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Author(s)	Sato, Ayako; Ueda, Hiroshi; Fukaya, Masahiro et al.
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Sexual Differences in Homing Profiles and Shortening of Homing Duration by Gonadotropin-Releasing Hormone Analog Implantation in Lacustrine Sockeye Salmon (*Oncorhynchus nerka*) in Lake Shikotsu

Ayako Sato¹, Hiroshi Ueda^{1*}, Masahiro Fukaya^{1,2}, Masahide Kaeriyama³,
Yonathan Zohar⁴, Akihisa Urano⁵ and Kohei Yamauchi²

¹Toya Lake Station for Environmental Biology, Faculty of Fisheries,
Hokkaido University, Abuta, Hokkaido 049-57, Japan

²Department of Biology, Faculty of Fisheries, Hokkaido University,
Hakodate, Hokkaido 041, Japan

³Research Division, National Salmon Hatchery, Fisheries Agency of Japan,
Sapporo, Hokkaido 062, Japan

⁴Center of Marine Biotechnology, University of Maryland Biotechnology Institute,
Baltimore, Maryland 21202, USA

⁵Division of Biological Sciences, Graduate School of Science, Hokkaido University,
Sapporo, Hokkaido 060, Japan

ABSTRACT—Adult sockeye salmon (*Oncorhynchus nerka*) in Lake Shikotsu were captured in September, October and November adjacent to their natal hatchery prior to spawning. They were sampled for hormones, tagged and released in the center of lake. Fish were again sampled at recapture to characterize changes in steroid hormone levels in individual migrants as well as homing percentage and duration in each month. All males returned faster than females early in the breeding season, although a half of the tagged males did not return to the natal site late in the season (November). A high percentage of females always returned, and homing duration shortened late in the season. In males, the shortening of homing duration coincided with an increase in serum testosterone (T) and 11-ketotestosterone levels. In females, the shortening of homing duration corresponded to an elevation of serum T and 17 α ,20 β -dihydroxy-4-pregnen-3-one (DHP) levels, and a drop in serum estradiol-17 β levels. Sustained administration of gonadotropin-releasing hormone analog (GnRHa, via implants) in September greatly shortened homing duration, especially in females. GnRHa treatment caused a dramatic increase in serum DHP levels in both sexes on average. Individual GnRHa-treated males which rapidly returned, however, showed higher serum T levels and lower serum DHP levels than slower returning males. The present study indicates sexual differences in homing profiles as well as shortening of homing duration following GnRHa implantation in lacustrine sockeye salmon in Lake Shikotsu which may be reflective of changes in serum steroid hormone levels.

INTRODUCTION

Anadromous salmonids have an amazing ability to return to their natal site for reproduction. Salmonid homing behavior is considered to be closely related to gonadal maturation which is regulated by endocrine systems, mainly the hypothalamo-pituitary-gonadal axis. Briefly, gonadotropin-releasing hormone (GnRH) controls gonadotropin (GtH) release from the pituitary gland. GtHs induce steroidogenesis in the gonads, and these steroid hormones stimulate gametogen-

esis and final maturation. Despite many studies on the changes in steroid hormone profiles during various kinds of migration in salmonids (Ueda and Yamauchi, 1995), the precise roles of steroids in salmonid homing behavior are still unclear. One of the reasons for this uncertainty is the lack of a suitable model system to examine homing behavior in salmonid fishes.

In Lake Shikotsu, Hokkaido, Japan, juvenile sockeye salmon (*Oncorhynchus nerka*) are released from a hatchery into the lake and attain full growth in 3–5 years. These fish then return to their natal hatchery to breed with high homing accuracy. Although the active breeding period starts in the middle of October, some maturing adults gather near the shore of the hatchery as early as September, and stay there until

* Corresponding author: Tel. +81-142-75-2651;
FAX. +81-142-75-2943.

November. Intensive experiments to clarify the homing mechanisms of salmonids can be carried out using these returners over a 3 month period. Typically, fish are captured adjacent to the hatchery, tagged and released in the center of lake. When tagged fish return, they are recaptured and homing duration and percentage are calculated.

