Prolactin Kinetics during Freshwater Adaptation of Mature Chum Salmon, Oncorhynchus keta

Authors: Ogasawara, Tsuyoshi, Sakamoto, Tatsuya, and Hirano, Tetsuya

Source: Zoological Science, 13(3) : 443-447

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.13.443

ZOOLOGICAL SCIENCE **13**: 443–447 (1996)

Prolactin Kinetics during Freshwater Adaptation of Mature Chum Salmon, *Oncorhynchus keta*

Tsuyoshi Ogasawara[1], Tatsuya Sakamoto[2,3] and Tetsuya Hirano[2,*]

[1] *Faculty of Science, Kanagawa University, Hiratsuka, Kanagawa 259-12, and*
[2] *Ocean Research Institute, University of Tokyo, Nakano, Tokyo 164, Japan*

ABSTRACT—When mature chum salmon (*Oncorhynchus keta*) caught in Otsuchi Bay (salinity 34 ppt), northern Honshu Island, Japan, were transferred directly to fresh water, they attained normal plasma ion levels within 24 hr. Plasma prolactin remained low in the fish kept in seawater. On transfer to fresh water, a significant increase in plasma prolactin concentration was seen only in females, but not in males. Metabolic clearance rate (MCR) and secretion rate of prolactin were calculated from its plasma levels after intra-arterial injection of chum salmon prolactin into chronically-cannulated fish. In both males and females, a significant increase in MCR was seen after transfer to fresh water, indicating that prolactin is involved in freshwater adaptation in both sexes. In females, the secretion rate increased significantly after 6 days in fresh water. No such change in the secretion rate was seen in males. It seems likely that prolactin secretion is affected by reproduction-related changes occurring in mature females in fresh water.

INTRODUCTION

Among seven species of Pacific salmon, chum (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*) are unique in spawning close to the sea and migrating to the sea soon after yolk absorption (see Groot and Margolis, 1991). In northern Honshu Island, Japan, chum salmon complete gonadal maturation while in the bay, and spawn sometimes less than 1 km from the river mouth. When mature chum salmon caught in the bay were transferred to fresh water, an increase in plasma prolactin concentration was seen only in females, although both males and females adapted well to fresh water (Hirano *et al.*, 1985, 1990). Prolactin may play important roles in osmoregulation in both sexes and/or in final maturation in females. On the other hand, mature fish of both sexes caught in the bay failed to survive in seawater for more than a week, with increased plasma osmolality due mainly to the increase in Na and Mg concentrations. Plasma prolactin remained low in seawater fish, suggesting that increased secretion of prolactin, a freshwater-adapting hormone, is not the cause of maladaptation to seawater (Hirano *et al.*, 1990). In general, the plasma level of a hormone is a result of equilibrium between the rate of secretion and the rate of clearance from the circulation. The present study was undertaken to clarify the roles of prolactin in mature chum salmon by measuring metabolic clearance rate of prolactin during acclimation to fresh water, with special reference to the difference between males and females.

MATERIALS AND METHODS

Mature chum salmon (*Oncorhynchus keta*) of both sexes, males weighing 2-3 kg and females 3-4 kg, were caught by a set-net in Otsuchi Bay, a small semi-enclosed bay on the Pacific coast of northern Honshu Island, Japan. Since their spawning ground in the Otsuchi River is less than 1 km from the river mouth, the fish in the bay have almost completed gonadal maturation in late November and early December, ovulation in most females and partial spermiation in males (see Hirano *et al.*, 1978, 1990). Fish were transported for 30 min in an oxygenated tank containing seawater to the Otsuchi Marine Research Center of the Ocean Research Institute, University of Tokyo.

The first experiment examined the differences in plasma prolactin and other plasma parameters between males and females after transfer from seawater to fresh water. Three hours after arrival at Otsuchi Marine Research Center, they were anesthetized with 0.05% tricaine methane sulfonate (Sigma, St. Louis, USA), and about 4 ml blood were taken from the caudal vessels with a hypodermic syringe. The fish were marked individually by clipping various parts of the fin and were then transferred to running freshwater (8°C) or seawater (34 ppt, 11°C) tanks. Males and females were kept in separate tanks. Blood samples were also taken 1, 4, 8 and 24 hr and 3, 5 and 7 days after transfer to fresh water. In accordance with our previous observations (Hirano *et al.*, 1990), most of the fish kept in seawater failed to survive for more than 3 days. Thus, their plasma samples were taken only 1, 4, 8 and 24 hr after transfer.

