持続的な高･低水温,長･短日長下での雌キンギョの再成熟

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Rematuration of Female Goldfish under Continued Warm or Cool Temperature in Combination with Short or Long Photoperiod*[1]

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Under natural conditions, some fish spawn in July-August but the majority spawn in the following March-June. The plasma E_2 was at high levels in July and March-April, very low in January-February and rather high in other months.

When transferred from natural daylength at 24°C to 12L or 16L combined with 24°C on June 22, only a few fish spawned at 24°C/12L with the majority at 24°C/16L in the first 2 months. Then, almost all fish stayed immature for about 11 months. Plasma E_2 was low and GtH rather high on both regimes.

When transferred to 16°C/12L or 16°C/16L, most fish spawned 1–2 times in the first 2 months. At 16°C/16L spawnings were repeated till the 16th month, although intervals were lengthened, while at 16°C/12L spawnings were interrupted for 4–5 months and then resumed. At 16°C/12L, plasma E_2 was low in the first six and last 3 months but were high in other months. At 16°C/16L, the E_2 fluctuated at rather high levels. The plasma GtH in individual fish in both regimes fluctuated frequently at high levels. At the end, most fish in both regimes were immature and the plasma E_2 and GtH were rather low.

Several authors have studied the effect of environmental factors on the gonadal maturation of goldfish. Kawamura and Otsuka[1] noted that both warm temperatures and a long photoperiod were necessary for inducing maturation in goldfish, whereas Fenwick[2] reported that low temperatures and long photoperiods stimulated gonadal maturation only in spring. Recently, we found that when kept at 24°C in autumn or early winter, female goldfish matured and spawned at 16L but remained immature at 12L. At 16°C, however, ovarian maturation occurred regardless of photoperiod.[3,4] The females remained immature at 24°C/12L for one year starting from July, while the fish matured and spawned at 16°C/12L.[5,6] We have also noticed that the critical daylength for ovarian maturation at 24°C lies between 13 and 14L in autumn, and the temperature level below which the female goldfish mature regardless of photoperiod exists between 18 and 21°C.[6] The tempo of maturation was slower in the female goldfish than in the male goldfish.[7,8]

The aims of the present study are as follows: how often can spawning occur under long term (12–17 months) exposure to 24°C or 16°C combined with 12L or 16L; are these regimes applicable to aquaculture; and how wide are individual differences under fixed conditions?

Material and Methods

Two-year old goldfish of the comet variety (weighing 29–45.5 g) were purchased from a fish farm near Nagoya on Apr. 25. They were induced to spawn by the administration of HCG as described before.[3] The fish were kept at 24°C in outdoor flow-through tanks under natural day length until use.

Starting on June 22, 5 groups of goldfish each comprising 25 males and 25 females were exposed to five different regimes: 24 or 16°C combined with 12 or 16L, or natural conditions. Individual fish were marked by fin clipping.

The natural condition group was kept in an outdoor tank (1.5×1×0.5 m) with a slight water exchange so that the tank simulated natural conditions. Other groups were transferred to either cool water tanks (2.5×1×0.7 m) or warm water tanks (2.4×1×0.5 m), which were supplied with well water of 16±1°C or 24±1°C at 1 to 1.5 *l*/s. The tanks were covered and illuminated for the given photoperiods with two 40 watt tubes for the

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cool water and two 20 watt tubes for the warm water. Experiments were performed for 12 moths on cool temperature regimes.

Every 15 days and after spawning, the body weight and changes of belly (softness and enlargement) were recorded. Blood samples were taken monthly from the caudal vasculature of the same 7 indiviudal fish in each group. The occurrence of spawning was shown by eggs attached to the spawning nests kept submerged in the water. Spawned fish were identified by individual checking. Egg hatchability was examined after each spawning by collecting 100 eggs and keeping them in a well aerated aquarium at room temperature. Some fish were occasionally sacrificed for their gonadal examination. All fish were examined at the end of the study. Gonads were fixed in Bouin's solution and then subjected to histological studies.

