キンギョのGtHサージおよび排卵におよぼす光周期と松果体摘除の影響

誌名	日本水産學會誌
ISSN	00215392
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巻/号	55巻12号
掲載ページ	p. 2099-2103
発行年月	1989年12月

Nippon Suisan Gakkaishi 55(12), 2099-2103 (1989)

Effects of Photoperiod and Pinealectomy on the Gonadotropin Surge and Ovulation in Goldfish *Carassius auratus*

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(Received June 13, 1989)

Ovulation was induced in sexually mature female goldfish *Carassius auratus* maintained at 12°C under 15L-9D by raising the water temperature to 20°C under various photoperiods. The fish were checked every 2 or 3 h, and those which had ovulated were blood-sampled to determine the timing of the GtH surge. In Experiment 1, 2 groups of fish (n=15 each) were placed under photoperiods fixed at 15L-9D. In Group A, lights on was 0400 h, and in Group B, lights on was 1600 h. Ovulation occurred during the dark-phase in both groups. In Experiment 2, the photoperiod was changed from 15L-9D (lights on 0400 h) to continuous light (Group C; n=21) or continuous darkness (Group D; n=18) after the water temperature increase. In both groups, ovulations were observed at a time corresponding to the dark-phase of the acclimation photoperiod, however, in Group D, ovulation was slightly delayed when compared to Group C. In both Experiments, the GtH surge was observed only in ovulated fish. In Experiment 3, mature females were reared under 14L-10D (lights on 0430 h) and either pinealectomized (PINX, Group E, n=19) or sham operated (Group F, n=21). The water temperature was increased one week following the operation. Fish from Group F ovualted during the dark-phase, whereas fish from Group E ovulated randomly. The GtH surge was observed in the ovulated fish from both groups.

These results indicate that photoperiod plays an important role in determining the onset time of the preovulatory GtH surge. The pineal gland is somehow involved in the mechanism regulating this photoperiodic response.

Many investigators have studied the influences of environmental factors on reproductive activity in fish, particularly, the effects of photoperiod and water temperature on gonadal development.[1] Relatively few studies, in contrast, have focused on determining what external factors induce the ovulatory gonadotropin (GtH) surge and subsequent ovulation. In an early study, Yamamoto *et al.*[2] demonstrated in goldfish *Carassius auratus* that ovulation was induced within one or two days by raising the water temperature from an inhibitory temperature of 12°C to 20°C. Aquatic vegetation acts as a spawning substrate and a highly effective stimulus for ovulation.[3]

Recently, the occurrence of a preovulatory GtH surge has been demonstrated in goldfish,[4-6] common crap *Cryprinus carpio*[7] and the kanehira-bitterling *Acheilognathus rhombea*.[8] In these species, the ovulatory GtH surge begins in the latter half of the light-phase, and ovulation occurs in the dark-phase around the time of the peak of the surge. In common carp, the onset time of the GtH surge has been demonstrated conclusively by Santos *et al.*[9] in our laboratory to be determined by a photoperiodic cue.

In the present study, we investigated the effects of photoperiod on the timing of the GtH surge and ovulation in goldfish. Since the pineal gland mediates photoperiodic information and influences daily activity rhythm,[10-14] we also examined the possible involvement of the pineal gland in the photoperiod-sensitive regulatory mechanism which determines the ovulation time in this species.

Materials and Methods

Experiment 1

Sexually mature female goldfish were kept in a stock tank under natural conditions until the experiments began in April. Thirty fish weighing 25–50 g were transferred to 80-*l* expeimental aquaria kept at 12°C (7 or 8 fish per aquarium). The fish were divided into two groups: Group A (n=15) was subjected to 15L-9D, lights on at 0400 h (normal photoperiod); Group B (n=15) was subjected to 15L-9D lights on at 1600 h

