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Source: Zoological Science, 13(5) : 737-745

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.13.737

ZOOLOGICAL SCIENCE **13**: 737–745 (1996)

Effects of Osmotic Stimulation on Expression of Neurohypophysial Hormone Genes in Pre-Spawning Chum Salmon

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ABSTRACT—Changes in expression of vasotocin (VT) and isotocin (IT) genes were analyzed in chum salmon during the last stages of spawning migration. Pre-spawning chum salmon were caught at following four locations in the Sanriku coast of the Pacific Ocean in Japan: 1) the off-coast area north to the Otsuchi Bay, 2) the mouth of the Otsuchi Bay, 3) inside of the Otsuchi Bay, and 4) the place 500 m upstream to the mouth of the Otsuchi River. In addition, effects of hypo-osmotic stimulation by transition from sea water (SW) to freshwater (FW) were examined in animals caught at the mouth of the Otsuchi Bay. The levels of VT and IT mRNAs in the forebrains were determined by Northern blot analysis. The plasma osmolality and the levels of Na^+ and Cl^- were also analyzed. Expression patterns of VT and IT genes were different between the males and the females. In the males, VT and IT gene expression were maintained essentially at the same levels from the off-coast area to the Otsuchi River. In contrast, in the females, the level of VT-I mRNA was significantly increased in the fish caught at the mouth of the bay. After entering the bay, the level of VT-I mRNA was decreased and maintained at a low level through the final stages of spawning migration. Such sexual difference in VT and IT gene expression found in the field fish was further analyzed by a SW to FW transition experiment, in which fish were divided into two groups, those retained in SW and others replaced with FW. In the FW-replaced fish, the levels of VT and IT mRNAs were decreased in both sexes, although much more conspicuous in the females. In the SW-retained animals, changes in the levels of VT and IT mRNAs were sexually different. The levels of VT and IT mRNAs were increased in the males, whereas they were decreased in the females, when compared to the initial levels just before the experimental treatments. These results suggest that regulation of VT and IT gene expression is sexually dimorphic in pre-spawning chum salmon.

INTRODUCTION

Vasotocin (VT) and isotocin (IT), neurohypophysial hormones in teleosts (Acher *et al.*, 1961, 1962), can be involved in salmon spawning migration, since they presumably have osmoregulatory and reproductive roles in teleosts (Urano *et al.*, 1994). Osmoregulatory mechanisms are very important for salmonids, because many salmonid species migrate to the ocean after hatch in freshwater, and re-enter the natal river to spawn. Osmoregulation during such salmonid migratory behavior between freshwater (FW) and sea water (SW) involves various hormones, including prolactin, cortisol and growth hormone (Hirano, 1991). VT may have an important role in FW adaptation in rainbow trout (Hyodo and Urano, 1991). IT was also suggested to have a physiological role in teleost osmoregulation (Chester Jones *et al.*, 1969; Maetz *et al.*, 1964).

The involvement of neurohypophysial hormones in spawning and parturition have been reported in some teleosts (see for review, Maetz and Lahlou, 1974). Spawning in oviparous fish and parturition in viviparous fish were induced by application of VT and/or IT. In these studies, contraction of the genital tracts was observed with a physiological dose of VT or IT. These facts support the above idea that neurohypophysial hormones can be involved in salmon spawning migration. However, physiological status are conspicuously different between juveniles in down river migration and matured fish in spawning migration, because spawning migration is accompanied with sexual maturation. Actually, our previous reports indicated that the changes in VT and IT gene expression in response to hypo-osmotic stimulation were not coincident between young rainbow trout (Hyodo *et al.*, 1991) and pre-spawning chum salmon (Hiraoka *et al.*, 1995). Furthermore, we found that, in chum salmon, the patterns of

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changes in VT and IT gene expression were different between pre-spawning males and females. Arising question here is whether the sexually different patterns of VT and IT gene expression is a general biological phenomenon in pre-spawning chum salmon, since salmon stocks in different rivers may have diverse genetic backgrounds. If the sexual difference is a general and repeatable phenomenon even experimentally, then the next question is whether VT and IT act on osmoregulation or reproduction.

To clarify the above questions, changes in VT and IT gene expression were examined in pre-spawning chum salmon whose natal river, the Otsuchi River, is different from the Ishikari River in our previous study. In the first experiment in 1993, animals were caught at four locations in the Sanriku coast of the Pacific Ocean, and the relative levels of VT and IT mRNAs in the forebrains were determined by Northern blot analysis to see naturally occurring temporal changes in the field during the last stages of spawning migration. The second experiments were conducted in both 1993 and 1994 to see whether the changes in VT and IT gene expression observed in the field animals are experimentally reproducible in aquaria. In this experiments, salmon were divided into two groups, animals retained in SW-aquaria and those whose environmental water was replaced with FW, and effects of these treatments on the levels of VT and IT mRNAs were examined also by Northern blot analysis.

Probably because of genomic tetraploidization, two types of cDNAs were obtained for precursors of VT (VT-I and VT-II) and IT (IT-I and IT-II) in chum salmon (Hyodo *et al.*, 1991). Hence we analyzed expression of all 4 genes. A new method developed by us in which the levels of mRNAs are quantitatively determined by use of single strand sense DNAs as the standards (Hiraoka *et al.*, 1995) was applied for the analysis of the samples in 1994.

