光周期によるコイのGtHサージ開始時刻の決定

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Photoperiodic Determination of Preovulatory Gonadotropin Surge Onset Time in the Carp *Cyprinus carpio*

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Four experiments were conducted to clarify the effects of photoperiod on the onset time of the preovulatory GtH surge in the female carp *Cyprinus carpio*. After acclimation together with males under photoperiods of 12L–12D (Group A, lights-on 0600–1800 hours; Group B, 1800–0600 hours) in Experiment 1, 18L–6D (Group C, lights-on 0000–1800 hours; Group D, 0600–2400 hours) in Experiment 2, and 16L–8D (lights-on; 1600–0800 hours) in Experiments 3 (Group E and F) and 4 (Group G and H), water temperature was raised from 16 to 24°C beginning at 1730 hours to induce ovulation and spawning.

In Experiments 1 and 2, a preovulatory GtH surge initiated in the latter part of the light-phase in each photoperiod group after the water temperature was increased irrespective of the starting time of temperature elevation, and ovulation was observed mainly during the dark-phase. In Experiment 3, the light-phase period was changed in Group F from 1600–0800 to 0400–2000 hours after increasing the temperature so that the dark-phase was abruptly inserted after a 4-h light-phase. This change resulted in a delay of the preovulatory GtH surge. In Experiment 4, the GtH surge began at the same clock time as in Experiments 1 and 2 corresponding to the latter part of the light-phase of the acclimation photoperiod even though the photoperiod was changed to 24L and 24D in Group G and H, respectively, after increasing water temperature.

These results indicate that female carp have a reproductive circadian rhythm and the onset time of the preovulatory GtH surge is determined by a photoperiodic cue.

Both environmental and physiological factors are considered important cues triggering the final oocyte maturation and ovulation in teleosts. In several species of teleosts, ovulation and oviposition occur at certain time of the day and their onset times are changeable by manipulating the photoperiod.[1–10]*2,3 Furthermore, in goldfish,[6–8] bitterling,[9] and carp,[10] the preovulatory gonadotropin (GtH) surge occurs in the latter part of the light-phase, suggesting a photoperiodic determination of onset time of the GtH surge which induces final oocyte maturation and ovulation. There is, however, no direct evidence supporting this hypothesis in teleosts. Therefore, in this paper we investigated the influence of photoperiod on the onset time of the GtH surge in the common carp *Cyprinus carpio*.

Material and Methods

Fish

A total of 56 mature female carp weighing 1–2.5 kg were used in this investigation. The fish were selected on May 2, 1983 and June 4, 1984 from a stock which had been reared in an outdoor pond (5×2×0.6 m) at 16°C under natural daylength at the Fisheries Experimental Station (Yoshida, Shizuoka Prefecture, Tokyo University of Fisheries). These fish were transported to the Fisheries Laboratory (Maisaka, Shizuoka Prefecture, The University of Tokyo) and used for the following experiments.

Experiments

Four experiments were conducted during May 2–24, 1983 and June 4–25, 1984. Each experi-

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*2 K. Sakai, M. Nomura and A. J. G. Santos, Oral presentation at the Autumn Meeting of the Japanese Society of Scientific Fisheries on Oct. 18, 1980 (Fukuoka). Abstract No. 407.

*3 M. Nomura, A. J. G. Santos, K. Sakai, T. Kataoka and M. Ishii, Oral presentation at the Autumn Meeting of the Japanese Society of Scientific Fisheries on Oct. 7, 1981 (Tsu). Abstract No. 305.

ment consisted of two groups of seven females and four males. Females were acclimated in two types of indoor tanks (dimensions in m; $3\times1.5\times0.5$ or $2\times1\times0.5$) for 2–3 weeks, and males in indoor tanks ($2.7\times1\times0.5$ m) for a week at 16°C under the following artificially-controlled photoperiods: 12L-12D (Group A, lights-on 0600–1800 hours; Group B, 1800–0600 hours) in Experiment 1, 18L-6D (Group C, lights-on 0000–1800 hours; Group D, 0600–2400 hours) in Experiment 2 and 16L-8D (Groups E to H, lights-on 1600–0800 hours) in Experiments 3 and 4.

