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Citation	北海道大學水産學部研究彙報, 36(4), 163-169
Issue Date	1985-12
Doc URL	http://hdl.handle.net/2115/23886
Type	bulletin (article)
File Information	36(4)_P163-169.pdf



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Changes in Serum Levels of Estradiol-17 β and Testosterone in the Japanese River Lamprey, *Lampetra japonica*, in the Course of Sexual Maturation

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Abstract

Serum levels of estradiol-17 β and testosterone were measured by specific radioimmunoassay methods in adult female and male Japanese river lampreys, *Lampetra japonica*, in various phases of sexual maturation during their spawning migration. Serum estradiol levels in female lampreys elevated, during the first half of the anadromous migration, concurrently with a gradual increase of gonadosomatic indices accompanying a gradual progress of vitellogenesis in ovarian oocytes. A considerably high level of serum estradiol was observed to occur in females at a later period of vitellogenesis. At the spawning period, serum estradiol levels in females fell to the level found at an early vitellogenic phase. Serum estradiol in male lampreys followed a similar pattern of change to that of females in the course of upstream migration, with nearly the same levels as in females, but retained a significantly higher level in spermiating males compared with that in females before and after ovulation. Testosterone was in no case detectable in the sera of both males and females of the lamprey. These findings were discussed in terms of possible physiological roles played by the sex hormones in reproduction of the lamprey.

In lampreys like other nonmammalian vertebrates, sex hormones are considered to be implicated in various aspects of reproduction such as the development of gonads and secondary sex characters (for review see Hardisty, 1979, and Gorbman, 1983). There remain, however, some problems to be solved in order to establish the roles of sex hormones in the reproduction of lampreys. Circulating estradiol-17 β has been identified in male sea lampreys, *Petromyzon marinus*, at concentrations similar to that found in females (Katz *et al.*, 1982; Sower *et al.*, 1985). Plasma levels of the estrogen covaried in males and females of the sea lamprey during the prespawning period (Sower *et al.*, 1985). Circulating testosterone has also been shown to occur at relatively low concentrations in the sea lamprey of both sexes (Weisbart *et al.*, 1980; Sower *et al.*, 1985), but some authors have failed to detect the androgen in the blood of the same species of lampreys (Katz *et al.*, 1982). Although the recent study of Sower *et al.* (1985) has revealed interesting changes of plasma sex hormones in female and male sea lampreys during the prespawning and spawning periods, information about the profile of circulating sex hormones in lampreys seems still to be too slight to correlate it exactly with various aspects of reproduction, and the study needs to be extended to various species of lampreys

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other than the sea lamprey.

The Japanese river lamprey, *Lampetra japonica*, goes downstream to the sea after spending the larval, metamorphosis, and young adult stages in the river (Fukayama and Takahashi, 1982). Maturation of the gonad may begin in female lampreys at the marine parasitic phase and continues through the period of anadromous spawning migration lasting for about ten months. So far as we know, no report has been concerned with circulating sex hormones in the Japanese river lamprey. In the present study, serum levels of estradiol-17 β and testosterone were measured by specific radioimmunoassay methods in female and male Japanese river lampreys at various times of the spawning migration, and the change was considered in relation to their sexual maturation.

Material and Methods

Adult males and females of the Japanese river lamprey, *Lampetra japonica*, were collected by commercial fishermen in the course of their upstream spawning migration in the Ishikari River, central Hokkaido, during a period from November 1983 to June 1984. They measured about 43-53 and 42-53 cm in total length for males and females, respectively. In addition, 2 males and 7 females were captured in the Sea of Japan in 1984, and 2 males and 2 females among them, measuring 41.2 and 44.7 cm in mean total length, respectively, were successively kept alive for serum steroid determinations. They were transported to the laboratory as soon as possible and subjected to subsequent treatments.

Blood was taken from each lamprey under anesthesia by cutting off the caudal peduncle, and was collected in glass capillary tubes to be clotted at room temperature. Serum was then separated by centrifugation and stored at -45°C until steroid analyses. Gonads of lampreys were dissected out and weighed for calculation of gonadosomatic index (GSI, gonad weight/body weight $\times 100$). For histological determination of the sexual maturity of each lamprey, pieces of gonads were fixed in Bouin's fluid, sectioned at 4-6 μm in thickness, and stained with Delafield's hematoxylin and eosin.

