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Circadian Rhythm in the Photosensitive Development of the Ovary in the Mosquitofish, *Gambusia affinis affinis* (Baird et Girard)

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Abstract

Adult female mosquitofish, *Gambusia affinis affinis*, with regressed ovaries were subjected to 6 hours of continuous light plus an additional 2 hours of light given at different times during the dark period of a 24-hour cycle, at 23~24°C in the post-spawning season. When the additional 2 hours of light fell between the 12th and the 14th hour, counting the onset of main light phase as 0 hour, ovarian development was induced. Stimulation of ovaries was also induced in the fish exposed to a 16L–32D or a 8L–28D light cycle, though it did not occur in the fish maintained under a short photoperiod of 8L–16D.

In the female mosquitofish which had regressed ovaries, a 8-hour light period coupled with dark periods of varying durations did not stimulate ovarian development in cycle lengths of 24, 48 and 72 hours, but caused ovarian maturation in cycles of 12, 36 and 60 hours. The above results suggest the presence of a photo-inducible phase for induction of ovarian maturation which has a circadian rhythm in its sensitivity to light stimulus. The significance of a circadian mechanism for photoperiodic measurement in the mosquitofish was discussed.

Environmental control of teleost reproductive cycles has been reviewed by Pickford and Atz[1] and recently by de Vlaming[2][3], Peter and Hontela[4], and Peter and Crim.[5] Photoperiod and temperature, among the factors concerned, have been considered to be much important in the regulation of teleost reproductive cycles.

Recently, the presence of a photosensitive phase in photosexual responses of fishes has been demonstrated in the stickleback, *Gasterosteus aculeatus*[6],[7], the Indian catfish, *Heteropneustes fossilis*[8], the medaka, *Oryzias latipes*[9], the honmoroko, *Gnathopogon elongatus caerulescence*[10], and the bitterling, *Rhodeus ocellatus ocellatus*[11]. In birds, the presence of a photosensitive 'circadian' rhythm for sexual cycling has been demonstrated in some species[12]–[15]. In fishes, Baggerman was the first to suggest the presence of a similar rhythm.

The mosquitofish, *Gambusia affinis affinis*, is a freshwater fish natively distributed in the southern part of North America. This species was introduced to many countries in the 20th century for the purpose of mosquito control. In Japan, it was first introduced in the 1910's, and is now distributed mainly in the Kanto Plain, the Ryukyu Islands and other places.[16] In the previous report by Medlen[17], the annual reproductive cycle of this species was discussed in terms of seasonal changes of temperature of ambient waters. However, Sawara[16] showed

that the daylength was the dominant factor controlling the ovarian activity of the mosquitofish under optimal temperature. Hubbs[18] also expected the daylength to play an important role. Accordingly, it is interesting to examine whether or not a rhythm of the photosensitivity may play an important role in the daylength measurement regarding the photosexual response of the mosquitofish as well as other photoperiodic species of teleosts. If the timing of breeding in the mosquitofish is regulated by a circadian rhythm, it may well be a photosensitive rhythm. The present study was carried out to ascertain the presence of such a rhythm as a basis for sexual photoperiodism in the mosquitofish.

Material and Methods

Female mosquitofish, *Gambusia affinis affinis*, used in the present study were obtained from the stock cultured under natural conditions in an indoor pond set in the campus of the Faculty of Fisheries, Hokkaido University (long. 41°49'N and lat. 140°45'E). Adult fish ranging from 2.3 to 3.1 cm in body length were used. Spawning season of this population extends from May through October in this habitat.

Two experiments were performed during the post-spawning period of 1978 and 1979. Prior to the biginning of these experiments, fish were maintained for about a week in an air-conditioned room where water temperature was regulated at $23 \sim 24°C$ and light condition was adjusted to 8 hours of light (8L: light on from 0600 to 1400) and 16 hours of darkness (16D: light off from 1400 to 0600). In both experiments, each group of fish was kept in a 60-liter glass aquarium with some males and floating vegetation, and was fed on a commercial fish pellet *ad libitum*. Illumination was provided by a 20-W fluorescent lamp, and the light intensity was conditioned at about 1500 lux at the level of water surface. Water temperature was kept constant at $23 \sim 24°C$ for all groups during the course of experiments. Experimental photoperiodic regimens used in the present study were as follows.

