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**Ovarian Development and Changes in the Serum Vitellogenin
Levels in the River Sculpin, *Cottus hangiongensis*,
during an Annual Reproductive Cycle**

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Abstract

Annual changes in the ovarian development and serum vitellogenin concentration were investigated in the river sculpin, *Cottus hangiongensis*, sampled monthly from a river in southern Hokkaido, Japan. Ovarian development started advancing from summer and continued during the winter months until March with a maximum mean gonadosomatic index (GSI) of 15.99%. Hepatosomatic index (HSI) was also highest in March with a range of 5.13-5.95%. Spawning season usually occurred from April to May. Annual changes in serum vitellogenin level correlated very well with the patterns of GSI and HSI, as well as histological changes of the ovary. However, high serum vitellogenin was maintained in March and April.

Introduction

Among the several species of river sculpins distributed in southern Hokkaido, Japan, *Cottus hangiongensis* Mori spawns in the lower reaches of rivers during spring (Sato and Kobayashi, 1953; Goto, 1981). This species is polygynous (Goto, 1987a), and some males inhabiting the upper reaches of a river in southern Hokkaido remain sexually immature during the spawning period, although they attain larger body size than mature males found in the spawning ground (Goto, 1974, 1987b).

Histological investigations on the gonad of this river sculpin have mainly dealt with males. These include studies on mature and immature testes of adult males during the spawning period (Goto, 1974; Usui and Goto, 1987) and annual changes in the testicular activity (Quintio et al., 1988). The latter also discussed the occurrence of aberrant spermatids which resulted in the formation of spermatid masses.

There have been no reports on the histology of the ovary of this species. However, studies on the ovarian development during an annual reproductive cycle of *C. bairdi* (Hann, 1927) and two types of *C. nozawae* (Goto, 1978) were conducted.

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In the present study, we observed the histological changes of the ovary of *C. hangiongensis* during an annual reproductive cycle. Changes of serum vitellogenin concentrations during the annual cycle were also investigated.

Materials and Methods

Samples of female river sculpin, *Cottus hangiongensis*, were collected using a dip net once every month from July 1986 to June 1987 at their spawning ground in the Tobetsu River, near Hakodate in southern Hokkaido, Japan. Sample size was limited to 2-4 adult females per month in order to minimize impact on the limited population of this species in the sampling area. Collection was usually conducted between 10:00 and 13:00 hours around the middle of each month. The surface water temperature of the sampling site was measured to the nearest 0.2°C.

The sampled fish ranged from 7.1 to 18.8 g (mean 12.8 g) in body weight, from 7.9 to 12.1 cm (mean 10.3 cm) in total length and from 6.1 to 9.9 cm (mean 8.6 cm) in standard length, which always exceeded the minimum size at which females of the river sculpin attain their first maturity (Goto, 1984). After anesthetizing the fish with a solution of *p*-aminobenzoate, body weight, total length, and standard length of each fish were measured, and blood was taken from the caudal vasculature by cutting off the tail. The blood was then centrifuged and the serum was stored at -40°C until analysis. The ovary was then dissected out, weighed, and preserved in Bouin's fluid. Tissues were dehydrated in an alcohol series and embedded in paraffin. Representative 7 μm cross-sections were stained with Delafield's hematoxylin and eosin. Gonadosomatic index (GSI) was calculated as :

