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Inhibitory Effects of Testosterone on Downstream Migratory Behavior in Masu Salmon, *Oncorhynchus masou*

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ABSTRACT—The effects of testosterone (T) on downstream migratory behavior of yearling masu salmon, *Oncorhynchus masou*, were studied during its downstream migratory period using artificial raceways. In experiment 1, 22 and 19 smolts were implanted with a medical silicone tube capsules containing 500 μ g of T or vehicle only. These groups were transferred together to the upper pond of artificial raceway which was connected to the lower pond through fish-way. Downstream migratory behavior was then observed for 1 week. In experiment 2, T500 μ g-, T50 μ g-, and T5 μ g-treated smolts, control smolts, and precociously mature males were transferred to the upper pond of the raceway and downstream migratory behavior was observed for 2 months. In experiment 3, 40 smolts were implanted with capsules containing 500 μ g of T or vehicle only. These groups were transferred separately to the upper ponds of raceways and downstream migratory behavior was observed for 3 weeks. In each experiment, injection of T caused increases in plasma T levels within physiological levels. In experiment 1, frequency of downstream migratory behavior was 89.5% in the control group and 31.8% in the T500 μ g-treated group. In experiment 2, the frequency was lower in the T500 μ g- and T50 μ g-treated groups than in the control group. T5 μ g implantation failed to inhibit downstream migratory behavior. Precocious males were not observed to go down the raceway. In experiment 3, frequency of downstream migratory behavior of the control group and the T500 μ g-treated group was 100 and 40%, respectively. In each experiment, plasma levels of T in T500 μ g-treated smolts which did not show downstream migratory behavior was higher than those of migrants. These results indicate that the downstream migratory behavior of masu salmon smolts is inhibited by physiological levels of T.

INTRODUCTION

In general, yearling masu salmon, *Oncorhynchus masou*, begin downstream (seaward) migration after parr-smolt transformation in spring (Kubo, 1974, 1980; Utoh, 1976, 1977; Kiso, 1995). However, precocious males that matured in under-yearling autumn show neither parr-smolt transformation nor downstream migration during the following spring, and they remain in a particular river until spawning season in autumn. It is noteworthy that complete castration of under-yearling precocious male masu salmon could induce the parr-smolt transformation in the following spring, whereas sham-operated and partially castrated under-yearling precocious males did not become smolts (Aida *et al.*, 1984). Yamazaki *et al.* (1973) and

Ikuta *et al.* (1985, 1987) have revealed that morphological and physiological changes in parr-smolt transformation of masu salmon smolts, such as silvering of the skin and hypomostoregulatory ability, were inhibited by administration of sex steroids, such as testosterone (T), 11-ketotestosterone (11-KT), estradiol-17 β (E₂), and 17 α -methyltestosterone (MT). From these reports, it is apparent that the physiological processes of parr-smolt transformation in yearling precocious males is inhibited by sex steroids. However, it remains unclear as to why precocious masu salmon do not show seaward migration.

In masu salmon, males including precocious individuals exhibit drastic increases in plasma levels of T, 11-KT, and 17 α ,20 β -dihydroxy-4-pregnene-3-one (DHP) during testicular maturation, and females exhibit significant increases in plasma levels of T, E₂, and DHP during ovarian maturation (Munakata *et al.*, unpublished data). Among these sex ste-

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roids, T, the common precursor hormone of 11-KT (in males) and E₂ (in females), begins to increase in May, the period of smolting and downstream migration, earlier than do other sex steroids in both sexes. These phenomena have led us to the hypothesis that T is an important factor inhibiting downstream migratory behavior in precocious masu salmon. Therefore, in the present study we attempted to investigate the effects of T on downstream migratory behavior using yearling masu salmon smolts and artificial raceways.

MATERIALS AND METHODS

Masu salmon

Yearling masu salmon, *Oncorhynchus masou*, were used as experimental fish. The fish were taken from a population which was reared from eggs at the Nikko Branch, National Research Institute of Aquaculture (NRIA). The origin of the fish strain was wild stocks which had migrated into the Shiribetsu River, Hokkaido, in the autumn of 1980. Eggs were artificially fertilized in October and were observed to hatch in December at the NRIA. Before the experiment was begun, fish were reared in flow-through concrete ponds (4 m x 1 m with water depth of 0.5 m) under natural photoperiod providing spring water at 9.5±0.5°C, and were fed commercial trout pellets twice per day at a level of 2% body weight. In this strain, most of the underyearling and yearling males mature precociously (Amano *et al.*, 1993), while some yearling immature males and most females exhibit parr-smolt transformation in spring. Both sexes mature in autumn at 2⁺ years.