The blood plasma was immediately separated by centrifugation at 10,000 rpm for 60 min at 5°C, and kept frozen at -80°C until analyses. Plasma levels of prolactin were measured by radioimmunoassay (Hirano *et al.*, 1985). Plasma concentrations of Na, Ca and Mg were measured by an atomic absorption spectrophotometer (Hitachi 203).

In the second experiment, changes in metabolic clearance rate

[3] Present address: National Research Institute of Fisheries Science, Fukuura, Kanazawa-ku, Yokohama 236, Japan
[*] To whom all correspondence should be addressed.

(MCR) of prolactin were examined during freshwater acclimation. The mature salmon caught in the bay were kept in seawater for 24 hr, and then cannulated via the dorsal aorta as described by Ishimatsu *et al.* (1988). The cannulated fish were kept in seawater for further 24 hr, and transferred to fresh water. Prolactin MCR was measured 24 hr after the transfer, and also in the fish kept in seawater. Some fish were transferred to fresh water without cannulation. They were cannulated 4 days after the transfer, and MCR was measured after 6 days.

The MCR was measured largely following the procedures employed for the measurement of MCR of growth hormone and prolactin in rainbow trout and coho salmon (Sakamoto *et al.*, 1990, 1991). At first, a 0.2-ml blood sample was removed to monitor basal concentration of prolactin. Then, 0.2-0.3 ml prolactin solution (chum salmon prolactin, provided by Prof. H. Kawauchi of Kitasato University), containing appropriate doses (7.5 μg/kg), was rapidly injected through the cannula. The cannula was immediately flushed with 0.6 ml saline. Blood samples (0.2 ml) were taken at 15 min (initial), and 0.5, 1, 2, 3, and 5 hr after the injection. Calculation of MCR was carried out as described by Sakamoto *et al.* (1990). The secretion rate of prolactin was calculated by multiplying the MCR by the endogenous concentration of prolactin.

Statistical significance was examined by Duncan's new multiple range test (Duncan, 1955) and Student's *t*-test, where appropriate.

RESULTS

The initial levels of plasma Na of the mature chum salmon caught in Otsuchi Bay were extremely high, about 210 mM in both sexes, indicating probable stress and maladaptation to seawater. When the fish were transferred to fresh water, plasma Na levels decreased gradually to about 160 mM during the first 24 hr; these levels were maintained thereafter. There was no difference between males and females (Fig. 1). Slight but significant reduction in plasma Ca and Mg levels was also seen during the first 24 hr (data not shown).

As shown in Fig. 2, plasma prolactin levels remained low in the fish kept in seawater. When females were transferred to fresh water, plasma prolactin started increasing after 4 hr, reached a peak level (7 ng/ml) after 24 hr, and then gradually decreased to about 3 ng/ml after 5 days. In contrast, no significant change was seen in males either in fresh water or in seawater.

There was no apparent difference between males and females in the disappearance rate of intra-arterially injected