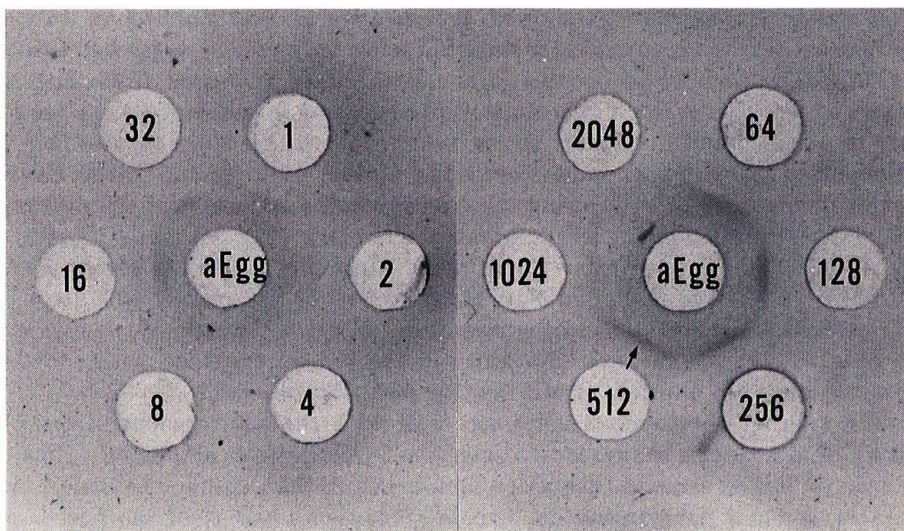


Fig. 1. A double immunodiffusion of serial dilutions of female *Cottus hangiongensis* serum. Numbers represent the dilution factor of the well. aEgg, antiserum of egg extract of the rockfish, *Sebastes taczanowskii*. Arrow shows the clearly noticeable precipitin line.

$$\text{GSI (\%)} = \frac{\text{gonad weight}}{\text{body weight}} \times 100$$

The liver was also dissected out and weighed in order to calculate the hepatosomatic index (HSI), which was computed as :

$$\text{HSI (\%)} = \frac{\text{liver weight}}{\text{body weight}} \times 100$$

The dilution titer of vitellogenin was measured using the double immunodiffusion method (Ouchterlony, 1953). The antiserum used was raised against an egg extract of the rockfish, *Sebastes taczanowskii*, injected into a rabbit. A clear precipitin line was observed with the serum of *C. hangiongensis* against antiserum of *S. taczanowskii* (Fig. 1). Serial dilutions of the sculpin serum were placed in different wells and the dilution titer was determined at the dilution where the precipitin line was still clearly noticeable as shown in Fig. 1.

Results and Discussion

The GSI and HSI of *C. hangiongensis* underwent regular changes during the year (Fig. 2). Mean GSI was lowest in August (0.61%) and gradually increased until February (9.17%), then abruptly increased reaching a prominent peak in March (15.99%). GSI was still relatively high in April ranging from 11.28% to 15.81% after which a sudden drop to 3.74% occurred in May.

A similar pattern was observed in the change of mean HSI. Mean HSI was lowest in August (1.72%), then slowly increased reaching a peak in March with a range of 5.13–5.95%, and later decreased from April to June with a mean of 5.15% and 2.64%, respectively.

Histological observations showed that ovaries of fish sampled in July had mostly oocytes at the peri-nucleolar stage but a few yolk vesicle stages were also seen (Fig. 3a). The number of oocytes at the latter stage increased from August to October while those at the former stage were decreasing in number. A section of an ovary of a fish caught in October shows mostly yolk vesicle stage oocytes (Fig. 3b). In November, primary yolk stage oocytes had already occurred and further development of the ovary was seen during the winter months. Oocytes at the primary to tertiary yolk stages were most abundant in February (Fig. 3c) while those at the yolk vesicle and peri-nucleolar stages were minimal. Spawning season usually occurred from April to May. In May, many oocytes at the tertiary yolk stage were still observed while the other stages were few. Moreover, atretic oocytes were also seen (Fig. 3d). Then in June, the ovary of the fishes were filled with oocytes at peri-nucleolar stage and few atretic oocytes were also observed.

The ovarian development correlates well with the development of testicular activity of this species as previously described by Qunitio et al. (1988). However, the peak of GSI in females occurs one month earlier than that in the male. As in the male, gonadal development in female *C. hangiongensis* is of the post-nuptial type.