Expeiment 1.

Experimental photoperiodic schedules used in the experiment 1 are summarized in Fig. 1. The experiment lasted for 7 weeks from late October to late December 1978. Group A was exposed daily to 8L–16D and served as control. Group B, C, D, E and F were subjected to a so-called "assymmetrical skeleton photoperiod". They were exposed to first a continuous 6-hour light period and then an additional 2-hour light phase given during the dark period at the 10th (B), 12th (C), 14th (D), 16th (E), and 18th (F) hour, respectively, counting the onset of the main light phase (0600) as 0 hour. Group G was exposed to a 48–hour cycle consisting of the same ratio of light and darkness (16L–32D) as in group A. In group H a light period was set during the first 8 hours of a 36-hour cycle.

Experiment 2.

Experimental photoperiodic schedules used in the experiment 2 are

Group Photoperiodic regime □ Light
Exp. 1 ■ Dark

A 8L–16D
B 6L–4D-2L-12D
C 6L–6D-2L-10D
D 6L–8D-2L-8D
E 6L–10D-2L-6D
F 6L–12D-2L-4D
G 16L–32D
H 8L–28D

0 24 48 72
Time in hours

Fig. 1. Experimental photoperiodic schedules used in the Experiment 1.

Group Photoperiodic regime □ Light
Exp. 2 ■ Dark

A 8L–4D
B 8L–16D
C 8L–28D
D 8L–40D
E 8L–52D
F 8L–64D
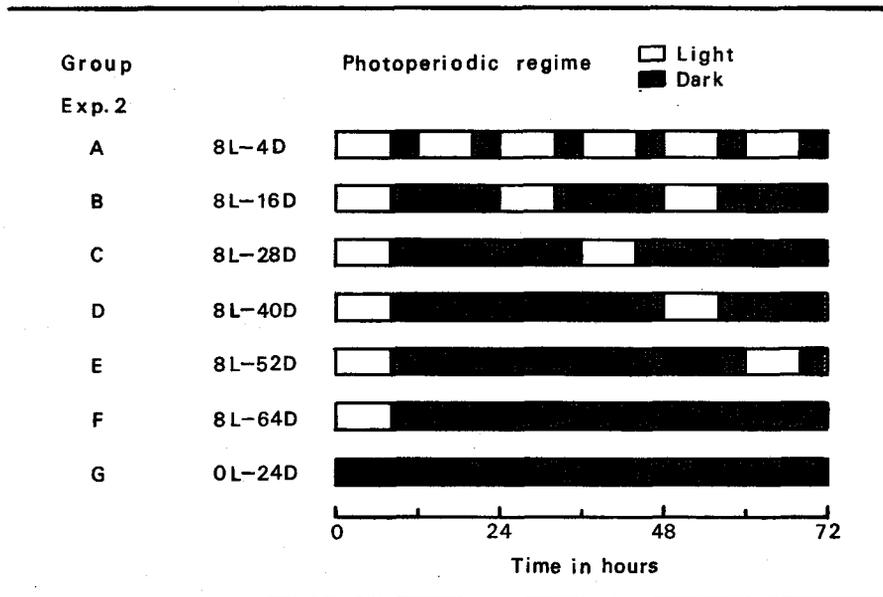G 0L–24D