The aims of the present study were to investigate changes in homing percentage and duration of lacustrine sockeye salmon in Lake Shikotsu on a monthly basis, and to measure serum levels of steroid hormones, including $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (DHP), estradiol- 17β (E_2), 11-ketotestosterone (11KT), and testosterone (T) in individual fish both at the time of initial capture (release) and recapture at the hatchery (return). The present study also examined the effects of GnRH analog (GnRH α) implantation on homing duration and percentage and on serum steroid hormone levels in fish caught in September. This is relevant since GnRH α treatment has been shown to be highly effective in inducing GtH release, ovulation and spermiation in other teleost fishes (Zohar, 1996), and therefore might be expected to be involved in the reproductive homing behavior of salmonids.

MATERIALS AND METHODS

Fish

Male and female adult lacustrine sockeye salmon of 3-5 years old were caught using a large stationary set net located near the shore of the hatchery (Fig. 1). Every month from September to November, 1996, 9-12 males and females were captured, anesthetized with 0.002% ethyl m-aminobenzoate methanesulfonate (Nakarai, Kyoto, Japan) and tagged with numbered disc. One ml of blood samples were collected from the caudal vasculature using a 1 ml syringe with 27 guage needle (Terumo, Tokyo, Japan) for radioimmunoassay (RIA) of steroid hormones. Tagged fish were held overnight in the hatchery and released in the center of Lake Shikotsu (7.5 km southeast of the hatchery; Fig. 1) in the following morning. The duration of time before the fish returned to the hatchery was determined by checking a small recapture set net (Fig. 1) on a daily basis. The percentage of homing was also calculated. After recapture, fish were anesthetized and the second 1 ml blood sample was collected.

GnRH α implantation

After tagging and blood sampling, 10 males and 10 females in September received a 2 mm implant capsule containing 75 μ g of GnRH α ([D-Ala⁶, Pro⁹NET]-GnRH) in an ethylene-vinyl acetate copolymer matrix (Zohar *et al.*, 1990; kindly donated by AquaPharm Technologies Corp., Columbia, Maryland, USA). The GnRH α implants were administered intramuscularly via a 17 guage needle. The GnRH α -implanted fish were subjected to procedures similar to those described above for the monthly sampled fish. The non-implanted fish sampled in September served as controls for this experiment.

RIA

Serum levels of steroid hormones (DHP, E_2 , 11KT, and T) were measured by the RIA methods of Young *et al.* (1983), Kagawa *et al.* (1982), Ueda *et al.* (1985), and Ueda *et al.* (1991). The intra-assay variances were 5.1%, 11.3%, 9.5%, and 6.5%, respectively; the sensitivity of the lowest detection level was 30 pg/ml in all assays.

Statistics

All data were expressed as means $\pm$ SEM. Statistical analyses were calculated by one-way factorial analysis of variance (ANOVA).

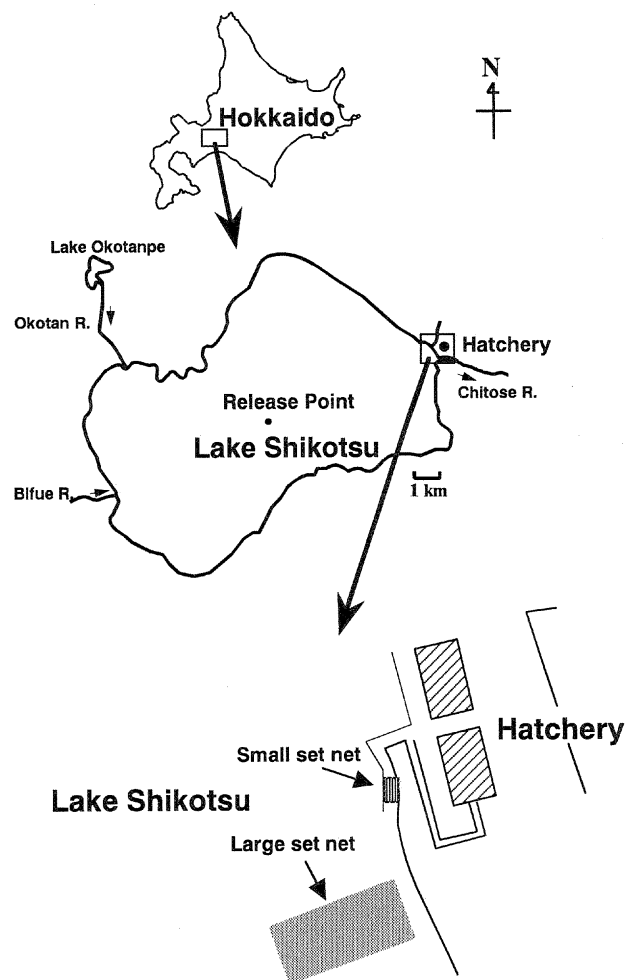


Fig. 1. Map of the Lake Shikotsu, Japan showing the release point and the hatchery (return point). Fish are first captured by the large set net near the shore of the hatchery, and later recaptured by the small set net at the mouth of the hatchery.