The pattern of dilution titer of vitellogenin in the river sculpin is shown in Fig. 4. Vitellogenin level started to increase in October and rapidly increased from January to March. Higher levels were maintained in March and April which

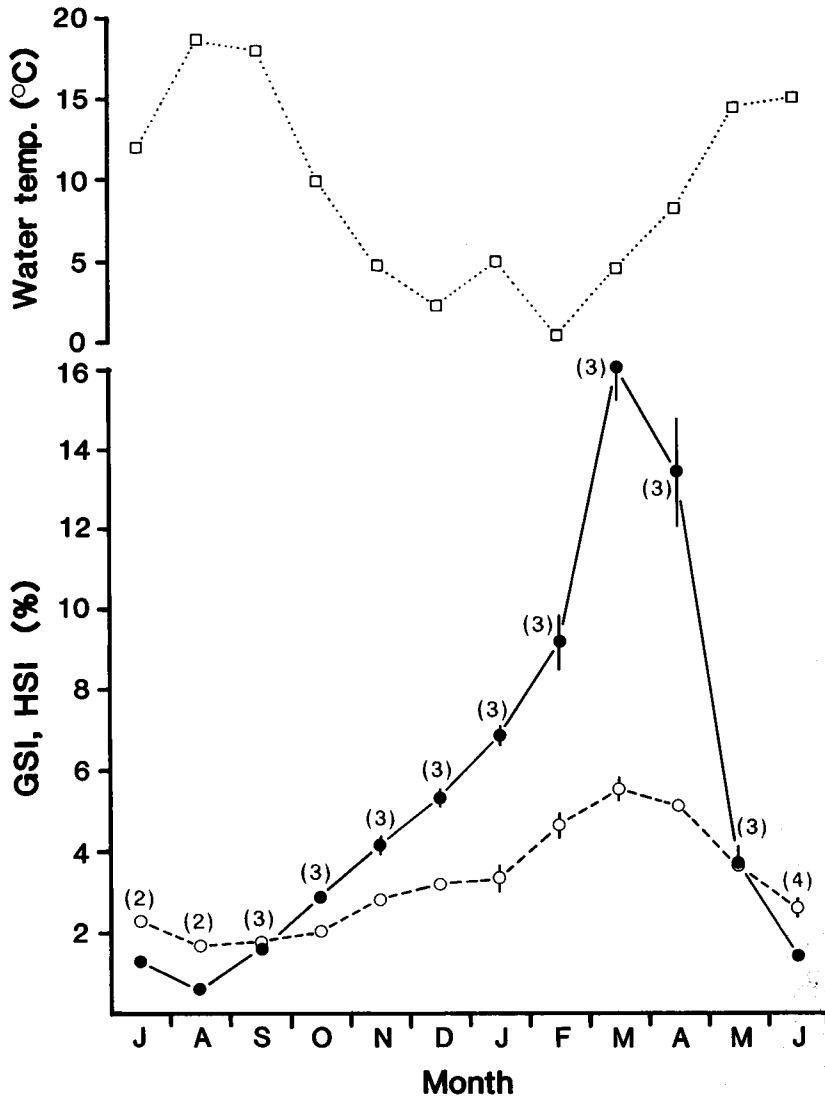


Fig. 2. Seasonal changes in the gonadosomatic index (GSI, ●—●) and hepatosomatic index (HSI, ○---○) of female *Cottus hangiongensis* collected from the Tobetsu River and water temperature (□-----□) of the sampling site.

decreased starting in May. The pattern of change in serum vitellogenin concentration of this species correlated very well with that of GSI and HSI, as well as the histological changes of the ovary.

When vitellogenic oocytes were abundant in the ovary, GSI, HSI and serum vitellogenin concentration were already high. The GSI pattern of this sculpin is similar to that of the small-egg type of *C. nozawae* (Goto, 1978), wherein the GSI of both species reached a peak in March. Simultaneously, HSI also reached a peak in