0 24 48 72
Time in hours
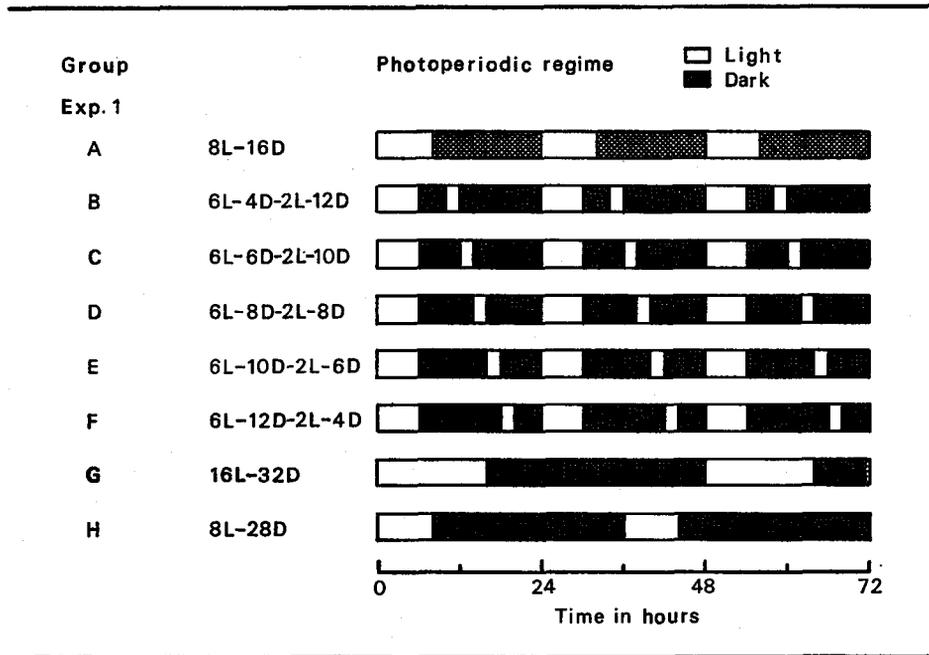
Fig. 2. Experimental photoperiodic schedules used in the Experiment 2.

summarized in Fig. 2. The experiment lasted for 6 weeks from early November to late December 1979. In this experiment a light period of 8 hours was coupled with a variety of dark periods to give cycles of 12(A), 24(B), 36(C), 48(D), 60(E), and 72(F) hours. Group G was exposed to continuous darkness. In all groups except group G, the first light period of each of the experimental cycles commenced at 0600.

To examine the ovarian condition, fish were sacrificed at the start and end of the experiments. After measuring the body length and body weight, fish were killed by decapitation, their gonads were excised, weighed and fixed in Bouin-Hollande solution. Then the ovaries which had no embryos were sectioned at 10 μm in thickness by the routine paraffin method, and stained with Delafield's hematoxylin and eosin for microscopic examinations.

In the present study, ovarian oocytes were histologically classified into two stages: oil drop stage and yolk globule stage. On the other hand, intraovarian embryos were divided into four types according to the descriptions by Sawara[16]: early embryo, eyed embryo, yolk embryo, and mature embryo. To determine developmental stages of embryos, those of *Xiphophorus* introduced by Tavolga[19] were applied to the mosquitofish. For further evaluation of the gonadal condition, gonadosomatic index (GSI) was calculated for each fish as a percentage of the ovarian weight to the total body weight. Statistical analyses for the two experiments were carried out by means of Student's t test.

Results

Experiment 1.

Values of GSI and stages of ovarian development of the mosquitofish in each group of the experiment 1 are presented in Figs. 3 and 4, respectively.

Initial control of the experiment 1 beginning in late October had regressed ovaries with oocytes staying at the oil drop stage. A 7-week exposure to experimental photoperiodic regimens did not alter the mean GSI value in group A in comparison with that of the initial control group. Ovaries of the fish of group A were still in the regressed condition, containing oocytes of the oil drop stage. Similarly, in the fish of groups B, D, E and F, no significant increases in GSI values were noticed. Ovaries of groups B, E and F still remained in the same condition as that of the control. However, many of the fish in group D had ovaries with oocytes of the yolk globule stage.

In Group C, a significant increase in GSI (P<0.001) over the control value was noticed. Fish of this group had ovaries with oocytes of the yolk globule stage or developing embryos. In groups G and H, GSI values were significantly greater than that of the control (P<0.001), and ovaries had yolk-laden oocytes or developing embryos.