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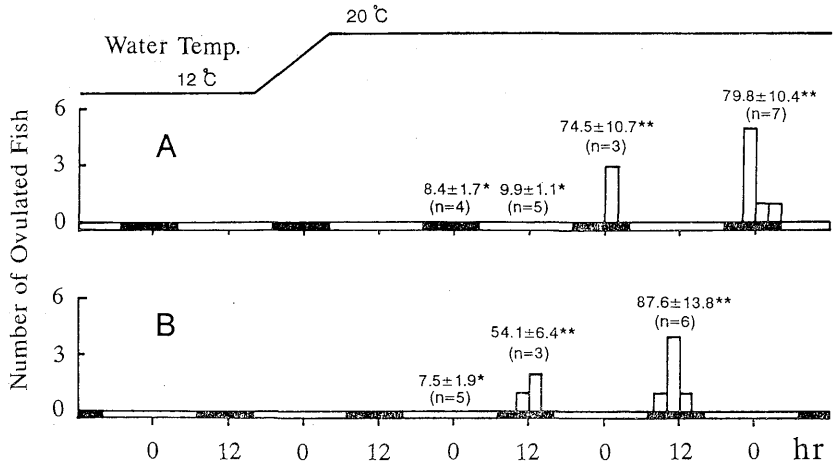

Fig. 1. The time of ovulation and the number of ovulated females in Group A and B in Experiment 1. Plasma GtH levels of non-ovulated females (*) at 0 and 12 h and those of ovulated females (**) are shown as the mean±S.E.. A, Group A; B, Group B.

(reversed photoperiod). Each aquarium was provided with a sand filter. The fish were fed once a day during the acclimation period with commercial trout pellets or *Tubifex* worms. After 3 weeks' acclimation, ovulation was induced by raising the water temperature to 20°C beginning at 1600 h over a period of 12 h (Fig. 1). Ovulation was checked every two hours during the dark-phase in both groups, and at 1200 and 0000 h during the light-phase in Group A and B, respectively. Blood samples were taken at 0000 and 1200 h from randomly selected fish and from fish which were confirmed to have ovulated. Ovulated fish were removed from the aquaria.

Experiment 2

Fish from the same stock as Experiment 1 were also used for this experiment. In May, 39 fish weighting 27–42 g were transferrred to 80-*l* experimental aquaria (9 to 10 fish per aquarium) kept at 12°C under 15L-9D (lights on at 0400 h). Each aquarium was provided with a sand filter, and the fish were fed as in Experiment 1. After 2 weeks' acclimation, artificial plants were added to the aquaria, and the water temperature was raised in the same manner as in Experiment 1. Prior to the water temperature increase, the fish were divided into two groups: Group C (n=21) was subjected to continuous light and Group D (n=18) to continuous darkness. The fish were checked every three hours from 1800 h after the water temperature increase to see if they had ovulated. Blood samples were taken from the fish confirmed to have ovulated, and from non-ovulatory fish at the end of the experiment. Ovulated fish were removed from the aquaria.

Experiment 3

Sexually mature goldfish were held in a tank maintained at 12°C under 14L-10D (linghts on at 0430 h) for 6 weeks from April to May when the experiment was conducted. After the acclimation 19 females were pinealectomized (PINX, Group E) and 21 females were sham-operated (Group F). After anesthetization with 0.005% ethyl-*p*-aminobenzoate, portion of the cranium above the pineal gland was cut with a dental drill. In the PINX group, the bone was raised, and the pineal gland removed by aspiration. In the sham fish, the cranium was cut, but the pineal gland was left intact. Following the operation, the wound was covered with dental cement.

One week after the operation, the 19 PINX and 21 sham fish were transferred to 40-*l* glass experimental aquaria (2 PINX and 2 sham per aquarium except for one aquarium which had 1 PINX and 3 sham) and kept at 12°C under 14L-10D (lights on at 0430 h). Each aquarium was provided with gravel, arificial plant, and a box filter. Ovulation was induced by raising the water temperature as in Experiment 1. The fish were checked for ovulation every three hours from 0900 h after the water temperature was increased. Blood samples were taken from the ovulated fish which were then removed from the aquaria.

Blood Sampling and Radioimmunoassay

Blood samples (150 μl) were taken from the

caudal vasculature with a heparinized syringe and needle after anesthetization with 0.02% tricaine methanesulfonate (TMS) for Experiment 1 and 2 or 0.005% ethyl-*p*-aminobenzoate for Experiment 3. Blood samples were centrifuged at 1500 g for 10 min, and the plasma stored at −20°C until it was assayed. During the dark-phase, blood sampling was conducted under the light of a dim red light lamp, invisible to goldfish.

Plasma GtH levels were measured by the radioimmunoassay for silver carp GtH developed by Kobayashi *et al.*[6] and validated for use in the goldfish.