RESULTS

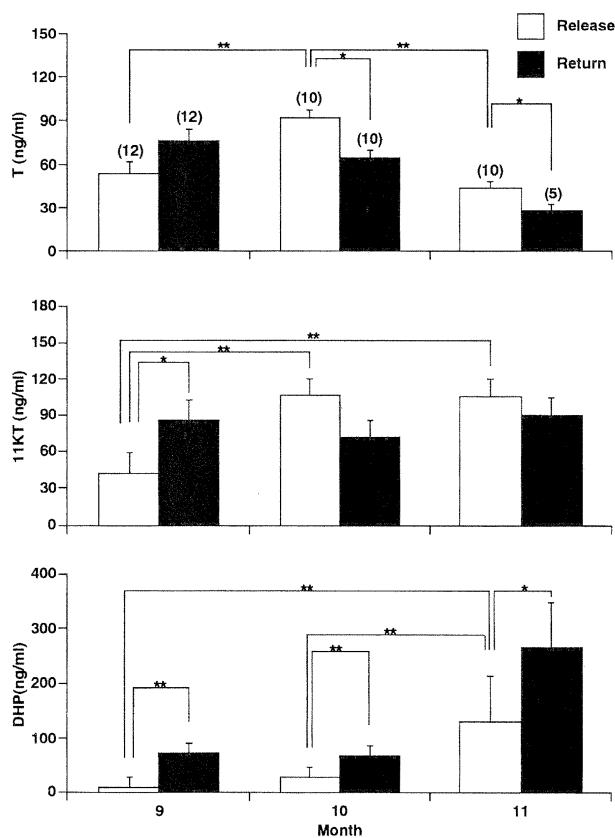
Monthly sample

Changes in homing percentage and duration from September to November are shown in Table 1. All males returned to the hatchery in September and October, but half of the tagged males did not return in November. In contrast, 78-90% of the females returned over the entire 3 month sampling period. Homing duration was significantly shortened from September to October in males and from October to November in females.

In males, serum T levels at the time of release in October were higher than those in September and November, and those at the time of return decreased in October and November from September levels (Fig. 2). Serum 11KT levels at the time of release were elevated in October and November over September levels. In September, 11KT was increased at the time of return over release levels. Serum DHP levels at the time of both release and return showed constant increases

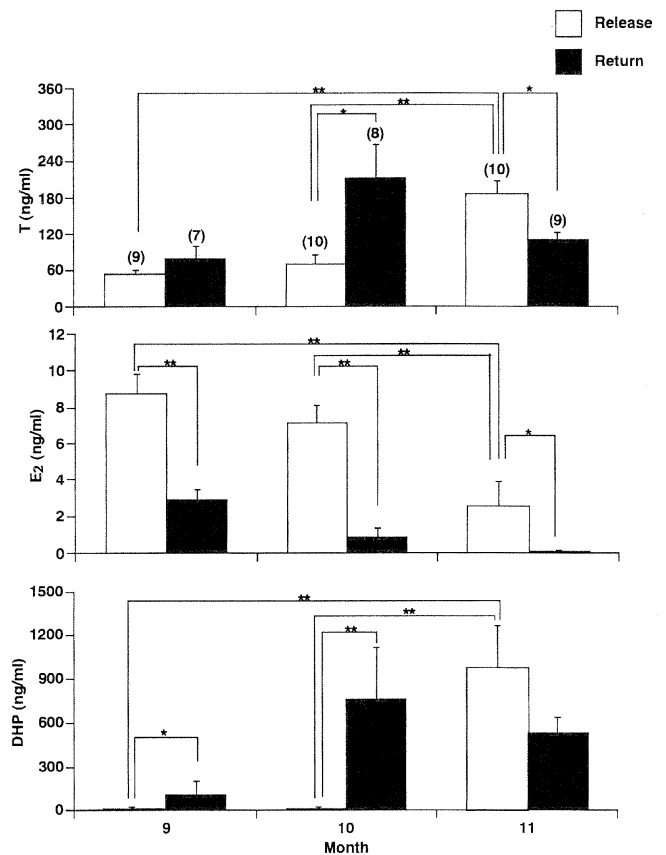
Table 1. Changes in homing percentage and duration of lacustrine sockeye salmon in Lake Shikotsu from September to November and GnRH α implantation (75 μ g/fish) in September