MATERIALS AND METHODS

Experiment 1: Changes in expression of VT and IT genes during spawning migration

Pre-spawning chum salmon, *Oncorhynchus keta*, of both sexes were captured at four areas in the Sanriku coast of the Pacific Ocean, the northern part of Honshu Island, Japan: 1) the off-coast area north to the Otsuchi Bay, 2) the mouth of the Otsuchi Bay, 3) inside of the Otsuchi Bay, and 4) the place 500 m upstream to the mouth of Otsuchi River. These areas are considered to be located on the migratory pathway of homing chum salmon to the Otsuchi River.

Collection of experimental animals

Off-coast chum salmon were fished in the Sanriku coast north to the Otsuchi Bay by salmon long lines during the cruise of the research vessel Tansei-Maru of Ocean Research Institute, University of Tokyo in the early to middle November of 1993. The long lines were set in the early morning before dawn, and were recovered within 1 hr after the set. Only actively moving fish were used for the determination of the levels of VT and IT mRNAs. They are referred to as Tansei fish.

After the research cruise of Tansei-Maru in the off-coast, matured chum salmon of both sexes were caught in the late November to the early December, 1993, by a salmon settled-net placed 1 km outside the Otsuchi Bay (ocean fish), a net settled in the bay close to the mouth of the Otsuchi River (bay fish), and a trap settled in the Otsuchi River 500 m upstream to the river mouth (river fish). The river fish were sampled in the field near the trap immediately after scoop from it. The ocean fish and the bay fish in the settled-net were transferred to oxygenated-SW aquaria (90 cm × 140 cm × 95 cm), and transported to the Otsuchi Marine Research Center, Ocean Research Institute, University of Tokyo. When arrived at the Research Center, fish were transferred to large aerated-running SW aquaria (290 cm × 150 cm × 100 cm, 12°C, 0.1 t/min), in each of which either 10 males or females were separately maintained. On the following day, blood and the brain tissues were collected. The maturity of fish was assessed by the gonadosomatic index, which is shown in Table 1 with the number of animals in the first experiments.

Table 1. Comparison of the gonadosomatic indices (gonad weight/body weight × 100) of pre-spawning chum salmon used in the Experiment 1. Animals were caught at 4 different places probably along the migratory pathway, and were referred to as Tansei fish, ocean fish, bay fish and river fish.

	Males	(n)	Females	(n)
Tansei	4.77 ± 0.32	(3)	15.57 ± 2.41	(3)
Ocean	4.97 ± 0.39	(7)	17.81 ± 0.87	(8)
Bay	4.74 ± 0.17	(7)	22.74 ± 1.11	(9)
River	3.74 ± 0.19*	(10)	21.80 ± 0.70*	(10)

*$p < 0.01$ compared to the ocean fish

Tissue preparation

Fish were anesthetized with 0.02% tricaine methane sulfonate (MS-222, Sigma), and measured of body weight and length. Blood was quickly sampled from the caudal vasculature. Then the fish were decapitated, and the forebrains which included mainly the telencephalon and the ventral diencephalon were taken out, frozen in liquid nitrogen and stored at -80°C. Entire magnocellular neurosecretory neurons were included in this tissue block. Total RNA was extracted from single forebrains by the acid guanidinium thiocyanate-phenol chloroform method (Chomczynski and Sacchi, 1987).

The blood taken from the caudal vasculature was centrifuged at 3000 rpm for 15 min to separate plasma. The plasma was stored at -20°C until the osmolality and the levels of Na^+ and Cl^- were measured. Plasma osmolality, and Na^+ and Cl^- levels were determined with a vapor pressure osmometer (Wescor 5500), ion analyzer (AVL 984-S, Graz, Austria) and chloridometer (Buchler), respectively.

Northern blot analysis

The 1/20 volume of total RNA (ca. 10 μg) extracted from a single brain was electrophoresed in a 1% agarose/formaldehyde, and transferred to Hybond-N^+ membrane (Amersham International plc, Amersham, Bucks, U.K.) according to the manufacturer's instruction.

cDNA probes were prepared by a random priming method using Multiprime DNA labeling system and [α-^{32}P]dCTP (Amersham) with chum salmon VT-I, VT-II, IT-I and IT-II cDNAs as templates. Hybridization with the labeled probe was performed in a 6 × standard saline citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), 5 × Denhardt's reagent and 100 μg/ml denatured yeast tRNA at 60°C for 16 hr. The membranes were washed twice with 0.1 × SSC/0.1% SDS at 60°C for 1 hr, and exposed to a Fuji imaging plate for 3 hr. Hybridization signals were analyzed by a Bioimaging analyzer (Fuji Photo Film Co., Ltd). For statistical analysis, Student's t-test and Duncan's multiple range test were applied after Bartlett's test for variance.

Experiment 2: Effects of osmotic stimulation on VT and IT gene expression

In the second experiment, environmental SW of salmon in aquaria was replaced with FW to see effects of hypo-osmotic stimulation on expression of VT and IT genes, primarily intending to do a model experiment in which sexually different patterns in VT and IT gene expression would be reproduced in pre-spawning fish. We considered that, if it is reproducible, then we can analyze roles of VT and IT in homing behavior of chum salmon, at least whether they are osmoregulatory or reproductive. Pre-spawning fish were thus stimulated in hyper- and hypo-osmotic environments to examine changes in expression of VT and IT genes.

Pre-spawning fish

Ocean fish were used in both preliminary tests in 1993 and systematic investigation in 1994. Fish were captured near the mouth of Otsuchi Bay and transported to the aquaria in early December as is described in Experiment 1. Most of experimental animals in 1993 showed SW silver color characteristic in SW fish, whereas those in 1994 had much or less nuptial color of maturing salmon. They were maintained for a day in a calm condition to be released from handling stresses by the capture and transportation, and then the initial controls (day 0) were sampled. Afterward, they were divided into two groups, those retained in SW and others whose environmental running SW was replaced with running FW (11°C, 0.1 t/min). The levels of Cl^- in the aquaria monitored by use of chloridometer (Buchler) showed that SW was almost completely replaced with FW within 1 hr.