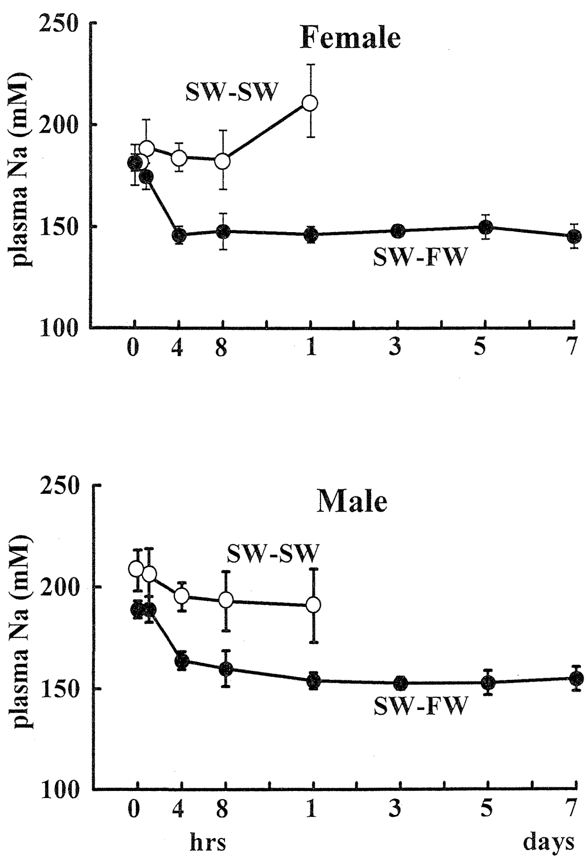

Fig. 1. Time-course of changes in plasma Na concentrations after transfer of mature chum salmon from seawater (SW) to fresh water (FW). Some fish were kept in SW. Results are expressed as means ± SEM (n=6). All the values in the FW-transferred fish (except 1 hr after the transfer) were significantly (P<0.01) different from the initial levels in SW (time 0).

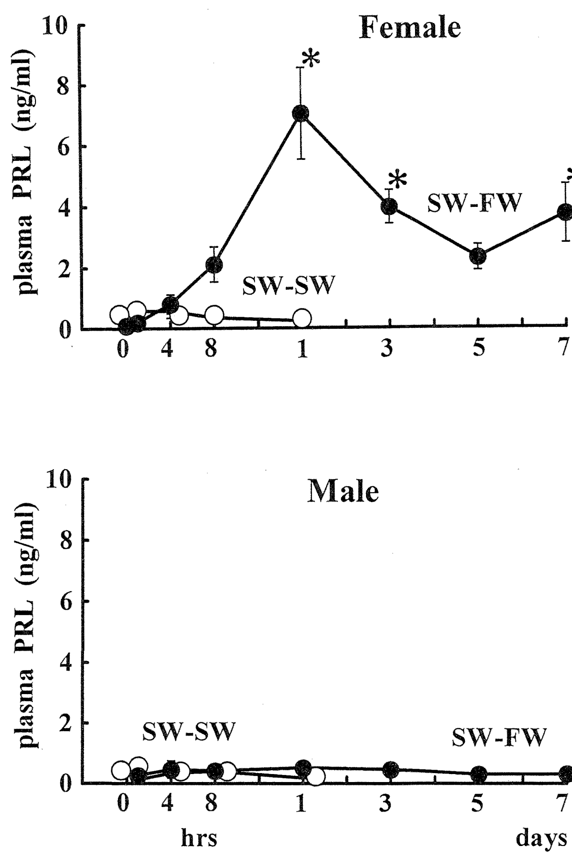

Fig. 2. Time-course of changes in plasma prolactin (PRL) concentrations after transfer of mature chum salmon (same as in Fig. 1) from seawater (SW) to fresh water (FW). Some fish were kept in SW. Results are expressed as means ± SEM (n=6). *Significantly different from the initial levels in SW (time 0) at 1% level.