It was concluded from the above results that, in the mosquitofish of the post-spawning period, ovarian development was induced when subjected to an additional 2 hours of light beginning with the 12th hour after the onset of the initial 6-hour light period, though a considerable response was also occurred in the fish when the
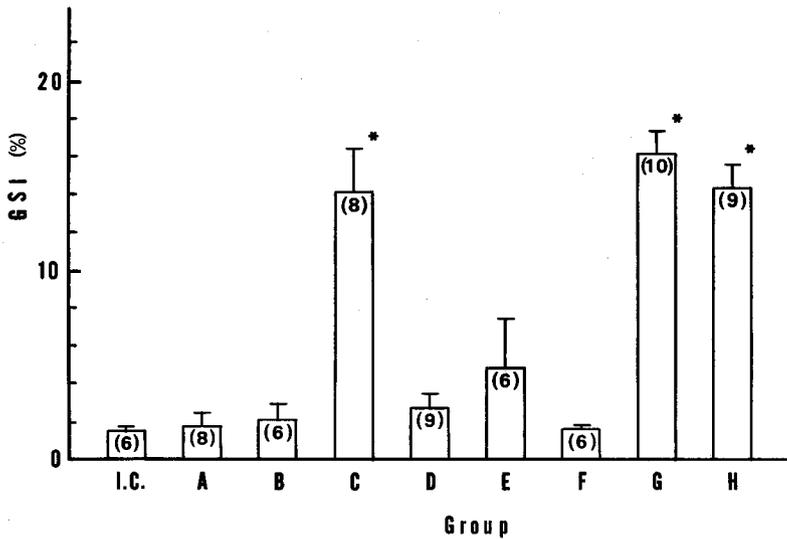
Fig. 3. GSI (ovary weight/body weight ×100) of the mosquitofish exposed to various photoperiodic schedules in the Experiment 1. I.C.; initial control group. Standard error of each mean is represented by vertical line. Figures in parentheses refer to the number of fish examined. *; significantly different from the control (A) at 0.001 percent level.
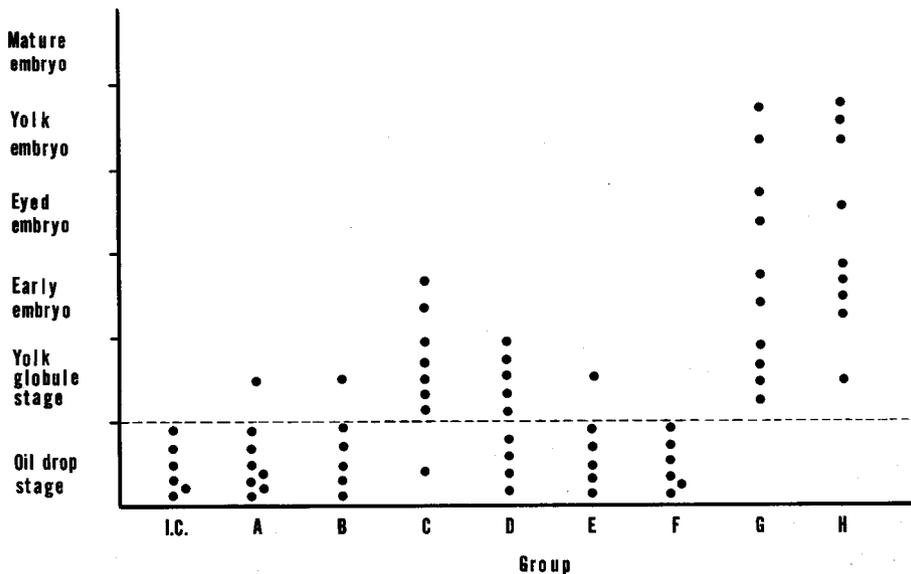

Fig. 4. Ovarian condition of the mosquitofish exposed to various photoperiodic schedules in the Experiment 1. I.C.; initial control group. One dot corresponds to one individual.

additional light pulse was given at the 14th hour after the start of the main light phase. Moreover, the experimental findings obtained in the fish reared under the 8L–16D, 16L–32D and 8L–28D light-dark cycles revealed that the gonadal maturation did not depend on the ratio between the length of light period and that of the dark period.

Experiment 2.

Values of GSI and stages of ovarian development of the mosquitofish used in the experiment 2 are presented in Figs. 5 and 6, respectively.

Fish of the initial control group sampled in early November had fully regressed ovaries containing oocytes of the oil drop stage. In group G subjected to continuous darkness, GSI value was the same level as that of the initial control. Ovaries of this group remained in a similar condition to that of the initial control. In Group A, C and E, GSI values increased significantly (P<0.001) as compared with that of the control. Ovaries of the former three groups contained developing embryos or yolk-laden oocytes. On the contrary, ovarian growth did not occur in the fish of groups B, D and F. GSI values in these groups were on a similar level to that of the control.