Experiment 1

In order to investigate the effects of testosterone (T) on the downstream migratory behavior of smolting fish, both T500µg-treated smolts and control smolts were transferred together into the same artificial raceway and the downstream migratory behavior was observed. On May 8 1995, 22 immature smolts were randomly selected and implanted with Silastic tube capsules (outer diameter 1.95 mm, inner diameter 1.47 mm, length 20 mm, Dow Corning Corp.) that contained 500µg of T. On the other hand, 19 smolts were implanted with the capsules containing vehicle only. Powder of T was dissolved in ethanol (200 mg/ml), diluted 1:9 in purified sesame oil, and 25 µl of T-oil solution was injected into each capsule. The ends of the capsule were sealed with Medical Adhesive (Silicone Type A, Dow Corning Corp.). After the fish was anesthetized in 50ppm ethyl-*p*-aminobenzoate solution, the capsule was implanted into the abdominal cavity through a 2 mm long incision. The adipose fin of the T500µg-treated fish was cut off for marking. After each group had been reared in 300-l circular tanks for 2 weeks, they were transferred together to the upper pond (2 m x 4 m with a water depth of 50cm) of an artificial raceway that was connected to lower pond (2 m x 8m with a water depth of 50 cm) through fish-way (ø20 cm x 4 m long) made of a P.V.C. half pipe. Flow rate and velocity of water in the fish-way ranged from 10 to 12 // sec and 75–85 cm/sec, respectively. Water temperature was kept at 9.5±0.5°C. In this experiment, downstream migratory behavior was observed for approximately 1 week from May 24 to 31. In each experiment, behavioral observation was continued as long as the number of migrants of control smolts increased. During the experimental period, fish were fed commercial trout pellets twice per day at a level of 2% body weight. Fish which went down the raceway were recaptured by a net trap (2.0 m x 0.7 m x 0.7 m) and were sampled every day at 0900h. Frequency of fish which showed downstream behavior was expressed as the percentage of the sum of migrants and non-migrants. Standard length (BL, cm) and body weight (BW, g) were measured and condition factor (CF) was calculated as BW x 100/BL³. Plasma and pituitary samples were taken from the fish in order to measure levels of T, gonadotropin (GTH) II, thyroxine (T₄), and triiodothyronine (T₃) by radioimmunoassay (RIA). At the end of the ex-

periment on May 31, non-migrants were also sampled as described above.

Experiment 2

In order to investigate the detailed involvement of T, downstream migratory behavior of T500µg-, T50µg-, and T5µg-treated smolts, control smolts, and precociously mature males was observed in the raceway. On April 26 1996, Silastic tube capsules containing T500µg, T50µg, and T5µg were implanted into 55 each of masu salmon smolts. Seventy controls and 77 precociously mature males were implanted with capsules containing vehicle only. Passive integrated transponder (PIT) tags (Prentice *et al.*, 1990a, 1990b) were also implanted into the experimental fish for group identification. The fish were reared in the upper pond of the raceway for two weeks. Flow rate and velocity of water in the fish way and water temperature were the same as described in experiment 1. Downstream migratory behavior was