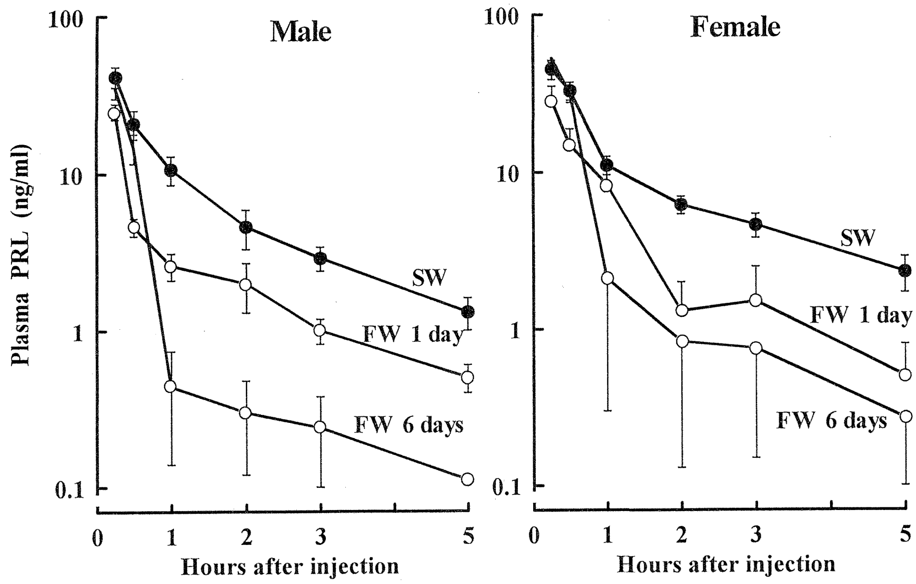

Fig. 3. Plasma clearance curves of salmon prolactin after intra-arterial injection of 7.5 µg/kg into mature salmon in seawater (SW), and after 1 and 6 days in fresh water (FW). Results are expressed as means ± SEM.

prolactin from the plasma. The initial levels were about 50 ng/ml in both cases. The disappearance rates were already greater 1 day after transfer to fresh water than the rates in seawater, and increased further after 6 days in fresh water (Fig. 3).

Figure 4 shows changes in plasma prolactin concentrations, metabolic clearance rate (MCR) and secretion rate during adaptation to fresh water. Basal prolactin levels in these fish, measured in the plasma immediately before the intra-arterial injection of prolactin, were low (less than 0.1 ng/ml) in the fish in seawater. In females, there was a tendency toward an increase after 1 day, and a significant increase after 6 days in fresh water. There was no significant change in males; only two fish showed detectable levels of prolactin (0.3 and 0.6 ng/ml) after 6 days.

On the other hand, the MCR increased significantly in males after 1 day in fresh water. The increase in the female was not significant, although there was no significant difference between the sexes. The MCR increased significantly after 6 days in both males and females. As a result of the increased turnover rate (MCR) and plasma concentration, the secretion rate of prolactin increased significantly after 6 days in fresh water in females, whereas no such increase was seen in males.

Plasma profiles of Na of these fish were essentially the same as in the first experiments (Fig. 1), decreasing significantly after 1 day, with levels maintained until 6 days

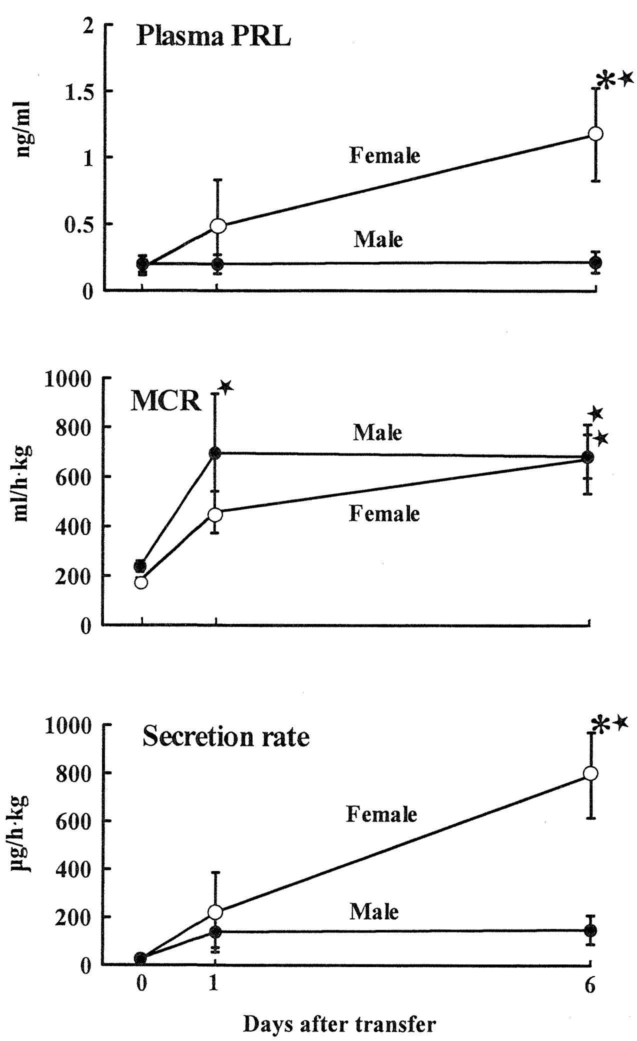

Fig. 4. Time-course of changes in plasma prolactin concentrations, metabolic clearance and secretion rates of prolactin after transfer of mature chum salmon (same as in Fig. 3) from seawater (time 0) to fresh water (FW). Results are expressed as means ± SEM. *,★ Significantly (P<0.01) different from the seawater levels (time 0) and from the corresponding levels in the male fish, respectively.

after the transfer in both sexes (data not shown).