In the preliminary experiment in 1993, FW fish were sampled on days 1 and 3, and SW fish on day 5 after the experimental treatment of either retaining or replacement. In 1994, the FW fish and the SW fish were sampled 1, 2 and 4 days after the experimental treatments. FW fish were additionally sampled on day 7. Matured fish caught in the Otsuchi Bay cannot survive in SW for more than a week, so that 7-day SW fish were not prepared. In addition, in 1994, SW salmon which had prominent silver color (silver fish) were simultaneously captured with matured salmon, similarly adapted to SW aquaria, and were sampled to compare with treated animals.

Tissue preparation

Fish were anesthetized with 0.02% MS-222 (Sigma), and were weighed and measured of body length. Then blood and the brains were collected as described in the Experiment 1. Blood was centrifuged at 3000 rpm for 15 min to separate plasma. Plasma was stored at -20°C until analyses. Total RNA was extracted from single brain tissues as described in the Experiment 1.

Quantitative Northern blot analysis

For quantitative analyses, single strand standard DNAs which have the same sequence of chum salmon VT-I, VT-II, IT-I, or IT-II mRNAs were prepared by the method previously described (Hiraoka *et al.*, 1995).

The 1/20 volume of total RNA (ca. 10 μg) extracted from a single brain was electrophoresed in a 1% agarose/formaldehyde gel with VT-I, VT-II, IT-I, or IT-II standard DNAs, and transferred to membrane as described above. Preparation of cDNA probes, hybridization, washes and exposure were similarly carried out as described in the Experiment 1. Hybridization signals were analyzed by a Bioimaging analyzer (Fuji Photo Film Co., Ltd). For statistical analysis, Student's t-test and Duncan's multiple range test were applied after Bartlett's test for variance.

RESULTS

Pre-spawning chum salmon which were used in the present study showed characteristic nuptial color of maturing animals in both males and females, although it was not fully developed in Tansei and ocean fish. Some of them showed silver color characteristic in SW fish. Most of the female ocean fish were not yet ovulated, whereas the females caught within the bay and in the river were completely ovulated. The incidence of ovulated fish in the Experiment 2 gradually increased up to day 4, on which almost all females sampled had ovulated eggs. In the males, the amount of milt obtained by pressing the abdomen was much less in the Tansei and ocean fish when compared to the bay and river fish.

In general, the levels of VT-I and IT-I mRNAs in the brain of chum salmon were higher than those of their counterparts, VT-II and IT-II mRNAs. Since the levels of mRNAs were relatively determined in the 1993 samples, such differences are not clear in Figs. 1 and 3, but apparent in Figs. 4 and 5 in which the levels of mRNAs in the 1994 samples are shown as ng DNA equivalent. The same differences were also observed in the animals captured in the Ishikari Bay, Hokkaido (Hiraoka *et al.*, 1995).

Experiment 1

Changes in expression of VT and IT genes

Patterns of changes in expression of VT and IT genes were different between the males and the females during the last stages of spawning migration from the Sanriku off-coast to the Otsuchi River (Fig. 1). No significant differences were

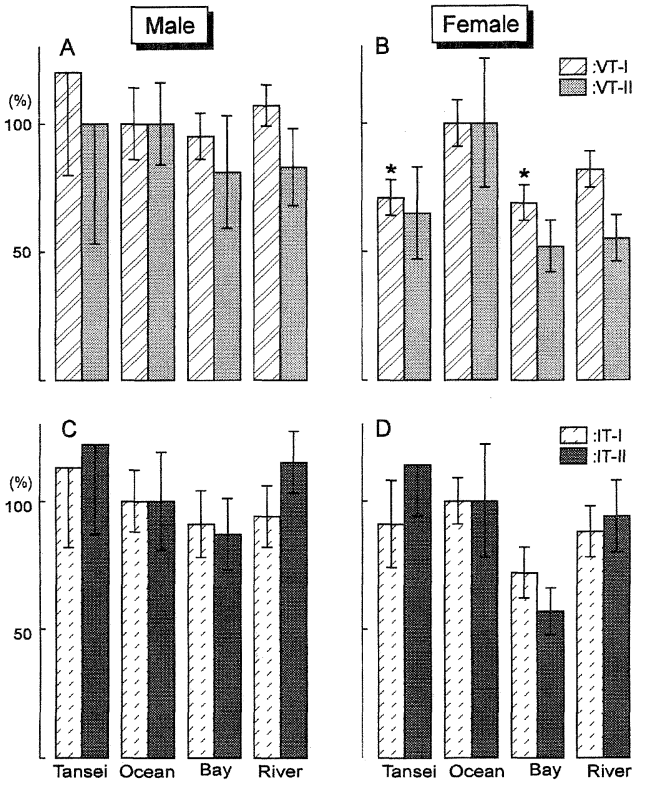

Fig. 1. The levels of VT and IT mRNAs in pre-spawning chum salmon during the last stages of spawning migration. The levels of mRNAs are shown as relative values by regarding the level in the ocean fish as 100%. A and C, males; B and D, females. Mean ± SEM (n=3–10, see Table 1 for the number of fish in each group). *, $p<0.05$ compared to the ocean fish.

seen in the levels of VT and IT mRNAs in the pre-spawning males. In contrast, in the females, the levels of VT-I mRNAs in the bay fish were significantly lower than those in the ocean fish. Furthermore, the levels of VT-I mRNA in the Tansei fish was lower than that in the ocean fish. These results indicate that expression of VT-I gene was transiently increased in the ocean females, although the physiological meaning is not known. Because of the technical limitations, the amounts of mRNAs could not be directly compared between the males and the females.