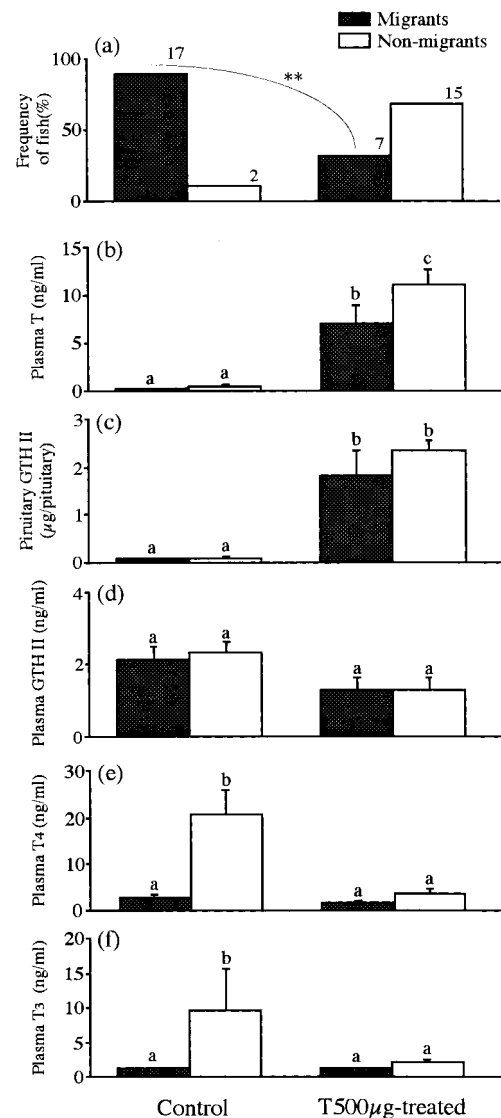


Fig. 1. Frequency of migrants and non-migrants, plasma levels of T, pituitary contents of GTH II, and plasma levels of GTH II, T₄, and T₃ in controls and T500µg-treated smolts. Figures beside columns indicate the number of migrants or non-migrants. Hormone levels are expressed as the mean and standard error. ** indicates significant difference at *P*<0.01. Differing letters represent significant differences at *P*<0.05 among all groups.

Table 1. Body length (BL), body weight (BW), and condition factor (CF) in the control and T500 µg - treated groups

Group	N	BL(cm)	BW(g)	CF	Sampling date
Initial data					
Control	19	14.81±0.14	36.30±0.92	1.12±0.02	May 8
T500µg-treated	22	14.80±0.14	38.85±1.11	1.11±0.02	May 8
Control (19)[†]					
Migrants	17	15.07±0.15 ^a	34.91±1.29 ^{ab}	1.01±0.02 ^a	May 25–31
Non-migrants	2	15.60±0.40 ^a	45.00±2.81 ^{bc}	1.18±0.02 ^b	May 31
T500µg-treated (22)[†]					
Migrants	7	14.71±0.35 ^a	32.27±2.93 ^a	1.00±0.02 ^a	May 25–31
Non-migrants	15	15.35±0.17 ^a	41.44±2.00 ^c	1.13±0.03 ^b	May 31

[†] Initial number.
Differing letters indicate significant differences (p<0.05) among groups.

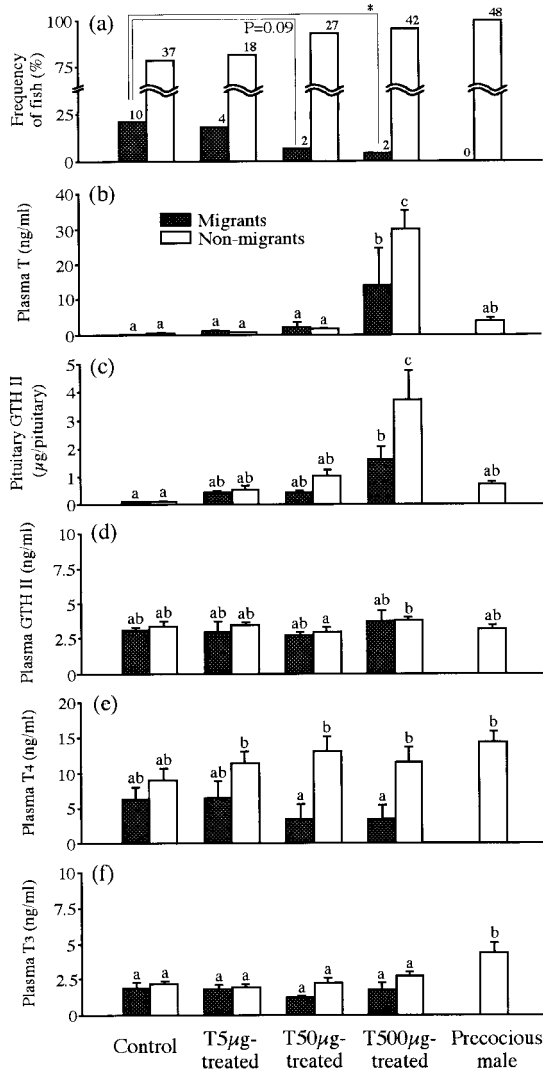


Fig. 2. Frequency of migrants and non-migrants, plasma levels of T, pituitary contents of GTH II, and plasma levels of GTH II, T₄, and T₃ in controls, T5µg-, T50µg-, T500µg-treated smolts and precocious males. Figures beside columns indicate the number of migrants or non-migrants. Hormone levels are expressed as the mean and standard error. * indicates significant difference at P<0.05. Differing letters represent significant differences at P<0.05 among all groups.