DISCUSSION

Returning chum salmon in northern Honshu Island, Japan, complete gonadal maturation while in the bay or before entering the river. In previous studies examining the hormones involved in freshwater adaptation of mature chum salmon, we had difficulty in maintaining the control fish alive in seawater (Hirano *et al.*, 1985, 1990). Mortality in seawater seems to result primarily from increased plasma osmolality. In close correlation with the increased plasma osmolality and electrolyte concentrations, plasma levels of cortisol and growth hormone increased in the fish kept in seawater. Both cortisol and growth hormone are putative seawater-adapting hormones, particularly in salmoninds (see Sakamoto *et al.*, 1993; McCormick, 1995); these hormones may be secreted in response to increased plasma osmolality. Plasma prolactin, on the other hand, remained low in the seawater fish, indicating that the increased secretion of prolactin, a freshwater-adapting hormone in fishes (see Hirano, 1986; Hirano *et al.*, 1987; Brown and Brown, 1987), is not the cause of maladaptation to seawater. When the mature fish caught in the bay were transferred to fresh water, an increase in plasma prolactin concentrations was seen only in females (Hirano *et al.*, 1985, 1990). A similar difference between sexes was observed in the present study; on transfer to fresh water, significant increase in plasma prolactin was observed only in females, although both males and females adjusted their plasma osmolality and electrolytes equally efficiently in fresh water. As plasma concentrations of the hormone are the results of an equilibrium between the secretion rate and metabolic clearance rate (MCR), a complete assessment of prolactin dynamics requires kinetic studies as in the present study.

As is clearly shown in the present study, a significant increase in prolactin MCR was seen after transfer of mature chum salmon to fresh water in both males and females, indicating that prolactin is involved in freshwater adaptation in both sexes. According to Sakamoto *et al.* (1991), transfer of the adult coho salmon from seawater to fresh water was followed after 2 days by a rise in plasma prolactin, which tended to stay at high levels for 2-3 weeks; prolactin MCR also increased significantly after 2-3 weeks in fresh water. In coho salmon, however, a sexual difference in prolactin dynamics was unclear, since the experiments were carried out on the fish about 2 months before the spawning period. Brewer and McKeown (1980) also reported a shorter half-life (increased MCR) of injected ^{125}I-labeled ovine prolactin in coho salmon transferred to fresh water than in the fish in seawater.

The MCR reflects the distribution, utilization, and degradation of the hormone. To our knowledge, no homologous radioreceptor assay for salmon prolactin has been established. According to Fryer (1979), binding of tilapia prolactin to tilapia kidney membranes was greater in freshwater-acclimated than in seawater-acclimated fish. In tilapia, two distinct prolactin forms have been characterized ($tPRL_{188}$ and $tPRL_{177}$) (Specker *et al.*, 1985; Yamaguchi *et al.*, 1988). Using recombinant tilapia prolactins, high specific binding of prolactin has recently been shown in the gills and kidney of tilapia, involving only one class of receptors, which binds $tiPRL_{188}$ with higher affinity than $tiPRL_{177}$ (Auperin *et al.*, 1994). Binding studies have also shown various modifications of prolactin receptor levels during seawater adaptation (Auperin *et al.*, 1995). More recently, a tilapia prolactin receptor gene has been cloned, and Northern blot analysis shows the existence of a single transcript in osmoregulatory organs such as the gills, kidney and intestine, as well as in the testis. Transfer of tilapia to brackish water resulted in a low but significant decrease of the prolactin receptor transcript level in the gills (Sandra *et al.*, 1995). The increased clearance of prolactin from blood after transfer of chum and coho salmon to fresh water may reflect increased prolactin binding to the receptors in osmoregulatory organs.

