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Gonadotropin-Releasing Hormone Analog and Sex Steroids Shorten Homing Duration of Sockeye Salmon in Lake Shikotsu

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ABSTRACT—Gonadotropin-releasing hormone analog (GnRHa), testosterone (T) or 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) was implanted in lacustrine sockeye salmon (*Oncorhynchus nerka*) to examine their effects on homing behavior prior to spawning. Maturing adult fish in Lake Shikotsu were captured by a set net adjacent to their natal hatchery in September and October, 1997. They were tagged, implanted with hormones and released at the center of lake. More than 70% of released fish returned to the hatchery. In September, GnRHa-implanted fish returned significantly earlier than the controls regardless of sexes. DHP also shortened homing duration in the females but not in the males. T tended to reduce homing duration in the males, however it did not have any significant effect in the females. In October, fish in all groups quickly returned within a few days after the release. Hence, the shortening effect of GnRHa on homing duration was not seen. The October fish may be already well primed by endogenous hormones. The present study showed that, in September, GnRHa consistently shortened homing duration, whereas sex steroid actions varied depending on sex in lacustrine sockeye salmon.

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) seems to function as a neuromodulator in addition to stimulating pituitary gonadotropin release. Administration of exogenous GnRH facilitates sexual behavior in many species (see Pfaff *et al*., 1987). In teleosts, GnRH-immunoreactive cells in male dwarf gouramis, *Colisa lalia*, are involved in the control of reproductive behavior (Yamamoto *et al*., 1997).

Salmonid homing behavior is considered to be closely related to gonadal maturation which is in turn regulated by endocrine systems, mainly the hypothalamo-pituitary-gonadal axis. 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. Part of the reason for this uncertainty is the variety of homing patterns in anadromous salmonids, and the lack of a suitable model system to examine homing behavior in salmonid fishes.

In Lake Shikotsu, Hokkaido, Japan, sockeye salmon, *Oncorhynchus nerka*, are released from a hatchery into the lake as juvenile and grow until maturation into 2-4 years. These fish then return with high homing accuracy to their natal hatchery to breed. In a recent study, homing profiles and shortening of homing duration by GnRH analog (GnRHa) were investigated in lacustrine sockeye salmon in Lake Shikotsu (Sato *et al*., 1997). The results showed that the shortening of the homing duration was accompanied with changes in the plasma steroid hormone levels, in particular higher levels of testosterone (T) in early male returnees and 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) in female returnees. However, it was not clear in the previous experiments whether sex steroid hormones stimulate homing behavior. Moreover, the shortening effect of GnRHa on homing duration was not clearly shown in the males.

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In the present study, GnRHa, T or DHP was implanted in maturing lacustrine sockeye salmon in Lake Shikotsu, and their effects on homing behavior to the spawning site were examined to clarify the above questions.

MATERIALS AND METHODS

Fish

Male and female adult lacustrine sockeye salmon of 3-5 years old were caught near the shore of the hatchery using a large stationary set net (Fig. 1). In each of September and October 1997, 50 males and 50 females were captured, anesthetized with 0.002% ethyl m-aminobenzoate methanesulfonate (MS222, Nakalai tesque, Kyoto, Japan) buffered with sodium bicarbonate, and tagged with a numbered disc. Tagged fish were held overnight in the hatchery, and released at the center of Lake Shikotsu, 7.5 km southeast of the hatchery (Fig. 1) in the next morning. The duration of time until the fish returned to the hatchery was determined by checking the large set net and a small recapture set net (Fig. 1) on a daily basis.

Hormone implantation

After tagging, 8-10 males and 8-10 females received a 2-mm implant capsule containing 75 µg of GnRHa, [Des-Gly^{10}, D-Ala^{6}, Pro^{9}]-GnRH ethyl amide in an ethylene-vinyl acetate copolymer matrix (Zohar *et al.*, 1990; Zohar, 1996) intramuscularly *via* a 17-gauge needle