Plasam estradiol-17β (E_2) levels were measured according to the method of Aida *et al.*[9] Intra-assay and interassay coefficients of variation were 2.3% ($n=6$) and 6.3% ($n=5$), respectively. Other details were described by Shimizu *et al.*[10]

The plasma gonadotropin (GtH) levels were measured as described by Kobayashi *et al.*[11] The intraassay and interassay coefficients of variation were 6.8% ($n=6$) and 8.6% ($n=5$), respectively. The plasma GtH of goldfish showed parallel competition curves to standard hormone.

Results

Natural Condition

Changes of temperature and photoperiod under natural conditions are shown in Fig. 1.

Number of spawnings of individual fish, and plasma E_2 and GtH changes of blood sampled individuals and final GSIs (gonad weight × 100/body weight) are shown in Fig. 2. Nine of 25 females spawned in the first two months. Spawnings resumed from the following March and most fish spawned once or twice by June. Eggs hatchabilities ranged between 76 and 82%.

On Aug. 29, 8 females were sacrificed. Their GSIs varied widely (Fig. 2). Four fish with GSIs over 4.7% had oocytes in yolk globule (exogenous yolk) stage. They were under atretia. Other fish had oocytes in either immature (yolkless) or early yolk vesicle (endogenous yolk) stage. At the end of experiment, fish with GSIs below 3.9% were either immature or under regression. One of these fish (No. 24) never spawned. The remaining 6 fish with GSIs over 5.9% were in yolk globule stage.

Most of the seven blood sampled fish (Nos. 1–7) showed a high peak of E_2 in late July. Then the E_2 levels dropped in August, and tended to increase slightly in October or November. The levels decreased again in December or January to stay at the lowest in February, but changed to a marked rise to ca. 2 ng/m*l* from March to April ($P<0.01$–0.05), resulting in the resumption of spawning. Thereafter, a general decline of E_2 level was observed.

Plasma GtH levels of most blood sampled fish were rather high (6–14 ng/m*l*) during summer through autumn with a peak in late August, and started to decrease in December, reaching the bottom in January and February. They were elevated markedly or less markedly in the following months.

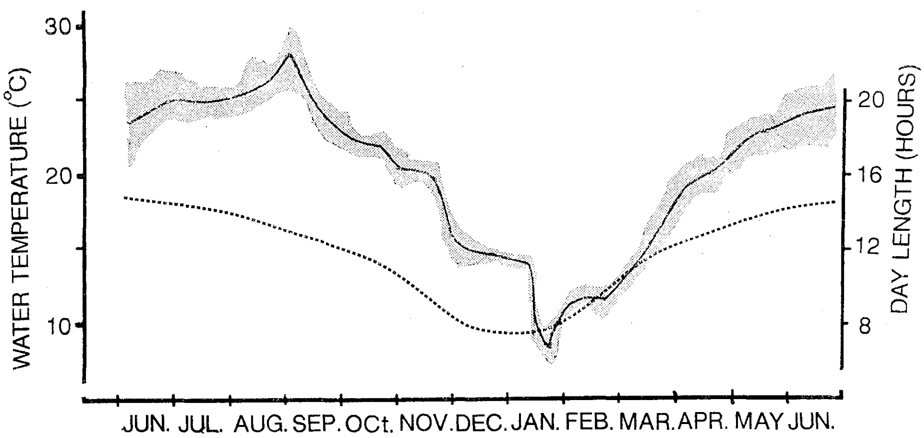

Fig. 1. Changes in day length and water temperature under natural conditions.