Statistical Analysis

The number of ovulated fish between groups were analyzed by the X^2 test. The Mann-Whitney U-test was used for statistical analysis of the plasma GtH levels and the distribution of ovulation time.

Results

Experiment 1

Ten fish ovulated in Group A and 9 in Group B during the experimental period (Fig. 1). The number of ovulated fish was not significantly different between Groups A and B. Ovulation always occurred during the dark-phases for both groups. The clock times of ovulation were significantly different ($P<0.01$) between groups. When the ovulation time of either group was shifted 12 h, however, these differences disappeared. Plasma GtH levels in the ovulated fish were significantly higher than those in the fish not yet ovulated (Guroup A, $P<0.01$; Group B, $P<0.01$).

Experiment 2

Thirteen fish out of 21 ovulated in group C, and 5 out of 18 in Group D (Fig. 2). The number of ovulated fish in Group C was larger than in Group D ($P<0.05$). Ovulation was observed between 2100 and 0300 h in Group C, and between 0000 and 1200 h Group D. The distribution of ovulation times was significantly different ($P<0.01$) between Groups C and D. Plasma GtH levels in the ovulated fish were high (Group C, 87.7±10.1 ng/m*l* (mean±S.E.); Group D, 93.4±14.9 ng/m*l*).

Experiment 3

Five fish out of 19 ovulated in Group E, and 15 out of 21 in Group F (Fig. 3). The number of ovulated fish in Group E was significantly smaller than in Group F ($P<0.01$). Fish in Group F ovulated between 2100 and 0600 h, but four fish in Group E ovulated during the light-phase when goldfish normally does not ovulate. Plasma GtH values in the ovulated fish were high (Group E, 73.5±20.5 ng/m*l* (mean±S.E.); Group F, 63.8±10.2 ng/m*l*).

Discussion

The present study showed that the preovulatory

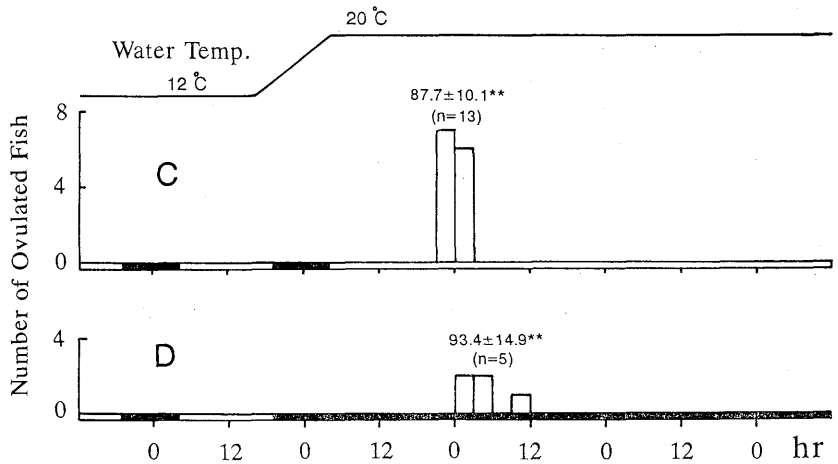

Fig. 2. The time of ovulation and the number of ovulated females in Group C and D in Experiment 2. All fish in Group C ovulated in the period corresponding to the light phase of acclimation photoperiod. In group D, ovulation time was rather delayed when compared to that of Group C. Plasma GtH levels of ovulated females (**) are shown as the mean±S.E.. C, Group C; D, Group D.

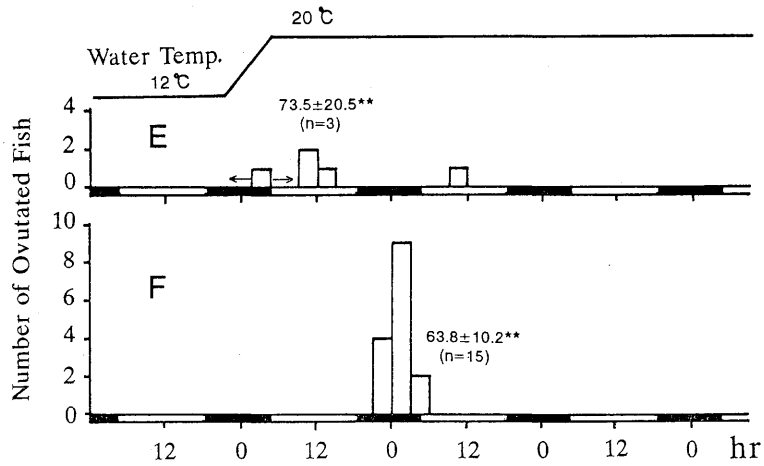

Fig. 3. The time of ovulation and the number of ovulated females in Group E and F in Experiment 3. In Group F, most of the females ovulated in the dark-phase, while in Group E, ovulation occurred randomly. Arrows indicate the period where ovulation for the first individual was speculated to have occurred. Plasma GtH levels of ovulated females (**) are shown as the mean±S.E.. E, Group E; F, Group F.