Changes in the plasma osmolality and the levels of Na^+ and Cl^-

The plasma osmolality and the levels of Na^+ and Cl^- measured in the present study (Fig. 2) generally coincided with those in the previous reports (Hirano *et al.*, 1990; Hiraoka *et al.*, 1995). However, in the ocean salmon, the plasma osmolality and the level of Na^+ in the females were significantly lower than those in the males. These values in the ocean females were also lower than those in the Tansei females. Although this decrease was coincident with the transient increase in the levels of VT-I mRNA mentioned above, the reason and meaning of this difference are not clear at present.

The values of plasma osmolality and levels of Na^+ and Cl^- in the male and female river fish showed similar levels in the FW-adapted fish, in spite of the fact that the area where the river fish were caught is rather brackish.

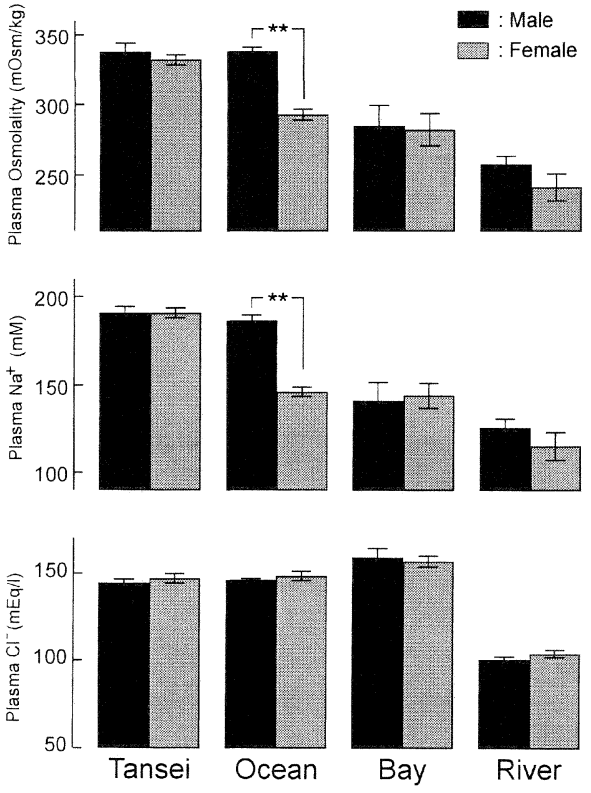

Fig. 2. The plasma osmolality and the levels of Na^+ and Cl^- in pre-spawning chum salmon during the last stages of spawning migration. Mean ± SEM (n=3–10, see Table 1 for the number of fish in each group). **, p<0.01 compared to the males.

Experiment 2

Effects of osmotic stimulation on VT and IT gene expression

In the preliminary SW to FW transition experiment in 1993, we found that changes in expression of VT and IT genes were somewhat different between the males and the females (Fig. 3). The levels of VT and IT mRNAs in the 5-day SW-retained and the 3-day FW-replaced males were essentially the same with those in the initial controls (Day 0), whereas those in the females were decreased in 5-day SW-retained and 3-day FW-replaced animals when compared to the initial controls, although the changes were not statistically significant (Fig. 3).

The levels of VT and IT mRNAs in the fish which were used for the SW to FW transition experiment in 1994 were quantitatively analyzed by using single strand sense DNAs as the standards. The patterns of changes in the levels of VT and IT mRNAs were apparently different between the males and the females (Figs. 4 and 5). In the females, the levels of

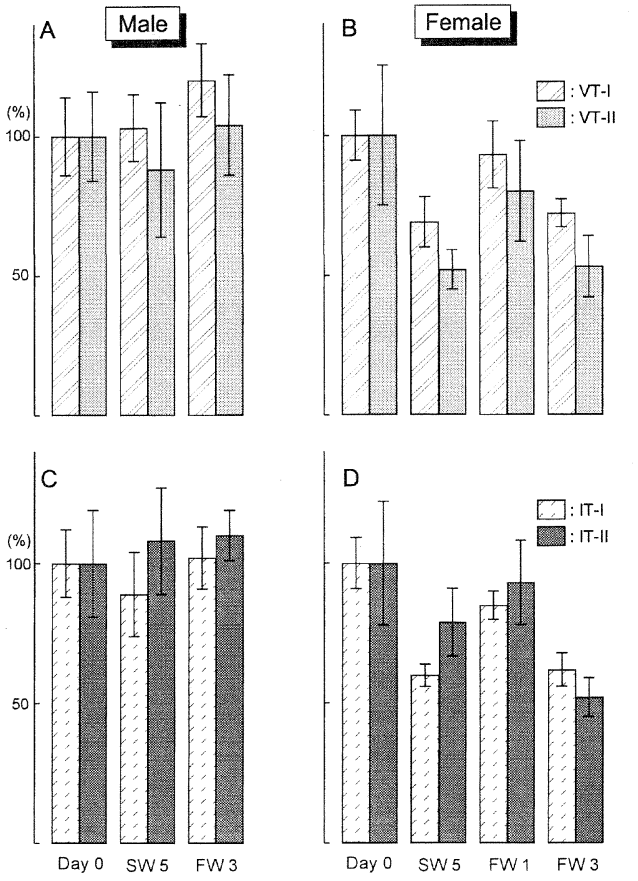

Fig. 3. Effects of SW-retaining and FW-replacement on the levels of VT and IT mRNAs in 1993 pre-spawning chum salmon caught near the mouth of Otsuchi Bay (ocean fish). Most of animals in this experiments showed silver color characteristics of SW fish. A and C, males; B and D, females. Mean ± SEM. N=9, except for the SW-retained males on Day 5 (n=8), FW-replaced males on Day 3 (n=7) and FW-replaced females on Day 1 (n=5).