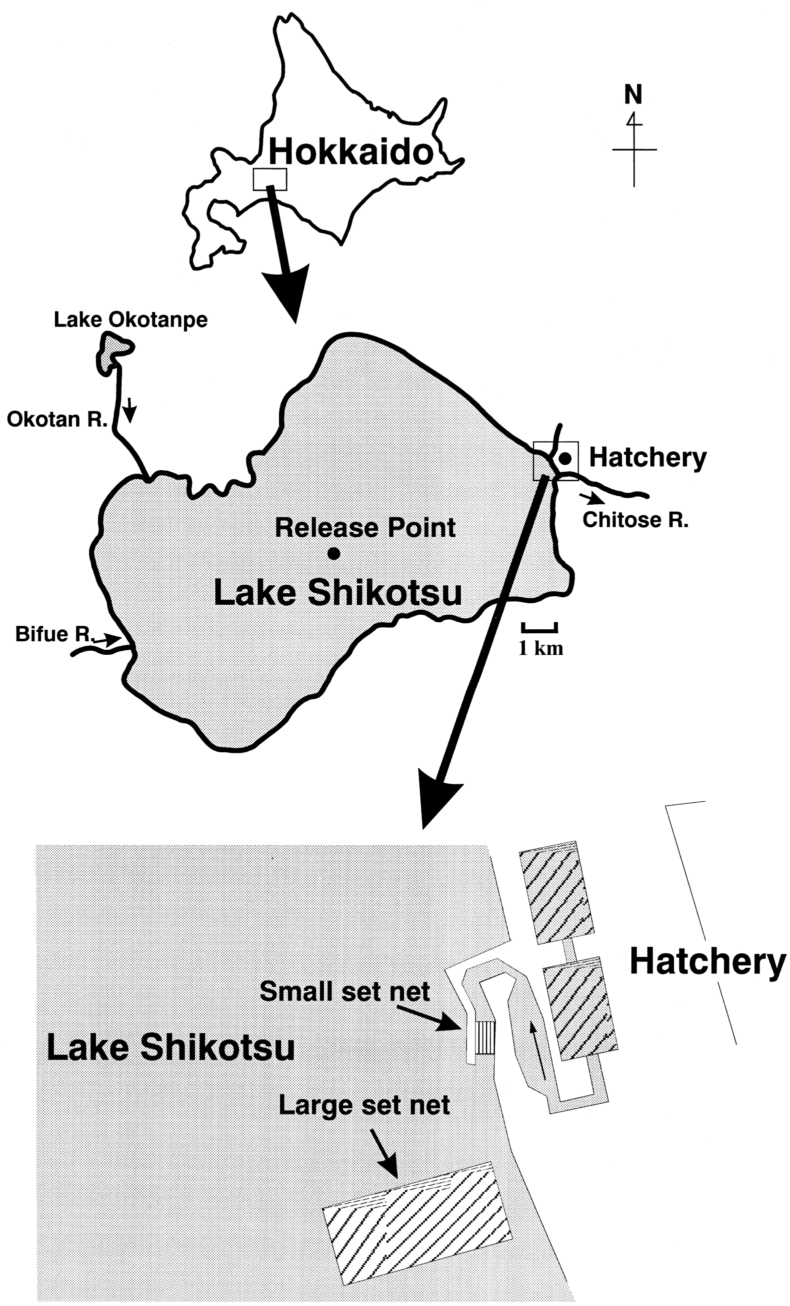

Fig. 1. Map of Lake Shikotsu, Japan showing the release point and the hatchery (return point). Fish were first captured by a large set net near the shore of the hatchery, and later recaptured by the large set net and a small set net at the mouth of the hatchery.

or a 2-cm implant capsule containing 200 µg of T or DHP in a methacrylate resin (Nisshin EM Corp., Tokyo, Japan) intraperitoneally. The hormone-implanted fish were subjected to procedures similar to those described above to determine homing duration. Animals which received an implant capsule containing only copolymer matrix served as GnRHa controls.

Statistical analysis

All data were expressed as mean ± SEM. One-way factorial analysis of variance (ANOVA) and Post-hoc tests were used as statistical analyses.

RESULTS

Homing percentage (returned/released × 100), body weight, fork length and gonadosomatic index (GSI) are shown in Table 1. There were no differences in homing percentages or body sizes among the groups. Fish in all groups returned to the hatchery with a high fidelity (70-100%) in both sexes over the sampling period.

The control fish, both males and females, took 20-30 days to return to the coast nearby the hatchery in September, whereas they required only several days to return to the spawning site in October (Fig. 2). This short homing duration is comparable to that observed in the previous experiment, i.e., three to five days were sufficient to return to the hatchery after release in October and November males and November females of Lake Shikotsu in 1996 (Sato *et al*., 1997).

Implantation of GnRHa consistently shortened the homing duration in both the males and the females (Fig. 2). In September, homing duration of GnRHa-implanted fish (males, 10.1 ± 2.4 days; females, 12.9 ± 1.8 days) were shorter than those of the controls (males, 20.4 ± 2.1 days; females, 29.3 ± 3.4 days). Nevertheless, in the October males and females implanted with GnRHa, reduced homing duration was not observed. As in the control males and females, the implanted fish homing duration was approximately five days, much shorter than in September GnRHa-implanted animals.

Effects of sex steroid hormones on homing behavior differed between the sexes. The T-implanted September males tended to return to the natal hatchery earlier than the control males (T-implanted males, 15.6 ± 3.1 days), however the effect of T-implantation was not clear in the females. As shown in Fig. 2, in the T-implanted September females, averaged homing duration seemed to be reduced, but there was no statistical significance. In contrast, the DHP-implanted September females returned to the spawning site earlier than the control females (DHP-implanted females, 17.4 ± 4.1 days), however, the homing duration of DHP-implanted males was nearly the same as the control males. The October fish implanted with sex steroid hormones showed similar short homing duration to that observed in the other groups.

