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Effects of Temperature and Daylength on Gonadal Development of a Goby, *Rhinogobius brunneus* (Orange Type)

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Abstract The effects of temperature and daylength on the initiation of sexual maturation of yoshinobori, *Rhinogobius brunneus* (orange type), were examined. In the natural habitat, onset and further progress of vitellogenesis and spermatogenesis were not synchronous among individuals. Precocious females started yolk accumulation in March and reached pre-spawning stage in late May. Spermatocytes were found in all male specimens in March, though meiotic figures could be seen as early as December in the testes of larger males. In late May, some larger precocious individuals reached the final stage of spermatogenesis. Many deposited egg masses and brood-caring males were observed in June. In experiments, combination of high temperature and long day alone elicited the onset of vitellogenesis. In the case of the testes, however, high temperature-long day regime was not the triggering factor of meiosis but only an accelerator. All the reproductive process of yoshinobori, from beginning of vitellogenesis to spawning, proceeds quickly under high temperature-long day conditions. Minimum temperature and daylength for the onset of female maturation is probably around 9°C and around 12 hours respectively.

Yoshinobori, *Rhinogobius brunneus* (orange type), is a gobiid fish abundant in the littoral of Lake Biwa and in its inlet rivers. It is reported to breed over a long period of time from April to October (Miura et al., 1966). One of the authors Takahashi (1982) has studied the environmental control of vitellogenesis of another gobiid fish isaza, *Chaenogobius isaza*, which also abundantly lives offshore of Lake Biwa and has a breeding season concentrated in a short period of spring. Her studies show that the subjective thermoperiod experienced by isaza as a result of their diel vertical migration controls their vitellogenesis. In the present investigation, we intend to examine the effects of temperature and daylength on the initiation of sexual maturation of yoshinobori.

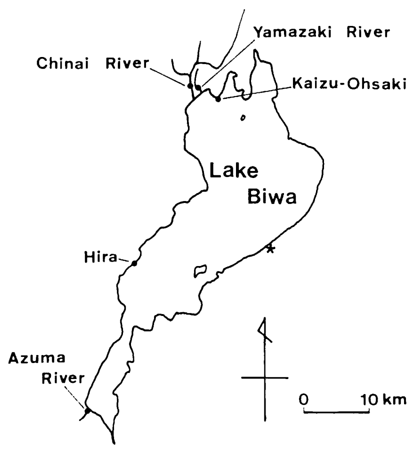

Fig. 1. Map of Lake Biwa showing the location of study sites. Asterisk, Shiga Prefectural Fisheries Experimental Station.

Materials and methods

To study the process of sexual maturation under natural condition, yoshinobori were observed and collected in the shore of Lake Biwa and its inlet rivers from December 3, 1984 to June 17, 1985. The stations are shown in Fig. 1. Collected specimens were fixed in situ in 10% formalin. Brought back to laboratory, gonads of the specimens were fixed again in Bouin's fixative.

Experiments to study the effects of temperature and daylength on the initiation of gonadal development were performed from October 1, 1980 to February 5, 1981 in Shiga Prefectural Fisheries Experimental Station located near Lake Biwa (35°15′N).

Two different temperature conditions were supplied by using the lake water (above 20°C at the beginning of October eventually dropping to near 5°C by February) and the subterranean water of