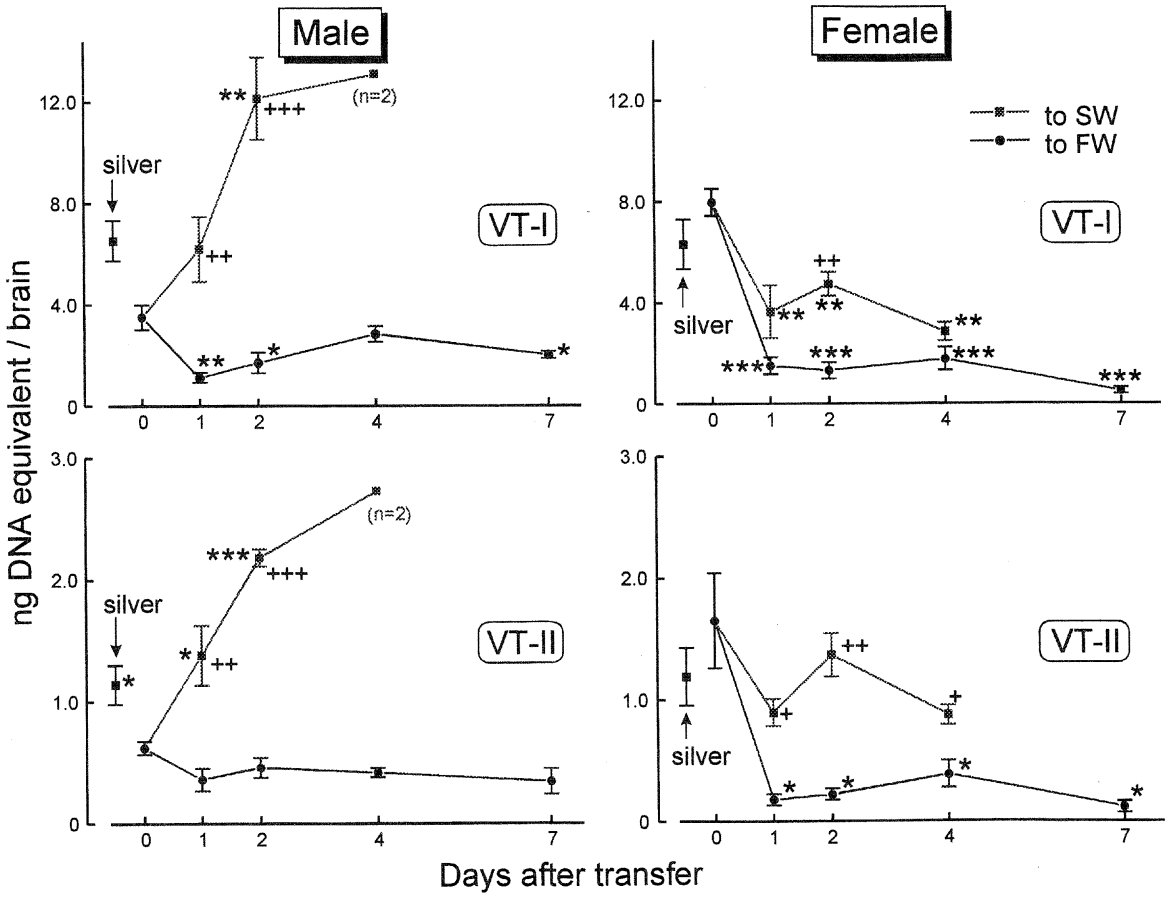

Fig. 4. Effects of SW-retaining and FW-replacement on the levels of VT-I and VT-II mRNAs in 1994 pre-spawning chum salmon (ocean fish). Mean ± SEM. N=6, except for the SW-retained females on Day 2 (n=7) and Day 4 (n=4), SW-retained males on Day 4 (n=2) and FW-replaced females on Day 7 (n=7). *, $p<0.05$; **, $p<0.01$; and ***, $p<0.001$ compared to the initial control (day 0). $^{+}$, $p<0.05$; $^{++}$, $p<0.01$; and $^{+++}$, $p<0.001$ compared to the FW fish.

VT and IT mRNAs were drastically decreased by 1 day after the replacement of SW with FW, and were maintained at the low levels up to 7 days after the treatment. Although the levels of VT and IT mRNAs were also decreased in the SW-retained females, this decrease was less than that in the FW-replaced females. Probably reflecting the transient increases in the levels of VT-I and VT-II mRNAs in the ocean females shown in the Experiment 1 (Fig. 1), those in the initial controls tended to be higher than those in the silver females.

In the SW-retained males, all of VT and IT mRNAs showed significant and gradual daily increases. In contrast, the levels of VT and IT mRNAs except for VT-II mRNAs were not conspicuously but significantly decreased in the FW-replaced males. The magnitudes of decreases compared to the initial controls are much smaller in the males than in the females, so that the levels of VT and IT mRNAs remained higher in the males than in the females 7 days after the FW-replacement.