Sex	Month & Treatment	Age*	BW (g)*	No. of released fish	No. of returned fish (%)	Duration (days)*
Male	Sep.	4.3 $\pm$ 0.1	187.1 $\pm$ 4.3	12	12 (100)	14.8 $\pm$ 5.7
	GnRH α	3.9 $\pm$ 0.3	177.4 $\pm$ 4.4	10	9 (90)	10.1 $\pm$ 3.7
	Oct.	3.9 $\pm$ 0.2	188.4 $\pm$ 6.8	10	10 (100)	5.8 $\pm$ 3.1**
	Nov.	4.1 $\pm$ 0.1	182.5 $\pm$ 3.3	10	5 (50)	3.8 $\pm$ 1.1
Female	Sep.	4.2 $\pm$ 0.2	175.9 $\pm$ 5.2	9	7 (78)	21.4 $\pm$ 8.8
	GnRH α	4.0 $\pm$ 0.5	171.8 $\pm$ 4.5	10	10 (100)	12.9 $\pm$ 4.4***
	Oct.	4.2 $\pm$ 0.1	171.0 $\pm$ 3.5	10	8 (80)	18.5 $\pm$ 8.1
	Nov.	4.1 $\pm$ 0.1	174.4 $\pm$ 5.6	10	9 (90)	3.1 $\pm$ 1.1**

* Mean $\pm$ SEM.** $P < 0.01$ compared with the previous month.*** $P < 0.01$ compared with monthly sampled fish in September.**Fig. 2.** Changes in serum steroid hormone levels of male lacustrine sockeye salmon from September to November in Lake Shikotsu. White and black bars represent the mean $\pm$ SEM of the indicated number of samples shown in parenthesis at the time of release and return, respectively. Significant differences at 5% (*) and 1% (**) levels are indicated.

over the 3 month sampling period (Fig. 2).

In females, serum T levels at the time of release in November were higher than those in September and October, while levels at the time of return were highest in October and decreased in November (Fig. 3). Serum E₂ levels at the time

**Fig. 3.** Changes in serum steroid hormone levels of female lacustrine sockeye salmon from September to November in Lake Shikotsu. White and black bars represent the mean $\pm$ SEM of the indicated number of samples shown in parenthesis at the time of release and return, respectively. Significant differences at 5% (*) and 1% (**) levels are indicated.

of release in November were lower than those in September and October, and those at the time of return showed constant decreases from the initial capture levels over the 3 month sampling period. Serum DHP levels at the time of release in November were much higher than those in September and

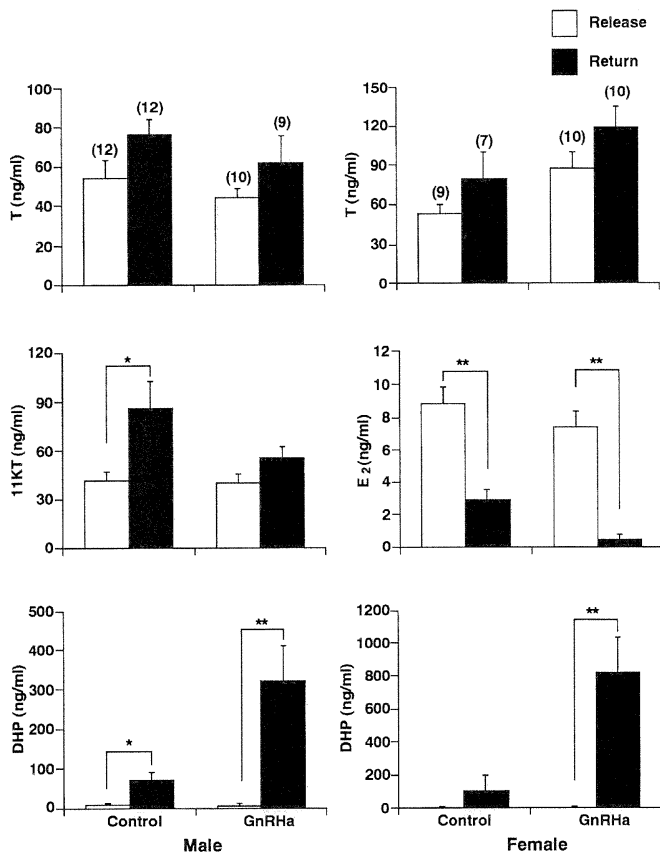


Fig. 4. Effects of GnRH α implantation on serum steroid hormone levels of male and female lacustrine sockeye salmon in Lake Shikotsu in September. White and black bars represent the mean $\pm$ SEM of the indicated number of samples shown in parenthesis at the time of release and return, respectively. Significant differences at 5% (*) and 1% (**) levels are indicated.