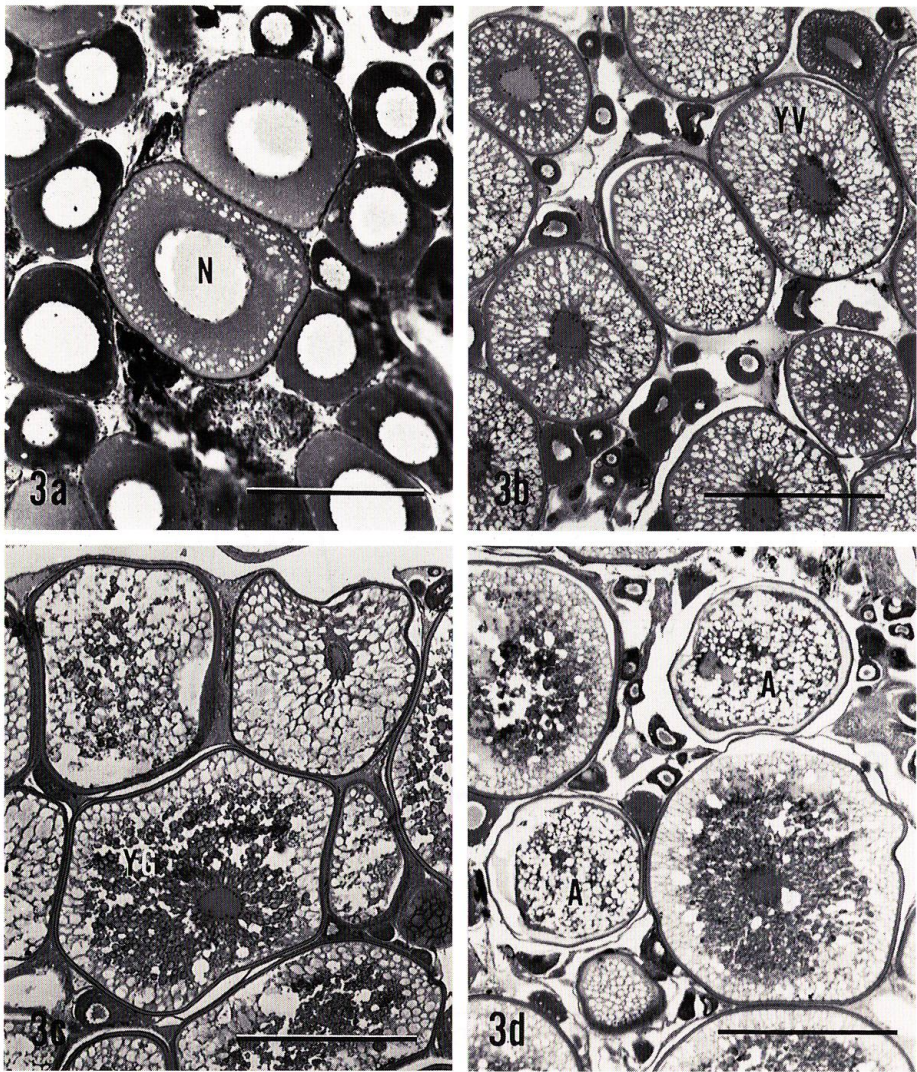


Fig. 3. Cross-sections of ovary of *Cottus hangiongensis*. (a) A section from a fish collected in July, showing oocytes mostly at the peri-nucleolar stage together with a few oocytes at the yolk vesicle stage. N, nucleus. Scale bar=200 μ m. (b) A section from a fish collected in October with oocytes mostly at the yolk vesicle stage. Scale bar=500 μ m. (c) A section from a fish collected in February showing abundance of oocytes at the primary to tertiary yolk stages. Scale bar=200 μ m. (d) A section of a fish collected in May, showing oocytes at the tertiary yolk stage together with some atretic oocytes. Scale bar=200 μ m.