The above results indicated that the gonadal growth was accelerated only when a light period of 8 hours was coupled with dark periods to give cycles of 12, 36 and 60 hours.
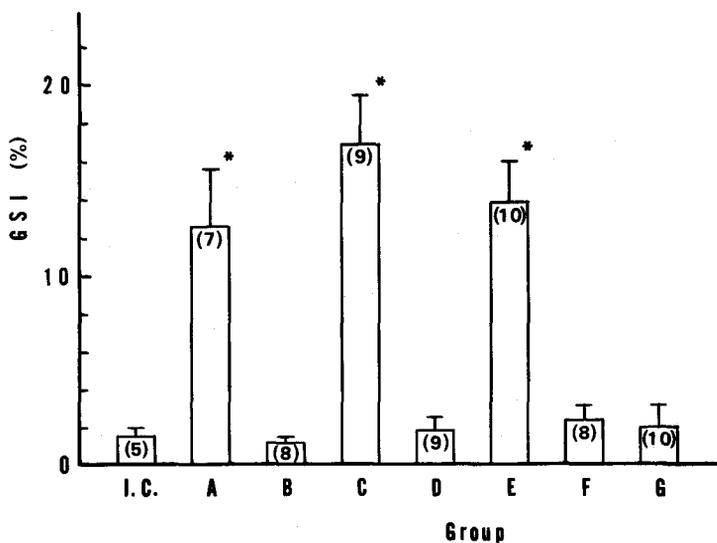

Fig. 5. GSI (ovary weight/body weight ×100) of the mosquitofish exposed to various photoperiodic schedules in the Experiment 2. I.C.; initial control group. Standard error of each mean is represented by vertical line. Figures in parentheses refer to the number of fish examined. *; significantly different from the value of group G at 0.001 percent level.
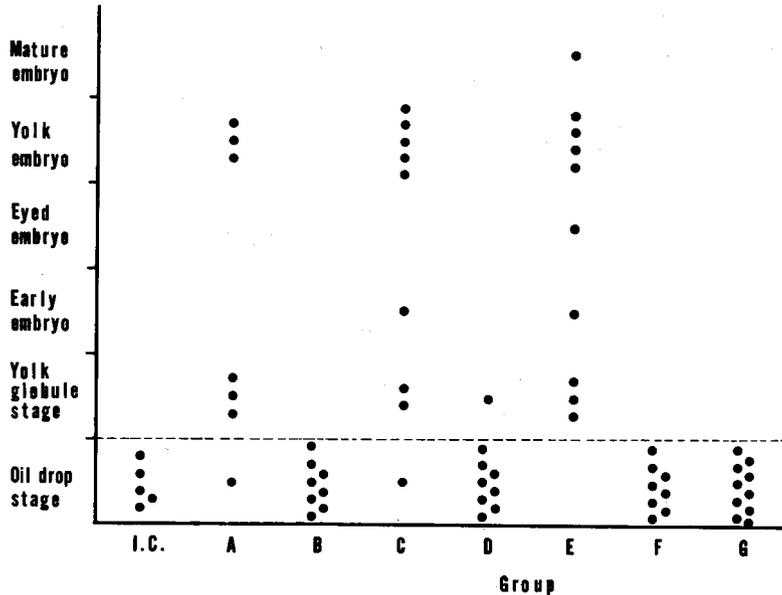
Fig. 6.   Ovarian condition of the mosquitofish exposed to various photoperiodic schedules in the Experiment 2.   I.C.; initial control group.   One dot corresponds to one individual.

Discussion

In the mosquitofish, the annual reproductive cycle has been considered to have a relation to the seasonal changes of water temperature.   Recently, Sawara[16] revealed that the threshold temperature for ovarian maturation in a natural population of the mosquitofish was presumed to lie between 17° and 21°C under long daylength.   Moreover, he showed that the critical daylength for the ovarian development lies between 12.5 and 13.0 hours.   His experimental evidence suggests that the daylength is the dominant factor controlling the ovarian activity in this species under optimal temperature.