DISCUSSION

In the present study, we examined the effects of GnRHa, T and DHP on homing behavior of lacustrine sockeye salmon in Lake Shikotsu. In September, GnRHa and sex steroids shortened homing duration in a sex dependent manner. GnRHa treatments were effective in shortening of homing duration in the males, whereas GnRHa and DHP were in the females. Although not significant, T tended to reduce homing duration in the males. It is noteworthy that the most effective treatment for shortening of homing duration was GnRHa-implantation. The effect was consistent in both males and females. The presence of GnRH may be crucial to stimulate homing behavior to the spawning bed, at least in lacustrine sockeye salmon.

Table 1. Effects of GnRH analog (75 µg/fish) and steroid hormone (200 µg/fish) implantation on homing percentage, fork length (FL), body weight (BW) and gonadosomatic index (GSI) of lacustrine sockeye salmon in Lake Shikotsu in September and October, 1997

Sex	Month	Treatment	No. of released fish	No. of returned fish (%)	FL(mm)*	BW(g)*	GSI*
Male	Sep.	Control	8	7 (87.5)	257.1 ± 1.6	191.7 ± 3.9	5.1 ± 0.4
		GnRHa	10	7 (70.0)	252.7 ± 2.0	193.4 ± 3.3	4.7 ± 0.3
		T	8	8 (100.0)	257.6 ± 1.7	194.0 ± 3.6	3.9 ± 0.3
		DHP	10	7 (70.0)	252.6 ± 2.2	184.9 ± 5.6	4.8 ± 0.2
	Oct.	Control	10	9 (90.0)	251.7 ± 1.8	191.4 ± 4.4	3.5 ± 0.2
		GnRHa	10	10 (100.0)	252.4 ± 2.4	197.1 ± 4.7	3.5 ± 0.1
		T	10	8 (80.0)	255.8 ± 2.2	203.7 ± 4.1	3.5 ± 0.1
		DHP	10	10 (100.0)	252.2 ± 2.2	197.0 ± 6.0	4.6 ± 0.1
Female	Sep.	Control	8	8 (100.0)	256.3 ± 3.0	188.3 ± 5.3	14.7 ± 0.8
		GnRHa	9	7 (77.8)	253.9 ± 2.0	185.1 ± 4.3	12.1 ± 0.9
		T	11	8 (72.7)	253.0 ± 1.6	170.7 ± 3.6	14.2 ± 1.2
		DHP	8	7 (87.5)	251.4 ± 2.3	179.2 ± 4.2	13.6 ± 1.2
	Oct.	Control	10	8 (80.0)	252.9 ± 1.5	183.3 ± 4.6	15.4 ± 0.5
		GnRHa	9	8 (88.9)	253.5 ± 1.6	183.4 ± 3.9	13.6 ± 1.1
		T	11	8 (72.7)	258.0 ± 3.1	192.5 ± 9.3	13.0 ± 1.6
		DHP	10	8 (80.0)	248.1 ± 2.6	174.2 ± 5.8	14.6 ± 0.6

* Mean ± SEM.