Water temperature was gradually raised from 16 to 24°C beginning at 1730 hr on Day 1 by stopping the cooler and supplying 24°C water (Fig. 1). In both Experiments 1 and 2, females and males were transferred to the experimental tanks ($3\times1.5\times0.5$ m) in the late afternoon on Day 0 and Day 1, respectively. In both Experiments 3 and 4, females and males were transferred to the tanks in the morning (early in the dark phase) and in the late afternoon (early in the light phase) on Day 1, respectively. Groups A, B, C, and D (Experiments 1 and 2) and Group E (Experiment 3) were maintained after raising water temperature under the same photoperiods as those used for acclimation, whereas the photoperiods for Groups F (Experiment 3), G, and H (Experiment 4) were abruptly changed to dark-phase during 2000–0400 hours, to 24D (continuous dark) and to 24L (continuous light), respectively. Water temperature was recorded throughout the experiments.

Blood Sampling

From females 1-m*l* blood samples were taken repeatedly under the schedules indicated in Figs. 2 to 5. During the sampling, the experimental room was maintained under the same lighting conditions in accordance with the light/dark phase of the experimental tanks. A dim light was used when sampling in the dark phase. The fish were anesthetized in 15 *l* of water containing 1.5% uretan (ethyl carbamate), and blood was taken from caudal vessels by using a heparinized 1-m*l* syringe with 22 G needle. Plasma was separated by centrifugation at 3000 rpm for 10 min and stored at −80°C until analysis.

GtH RIA

Plasma GtH levels were determined by RIA reported by Kobayashi *et al.*[8] in our laboratory.

Results

The number of ovulated fish at each sampling time is summarized in Fig. 1. Changes in the plasma GtH levels are shown in average with S.E.M. in Figs. 2 to 5.

Experiment 1

The fish were acclimated under 12L-12D, and the water temperature was rising at the commencement of the dark-phase in Group A and at the beginning of the light-phase in Group B as shown in Fig. 1. In group A, ovulation was observed in all the females during the second dark-phase after increasing the water temperature, whereas in Group B, all the females ovulated during the first dark-phase.

As indicated in Fig. 2, plasma GtH in the fe-

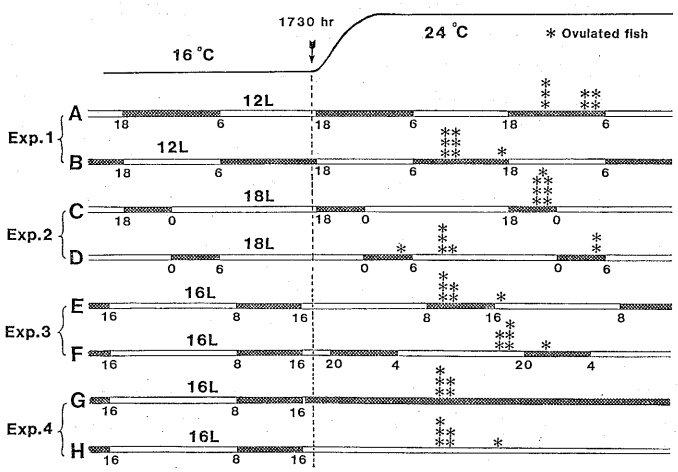

Fig. 1. The number of ovulated fish at each sampling time under various photoperiods after an increase in water temperature from 16 to 24°C beginning at 1730 hours (arrow).

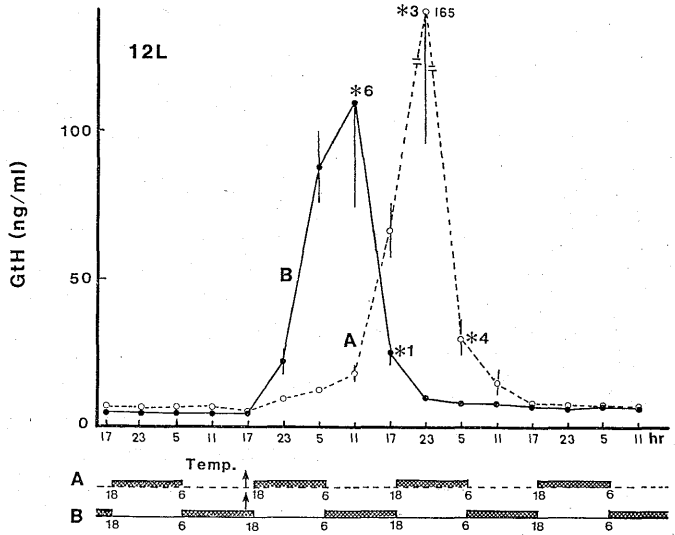

Fig. 2. Changes in plasma GtH levels of Group A (dotted line) and B (solid line) in Experiment 1. GtH values are indicated as mean±S.E.. Numbers beside asterisks indicate the number of ovulated fish. Arrows show the commencement of temperature elevation.