observed from May 8 through July 10. During the experimental period, fish were fed as described in experiment 1. Fish which went down the raceway were recaptured by a net trap (2.0 m×0.7 m×0.7 m) and were sampled every day at 0900h. BL and BW were measured and CF was calculated. Plasma and pituitary samples were taken from the fish that showed downstream migratory behavior in order to measure levels of T, GTH II, T₄, and T₃ by RIA. At the end of the experiment, non-migrants were also sampled.

Experiment 3

In order to examine whether intra-specific interactions between T500µg-treated smolts and control smolts influence the downstream migratory behavior or not, we observed the downstream migratory behavior of 40 control smolts and 40 T500µg-treated smolts in separate raceways. On April 23 1999, 40 immature smolts were randomly selected and implanted with Silascone tube capsules (outer diameter 1.5 mm, inner diameter 1.0 mm, length 30 mm, Kaneka Medics Corp.) that contained 500µg of T. On the other hand, 40 smolts were implanted with the capsules containing vehicle only. Powder of T was dissolved in ethanol (250 mg/ml), diluted 1:9 in purified sesame oil, and 20 µl of T-oil solution was injected into each capsule. The ends of the capsule were sealed with Medical Adhesive (Silicone Type A, Dow Corning Corp.). In this experiment, Silascone tube was used, since the production of Silastic tube was stopped. In general, characteristics of the Silascone tube capsule was considered to be almost similar to those of the Silastic tube capsule (Munakata *et al.*, unpublished data). The adipose fin of the T500µg-treated fish was cut off for marking. After each group had been reared in 300-l circular tanks for 2 weeks, they were transferred separately to the upper ponds of artificial raceways. Flow rate and velocity of water in the fish way and water temperature were the same as described in experiment 1. Downstream migratory behavior was observed from May 8 to 28. During the experimental period, fish were fed as described in experiment 1. Fish which went down the raceway were recaptured by a net trap (2.0 m×0.7 m×0.7 m) and were sampled every day at 0900h. BL and BW were measured and CF was calculated. Plasma samples were taken from the fish that showed downstream migratory behavior in order to measure plasma levels of T, T₄, and T₃ by RIA. At the end of the experiment, non-migrants were also sampled.

Hormone measurement

Plasma levels of T, GTH II, T₄, and T₃ were measured by RIAs described previously by Kobayashi *et al.* (1986), Lou *et al.* (1984), Aida *et al.* (1984), and Ikuta *et al.* (1987), respectively.

Statistics

The difference in the frequency of downstream migratory behavior from the control was analyzed by the χ²-test. Differences in mean plasma levels and pituitary contents of hormones, BL, BW, and CF

Table 2. Body length (BL), body weight (BW), and condition factor (CF) in the control, T5 µg-, T50 µg-, and T500 µg-treated smolts and precocious males

Group	N	BL(cm)	BW(g)	CF	Sampling date
Initial data					
Smolt	6	14.50±0.27	32.43± 1.50	1.09±0.02	April 26
Precocious male	6	15.18±0.85	47.77±10.3	1.24±0.12	April 26
Control (Smolt) (57) [†]					
Migrants	10	15.41±0.28 ^a	39.17± 2.34 ^{ab}	1.06±0.02 ^a	May 10-July 10
Non-migrants	37	15.84±0.22 ^{ab}	46.54± 1.33 ^{abc}	1.19±0.05 ^a	July 11
T5 µg-treated (27) [†]					
Migrants	4	14.68±0.39 ^a	33.45± 2.31 ^a	1.06±0.03 ^{ab}	May 10-July 10
Non-migrants	18	16.75±0.56 ^b	51.00± 1.87 ^c	1.14±0.05 ^a	July 11
T50 µg-treated (33) [†]					
Migrants	2	14.25±0.05 ^{ab}	29.20± 0.20 ^{ab}	1.01±0.04 ^{ab}	May 10-July 10
Non-migrants	27	15.74±0.18 ^{ab}	48.71± 1.93 ^{bc}	1.24±0.02 ^a	July 11
T500 µg-treated (46) [†]					
Migrants	2	14.25±0.35 ^{ab}	31.60± 5.80 ^{abc}	1.08±0.12 ^{ab}	May 10-July 10
Non-migrants	42	15.09±0.25 ^a	46.69± 1.14 ^{abc}	1.48±0.13 ^b	July 11
Precocious male (54) [†]					
Non-migrants	48	16.74±0.32 ^c	69.94± 3.41 ^d	1.46±0.06 ^b	July 11