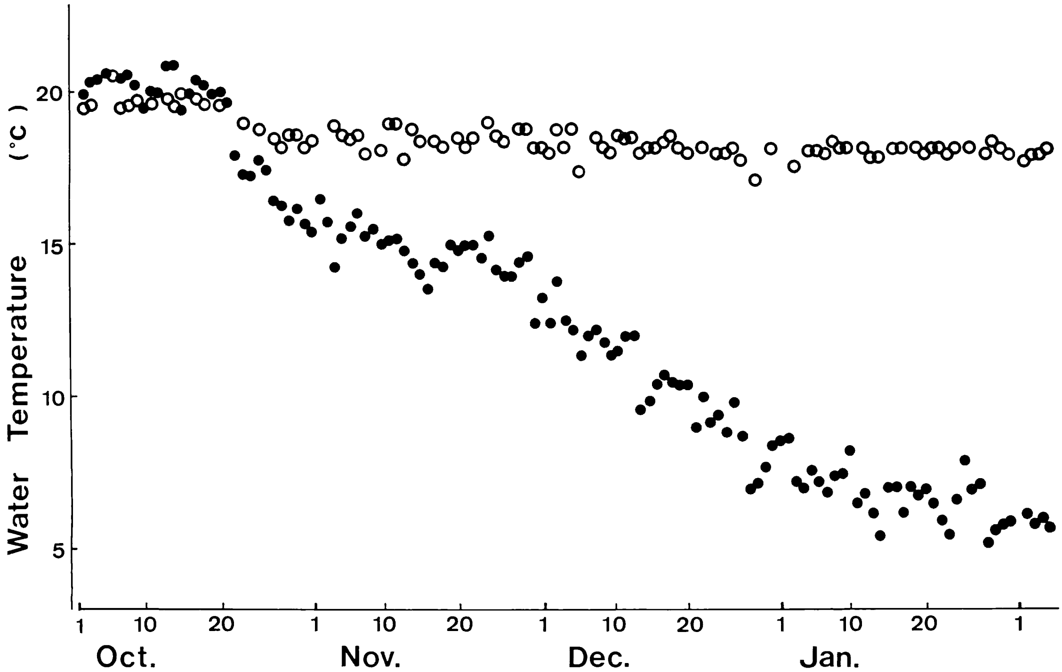

Fig. 2. Water temperature conditions in the experiments. Open circles, subterranean water; solid circles, lake water.

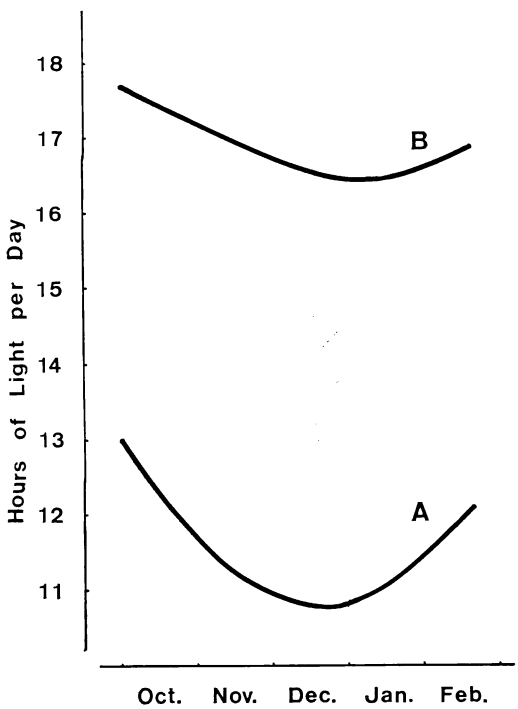

Fig. 3. Daylengths in the experiments. A, uncontrolled natural daylength; B, artificially elongated daylength.

relatively constant high temperature (18–20°C), which is equivalent to the water temperature of the lake shore in October or in June. These temperature conditions in the experiments are illustrated in Fig. 2.

The daylengths used were also two kinds, i.e. uncontrolled natural daylength (around 11L) and artificially elongated day length (around 17L) (Fig. 3). Additional light was provided by a 40-watt fluorescent lamp controlled to turn on at 16.00 and off at 23.00 and located about 30 cm above the water surface of two experimental tanks placed side by side. The four variants of temperature-daylength combinations were as follows:

Experiment I. Low temperature-short day regime: With lake water and natural daylength similar to natural fall-winter condition. Started on October 1, 1980.

Experiment II. High temperature-short day regime: With warm subterranean water and natural daylength. Started on October 1, 1980.

Experiment III. Low temperature-long day regime: Artificially elongated daylength combined with cold lake water. Started on December 5, 1980.

Experiments IV-1 and 2. High temperature-long day regime: With subterranean water and artificially elongated daylength simulating late spring-summer conditions. Started on October 1, 1980 (IV-1) and December 8, 1980 (IV-2).