Changes in the plasma osmolality and the levels of Na^+ and Cl^-

Changes in the plasma osmolality and the Na^+ and Cl^- levels differed between the males and the females that were retained in SW in the 1994 transition experiment (Fig. 6). Similar results were also observed in the 1993 experiment (data not shown). All of the parameters were well maintained at the same levels in the males up to 4-day SW retaining, however the mortality in this group was so high that only 2 fish from 7 could survive. In contrast, in the SW-retained females, all of the parameters showed gradual daily increase. The mortality in the SW-retained female group seemed to be not so high compared to the males. Both in the FW-replaced males and females, the plasma osmolality and the Na^+ and Cl^- levels were significantly decreased by 1 day after the FW replacement and were maintained at the same level during the experiment.

DISCUSSION

The present study in pre-spawning chum salmon which were caught at 4 locations on migratory pathway demonstrated that changes in expression of VT and IT genes were different between the males and the females during the last stages of spawning migration. Such sexually dimorphic gene expression coincided with our previous report on the Ishikari stock (Hiraoka *et al.*, 1995). The effects of osmotic stimulation on expression

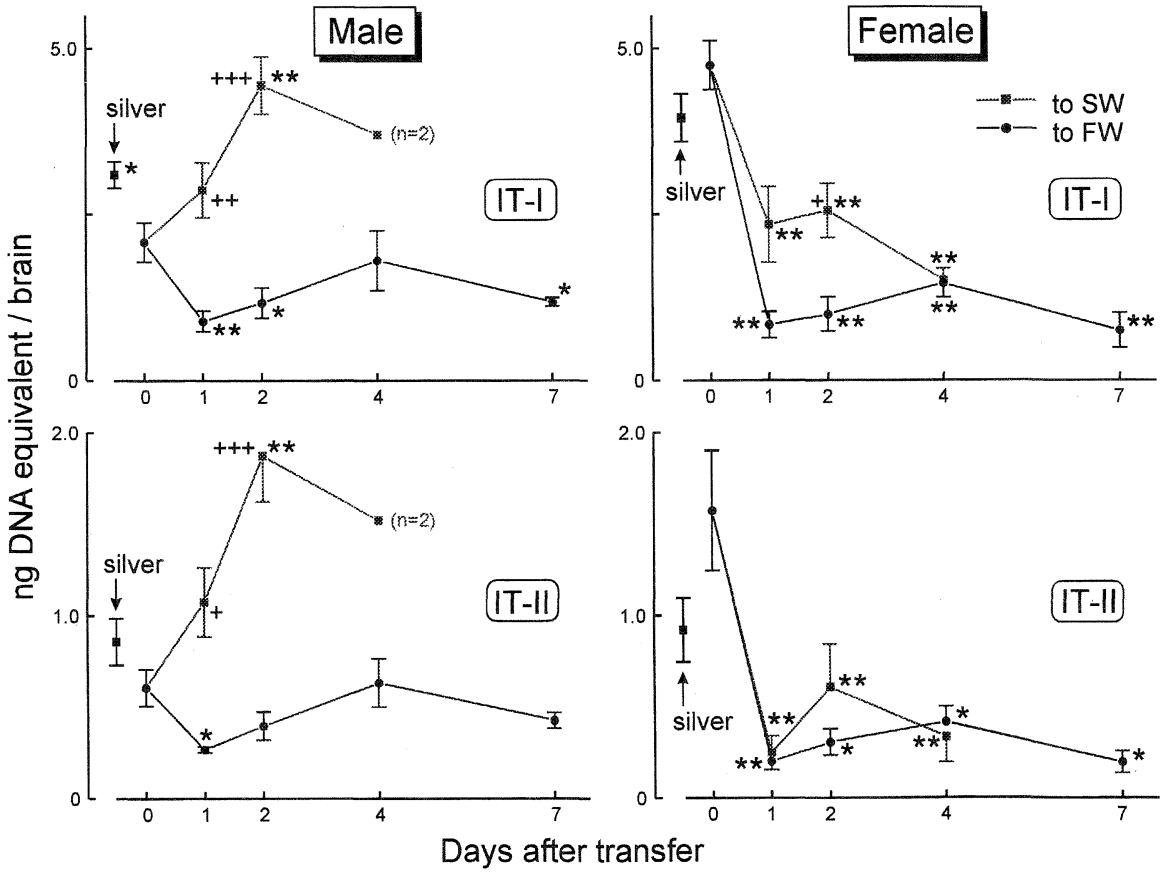

Fig. 5. Effects of SW-retaining and FW-replacement on the levels of IT-I and IT-II mRNAs in 1994 pre-spawning chum salmon (ocean fish). Mean ± SEM (n=5–9). The numbers of fish were the same with those shown in Fig 4. *, $p<0.05$ and **, $p<0.01$ compared to the initial control (day 0). $^{+}$, $p<0.05$; $^{++}$, $p<0.01$; and $^{+++}$, $p<0.001$ compared to the FW fish.

of VT and IT genes were also different between the males and the females captured near the mouth of Otsuchi Bay. Therefore, we consider that such sexual difference in VT and IT gene expression naturally occur in pre-spawning chum salmon in the field. We further showed that these sexual differences were reproducible by the experimental treatment. These facts suggest that the sexual dimorphism in VT and IT gene expression may be physiologically important phenomena in pre-spawning chum salmon.