[†] Initial number.

Differing letters indicate significant differences ($p < 0.05$) among groups.

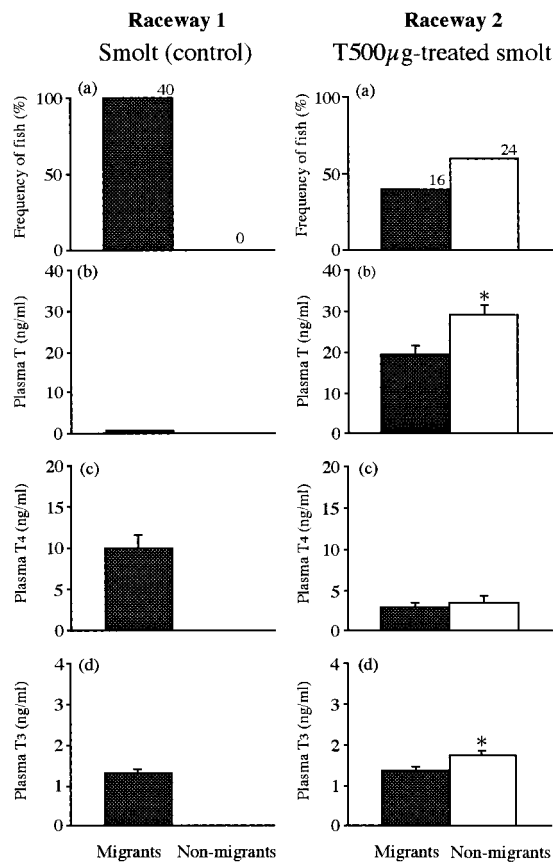


Fig. 3. Frequency of migrants and non-migrants, and plasma levels of T, T₄, and T₃ in the control group (raceway 1) and the T500µg-treated group (raceway 2). Figures beside columns indicate the number of migrants or non-migrants. Hormone levels are expressed as the mean and standard error. * indicates significant difference at $P < 0.05$.

between and among groups were analyzed by the Student's *t*-test and ANOVA followed by Fisher's PLSD, respectively, using StatView version 4.5 software (Abacus Concepts, Inc., Berkeley, California, USA).

RESULTS

Experiment 1

Frequency of downstream migratory behavior in the control group and the T500µg-treated group was 89.5% and 31.8%, respectively (Fig. 1 a). The frequency in the T500µg-treated group was lower ($P < 0.01$) than that of the control group. Downstream movement was observed mainly during night. Plasma T levels and pituitary GTH II contents in migrants and non-migrants of the T500µg-treated group were higher ($P < 0.05$) than those in migrants and non-migrants of the control group (Fig. 1 b, c). In the T500µg-treated group, plasma T levels in non-migrants were higher ($P < 0.05$) than those in migrants. No significant difference was observed in plasma GTH II levels among migrants and non-migrants of both groups (Fig. 1 d). Plasma levels of T₄ and T₃ in non migrants in the control group were higher ($P < 0.05$) than those in migrants of the control group and migrants and non-migrants of the T500µg-treated group (Fig. 1 e and f). BW and CF in migrants of the control and T500µg-treated groups were lower ($P < 0.05$) than those in non-migrants of the T500µg-treated group. (Table 1).

Experiment 2

Frequency of downstream migratory behavior in the control, T5µg-, T50µg, T500µg-treated, and precocious male groups were 21.3, 18.2, 6.9, 4.5, and 0%, respectively (Fig. 2a).

