than the GSI of the control group. On the contrary, GSI in group V remained at nearly the same level as that in the control group.

Table 2. *Gonosomatic indices (GSI) in control and experimental groups of the bitterling.*

Group	GSI*
Initial control	2.07±0.25
Control	1.97±0.17
I	1.57±0.17
II	4.07±0.56**
III	5.35±0.69***
IV	5.79±0.60***
V	2.05±0.08

* Mean±SE
** Significantly greater than control ($P<0.02$).
*** Significantly greater than control ($P<0.01$).

Changes in the composition of ovarian oocytes

In the present study, ovarian oocytes were classified into the following four categories; peri-nucleolus stage, yolk vesicle stage, yolk globule stage and atretic stage. Percentage distributions of oocytes of these stages in control and experimental groups are given in Table 3. At the start of experiment, ovaries of the initial control fish contained no oocytes of the yolk globule stage, 37.8% of ovarian oocytes being at the yolk vesicle stage as the most advanced one. In the control group, ovaries were in a similar state of development to those of the initial control group, the most advanced oocytes of the yolk vesicle stage accounting for 45.7% of all ovarian oocytes. In the experimental groups I and V, too, yolk-laden oocytes were not found, though there was a slight decrease in the percentage of oocytes of the yolk vesicle stage in group I. On the contrary, oocytes of the yolk globule stage appeared in the ovaries in experimental groups II, III and IV, being the highest in percentage in group IV (8.3%). All the fish in group IV showed an active vitellogenesis in their ovaries. In 3 out of 7 fish in group II and in 2 out of 6 fish in group III, however, the most advanced ovarian oocytes still remained at the yolk vesicle stage.

The present data appear to coincide well with the results of GSI. Accordingly

it is concluded that, when an additional light period of 2 hours was given beginning at the 12th, 14th or 16th hour after the onset of an initial 6-hour light period, the maturation of ovaries can be strongly stimulated. Moreover, the sensitivity of ovaries to photostimulation appears to be maximum when an additional light period of 2 hours is given between the 16th and 18th hour during the 24-hour cycle.

Table 3. *Stage composition of ovarian oocytes in control and experimental groups of the bitterling subjected to various experimental conditions.*

Group	Percentage of oocytes at each stage*			
	Peri-nucleolus stage	Yolk-vesicle stage	Yolk-globule stage	Atretic stage
Initial control	62.2±7.0	37.8±7.0		
Control	54.3±10.0	45.7±10.0		
I	71.4±5.8	28.6±5.1		
II	50.5±7.0	43.6±6.2	5.7±4.7	0.2±0.2
III	56.0±7.0	37.8±6.0	6.1±3.0	0.1±0.2
IV	54.4±7.8	37.1±6.2	8.3±2.9	0.2±0.5
V	59.2±0.7	40.8±0.7		

* Mean±SD

Changes in ovipositor indices

Results of measurements of the ovipositor index (OI) are summarized in Table 4. The OI was 0.080 in the initial control group. After 8 weeks the index attained to 0.102 in the control group, but the change was statistically insignificant. In the experimental groups I and V, the indices increased, though not significantly, to 0.115 and 0.084, respectively. On the other hand, the fish of groups II, III and IV revealed marked increases in the OI. In these groups, some females came to ovulate and showed a remarkable elongation of their ovipositors, which were 0.831 and 0.983 in the OI in groups III and IV, respectively, when the fish were sacrificed at the end of the experiment. Therefore, the fish that had not ovulated at the time of sacrifice were used for the comparison of the OI among the

Table 4. *Ovipositor indices (OI) of control and experimental groups of the bitterling.*

Group	No. of female	OI*
Initial control	6	0.080±0.013
Control	5	0.102±0.016
I	6	0.115±0.005
II	7	0.213±0.021**
III	4	0.208±0.018**
IV	7	0.289±0.044**
V	5	0.084±0.021

* Mean±SE

** Significantly greater than control ($P<0.01$).

experimental groups; the mean OI were 0.213, 0.208 and 0.289 for groups II, III and IV, respectively. These values were significantly greater than those of the control groups ($P<0.01$).