In the present study, as a result of the increased turnover rate and plasma concentration, the secretion rate of prolactin increased significantly after 6 days in fresh water only in females but not in males, suggesting that prolactin secretion may be affected by reproduction-related changes in mature females in fresh water. In accordance with present observations, Suzuki *et al.* (1987) reported that prolactin secretion from organ-cultured pituitaries of mature chum salmon caught in the river was greater in females than in males. Estradiol is known to induce activation of prolactin cells in the goby (Nagahama *et al.*, 1975), and stimulate prolactin release from tilapia pituitary *in vitro* (Barry and Grau, 1986). Gonads are one of the important targets for prolactin action (see Hirano, 1986). Edery *et al.* (1984) demonstrated specific binding of ovine prolactin predominantly in the gonads and liver of the tilapia. Tilapia prolactin has been shown to stimulate estradiol production in early vitellogenic oocytes of the guppy (Tan *et al.*, 1988). On the other hand, plasma testosterone levels in male killifish were increased following injections of salmon prolactin, and the testes of rainbow trout produced more testosterone when exposed to salmon prolactin (Singh *et al.*, 1988). More recently, Rubin and Specker (1992) reported stimulatory effects of homologous prolactins on *in vitro* production of testosterone by tilapia testes. These observations exemplify prolactin actions on both female and male gonads in teleosts.

In contrast to most teleost species, salmonid fishes posses naked ovaries, and oviducts are lacking. The ovulated eggs lie free in the body cavity filled with coelomic fluid, and can be forced through the genital pore with slight pressure. Thus, the greater secretion rate of prolactin in mature female chum salmon may also be attributable to the modified hydromineral balance owing to secretion of a large amount of coelomic fluid in association with ovulation, the volume of coelomic fluid being greater than the volume of the seminal fluid in the male (Hirano *et al.*, 1978; Morisawa *et al.*, 1979). According to Avella *et al.* (1991), chronic stress resulted in an elevation of plasma prolactin in coho salmon. In our previous study, plasma cortisol levels were significantly higher in the female chum salmon in the river than those in the male river fish, indicating that the mature female may be under severe stress (Hirano *et al.*, 1985). Stress may also affect MCR as well as secretion rate of prolactin.

ACKNOWLEDGMENTS

We are grateful to Prof. Howard A. Bern, University of California at Berkeley for his continued interest and encouragement throughout the course of our study and also for critical reading of the manuscript. We are also indebted to the staff of Otsuchi Marine Research Center, Ocean Research Institute, University of Tokyo for their generous help in obtaining the mature chum salmon and providing facilities for the experiments. Thanks are also due to Ms. Sanae Hasegawa for her help in measuring plasma ion and osmolality and also in preparation of the manuscript. We thank Prof. Hiroshi Kawauchi of Kitasato University for kind provision of chum salmon prolactin. This study was supported in part by grants-in-aid for scientific research from the Ministry of Education and also from the Fisheries Agency, Japan.

REFERENCES

Auperin B, Rentier-Delrue F, Martial JA, Prunet P (1994) Characterization of a single prolactin (PRL) receptor in tilapia (*Oreochromis niloticus*) which binds both PRLI and PRLII. J Mol Endocrinol 13: 241-251

Auperin B, Rentier-Delrue F, Martial JA, Prunet P (1995) Regulation of gill prolactin receptor in tilapia (*Oreochromis niloticus*) after a change in salinity or hypophysectomy. J Endocrinol 145: 213-220

Avella M, Schreck CB, Prunet P (1991) Plasma prolactin and cortisol concentrations of stressed coho salmon, *Oncorhynhus kistch*, in fresh water and salt water. Gen Comp Endocrinol 81: 21-27

Barry G, Grau EG (1986) Estradiol-17β and thyrotropin- releasing hormone stimulate prolactin release from the pituitary gland of a teleost fish *in vitro*. Gen Comp Endocrinol 62: 306-314

Brewer KJ, McKeown BA (1980) Prolactin regulation in the coho salmon, *Oncorhynchus kisutch*. J Comp Physiol B 140: 217-225

Brown PS, Brown SC (1987) Osmoregulatory actions of prolactin and other adenohypophysial hormones. In "Vertebrate Endocrinology: Fundamentals and Biomedical Implications Vol 2" Ed by PKT Pang, MP Schreibman, Academic Press, San Diego, pp 45-84