The sexually different VT gene expression found in the present study may be accounted for by effects of steroid hormones on transcriptional regulation of VT genes, because three estrogen responsive elements and four glucocorticoid responsive elements are present in the 5'-upstream region of the VT-I gene in chum salmon (Satomi *et al.*, 1994). In the previous study, the serum concentrations of steroid hormones were determined in chum salmon during spawning migration (Ueda *et al.*, 1984). The level of estradiol-17β in the females caught in the Ishikari Bay was significantly higher than that in the fish caught in the river. Such drastic change in the estradiol-17β concentration can induce the sexual difference of VT gene expression. Furthermore, in matured chum salmon, the level of plasma cortisol in females was higher than that in males (Hirano *et al.*, 1990). Because the activity of vasopressin synthesis is repressed by glucocorticoid in the paraventricular nucleus of the rat (Davis *et al.*, 1986), it is possible that the high concentration of plasma cortisol also repress VT gene expression in FW females.

In the female chum salmon, the levels of VT and IT mRNAs were decreased in the final stages of spawning migration. Such decrease was also observed in the FW-replaced females and in the SW-retained females in both 1993 and 1994. It was reported that the levels of vasopressin and oxytocin mRNAs were increased by the stimulation which elevates the plasma osmolality and Na^{+} level, such as sodium loading and water deprivation in the rat supraoptic and paraventricular nuclei (Hyodo *et al.*, 1988, 1989), and that the neurosecretory cells derived from the rat supraoptic nucleus were activated by osmotic stimuli (Stéphane and Charles, 1994). Fish magnocellular neurons are also responsive to osmotic stimuli (see Urano *et al.*, 1994). However, the intensity of VT mRNA hybridization signals in magnocellular neurons in the nucleus preopticus magnocellularis were decreased after transfer from FW to 80% SW in immature rainbow trout, suggesting that VT may have a physiological role in salmonid osmoregulation, particularly in adaptation to a hypo-osmotic

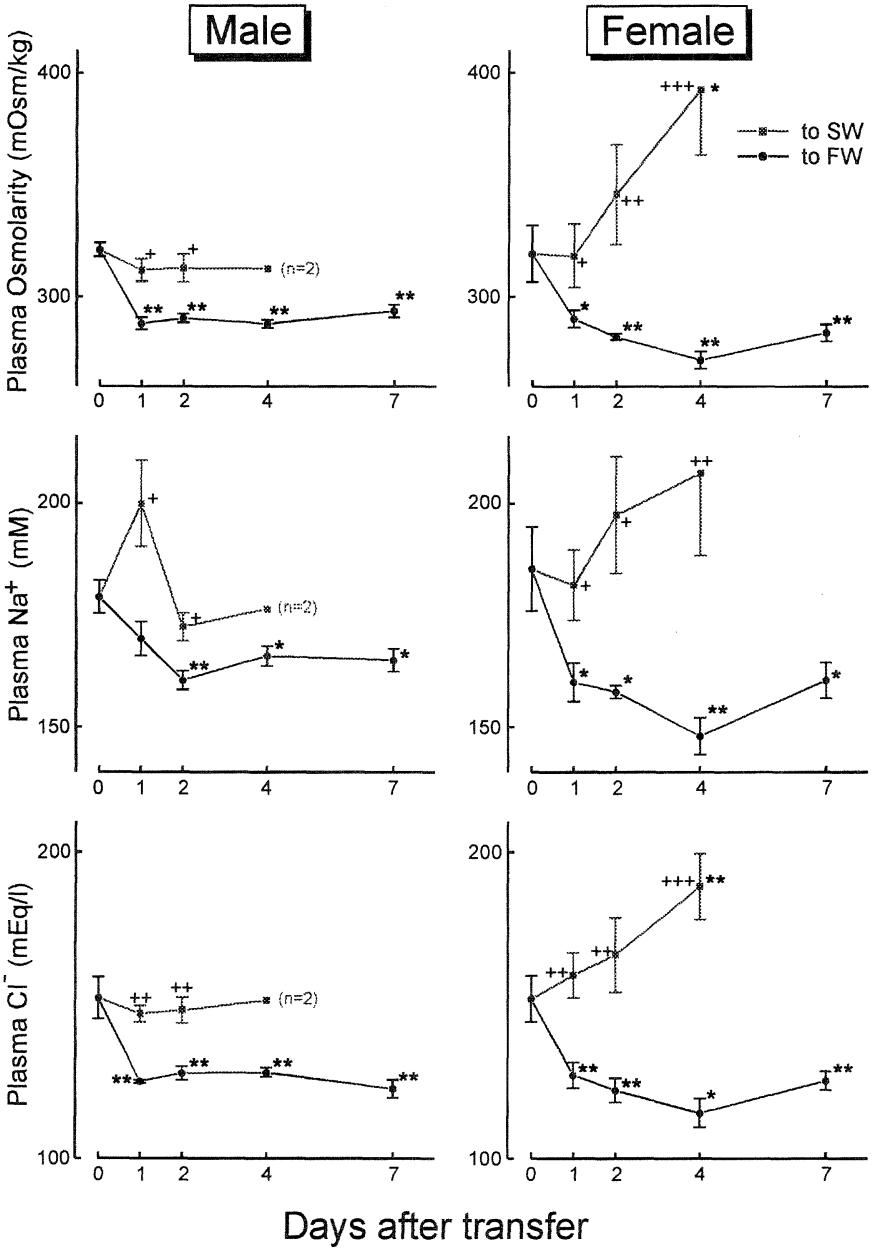

Fig. 6. Changes in the plasma osmolality and the levels of Na^+ and Cl^- in 1994 pre-spawning chum salmon. The values in the silver fish were almost the same with those in the initial control, so that not shown in the figure. Mean ± SEM (n=2–7). *, $p<0.05$ and **, $p<0.01$ compared to the initial control (day 0). $^+$, $p<0.05$; $^{++}$, $p<0.01$; and $^{+++}$, $p<0.001$ compared to the FW fish.

environment (Hyodo and Urano, 1991). This discordance in the responses to osmotic stimulation may be caused by the difference in magnitudes of gonadal development, because the physiological status are conspicuously different between immature and matured fish, for example, the endocrine systems involved in sexual development are widely changed, and also sex steroid hormones are well known to modulate expression of various genes.