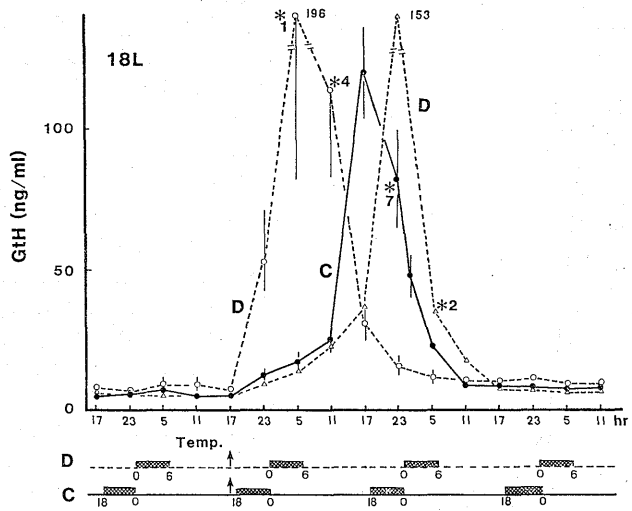

Fig. 3. Changes in plasma GtH levels of Group C (solid line) and D (dotted line) in Experiment 2. GtH values are indicated as mean±S.E.. Numbers beside asterisks indicate the number of ovulated fish. Arrows show the commencement of temperature elevation.

males of Group A increased gradually during the first dark-phase and earlier part of the following light-phase and showed a distinct rise (surge) during the latter part of the light-phase. The highest GtH values and occurrence of ovulation were observed during the second dark-phase; GtH levels then decreased quickly to basal values during the next light-phase. In Group B, plasma GtH showed a small increase following the elevation in the water temperature in the earlier part of the first light-phase, and the surge occurred during the latter part of the light-phase. Again the highest GtH values and occurrence of ovulation were observed during the dark-phase, and GtH decreased to basal values during the next light-phase.

Experiment 2

The fish were acclimated under 18L-6D, and the water temperature was rising at the start of the dark-phase in Group C and in the latter part of the light-phase in Group D, as shown in Fig. 1. In Group C, ovulation was observed in all the

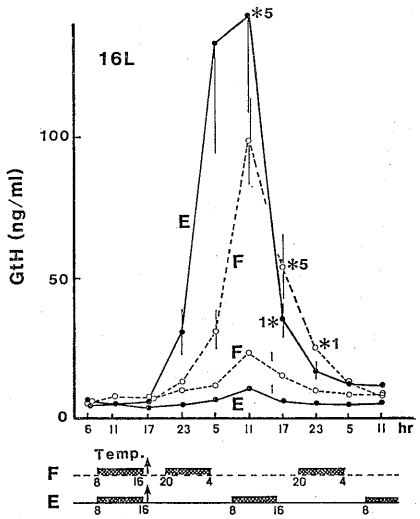

Fig. 4. Changes in plasma GtH levels of Group E (solid line) and F (dotted line) in Experiment 3. GtH values are indicated as mean±S.E.. Numbers beside asterisks indicate the number of ovulated fish. One fish in each group failed to ovulate. Arrows show the commencement of temperature elevation.

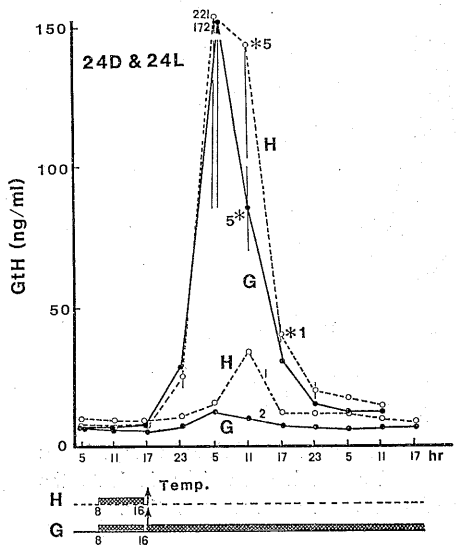

Fig. 5. Changes in plasma GtH levels of Group G (solid line) and H (dotted line) in Experiment 4. GtH values are indicated as mean±S.E.. Numbers beside asterisks indicate the number of ovulated fish. One fish in Group H and two in Group G failed to ovulate. Arrows indicate the commencement of temperature elevation.

females in the second dark-phase, whereas in Group D, ovulation occurred in the middle part of the second light-phase in 5 fish and in the second dark-phase in 2 fish.

Plasma GtH in the females of Group C increased slowly during the first dark-phase following the elevation in water temperature, and the surge commenced in the latter part of the next light-phase. In Group D, the GtH surge began in five females immediately after the commencement of the temperature elevation, but in the remaining two females the surge started about 24 h later at the same clock time in the light-phase.