October, and those at the time of return increased in September and October (Fig. 3).

GnRH α implantation

The effects of GnRH α implantation on homing percentage and duration are shown in Table 1. No significant difference was observed in homing percentage in either sex as a result of implantation, whereas homing duration was shortened significantly in females.

GnRH α implantation did not cause any significant differences in serum T and 11KT levels in males or T levels in females either at the time of release or return. GnRH α induced a large elevation of serum DHP levels in both sexes at the time of return over initial capture levels (Fig. 4).

The relationship between serum steroid hormone levels in individual GnRH α -implanted and control fish at the time of return and homing duration revealed that serum T levels in rapidly returning GnRH α -implanted males were significantly higher than those in slowly returning individuals ($p < 0.05$; Fig. 5). Serum DHP levels in slowly returning males were significantly higher than those in rapidly returning males ($p < 0.05$).

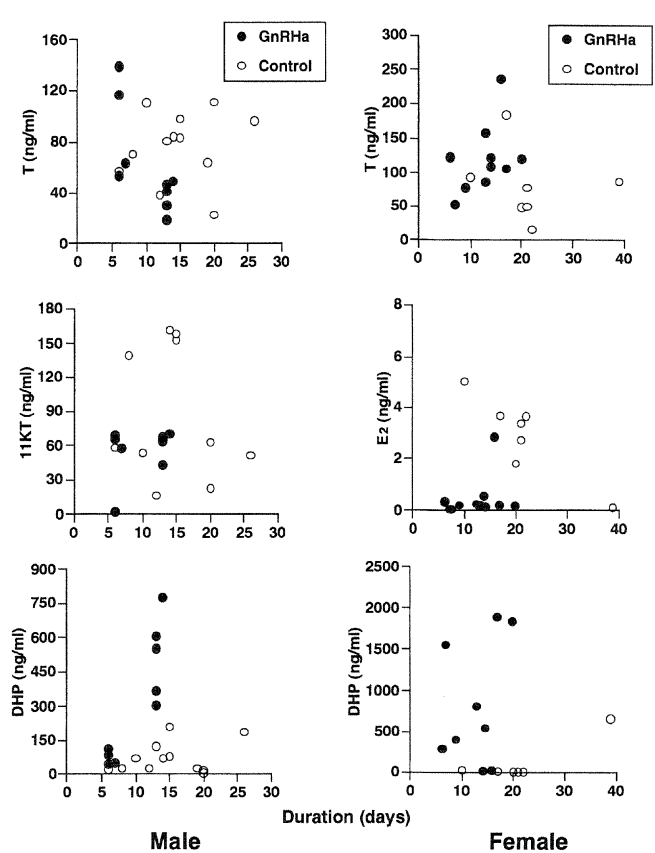


Fig. 5. Relationship between serum steroid hormone levels at the time of return and homing duration in individual GnRH α -implanted (closed circle) and control (open circle) lacustrine sockeye salmon in Lake Shikotsu.

In contrast, obvious relationships between hormone levels and behavior were not observed in control males and GnRH α -implanted or control females (Fig. 5).

DISCUSSION

The present study demonstrates for the first time that homing percentage and duration of lacustrine sockeye salmon in Lake Shikotsu are different between males and females during the spawning period. Males returned to the natal site faster than females early in the spawning season, but half of the tagged males did not return late in the season. In contrast, 78-90% of females always returned, and homing duration was significantly shortened late in the season. Studying the sex ratio of chum salmon on the spawning ground, Bakkala (1970) reported that males predominated early and females late in the spawning run. Although male salmonids do not show any territorial behaviors, they maintain high levels of aggressive behavior to compete for access to females (Jones, 1959) suggesting that early returning males might accrue some benefits in securing females for breeding. The present drastic re-

duction of male homing percentage late in the season may be interpreted in two ways; 1) some males prefer to go other unsampled breeding sites to find females, 2) some males are prevented from returning to the natal site by their early death. A constant "lose" of a few non-homing females throughout the spawning period may be related to the following two population-level hypotheses; 1) the conservative protection of these individuals' strain from the disruption of being captured at their natal spawning site, 2) the enhancement of their strain arising from a wide spawning distribution within the lake.