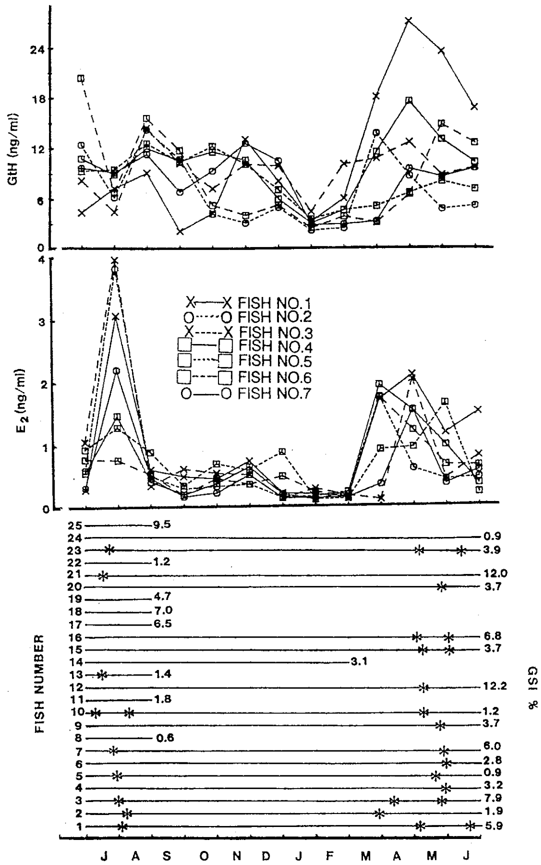

Fig. 2. Sequential plasma E_2 and GtH changes in individual female goldfish under natural condition. Each asterik denotes ovulation or spawning.

Warm Temperature and Short Photoperiod (24°C/12L)

As shown in Fig. 3, four of 25 fish spawned in July and August. Egg hatchabilities were 67–75%. Thereafter, none spawned and no distinct morphological changes of the bellies were observed.

The fish sampled in late February, and those sacrificed at the end (Fig. 3) had small ovaries (GSI<3.1%) which comprised only immature oocytes.

Except in a few fish, plasma E_2 stayed low (<1.0 ng/m*l*). Plasma GtH level, however, fluctuated widely (0.8–15 ng/m*l*).

Warm Temperature and Long Photoperiod (24°C/16L)

In the first 3 months, 22 of 25 fish spawned heavily one once or twice (Fig. 4). Thereafter, only fish Nos. 3, 10, and 19 spawned 3, 1, and 2

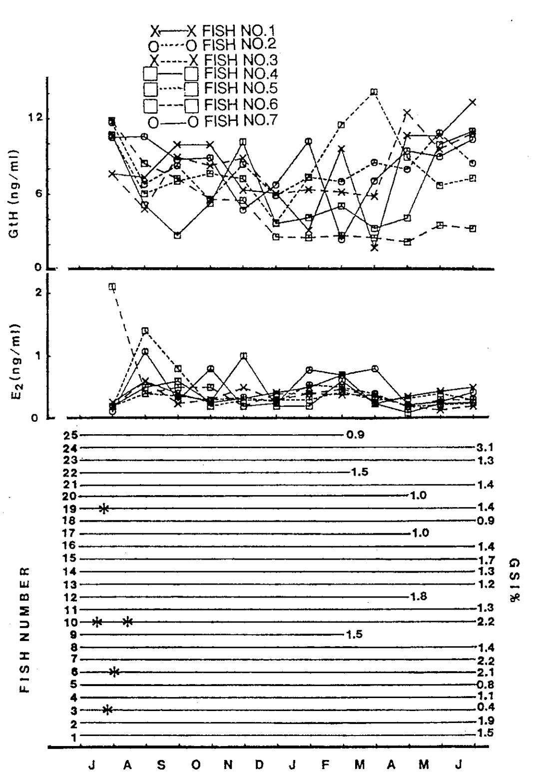

Fig. 3. Sequnetial plasma E_2 and GtH changes in individual female goldfish under continuous 24°C/12L condition. Each asterik denotes ovulation or spawning.

times, respectively. Throughout the experiment, the egg hatchabilities ranged between 78 and 82%.