Fish used in the experiments were immature 0-year fish which were collected from the overflows of the fish ponds in their ascending migration from Lake Biwa. On October 1, 1980, 50–70 specimens (males and females) were distributed in each of the three 70 *l* experimental tanks (Experiments I, II and IV-1). The specimens in Experiments III and IV-2 were derived from Experiment I. Fish were fed with a blend of artificial foods for carp and eel.

A part of specimens were removed each month and fixed in Bouin's fixative. To determine the stage of gonadal development, the fixed gonads of specimens, both natural and experimental, were sectioned by the usual paraffin method and stained with Mayer's haematoxylin-eosin.

Results

Ovaries studied were classified into six developmental phases represented by the stage of the most developed oocyte group because the oocyte development in a group was synchronous (group synchronism). The six phases are as follows:

Phase 1. Yolkless stage: Ovaries are occupied with germ cells up to the peri-nucleolus stage.

Phase 2. Yolk-vesicle emergence: The most developed oocytes have reached yolk-vesicle stage.

Phase 3. Beginning of yolk accumulation: The most developed oocytes have reached primary yolk stage.

Phase 4. Active yolk accumulation: The developing oocytes are in secondary yolk stage, i.e. yolk globules are actively accumulated throughout the cytolplasm.

Phase 5. Pre-spawning: The developing oocytes are in tertiary yolk stage or already in migratory-nucleus stage to ripe stage. Yolk accumulation is almost completed and adherent filaments are formed on the outside of oocytes.

Phase 6. Post-spawning and regressing: Post-ovulatory follicles and/or atretic follicles are found among the young yolkless oocytes. The stroma of the ovary appears disorganized.

The testis of yoshinobori is a lobular one (Billard et al., 1982). In testes examined in this study, five phases were distinguished according to the following histological characteristics:

Phase 1. Inactive stage: Lobules are small in diameter. Lobule walls contain only spermatogonia.

Phase 2. Beginning of spermatogenesis: Cysts of spermatocytes and spermatids are found along the lobule wall but rare.

Phase 3. Active spermatogenesis: All spermatogenetic stages can be seen abundantly.

Phase 4. Later phase of spermatogenesis: Frequency of meiotic figures has passed the peak. Spermatocytes in lobule walls have decreased. Lobules are large and expanded with sperm.

Phase 5. Full ripe: Lobules are filled with spermatozoa. Lobule walls have become thin and contain few spermatogonia.

The developments of the gonads under natural conditions. The developmental phases of the gonads of yoshinobori under natural conditions from December 3, 1984 to June 17, 1985 are shown in Fig. 4. Samples were collected from lake shores and three inlet rivers. Onset and further progress of vitellogenesis and spermatogenesis were not synchronous among individuals. Ovary of Phase 2 or 3, early signs of yolk accumulation, was found in one of the two specimens caught in the Yamazaki River on February 20 and five of the eight in the same river on March 19, when the water temperatures were 8.5°C and 9.6°C respectively, a little higher than other stations (6.8°C and 7.0°C). Precocious females reached pre-spawning stage in late May. To complete oogenesis from the onset of yolk accumulation, it seemed to take around two months in this season.

In the ovaries of the second largest specimen collected from the Yamazaki River on May 25 and the second largest one from the same river on March 19, atretic follicles and the disorganized stromata with hypertrophic granulosa cells still remained. Postovulatory empty follicles, however, were not found and the atretic follicles had already undergone strong degeneration and absorption. These features indicate that the specimens had probably spawned last year and had almost recovered at that time. The exact times of the preceding spawnings, however, were not known. Females just after spawning were not found on May 25.