Thus, the groups which were given the interruption of 2-hour light from the 12th and 16th hour during the 18-hour dark period showed significant increases in the ovipositor indices in parallel with the development of their ovaries. There exists a close relationship between the degree of ovarian maturation and the length of ovipositors.

Discussion

In many teleost fishes, the photoperiod is an important environmental factor which regulates the cyclic alteration of reproductive activities[1)-3)]. Many workers have suggested that, in teleost species of the so-called long-day type, a long photoperiod accompanied with a warm temperature accelerate the ovarian maturation[2)3)]. Females of the bitterling, *Rhodeus ocellatus ocellatus*, are truly photoperiodic fish, since the stimulated development of their ovary is effectively brought about in the fish kept under a long photoperiod combined with a warm temperature during the post-spawning season[8)].

The results of the present study suggest that the females of the bitterling can respond with an accelerated ovarian development to the light stimulus given during the dark period. It was indicated in the present study that, under warm temperature, 8 hours of continuous light alternating with 16 hours of continuous darkness per day was ineffective in stimulating the ovarian development. However, when the light period of 8 hours was broken up into 6 hours of continuous light plus 2 hours of light which was set at a time from the 12th to 16th hour during the dark period of the 24-hour cycle, a considerable ovarian development was induced. This may probably indicate that the photosensitive time area exists in the photosexual response of the bitterling used in the present study.

In the stickleback, *Gasterosteus aculeatus*, Baggerman[4)5)] first reported the presence of photosensitivity for reproductive activities. The earlier work of vanden Eeckhoudt[9)] who examined the ovarian development in the stickleback subjected to an intermittent photoperiod seems likely to support Baggerman's result. The results of the present experiment show that the total amount of light *per se* is less important than the manner by which the light is applied. Besides, the results suggest that the sensitivity of ovaries to photostimulation is extremely low for the first 12 hours during the 24-hour cycle, and that it then increases gradually from the 12th hour to reach a maximum around the 16th hour. Finally, the sensitivity drops abruptly to a low level at or after the 18th hour of the 24-hour cycle. In other photoperiodic species such as the medaka, *Oryzias latipes*[7)], and the Indian catfish, *Heteropneustes fossilis*[6)], similar responses have been reported to occur.

Investigators working on avian species such as the house finch[10)11)] and the quail[12)] suggest that the sensitivity to light in the photosexual response occurs when based on the endogenous circadian rhythm. According to Bünning[13)14)], endogenous circadian rhythms are in some manner involved in measuring the length of photoperiod. His hypothesis envisages a circadian rhythm consisting of two

half-cycles each of approximately 12-hour duration, one of which is the light-requiring, photophil phase, and the other the dark-requiring, scotophil phase. The photoperiodic induction of a process requiring long days occurs only when the duration of light period extends into the scotophilic part of the cycle[14]. The present investigation shows that the photo-inducible phase of gonad stimulation may lie in the duration of the latter 12 hours of a photosensitive daily rhythm in the female *Rhodeus ocellatus ocellatus*. This does not prove, however, the involvement of an endogenous circadian rhythm in the photosexual response of the bitterling. Baggerman[5] states that the stickleback indeed possesses a truly endogenous, circadian rhythm in the photosensitive changes of reproductive activity. In any way, the bitterling seems to possess a mechanism of a photosensitive nature for regulating its annual reproductive cycle, as is the case for the stickleback, the Indian catfish and the medaka.

Follett and Sharp[12] consider that the initial main light period in interrupted-night experiments seem to act as an entraining agent for the circadian rhythm while the additional light period given during the dark period plays the role of an inducer for photoperiodic responses. In the present study, the first 6 hours of light may act as an entraining agent for a daily rhythm and the additional 2 hours of light as an inducer for the photosexual response in the bitterling. However, the exact nature of both the entraining and inducing phases in the daily rhythm of photosensitivity still remains to be clarified especially regarding their minimum lengths of light period that are effective to induce a photosexual response in fishes.

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