The fish sampled in late February and April were immature (GSI<1.9%). At the end, 4 (GSI>6.4%) of 19 fish were in yolk globule and the others (GSI<1.2%) in immature stages (Fig. 4).

Plasma E_2 levels were rather high in fish Nos. 6 and 7, but low in the remaining 5 fish in late July (Fig. 4). Fish No. 3 spawned 4 times during the experiment and its plasma E_2 fluctuated at high levels (0.8–3 ng/m*l*) after November. The E_2 levels of others were low after September. Plasma GtH in most females fluctuated at rather high levels during the exposure.

Cool Temperature and Short Photoperiod (16°C/12L)

In the first three months, 15 of 25 females spawned (Fig. 5). None spawned between Aug. 9 and Dec. 12. Spawnings of individual fish resumed again from mid December. By the following October, 23 of 25 fish spawned, each 1–4 times.

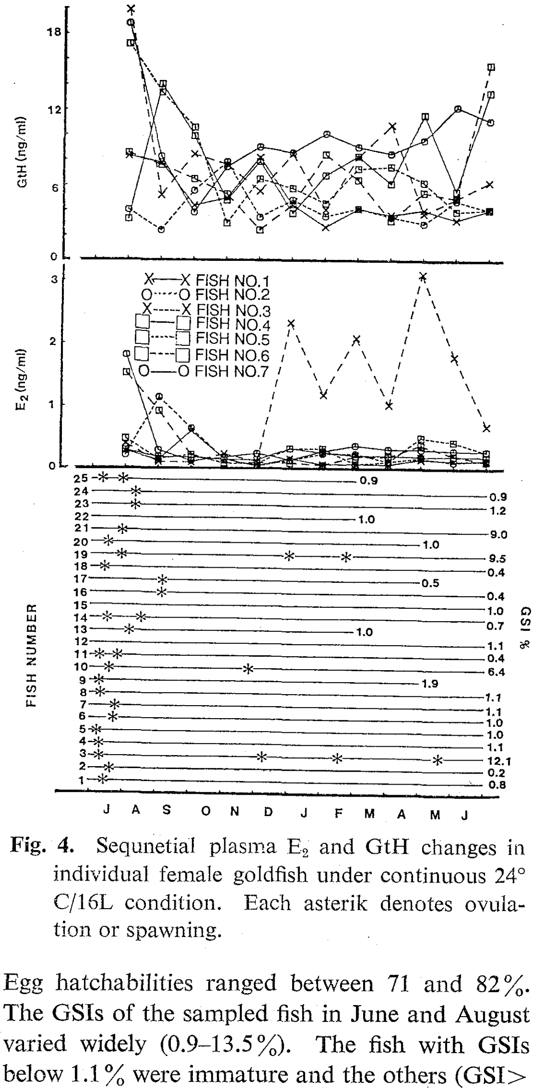

Fig. 4. Sequnetial plasma E_2 and GtH changes in individual female goldfish under continuous 24°C/16L condition. Each asterik denotes ovulation or spawning.

Egg hatchabilities ranged between 71 and 82%. The GSIs of the sampled fish in June and August varied widely (0.9–13.5%). The fish with GSIs below 1.1% were immature and the others (GSI> 5.1%) were in yolk globule stage. At the end, 3 (GSI>5.0%) of 15 fish were in yolk globule and the others (GSI<3.2%) in either yolk vesicle or immature stage.

The plasma E_2 levels stayed low in the first 6 months (Fig. 5). Then, the levels rose in most individuals and fluctuated 2–3 times, finally settling down for about 4 months. The GtH fluctuated at rather low levels in the first three and last four months but at high levels in between.

Cool Temperature and Long Photoperiod (16°C/16L)

Except in the last month, spawnings were observed several times each month (Fig. 6). Each fish spawned 2–6 times, although 3 of 25 females remained immature throughout. Egg hatchability ranged between 75 and 83%.