Serum estradiol and testosterone concentrations were determined by radioimmunoassay according to the methods of Kagawa *et al.* (1981, 1982). Anti-estradiol-17 β -6-CMO-BSA antiserum was obtained from Teikoku Hormone Mfg. Co., Tokyo, and anti-testosterone-3-CMO-BSA antiserum from the National Institute for Basic Biology, Okazaki. The anti-estradiol antiserum cross-reacted with estradiol-17 β , estrone, estriol and testosterone at 100.00, 3.20, 1.77 and 0.29% levels, respectively. The anti-testosterone antiserum cross-reacted with testosterone, 11-ketotestosterone and androsterone at 100.00, 1.40 and 0.77% levels, respectively. (2, 4, 6, 7- ^3H) Estradiol-17 β and (1, 2, 6, 7- ^3H) testosterone, both obtained from Amersham Japan K.K., were used as antigens in the radioimmunoassay systems used.

The radioimmunoassays were validated by assaying replicate aliquots of lamprey serum pools to which known amounts of cold steroids had been added. The regression line observed between the estimated and expected values was $y=0.89x+2.98$ ($r=0.96$, $P<0.001$) for estradiol-17 β and $y=0.88x-0.32$ ($r=0.78$, $P<0.025$) for testosterone. BSA-PBS with different concentrations of the steroids was similarly assayed for comparison, and the regression line formed between the observed and

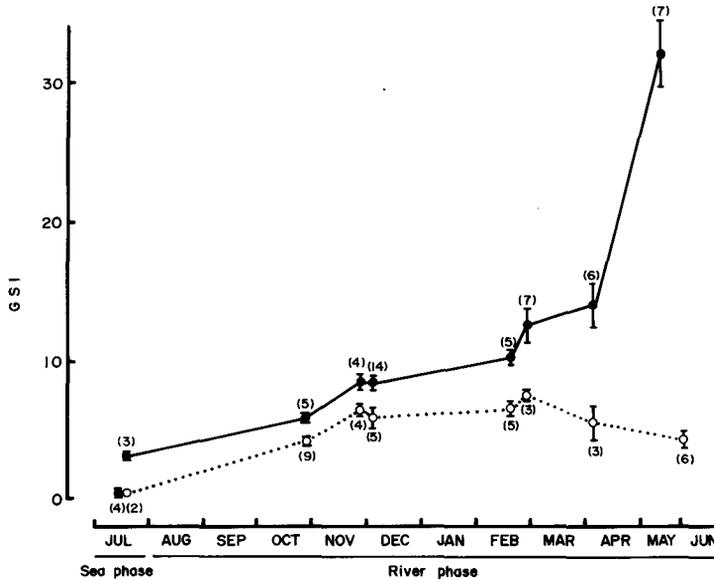


Fig. 1. Changes of gonadosomatic indices (GSI) of female (●) and male (○) Japanese river lampreys during their anadromous spawning migration. Numerals in parentheses indicate the number of lampreys examined, and vertical bars show SEM.

expected values was $y=0.95x+0.01$ ($r=0.96$, $P<0.01$) for estradiol-17 β and $y=1.62x-0.65$ ($r=0.92$, $P<0.05$) for testosterone. It was thus confirmed that the estimated levels of the steroids in lamprey serum were correlated very closely with the amounts of steroids added, indicating the accuracy of the assays using lamprey serum as the sample.

Comparisons of data for serum steroid levels, using Student's *t*-test or Cochran-Cox test, were considered statistically significant if $P<0.05$.

Results

Changes of GSI values of the lamprey of both sexes during their spawning migration are shown in Fig. 1. Four out of 7 female lampreys captured in the sea were sexually immature with previtellogenic oocytes in the ovary measuring 1.22 ± 0.16 in GSI. The remaining 3 females had already started vitellogenesis in ovarian oocytes, showing a higher GSI value of 3.21 ± 0.08 . During the period of upstream migration, mean GSI values in female lampreys increased gradually as vitellogenesis progressed, reaching 14.03 ± 1.49 in April when ovarian oocytes were near to the end of exogenous vitellogenesis. Then there occurred a sharp increase in GSI value in female lampreys at the spawning period, and the increase of GSI values was well in accord with an increase in the absolute weight of ovaries which averaged 29.6 and 60.1 g in April and July, respectively.

Two males captured in the sea had testes with many spermatogonia undergoing mitotic proliferations, revealing GSI value of about 1.17. Testes of male lampreys captured in the river in October were still at the spermatogonial multiplication stage

Table 1. Serum concentrations of estradiol-17 β in adult female and male Japanese river lampreys measured at various times of their anadromous spawning migration.