The present results in the pre-spawning females showed that, after transient increase, expression of VT and IT genes were repressed in both SW and FW, indicating that osmotic stimuli are not critical to modulate expression of VT and IT genes. Such changes in VT and IT gene expression may be important for reproduction or reproductive behavior rather than osmoregulation in the final stages of spawning migration, because ovulation appeared to occur following these events. In the river females, plasma prolactin increased greatly 1 day after SW to FW transition, and the elevated levels were maintained for 3 to 5 days afterward (Hirano *et al.*, 1990). Because prolactin is well established as the FW-adaptation hormone in teleosts (Hirano, 1991), adaptation to a hypo-osmotic environment in the pre-spawning females is probably regulated by prolactin rather than by VT or IT.

The levels of VT and IT mRNAs in the males were

generally higher than those in the females in the final stages of spawning migration. This phenomenon was confirmed in the males maintained in FW for 7 days, as well as in the Ishikari stock (Hiraoka *et al.*, 1995). Similar or even higher levels of VT and IT mRNAs on day 0 in the females may be due to the transient increase in their gene expression mentioned above. It is well known that innervation of vasopressin or VT immunoreactive (ir) fibers in some brain loci are abundant in the males compared to the females (Van Leeuwen *et al.*, 1985; Boyd *et al.*, 1992). Such abundant vasopressin-ir innervation are probably supported by androgens (Van Leeuwen *et al.*, 1985; Miller *et al.*, 1992). Interestingly, in prairie voles (*Microtus ochrogaster*), the number of neurons in the bed nucleus of the stria terminalis labeled for vasopressin mRNA was higher in males than in females (Wang *et al.*, 1994), and further central vasopressin pathways have been implicated in the mediation of paternal behavior, selective aggression and affiliation. The frequency of attack and threat behaviors was decreased after treatment with vasopressin antagonist (Winslow *et al.*, 1993). In our results from the ocean to the river, the levels of VT mRNA in the males were maintained nearly at the same level, whereas those in the females were decreased. These facts may indicate an involvement of VT in the control of male spawning behavior.

In the pre-spawning males, expression of VT and IT genes seemed to be slightly repressed in FW, although the magnitude was weaker than that in the females. Such a repressing mechanism may be inactivated or rather suppressed in the SW-retained males, in which the levels of VT and IT mRNAs were gradually elevated. If this idea is true, then the increase in the levels of VT and IT mRNAs seems to be partly accounted for by inactivation of this repression, since the expression of VT and IT genes in the males seems to be maintained rather active even in FW when compared to the females. A plausible alternate explanation for the increase in VT and IT mRNAs in the SW males is that VT and IT function as SW-adaptation hormones or antidiuretic hormones in matured chum salmon. However, this hypothesis cannot explain the facts that the levels of VT and IT mRNAs were rather high in the male river fish compared to the bay fish in the Ishikari stock (Hiraoka *et al.*, 1995), and that those in the males seemed to be not affected in the present SW to FW transition study in 1993.

In the latter experiment mentioned above, the pattern of changes in the levels of VT and IT mRNAs differed from the results in 1994, despite the coincidence with those in the field animals in the same year. The mRNA levels in the males were not elevated in SW, nor decreased in FW. In addition, the initiation of decreases in the levels of mRNAs was postponed for about 2 days in the females in 1993, when compared to that in 1994 (see Figs. 3, 4 and 5). Such discordance between 1993 and 1994 may be derived of the difference in sexual maturity, because the salmon in 1993 had silver color, whereas the animals in 1994 showed nuptial color. Effects of sexual maturity on responsiveness of VT and IT gene expression to osmotic stimuli remained to be clarified.

In conclusion, changes in expression of VT and IT genes were different between the pre-spawning males and females. In the males, the changes in expression of VT and IT genes were essentially at the same level during spawning migration. The levels of VT and IT mRNAs were maintained at the similar level or decreased by the hypo-osmotic stimulation. In contrast, in the females, the levels of VT and IT mRNAs were tended to decrease from the Sanriku off-coast to the Otsuchi River, and decreased by both the hyper- and hypo-osmotic stimulation. Such sexually dimorphic gene expression should be natural phenomena in pre-spawning chum salmon. Since these sexual differences were reproducible by the experimental treatments, the sexual dimorphism of VT and IT gene expression may be a physiologically important phenomenon in pre-spawning chum salmon. The control of VT and IT gene expression should be different between the males and the females during the last stages of pre-spawning migration.

ACKNOWLEDGMENTS

We expressed our gratitude to colleagues in our laboratory, to Dr. Yasuaki Takagi and Mr. Kouichi Morita, Otsuchi Marine Research Center, and to the crew of the research vessel Tansei-Maru, Ocean Research Institute, University of Tokyo, for their help in sampling efforts. We are also grateful to the staffs and members, Laboratory of Physiology, Ocean Research Institute, University of Tokyo, for their kind help in measuring plasma osmolality and Na^+ concentration. This study was supported in part by Grants-in-Aid from the Fisheries Agency, and the Ministry of Education, Science, Sports and Culture, Japan.

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(Received April 2, 1996 / Accepted July 25, 1996)