The development of testes started earlier and went on slower than that of ovaries. In the testes of the larger males, meiotic figures could be seen

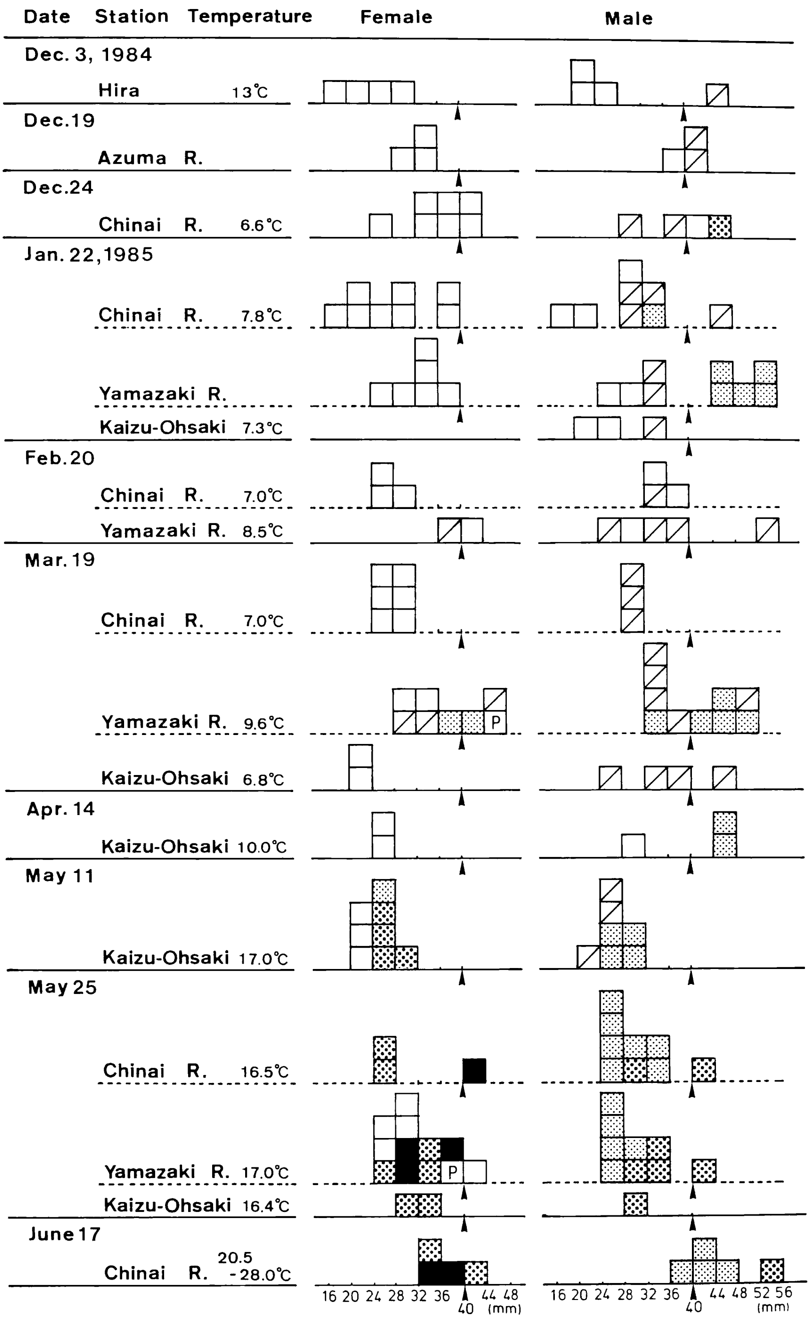

Fig. 4. Gonadal development of yoshinobori in the natural habitat. Each square shows one individual. Horizontal axes indicate size (standard length) of fish in mm. Arrows indicate 40 mm. For symbols see Fig. 5.

as early as in December. The smaller males, however, seemed to begin spermatogenesis later. It was not before March that the spermatocytes could be found in all specimens. In late May, some larger precocious individuals reached the final stage of spermatogenesis.