Date of capture	Serum estradiol-17 β level (ng/ml)		Remarks
	Female	Male	
20 Jul. 1984	0.44 \pm 0.08 (2)*	0.79 \pm 0.16 (2)*	captured in the sea
25 Nov. 1983	1.25 \pm 0.27 (4)	1.85 \pm 1.27 (3)	captured in the river
3 Dec. 1983	1.06 \pm 0.18 (7)	1.03 \pm 0.32 (3)	"
18 Feb. 1984	3.22 \pm 0.96 (5)	4.52 \pm 1.26 (5)	"
24 May 1984	0.51 \pm 0.18 (2)	—	after spawning
2-10 Jun. 1984	0.96 \pm 0.33 (9)	—	ready to ovulate
4-14 Jun. 1984	—	2.73 \pm 0.38 (3)	spermiating

* Numerals in parentheses indicate the number of lampreys measured.

but showed a steady increase in mean GSI value measuring 4.20 ± 0.22 . Appearance of spermatocytes was first noticed in the testis examined in February when mean GSI value was 6.72 ± 0.31 . Males sampled in April were observed to be undergoing active spermatogenesis and spermiogenesis, but tended to lower their mean GSI value to 5.64 ± 1.16 . Mean GSI value as well as mean absolute weight of the testis became smaller in sexually matured males than in sexually maturing males observed at the preceding period.

Serum concentrations of estradiol-17 β showed some change both in female and male lampreys during their spawning migration (Table 1). Estradiol was apparently detectable, though low in level, in the serum of vitellogenic females captured in the sea. Serum estradiol levels were significantly higher in females migrating in the river in November and December when vitellogenesis in ovarian oocytes was proceeding further. In late February, with the advancement of vitellogenesis, serum estradiol levels in females became much higher than those found in December. A significant decrease in serum estradiol levels was observed to occur in females at the spawning period, but levels were still comparable to those in vitellogenic females captured in the sea.

Estradiol-17 β occurred also in the serum of all male lampreys examined, including those captured in the sea (Table 1). Serum estradiol levels of male lampreys followed a closely similar pattern of change to those of females through the period of sexual maturation, though individual variations in the level of the males appeared somewhat larger than those of the females. Serum estradiol in males tended to be retained at slightly higher levels than in females, and attained the largest level of 4.52 ± 1.26 ng/ml in February, simultaneously with that in females, when meiotic changes of testicular germ cells occurred. A drop in serum estradiol levels was observed also in males at the spawning period, but the level was significantly higher ($P < 0.01$) in spermiating males as compared with that in females ready to ovulate.

Unexpectedly, testosterone was never detectable in the serum of male and female lampreys examined, being, if present at all, below the minimum level of detection (30 pg/ml) in the radioimmunoassay system used in the present study.

Discussion

The results of the present study indicate that, in the Japanese river lamprey, *Lampetra japonica*, serum levels of estradiol-17 β in adult females changed during their anadromous spawning migration. In the female, vitellogenesis in ovarian oocytes, which had started to occur at some time of their marine life, proceeded through the period of upstream migration. This was accompanied by a gradual and steady increase in GSI values until April when exogenous vitellogenesis in ovarian oocytes was nearing completion. An elevation of serum estradiol levels in females appeared to occur concurrently with the increase in GSI values. Pickering (1976) has experimentally demonstrated that, in the river lamprey *Lampetra fluviatilis*, estradiol may increase the weight of ovaries by stimulating yolk production in oocytes. These findings may suggest a possible relationship of circulating estradiol to the maturation of ovaries in lampreys. A female-specific serum protein, possibly vitellogenin, was immunologically detected in the blood of vitellogenic females of both the Japanese river lamprey and the sand lamprey, *Lampetra reissneri*, by our previous study (Fukayama *et al.*, 1985), and the occurrence of the protein was successfully induced by the treatment of adult male sand lampreys with estradiol-17 β (Fukayama *et al.*, unpublished observation).

Evenett and Dodd (1963) and Larsen (1974) have suggested that the development of secondary sex characters in female *L. fluviatilis* may be dependent on sex hormones. According to our observations on the Japanese river lamprey, the appearance of female post-anal fins and a change in the form of dorsal fins occur in some females in April just prior to the spawning period. A high level of circulating estradiol observed in females in February is likely to act to stimulate the development of such female sex characters. Piavis *et al.* (1975) also suggested that, in the sea lamprey, *Petromyzon marinus*, circulating estradiol measured by radioimmunoassay tended to increase with increasing maturity scored on the degree of development of secondary sex characters.