In the latter July and August, GSIs ranged widely (Fig. 6). The fish were divided into immature (GSI<1.4%) and yolk globule (GSI>7.2%) stages. At the end, 5 fish (GSI>6.2%) were in yolk globule and 14 (GSI<2.2%) in immature stages.

Plasma E_2 were generally high in the first 3 months. Thereafter the E_2 levels became gradually low. The E_2 rose again in some fish during the last five months. Plasma GtH were usually high and showed several wide fluctuations during the experiment.

Discussion

Under natural conditions, vitellogenesis took place actively in July and some fish even spawned (Fig. 2), which was unusual as the goldfish normally move into post-spawning in late June.[3] Before this experiment, the fish had been kept at 24°C and the natural daylength which possibly allowed continuation of vitellogenesis. Changes of gonads observed in September and thereafter were common to those described for the yearling and 2–3 year old goldfish.[3,7] Individual differences were noted as a few fish did not spawn while some others spawned 1–3 times. High levels of plasma E_2 in July and the following March-May (Fig. 2) signify active vitellogenesis occurring in these periods. Plasma GtH levels were high in August and the following March-June. This was probably due to the high temperature in summer and rising temperature in spring (Fig. 1). The temperature dependency of GtH secretion has already been reported.[8,12]

Under 24°C/12L, only a few fish that had shown large bellies before spawned in the first 3months, while most fish with hard bellies did not spawn. The plasma E_2 levels were low and all sacrificed fish were immature during and at the end of the experiment (Fig. 3). We also noticed that the yearling and 2–3 year old female goldfish remained suppressed in summer, autumn, and early winter when they were transferred from natural condition to 24°C/12L and reared for 1 to 2 months. In autumn the critical photperiod locates between 13L and 14L at 24°C.[4] Female goldfish also remained immature when kept at 24°C/12L for about one year starting from July.[1,5] These results definitely indicate that the female goldfish can be kept suppressed at 24°C/12L throughout the year.

Under 24°C/16L, most females spawned in the first 3 months and their egg hathcabilities were almost comparable to those of the natual condition

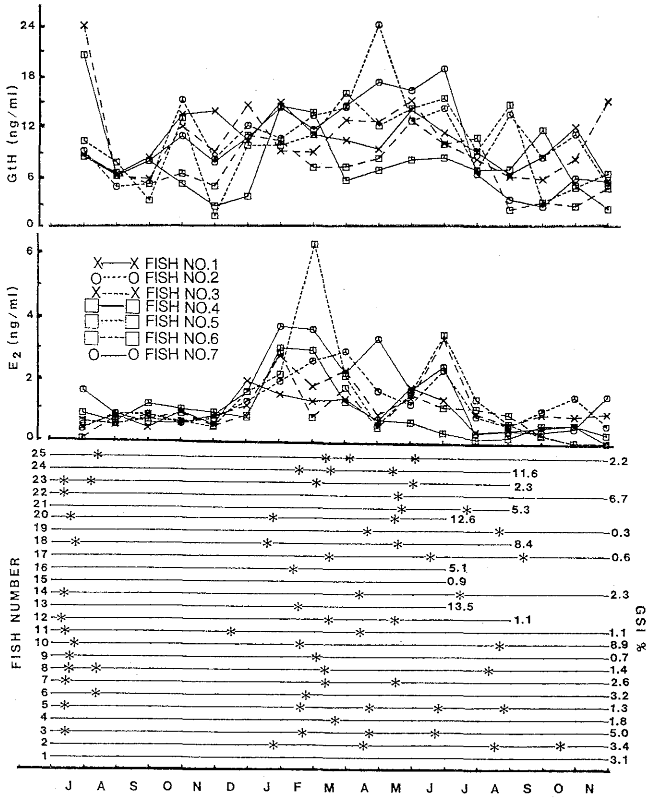

Fig. 5. Sequnetial plasma E_2 and GtH changes in individual female godfish under continuous 16°C/12L condition. Each asterik denotes ovulation or spawning.

group. Thereafter, however, most fish remained immature with very low plasma E_2 levels, although the GtH levels were rather high (Fig. 4). Perhaps, the ovarian endocrine function became so seriously suppressed after the hyperactivity in the first 3 months. It may further be suggested that 24°C was rather too high for the 16L to play any effective role in the initiation of the remeturational cycle.