Table 3. Body length (BL), body weight (BW), and condition factor (CF) in the control (Raceway 1) and T500 µg -treated groups (Raceway 2)

Group	N	BL(cm)	BW(g)	CF	Sampling date
Initial data	10	14.59±0.23	35.00±1.58	1.11±0.01	April 23
Raceway 1					
Control	(40) [†]				
Migrants	40	14.56±0.12	33.45±0.76	1.08±0.01	May 8–14
Raceway 2					
T500 µg-treated	(40) [†]				
Migrants	16	14.07±0.72 ^a	29.28±1.19 ^a	1.04±0.01 ^a	May 8–13
Non-migrants	24	14.89±0.16 ^b	37.96±1.34 ^b	1.14±0.01 ^b	May 28

[†] Initial number.

Differing letters indicate significant differences ($p < 0.001$) between groups.

Frequency was lower in the T500µg- ($P=0.02$) and T50 µg-treated ($P=0.09$) groups than that in the control group. T5µg implantation failed to inhibit downstream migratory behavior ($P=0.76$). Precocious males did not go down the raceway. Plasma T levels in migrants and non-migrants of the T500µg-treated group were higher ($P < 0.05$) than those in migrants and non-migrants of the control, T5µg-, and T50µg-treated groups (Fig. 2 b). Plasma T levels and pituitary GTH II contents of non-migrants in the T500µg-treated group were higher ($P < 0.05$) than those of migrants in the T500µg-treated group and non-migrants in the precocious male group (Fig. 2 b and c). There was no large difference in plasma GTH II levels (Fig. 2 d). In the T50µg- and T500µg-treated groups, plasma T₄ levels of migrants were lower ($P < 0.05$) than those of non-migrants (Fig. 2 e). On the other hand, no significant difference was observed in plasma T₃ levels between migrants and non-migrants within each group (Fig. 2 f). However, plasma T₃ levels in non-migrants of precocious male group were higher ($P < 0.05$) than those in the other groups. BL and BW in migrants of the T5µg-, T50µg-, and T500µg-treated groups were lower ($P < 0.05$) than those in non-migrants of precocious male group (Table 2).

Experiment 3

Frequency of downstream migratory behavior in the control group (raceway 1) and the T500µg-treated group (raceway 2) was 100% and 40%, respectively (Fig. 3 a). The frequency in the T500µg-treated group was significantly lower ($P < 0.001$) than that of the control group. Downstream movement was observed mainly during night. Plasma T levels of migrants and non-migrants in the T500µg-treated group were higher ($P < 0.05$) than those in migrants of the control group (Fig. 3 b). In the T500µg-treated group, plasma T levels of non-migrants were higher ($P < 0.05$) than those of migrants. Plasma T₄ levels in migrants of the control group were higher ($P < 0.05$) than those in migrants and non-migrants of the T500µg-treated group (Fig. 3c). There was no large difference in plasma T₃ levels among migrants in the control group and migrants and non-migrants in the T500µg-treated group. In the T500µg-treated group, however, plasma T₃ levels of migrants were lower ($P < 0.05$) than those of non-migrants

(Fig. 3 d). In the T500 µg-treated group, BL, BW, and CF of migrants were lower ($P < 0.001$) than those of non-migrants (Table 3).

DISCUSSION

In experiments 1 and 3, frequency of downstream migratory behavior was lower in the T500µg-treated group than that in the control group. In experiment 2, the frequency of downstream migratory behavior was lower in the T500µg- and T50µg-treated groups than in the control group. From these results, it is apparent that T inhibits downstream migratory behavior in masu salmon smolts. In these experiments, plasma levels of T in the T500µg-treated groups increased and ranged between 0.67 and 42.66 ng/ml. In an earlier study, it was demonstrated that plasma levels of T in mature masu salmon increased by nearly 30 ng/ml in males and 60 ng/ml in females, respectively (Munakata *et al.* unpublished data). These results imply that downstream migratory behavior of smolting fish could be inhibited by physiological levels of T.