In many other teleost fishes, too, the photoperiod is an important environmental factor which regulates the cyclic alteration of reproductive activities[2]-[5]. Recently, the presence of a photo-inducible phase has been demonstrated for photoperiodic responses in some fishes, as indicated first by Baggerman[7] in the stickleback, *Gasterosteus aculeatus*.

The results of the present study also suggest that female mosquitofish can respond with an accelerated ovarian development to the light stimulus given during the dark period.   It was indicated in the present study that 8 hours of light alternating with 16 hours of darkness in a 24-hour cycle was ineffective in stimulating the ovarian development.   However, the sensitivity of ovaries to photostimulation was shown to be extremely low for the first 12 hours during the 24-hour cycle, then occurred from the 12th to the 14th hour and decreased from the 14th hour to around the 16th hour.   This may probably indicate the existence of a

photo-inducible phase within the latter half of the 24-hour photoperiod. This finding supports Sawara's results[16] that the critical daylength lies between 12.5 and 13.0 hours.

The results of the night interruption experiment indicate that the photo-inducible phase may play an important role in photo-stimulation of the ovary in this species. These are consistent with the results of earlier experiments in the stickleback, *Gasterosteus aculeatus*[7], the Indian catfish, *Heteropneustes fossilis*[8], the medaka, *Oryzias latipes*[9], the honmoroko, *Gnathopogon elongatus caerulescence*[10], and the bitterling, *Rhodeus ocellatus ocellatus*[11], which demonstrate that gonadal growth can be induced by the night interruption.

In the stickleback[7], gonadal maturation was not accelerated under a 8L–16D cycle but caused under a 16L–32D cycle, although these two light cycles had the same ratio of light to darkness (1:2). This fact indicates that the ovarian development does not depend on the ratio between light and dark periods. Moreover, in the 24-hour light-dark cycle with a main light period in the first 6 hours, gonadal maturation of the stickleback was most stimulated when a 2-hour light pulse fell in the dark period between the 14th and the 16th hour after the onset of the main light phase. From these results, Baggerman concluded that gonadal photoperiodic responses of the stickbleback were based on a daily rhythm (circadian rhythm) of sensitivity to light.

When the mosqutitofish were exposed to photoperiodic regimens of 8L–16D, 8L–40D and 8L–64D, ovarian development did not occur. When subjected to 8L–4D, 8L–28D and 8L–52D, however, the fish showed a significant development of their ovaries. These results indicate that there exists in the mosquitofish an endogenous circadian rhythm with a periodicity of about 24 hours, and that a photo-inducible phase is present in the latter half of the cycle. When light is given at the proper phase of the rhythm gonadal recrudescence occurs, and when light is given at an incorrect phase of the rhythm no response is elicited. It is not the duration of light period which is important but the time where light falls relative to the photo-inducible phase of the rhythm. Similar result was reported in the honmoroko, *Gnathopogon elongatus caerulescence*, by Khiêt[10]. He showed that gonadal recrudescence did not occur in the fish reared under 12L–12D, 12L–36D and 12L–60D photoperiods, but was elicited in those exposed to 12L–24D and 12L–48D.

The concept of circadian rhythm was proposed by Bünning[20][21] in plants for the mechanism of measuring the length of photoperiod. He states that the photoperiodic efficiency of any given light-dark cycle depends primarily on which portion of an underlying circadian sensitivity rhythm is illuminated. His hypothesis envisages a circadian rhythm of cellular function consisting of two half-cycles each of approximately 12-hour duration, one of which is a light-requiring, photophil phase, and the other a dark-requiring, scotophil phase. Photoperiodic induction of a process requiring long days occurs only when the duration of the natural photoperiod extends into the scotophilic part of the rhythm. Pittendrigh and Minis[22] have presented a more explicit version of this hypothesis, stressing a dual function of the whole light cycle in acting both as the entraining agent for circadian oscillation and as the photoperiodic inducer.

The photo-inducible phase is daily and may be driven by the circadian oscillator

which is set by external lighting schedules, suggesting that an "external coincidence" model[22] is applicable to explain the phenomenon. In the mosquitofish, too, photoperiod may act as an important factor for the time measurement and the induction of gonadal maturation. However, there is the paucity of experimental data concerning photoperiodic response mechanism in fishes when compared with other animals, and further investigations are necessary to elucidate the problem.

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