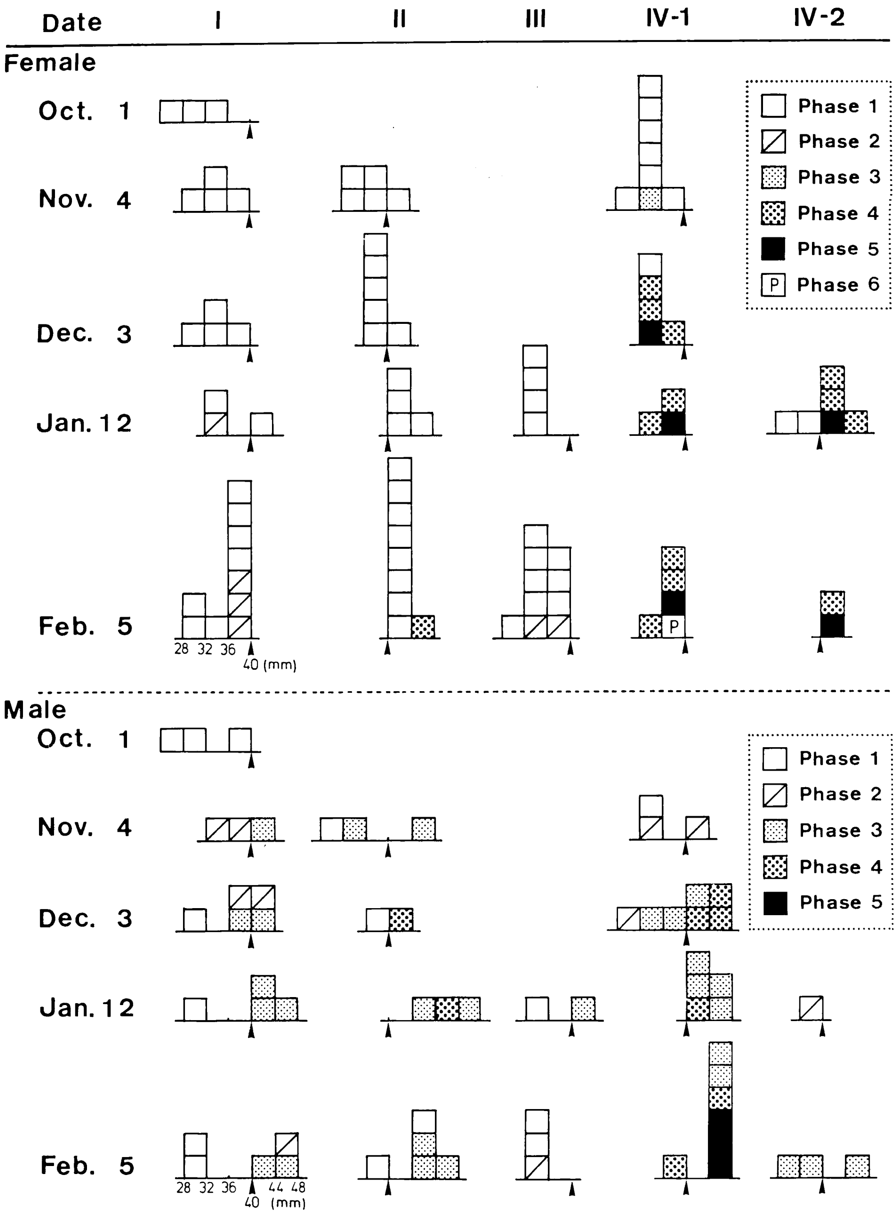

Fig. 5. Gonadal development of yoshinobori in the experiments.

Many deposited egg masses and brood-caring males were observed on June 17, 1985 in Chinai River. Under one stone, a pair and an egg mass were found. The egg mass consisted of two developmental stages, eyed and non-eyed eggs. The eyed eggs began hatching when they were taken out of water. Testes of the male were identified to be in Phase 4, i.e. later phase of spermatogenesis. Ovaries of the female were in Phase 5, i.e. the oocytes were in ripe stage and a few of them had been ovulated.

Results of temperature-daylength experiments. Fig. 5 shows the results of these experiments. Temperature-daylength condition of Experiment I was similar to natural fall-winter condition. Progress of vitellogenesis and spermatogenesis of Experiment I, therefore, were also similar to that of natural populations of yoshinobori shown in Fig. 4.