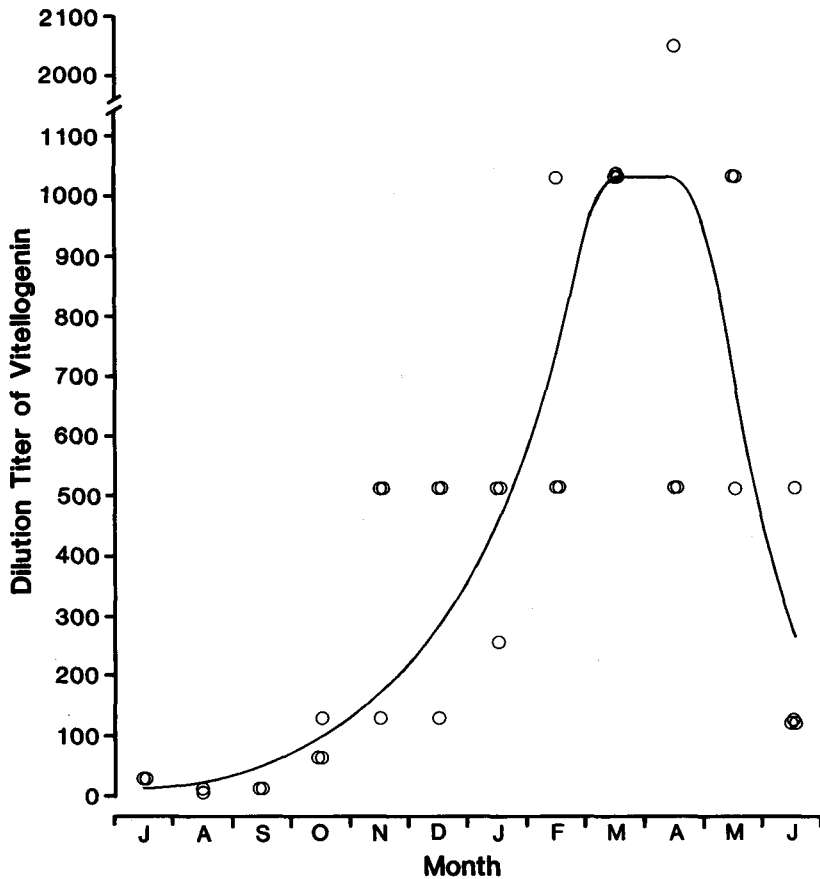


Fig. 4. Seasonal changes in dilution titer of serum vitellogenin of *Cottus hangiongensis* collected from the Tobetsu River.

March just before the onset of spawning season in *C. hangiongensis*. Moreover, serum vitellogenin concentration was also maximal at this period which indicates that the liver has a role in vitellogenin production (for review, see Ng and Idler, 1983). However, serum vitellogenin concentration remained at high levels until April of the spawning season while GSI and HSI slightly decreased in April and then abruptly decreased thereafter. The slight decrease in GSI may be due to partial release of ovulated oocytes and maintenance of high vitellogenin levels demonstrates that vitellogenesis is still undergoing in other ovarian oocytes. Goto (1987a) suggested that some females of this river sculpin spawn twice during the spawning season unlike in other sculpins which are only once, such as in *C. bairdi* (Simon and Brown, 1943; Brown, 1982), *C. nozawae* (Goto, 1982) and *C. amblystomopsis* (Goto, 1983). Multiple spawning per year has also been reported in the bullhead, *C. gobio*, which inhabits a stream in southern England (Fox, 1978).

Our observations on the annual changes of serum vitellogenin concentration of *C. hangiongensis* is similar to that of the loach, *Misgurnus anguillicaudatus*, as

reported by Teranishi et al. (1981). Female loach also spawn several times during the season and in this connection high levels of vitellogenin were also maintained during the spawning period (Teranishi et al., 1981).

It is also interesting to note that onset of vitellogenesis in this river sculpin occurred when water temperature was high as in other temperate species that also spawn in spring (Lam, 1983). Vitellogenesis became very active when water temperature of the river had abruptly decreased and continued to be low during the winter months. Water temperature was 8.1–14.1°C during the spawning season in the spawning ground of the river sculpins sampled. Goto (1988) noted that the water temperature range, when spawning was observed in this species, was 11–12°C.

The process of ovarian development in *C. hangiongensis* undergoes a regular annual change which is similar to many fishes (Breder and Rosen, 1966; Ishida and Kitakata, 1982). Histologically, no abnormality could be observed in the ovary of this species unlike in the testis in which aberrant spermatids that resulted into spermatid masses had been observed (Quinitio et al., 1988). This suggests that there are some differences in sensitivity to certain environmental stimuli between the two sexes during the process of gonadal development.

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