GtH surge and ovulation in goldfish are synchronized with photoperiod. In Experiment 1, the reversed photoperiod caused an approximately 12 h shift of the ovulation time in the Group B fish compared with normal ovulation time observed in the Group A fish. This shift occurred even though the water temperature was raised at the same time for both groups. This result is consistent with the data of Stacey *et al.*.[3] The The high plasma GtH levels in the Group B ovulated fish are evidence that the GtH surge also shifted in response to the reversed photoperiod. Although the changes in plasma GtH levels were not traced in individual fish, the onset time of the ovulatory GtH surge in goldfish is probably determined by a photoperiodic cue, irrespective of the starting time of the water temperature elevation, as demonstrated in common carp by Santos *et al.*.[9]

The fish in Group C, held on continuous light, ovulated at a time corresponding to the dark-phase of their acclimation photoperiod. This result indicates that the preovulatory GtH surge, which always starts in the light-phase, proceeds to completion regardless if there is a subsequent peroid of darkness or not. Under continuous darkness the ovulation was also observed with the GtH surge, suggesting that the timing of the GtH surge was determined by their endogenous circadian rhythm. The ovulation was, however, delayed to some extent compared with that of the Group C fish maintained under continuous light. In a similar experiment in common carp,[9] the GtH surge and ovulation occurred under continuous darkness at a time corresponding to the dark-phase of the fish's acclimation photoperiod without any delay compared to the timing of these events under continuous light conditions. The differences in ovulation time between the goldfish and common carp under continuous darkness may be attributed either to differences in their circadian rhythms or to the differences in experimental procedure (carp were exposed to a short period of light-phase for blood sampling before elevation of water temperature).

In daily spawning marine teleosts, the kisu *Sillago japonica*,[15,16] red seabream *Pagrus major*[17] and tobinumeri-dragonet *Repromucenns beniteguri*[*1] spawning occurs at defined times every day under natural photoperiod conditions. Under continuous darkness, however, spawning of the kisu is delayed by approximately 15 min, each day.[*2] This result suggests that under continuous darkness the spawning time of the kisu and above mentioned seawater and freshwater species are determined by an endogenous circadian rhythm. Under natural photoperiod conditions, these species probably spawn at the defined time of day by

[*1] Zhu *et al.*: in preparation.
[*2] Furukawa *et al.*: in preparation.

entraining to the prevailing light-dark cycle.

In common carp, there was no signtificant difference in the number of ovulated females between fish held under continuous light or dark conditions. This suggests that the difference in the number of ovulated goldfish between Groups C and D may have been due to the unequal distribution of fully matured females between the two groups.

Four pinealectomized fish ovulated in the light-phase, which never occurs under natural conditions. In addition in these fish the GtH surge was not synchronized with photoperiod, suggesting that the pineal gland is involved in determining the ovulation time in goldfish. The pineal gland mediates photoperiodic information and influences daily activity rhythm in many vertebrates.[10-12] Daily changes in plasma melatonin levels have been demonstrated in some teleosts.[13,14] Asynchronous ovulation in Group E is possibly due to a lack of photoperiodic information being transferred from the pineal gland to the regulatory site of the GtH surge in the hypothalamus. Since the photoreceptor cells in the pineal gland secrete melatonin into the blood and send nervous signals to the central nervous system,[18,19] it remains to be clarified whether the secretion of melatonin, or transmission of nervous signals, or a synchronization of such photoperiodic information is the main effector in the regulation of the GtH surge in goldfish. Further investigation on the involvement of the pineal gland in the regulation of ovulation time is required.

Acknowledgments

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Euctation, Science, and Culture of Japan and by a Grant-in-Aid (Bio Media Program 89-II-2-3) from the Ministry of Agriculture, Forestry and Fisheries.

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