The sexual differences in homing behavior are considered to be reflected by different steroid hormone profiles between males and females. In males, the shortening of homing duration from September to October coincided significantly with increases in serum T and 11KT levels at the time of release, which were statistically analysed by the Spearman Rank Correlation (SRC; $p < 0.01$). The reduction of homing percentage was associated with decreased serum T levels and increased serum DHP levels at the time of release. In females, the shortening of homing duration from October to November corresponded significantly to the elevation of serum T and DHP levels, and the drop in serum E_2 levels at the time of release (SRC; $p < 0.01$). It is now widely accepted that the functional roles of these steroid hormones in salmonid gametogenesis are as follows: 11KT is active in spermatogenesis, E_2 is important to vitellogenesis, and DHP is active in final maturation (Nagahama, 1994). Although the precise role of T in gametogenesis has not yet been clarified yet, high serum levels of T have been detected in many salmonid fishes of both sexes during the spawning period (Lou *et al.*, 1986; Fitzpatrick *et al.*, 1986; Mayer *et al.*, 1992), and T has been considered to be a substrate for E_2 and 11KT biosynthesis (Kagawa *et al.*, 1982; Ueda *et al.*, 1984). During the spawning migration of salmonids, serum T levels are maintained at high levels and decline only after spawning (Truscott *et al.*, 1986; Ueda *et al.*, 1991; Slater *et al.*, 1994). Recently, the peak of plasma T levels in land-locked sockeye salmon of both sexes was observed at the time when they gathered at the mouth of their natal stream in Lake Chuzenji, Japan (Ikuta, 1996). In the present monthly observation, a significant relationship is also found between the shortening of homing duration and the increase of serum T levels in both sexes (SRC; $p < 0.01$).

The present study also shows that GnRHa implantation in adult sockeye salmon is highly efficient in shortening the homing duration, especially in females. The GnRHa treatment caused dramatic increases in serum DHP levels in both sexes. However, an interesting discrepancy was observed between rapidly and slowly returning individual males: rapidly returning males showed higher serum T levels and lower serum DHP levels than slowly returning males. The shortening of homing duration observed in the monthly sampling parallels the rapidly returning GnRHa-implanted males in a similar elevation of serum T levels. It is possible that serum T may function by causing the central nervous system to motivate the salmon to evoke homing behavior. Androgens are well-known to be involved in stimulating aggressive behavior in

teleost fishes (Villars, 1983), and serum T and 11KT are two major androgens which influence spawning behavior as well as the social dominance hierarchy (Kindler *et al.*, 1989; Cardwell and Liley, 1991; Pankhurst and Barnett, 1993; Brantly *et al.*, 1993). In salmonids, however, the relationship between steroid hormones and sexual behavior was reported to be complex and to be different between laboratory and field conditions (Cardwell *et al.*, 1996). The functional roles of serum T in salmonid homing behavior should be further investigated.

Salmon GnRH (sGnRH) producing neurons which contain granule-like structures were observed in the olfactory system of masu salmon (Kudo *et al.*, 1994), and cytophysiological changes of these neurons in chum salmon forebrain were examined before and after upstream migration (Kudo *et al.*, 1996). These studies suggest that sGnRH neurons in the olfactory system and those in the preoptic area might be functionally different. The former could be involved in olfactory functions, while the latter might act on gonadal functions during the early and final phases of upstream migration. The present GnRHa treatment revealed a clear influence on gonadal function in terms of the elevation of serum steroid hormone levels, but its influence on olfactory function was unclear. However, it is obvious that GnRH is involved in the reproductive homing behavior of lacustrine sockeye salmon.

In conclusion, homing profiles of lacustrine sockeye salmon in Lake Shikotsu, which offers a good model system for studying the homing mechanisms in salmonids, were sexually different during the spawning period, and these differences may be reflective of changes in serum steroid hormone levels, particularly that of serum T. GnRHa implantation induced shortening of homing duration accompanied by the dramatic elevation of serum DHP levels. Further, an interesting elevation of serum T levels was observed in rapidly returning males.

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