The level of serum estradiol in Japanese river lampreys was significantly lower at the spawning period than at the preceding vitellogenic period. A similar decrease in circulating estradiol has been reported to occur during the process of ovulation in the sea lamprey, *P. marinus* (Piavis *et al.*, 1975). A recent study of Sower *et al.* (1985) on the sea lamprey also showed the lowest level (about 0.6 ng/ml) of plasma estradiol attained at the time of ovulation. This level appears to be equal to that measured in female Japanese river lampreys before and after ovulation (0.51–0.96 ng/ml). Sower *et al.* (1985) consider the decrease in estradiol level in relation to the elimination of negative feedback inhibition of pituitary gonadotropin release at the time of ovulation. Female sea lampreys at the final spawning period displayed apparent fluctuations of plasma estradiol levels ranging from about 0.6 to about 2.8 ng/ml (Sower *et al.*, 1985). A possible occurrence of such a fluctuation in serum estradiol levels in the Japanese river lamprey might explain the wide individual variation of the levels found in February during the late vitellogenic period.

It is not unexpected that serum estradiol-17 β was detectable in male Japanese river lampreys at similar levels to that in females, since Katz *et al.* (1982) have already detected estradiol in male plasma of the sea lamprey, *P. marinus*, at a comparable level to that present in female plasma and Callard *et al.* (1980) have

revealed the capability of the testis of the sea lamprey to synthesize estradiol in an *in vitro* condition. It is quite interesting to note, however, that serum estradiol levels in male Japanese river lampreys during the upstream migration followed substantially the same pattern of increase as those in females, and that the level in spermiating males was lowered but remained significantly higher than that in females ready to ovulate. The finding seems to be quite consistent with the results of a study by Sower *et al.* (1985) on the change of plasma estradiol levels in female and male sea lampreys during their prespawning and spawning periods. It is probable that some environmental changes, which may act to cause the change of serum estradiol levels in female lampreys during sexual maturation, possibly by modulating pituitary gonadotropic activities (Sower *et al.*, 1983), may exert a similar influence on male lampreys resulting in the same profile of serum estradiol level in the male.

Whether the circulating estrogen in males has any physiological significance in the reproduction of lampreys is quite uncertain at present. Our previous observations indicated that the coelomic epithelium of the sand lamprey, *L. reissneri*, became thickened and ciliated with some indication of secretory activity in males as well as in females near and at the spawning period (Fukayama and Takahashi, 1983). Treatment of young adult sand lampreys of both sexes with estradiol was effective in inducing similar changes of the epithelial cells (Fukayama *et al.*, unpublished observation). The coelomic epithelium of the Japanese river lamprey also displayed hypertrophy and ciliation in both sexes by the spawning period. These findings lead us to suppose a physiological role of circulating estradiol in the change of the coelomic epithelium, which may favour the maintenance of gametes shed into the coelomic cavity, in the Japanese river lamprey of both sexes. Katz *et al.* (1982) suggest that "the equally high titers of estradiol in both sexes are related to the widespread occurrence of estradiol target cells in the brain and various organs of lampreys of both sexes."

In the present study, testosterone was never detected in any of the blood samples obtained from both male and female lampreys. Kime and Rafter (1981) could not reveal *in vitro* biosynthesis of testosterone from progesterone in the testis of migrating adult river lampreys, *L. fluviatilis*, and Katz *et al.* (1982) could not detect testosterone, by a specific radioimmunoassay, substantially in any blood sample obtained from sexually mature sea lampreys, *P. marinus*, of both sexes. However, Weisbart *et al.* (1980) and Sower *et al.* (1985) have demonstrated, by double-isotope derivative assay and radioimmunoassay, respectively, the occurrence of testosterone in the blood of migrating males and females of the sea lamprey. The discrepancy of the results might be ascribed to differences in the species and sexual maturity of animals examined and further in the specificity of the assay methods used in these studies. Weisbart *et al.* (1980) stated that the presence of a relatively high amount of testosterone (about 1 ng/ml) in sea lampreys may reflect the physiological importance of the hormone. In this context, it is interesting to refer the study of Larsen (1974), who showed the effectiveness of exogenous testosterone in causing the development of male secondary sex characters in the river lamprey, *L. fluviatilis*.

Acknowledgements

We thank Mr. S. Nishiuchi and the crew of the research ship Hoku-yomaru, Wakkanai Fisheries Experimental Station, Hokkaido, for their kind help in collecting adult specimens of the Japanese river lamprey in the sea. We are greatly obliged also to Dr. Y. Nagahama, National Institute for Basic Biology, Okazaki, and to Dr. A. Kambegawa, Teikyo University School of Medicine, Tokyo, for the donation of the antitestosterone antiserum for our study.

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