Experiment 3

The fish were acclimated under 16L-8D, and the water temperature was raised just after the commencement of the light-phase. In Group E, most of the females ovulated in the first dark-phase, whereas in Group F, where the dark-phase was shifted to 2000–0400 hours after increasing a water temperature, ovulation was delayed about 6 h when compared with Group E, as indicated in Fig. 1.

The GtH surge in the females of Group E was initiated in the latter part of the light-phase, whereas in Group F the start of the GtH surge was delayed about 6 h when compared with Group E.

Experiment 4

The fish in this experiment were also acclimated under 16L-8D, and the water temperature increase was begun in the period equivalent to the beginning of the light-phase in the acclimation photoperiod. After the elevation in temperature, in Group G and H, the photoperiod was shifted to 24D and 24L, respectively. Females in both groups showed synchronized GtH surges and ovulated during the period corresponding to the dark-phase in the photoperiod used for acclimation.

Discussion

In the present investigation, the preovulatory GtH surge in carp was initiated in the latter part of the light-phase after increasing the water temperature, irrespective of the onset time of the temperature elevation. When water temperature was increased during the dark-phase and earlier part of the light-phase, plasma GtH levels gradually rose above basal levels. In contrast, the GtH surge commenced immediately, when temperature was elevated during the latter part of the light-

phase as in Group D of Experiment 2. In that group, water temperature was raised during the latter part of the light-phase, and the GtH surge started right away in 5 fish, though in 2 other females it started 24 h later at the same clock time in the next light-phase after a gradual GtH increase. These results strongly indicate that the onset time of the GtH surge is determined by photoperiodic cues, and not by the onset time of the temperature elevation. The cue is probably completed in the latter part of the light-phase.

The 2 females in Group D, that failed to respond to the first photoperiodic cue, did respond to the next cue after 24 h. Therefore, it is suggested that although they were not in the physiological condition to respond when the first cue was given, within 24 h the condition was probably satisfied by a gradual GtH increase. The steroid hormone environment may be involved in the physiological condition, but further investigation is necessary.

The results in Experiment 4 show that even under constant dark (Group G) or light (Group H) the GtH surge can be initiated at the same clock time corresponding to the latter part of the light-phase of the acclimation photoperiod, suggesting that the carp has a circadian rhythm, and the cue inducing GtH surge is given from the rhythm. In Group F in Experiment 3, the onset time of GtH surge was delayed for about 6 h when compared with Group E. Since the both groups had been acclimated under the same photoperiod, this delay in the GtH surge of Group F was probably induced by changing the light-phase to the dark-phase 2.5 h after starting the temperature elevation, representing an abrupt insertion of dark-phase into the light phase 4 hours after the lights-on. The sudden change in the photoperiodic regime probably resulted in the shift of the circadian rhythm, thus inducing the delay in the GtH surge.

In goldfish,[6-8] bitterling,[9] and Carp,[10] plasma GtH levels begin to increase in the afternoon, peak at midnight, usually with ovulation, and then decrease to basal values in the morning. Thus the mechanism regulating the timing of the GtH surge appears similar in these cyprinid species. However, the GtH surge as found in the cyprinid fishes seems not to be present in the salmonid fishes,[11-14], since the latter teleosts maintain plasma GtH levels throughout the pre- and post-spawning period for about one month.

It has been reported in some teleost species that ovulation and spawning occur at certain time of the day, and the time of ovulation and spawning are influenced by photoperiod.[1-10)*2,3] From the present results summarized in Fig. 1, it is clear that female carp kept under various artificial photoperiods ovulate mostly during the dark-phase or in the period corresponding to the dark-phase in the photoperiod used for acclimation, after increasing the water temperature from 16 to 24°C. Most of the females ovulated synchronously in the first or second dark-phases, except in Group D, where ovulation was observed in the second light-phase in 5 females and in the second dark-phase in 2 females. In the former 5 females, the GtH surge began immediately with the commencement of temperature elevation without a gradual GtH increase, since the temperature was raised in the latter part of the first light-phase. In the latter 2 females, GtH surge started 24 h later after a gradual GtH increase. During a gradual GtH increase, the oocytes may develop to the maturational stage just before GVBD, and then maturation is completed in the second dark-phase with the GtH surge. The delay of ovulation in the 5 females probably resulted from the absence of a gradual GtH increase, although GtH surge occurred in the latter part of the light-phase.

In summary, these findings provide a clear evidence for the role of photoperiod in determing the onset time of the preovulatory GtH surge in the carp and represents fundamental information for the artificial control of the onset time of ovulation and spawning in teleosts.

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