In experiment 2, plasma levels of T in migrants and non-migrants of the T500µg-treated group were higher than those in migrants and non-migrants of the control, T5µg-, and T50µg-treated groups. Moreover, in the experiments 1–3, plasma T levels in non-migrants of the T500µg-treated groups were higher than those in migrants. These results indicate that high plasma levels of T were necessitated to inhibit the downstream migratory behavior of smolting fish, although the frequency of downstream migratory behavior in the T50µg-treated group was lower than that in the control group in experiment 2. In experiment 2, however, some of the T500µg-treated smolts showed downstream migratory behavior, and their plasma T levels (13.73±9.37 ng/ml) was significantly higher than those of precocious males (3.86±0.93 ng/ml) which did not show downstream migratory behavior. One possible explanation is that such high plasma T levels could not completely impair downstream migratory behavior of smolting fish. In masu salmon, it is generally known that precocious males differentiate from immature parr during underyearling mid-summer (Utoh, 1976, 1977; Amano *et al.*, 1993, 1999). In autumn underyearling precocious males show high plasma T levels

and spermiate. Although the values of GSI and plasma androgen levels of precocious males decrease during winter, these values increase again during yearling spring through autumn (Munakata *et al.*, unpublished data). Aida *et al.* (1984) demonstrated that castrated underyearling precocious males became smolts, whereas sham-operated precocious males did not become smolts. From these results, it is considered that continuous release of sex steroids during winter through spring is one factor inhibiting smoltification and downstream migration, although the levels were low. It is also probable that other sex steroids, such as 11-KT, E₂, and DHP, are involved in the inhibition of downstream migratory behavior as T. It has been previously demonstrated that various sex steroids increase during gonadal maturation in salmonid fishes (Ueda *et al.*, 1984; Yamauchi *et al.*, 1984; Truscott *et al.*, 1986; Liley *et al.*, 1986; Slater *et al.*, 1994; Frantzen *et al.*, 1997). Berglund *et al.* (1994) have reported that 11-ketoandrostendione (11-KA) reduces the tendency of downstream migratory activity in Atlantic salmon, *Salmo salar*. Mayer *et al.* (1994) reported that sexual behavior of castrated mature male rainbow trout, *O. mykiss*, was induced by DHP and 11-KA. 11-KT is also known to induce sexual behavior of teleosts (Kindler *et al.*, 1991; Stacey and Kobayashi, 1996; Kobayashi *et al.*, 1999). These findings seem to support our speculation that multiple sex steroids regulate the downstream migratory behavior.

In the present study, inhibitory mechanisms of downstream migratory behavior relating to the functioning of T was unclear. In experiment 3, all of the control smolts went down the raceway within 1 week (data not shown), suggesting that downstream migratory behavior of smolting fish occurs spontaneously. Therefore, in T-treated smolts and precocious males, it is considered that T inhibits the downstream migratory behavior by directly inhibiting the downstream migratory activity of individual fish. On the other hand, the results of experiment 2 suggest that downstream migratory behavior was affected by social stimuli, such as intra-specific interactions. In experiment 2, the frequency of downstream migratory behavior in the control and T5 μ g-treated groups were relatively low, even though the observation was continued for 2 months. Moreover, the frequency of downstream migratory behavior in the T50 μ g-treated group was significantly lower than that in the control group, though plasma T levels of non-migrants in the T50 μ g-treated group were significantly lower than those in migrants of the T500 μ g-treated group. In this experiment, some of precocious males showed territorial behaviors near an opening of fish-way and four corners in the upper pond (data not shown). On the contrary, most of smolting fish including T-treated smolts gathered in middle part in the upper pond. It has been revealed that sex steroids such as T induce territorial aggressiveness of teleosts (Villars, 1983; Ikuta, 1994). Based on these information, it is suggested that T inhibits the downstream migratory behavior of T50 μ g- and T500 μ g-treated smolts, and precocious males through stimulating their territorial aggressiveness. If so, these findings suggest further possibility that downstream migratory behavior of

smolting fish is stimulated by territorial aggressiveness of the precocious males in rivers.

In experiments 1 and 3, BW of non-migrants in the T500 μ g-treated groups was higher than those in migrants of the control and/or T500 μ g-treated groups. In experiment 2, BL and BW of non migrants in the precocious male group were also higher than those in migrants of the control, T5 μ g-, T50 μ g-, and T500 μ g-treated groups. In experiment 2, it was observed that part of the T500 μ g-treated smolts occupied upper part of the upper pond, while most of the control smolts located at lower part of the upper pond. It has been demonstrated that salmonid fishes show size-dependent dominance hierarchy in rivers (Nakano and Furukawa-Tanaka, 1994; Nakano, 1995). From these results, it is suggested that not only T but also BL and BW contribute to the tendency of T50 μ g and T500 μ g-treated smolts, and precocious males to remain in the upper pond of the raceway as dominant fish.