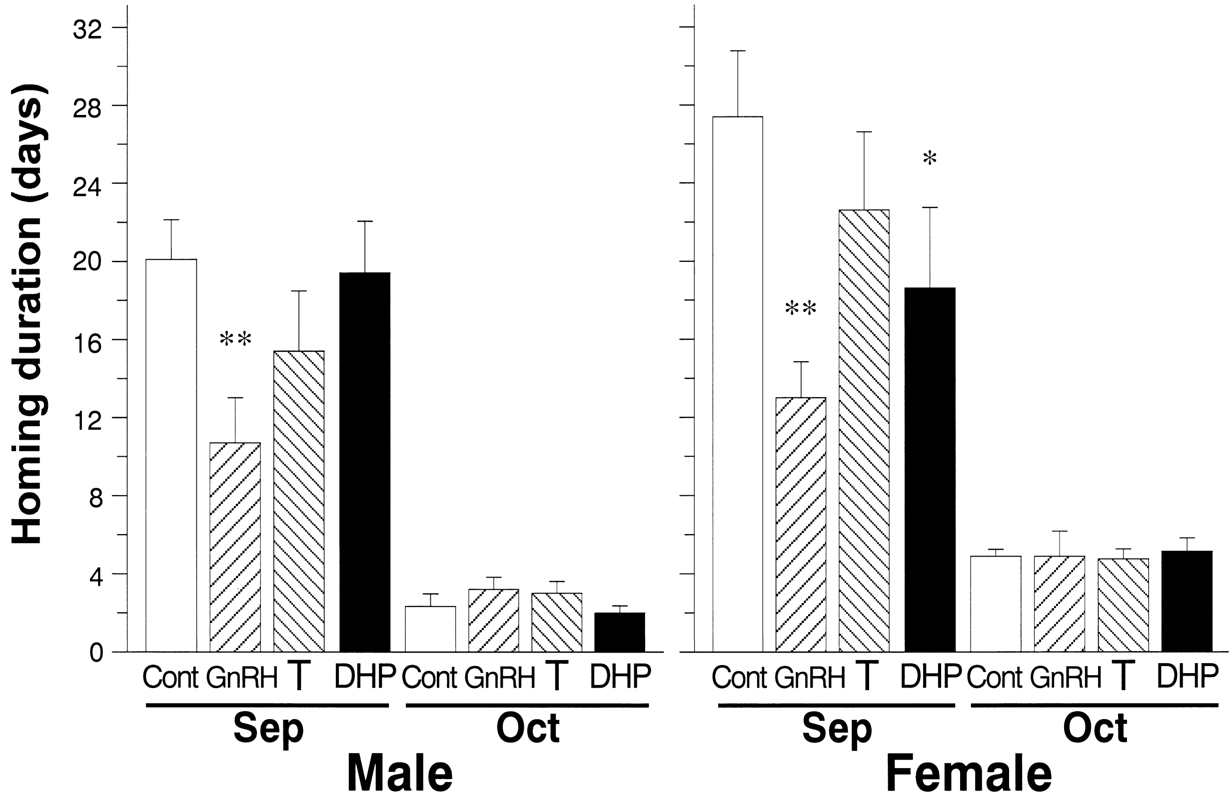

Fig. 2. Effects of GnRH analog (75 µg/fish) and steroid hormone (200 µg/fish) implantation on homing duration of lacustrine sockeye salmon in Lake Shikotsu in September and October, 1997. Cont, Control; GnRH, GnRHa-implanted; T, T-implanted; DHP, DHP-implanted. *, $P < 0.05$; **, $P < 0.01$ compared with control. Mean ± SEM.

In salmonids, as well as in many vertebrate species, GnRH-immunoreactive fibers are widely distributed in the hypothalamus, the pituitary, and in various other brain areas (see Gothilf *et al.*, 1996; Amano *et al.*, 1997). It is hypothesized that GnRH regulates GTH secretion from the pituitary as a hypothalamic neurohormone and brain functions as a neuromodulator. The former function in GTH secretion is supported by many previous papers as is reviewed by Amano *et al.* (1997), and the latter neuromodulatory role in teleosts is indicated by Yamamoto *et al.* (1997), as well as in amphibians (Urano, 1988).

A recent study demonstrated that shortening of homing duration with GnRHa in female lacustrine sockeye salmon was accompanied with increases in serum DHP levels (Sato *et al.*, 1997). In contrast, quickly returning males showed higher serum T levels and lower serum DHP levels compared to slowly returning males. The serum steroid hormone levels in the GnRHa-implanted fish (Fukaya *et al.*, in preparation) showed similar changes to those seen in the previous experiment (Sato *et al.*, 1997). The tendency to shorten homing duration by T-implantation in the males and apparent shortening of duration by DHP-implantation in the females seen in the present study thus supported and expanded the previous results.

Androgens stimulate aggressive behavior in teleost fishes (see Villars, 1983), while the levels of serum T and 11KT are correlated with spawning behavior as well as social dominance hierarchy (Kindler *et al.*, 1989; Cardwell and Liley, 1991; Brantly *et al.*, 1993; Pankhurst and Barnett, 1993). It is therefore possible that circulating T has functions in the central nervous system to motivate salmon homing behavior. Although DHP is known to be a maturation-inducing steroid in salmonids (Nagahama and Adachi, 1985), its function to the central nervous system has not yet been clarified. Central function of blood DHP should be further investigated including its actions regarding salmonid homing behavior.

In October, the fish in all groups quickly returned to the natal hatchery within a few days after the release. The shortening effect of hormones on homing duration was thus not clearly observed in the October fish. The most plausible explanation for this fact is that the October fish were already well primed by endogenous hormones. Homing behavior in fish primed by endogenous hormones may already be sufficiently motivated, or they may be refractory to additional hormonal stimulation. The previous study of GnRHa-implantation in 1996 (Sato *et al.*, 1997) showed that, GnRHa did not have a significant effect to reduce homing duration in September males; whereas the same experiment in 1997 (shown in the present report) showed a significant effect of GnRHa on homing duration in September but not October males. These facts suggest the presence of a sensitive or critical period to endogenous hormones, in particular to GnRH, in the salmonid neuroendocrine system.

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