In the females of Experiment IV-1, however, one of the eight specimens had already started vitellogenesis (Phase 3) after only one month of treatment (on November 4). Rapid vitellogenesis progressed thereafter. One more month later, on December 3, four out of the five sacrificed females had ovaries at Phase 4 or 5 and deposited eggs were found in the experimental tank. Females in Experiment IV-2 matured more rapidly than in Experiment IV-1, i.e. after one month of treatment (on January 12), four out of six specimens showed stage 4 or 5. Neither the high temperature (Experiment II) nor long daylength (Experiment III) promoted the vitellogenesis by itself. Combination of high temperature and long day alone elicited the onset of vitellogenesis.

In the case of the testes, temperature-daylength regimes had effects similar to that of the ovary. The testes exposed to high temperature-long day regime (Experiment IV-1) developed more rapidly than others. The effects on the testes, however, were not so evident as on the ovary. In most of the specimens, the spermatogenesis started already in November and proceeded slowly in winter even under low temperature and/or short day conditions. High temperature-long day regime was not the triggering factor of meiosis but only an accelerator. There were no distinct differences among Experiments I, II and III. The high temperature or long day had no effect separately.

The difference in fish body size between experiments was due to the growth after the starts of the experiments.

Discussion

The results of the experiments carried out in fall and winter indicate that yoshinobori mature under high temperature-long day regime. If this applies to the spring period when they start to mature in the field, the onset of maturation of yoshinobori in the natural habitat is likely to depend on the long day and high temperature of the spring.

In the case of isaza, vitellogenesis starts triggered by descending water temperature of the epilimnion in autumn and progresses throughout the winter. Then follows synchronous spawning under rising water temperature in spring. In many teleost fishes, as in the isaza, environmental factors required for the gonadal maturation progressively change with the phases of the annual reproductive cycle (de Vlaming, 1974; Breton and Billard, 1977; Hanyu et al., 1982; Beggerman, 1985).

On the contrary, all the reproductive processes of yoshinobori, from beginning of vitellogenesis to spawning, proceed within a short period of time under high temperature-long day condition. yoshinobori, consequently, can and probably do actually spawn asynchronously and repeatedly in a long span of breeding season, as is sometimes with other fishes (de Vlaming, 1972; Shiogaki, 1981; Hirose and Kubo, 1983; Grossman and de Vlaming, 1984; Kaneko and Hanyu, 1985).

The period of time required to complete maturation of yoshinobori from start of vitellogenesis, however, may vary according to seasons and conditions. It took about two months in Experiment IV-1 conducted in October and less than one month in Experiment IV-2 carried out in December and about two months in the observed natural spring populations under gradually rising water temperature.

Roughly speaking, maturation of yoshinobori depends on the size of individual as has been reported in isaza (Takahashi, 1974) and many other species (Alm, 1959). Precocious fish are larger ones. In Experiment II, high temperature was likely to promote feeding and, consequently, growth. However, maturation probably inhibited growth of fish in Experiments IV. The smaller yoshinobori probably grow abruptly and mature within a short period of time to spawn following the larger ones under spring-summer high water temperature and abundant foods.

The comparison of the temperatures of the Yamazaki River on February 20 and March 19 to other survey stations shows that the minimum temperature for the onset of vitellogenesis is probably around 9°C. The fact that vitellogenesis had not started in eight of the nine females on February 5 in Experiment II while in the natural habitat it started on February 20 indicates that minimum daylength for the onset of vitellogenesis lies around 12 hours.

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琵琶湖産ヨシノボリ（橙色型）の成熟に及ぼす水温と日長の影響

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琵琶湖産ヨシノボリ（橙色型）の成熟の開始は，自然条件下では個体によってまちまちであるが，雌では3月から卵黄形成を開始するものが見られる．雄では早いものでは12月ごろから成熟分裂を行っているものがあるが，多くは3月ごろから成熟を始める．6月には多くの産着卵と保育中の雄が観察された．異なる水温と日長を組みあわせた実験によると，雌では高温長日条件下でのみ卵黄形成が始まり，1–2か月で産卵に至る．雄では低温あるいは短日の下でも成熟分裂を行うものがあるが，高温長日条件で精子形成が促進される．雌の成熟に必要な水温と日長の最低値は9°C，12時間前後であると思われる．

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