These results obtained at 24°C point out that the photoperiodic response of female fish apparent during the first 2–3 months, disappears with prolonged exposure (compare Fig. 3 and Fig. 4). Since a few fish spawned at 24°C/12L in the first 2 months, and at 24°C/16L after November, individual differences under these regimes are also indicated.

Under 16°C regimes, the females spawned at both 12L and 16L in the first 2–3 months. Spawning continued at 16L while it ceasesd at 12L for a few months to resume thereafter (compare Fig. 5 with Fig. 6). Accordingly, patterns of changes in plasma E_2 and GtH levels differed between 12L and 16L. The present findings show that the same photoperiodic effect on maturations does exist at 16°C.

Under 16°C/12L or 16°C/16L, times of spawning differed greatly among individual fish. A few fish never spawned. Individual variation is thus indicated in readiness for rematuration. Individual differences were also clear for the plasma E_2 and GtH levels. At the end of the experiment, most fish were at the immature stage. On both regimes they had stopped spawning for 1–4 months, although a few fish with high GSIs were still in yolk globule stage. Possibly, in some fish the interruption of spawning was temporary and the spawning would have resumed if the experiment had been prolonged. However, the immature fish might no longer mature or a long period might be necessary for initiating the remeturational cycle.

In conclusion, at 16°C spawning took place several times at both 12L and 16L. At 24°C, most fish spawned at 16L and a few at 12L only in the first two months. Individual differences were wide: The tempo of maturation was diverse under the same external condition and some fish never spawned. Since egg hatchabilities in all regimes

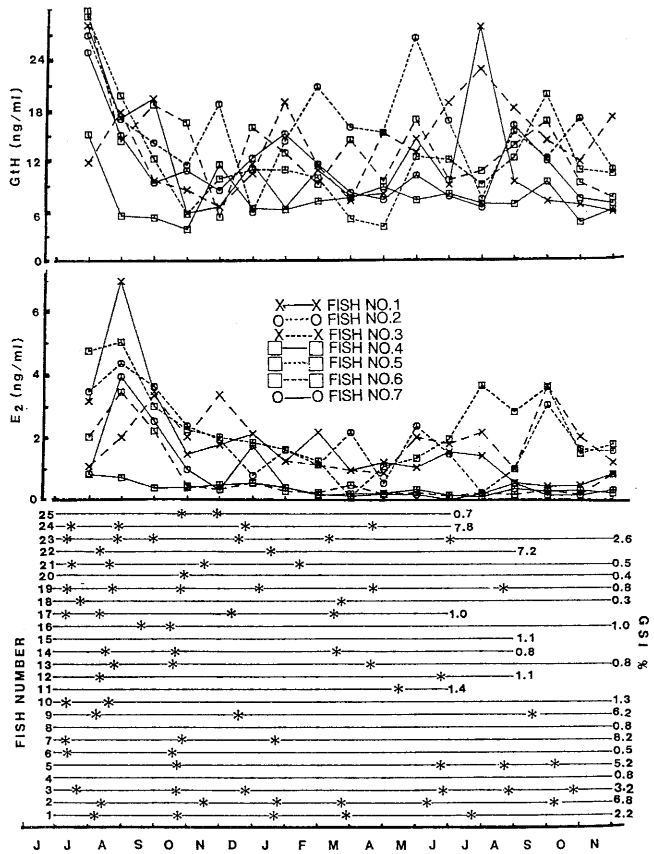

Fig. 6. Sequnetial plasma E_2 and GtH changes in individual female goldfish under continuous 16°C/16L condition. Each asterik denotes ovulation or spawning.

were almost comparable to those of control they are applicable in aquaculature practice.

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