In each experiment, T500 μ g-treated smolts, especially non-migrants, exhibited higher CF and dark skin color (data not shown). In experiments 1 and 2, migrants and non-migrants in T500 μ g-treated smolts had relatively high pituitary GTH II contents. These phenomena are in agreement with Ikuta *et al.* (1987). Therefore, it is suggested that T500 μ g and T50 μ g implantation inhibit downstream migratory behavior indirectly through inhibition of the physiological processes of parr-smolt transformation. In experiments 1 and 2, T500 μ g-treated smolts showed higher pituitary GTH II contents, indicating that T induced synthesis of GTH II in the pituitary gland. In T500 μ g-treated smolts, however, plasma GTH II levels did not increase. It is therefore likely that GTH II released into the plasma is not an important factor for the inhibition of downstream migratory behavior. Recently, it was revealed that treatment of MT induced the gene expression of gonadotropin releasing hormone (GnRH) in brain (Amano *et al.*, 1994a). Amano *et al.* (1994b) also reported that GnRH immunoreactive fibers were distributed in various parts in the brain. These reports suggest the possibility that sex steroid inhibit the downstream migratory behavior through affecting the GnRH neurone and other nervous systems in the brain.

Downstream migratory behavior was observed mainly during night-time, suggesting that photoperiod is one of the timing factors regulating downstream migratory behavior in masu salmon smolts. T₄ and T₃ have been considered to be stimulatory factors for downstream and upstream migratory behavior in anadromous and amphidromous fishes (Yamauchi *et al.*, 1985; Ueda *et al.*, 1984; Tsukamoto *et al.*, 1988; Iwata, 1995). In ayu, *Plecoglossus altivelis*, immature fish which exhibited upstream behavior in an artificial river had higher plasma T₄ levels than those which moved downward (Tsukamoto *et al.*, 1988). In hime (land-locked sockeye) salmon, *O. nerka*, smolting fish that begin downstream migratory behavior in artificial raceway have high plasma T₄ levels compared to the levels of non-migrants (Ikuta, 1994). From these reports it is indicated that migratory behavior was stimulated by T₄ released into the plasma. In the present study, however, plasma T₄ and T₃ levels in migrants tended to be

lower than those of the non-migrants in both the control and T-treated groups. Similar phenomena were also reported by Ewing *et al.* (1994) using rainbow trout. Since the fish which showed downstream migratory behavior were sampled at 0900h following downstream migratory behavior at night in this study, one possible explanation is that plasma levels of T_4 had already decreased after the completion of the behavior. This hypothesis is not yet far from established, but in our earlier study we have also revealed that masu salmon smolts sampled immediately after beginning downstream migratory behavior showed higher plasma T_4 levels than did non-migrants (Munakata *et al.*, unpublished data). Thus, a plasma T_4 surge is considered to be involved in triggering downstream migratory behavior.

In experiments 1 and 3, plasma T_4 levels in migrants and non-migrants of the T500 μ g-treated groups were lower than those of migrants (experiment 3) and non-migrants (experiment 1) in the control group. Since downstream migratory behavior is suggested to be triggered by plasma T_4 surge, these results suggest that T could have an ability to depress the secretion of T_4 into the plasma, and, hence these results imply that the inhibition of downstream migratory behavior in T-treated smolts resulted partly from such lower plasma T_4 levels. In experiment 2, however, plasma T_4 levels of non-migrants in T-treated groups and precocious male group were as high as those in the control smolts. Even though thyroid hormones are suggested to trigger downstream migratory behavior, the critical functioning of thyroid hormones in downstream migratory behavior is still not fully understood.

In summary, the present study clearly demonstrated that downstream migratory behavior of masu salmon smolt is inhibited by physiological levels of plasma T. However, such high plasma T levels could not completely impair downstream migratory behavior of smolting fish. It is likely that continuous release of T into the plasma is important in affecting inhibition of this behavior. Further investigation on the effects of other sex steroids on downstream migratory behavior is required.

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