Duncan DB (1955) Multiple range and multiple *F* tests. Biometrics 11: 1-42

Edery M, Young G, Bern HA, Steiny S (1984) Prolactin receptors in tilapia (*Sarotherodon mossambicus*) tissues: Binding studies using ^{125}I-labeled ovine prolactin. Gen Comp Endocrinol 56: 19-23

Fryer JN (1979) Prolactin-binding sites in tilapia (*Sarotherodon mossambicus*) kidney. Gen Comp Endocrinol 39: 397-403

Groot C, Margolis L (1991) Pacific Salmon. Life Histories. UBC Press, Vancouver, 564 pp

Hirano T (1986) The spectrum of prolactin action in teleosts. In "Comparative Endocrinology: Developments and Directions" Ed by CH Ralph, Alan Liss, New York, pp 53-74

Hirano T, Morisawa M, Suzuki K (1978) Changes in plasma and coelomic fluid composition of the mature chum salmon (*Oncorhynchus keta*) during freshwater adaptation. Comp Biochem Physiol 61A: 5-8

Hirano T, Ogasawara T, Bolton JP, Collie NL, Hasegawa S, Iwata M (1987) Osmoregulatory role of prolactin in lower vertebrate. In "Comparative Physiology of Environmental Adaptations" Ed by R Kirsch, B Lahlou, Kager, Basel, pp 112-124

Hirano T, Ogasawara T, Hasegawa S, Iwata M, Nagahama Y (1990) Changes in plasma hormone levels during loss of hypoosmoregulatory capacity in mature chum salmon (*Oncorhynchus keta*) kept in seawater. Gen Comp Endocrinol 78: 254-262

Hirano T, Prunet P, Kawauchi H, Takahashi A, Ogasawara T, Kubota J, Nishioka RS, Bern HA, Takada K, Ishii S (1985) Development and validation of a salmon prolactin radioimmunoassay. Gen Comp Endocrinol 59: 266-276

Ishimatsu A, Iwama GK, Heisler N (1988) *In vivo* analysis of partitioning of cardiac output between systematic and central venous sinus circuits in rainbow trout: a new approach using chronic cannulation of the branchial vein. J Exp Biol 137: 75-88

McCormick SD (1995) Hormonal control of gill Na^+,K^+-ATPase and chloride cell function. In "Fish Physiology Vol 14" Ed by CM Wood, TJ Shuttleworth, Academic Press, San Diego, pp 285-315

Morisawa M, Hirano T, Suzuki K (1979) Changes in blood and seminal plasma composition of the mature salmon (*Oncorhynchus keta*) during adaptation to freshwater. Comp Physiol Biochem 64A: 325-329

Nagahama Y, Nishioka RS, Bern HA, Gunther RL (1975) Control of prolactin secretion in teleosts, with special reference to *Gillichthys mirabilis* and *Tilapia mossambica*. Gen Comp Endocrinol 58: 436-442

Prunet P, Boeuf G, Bolton JP, Young G (1989) Smoltification and seawater adaptation in Atlantic salmon (*Salmo salar*): Plasma prolactin, growth hormone, and thyroid hormones. Gen Comp Endocrinol 74: 355-364

Prunet P, Boeuf G, Houdebine L-M (1985) Plasma and pituitary prolactin levels in rainbow trout during adaptation to different salinities. J Exp Zool 235: 187-196

Rubin DA, Specker JL (1992) *In vitro* effects of homologous prolactins on testosterone production by testes of tilapia (*Oreochromis mossambicus*). Gen Comp Endocrinol 87: 189-196

Sakamoto T, Iwata M, Hirano T (1991) Kinetic studies of growth hormone and prolactin during adaptation of coho salmon, *Oncorhynchus kisutch*, to different salinities. Gen Comp Endocrinol 82: 184-191

Sakamoto T, McCormick SD, Hirano T (1993) Osmoregulatory actions of growth hormone and its mode of actions in salmonids: A review. Fish Physiol Biochem 11: 155-164

Sakamoto T, Ogasawara T, Hirano T (1990) Growth hormone kinetics during adaptation to a hyperosmotic environment in rainbow trout. J Comp Physiol B 160: 1-6

Sandra O, Sohm F, de Luze A, Prunet P, Edery M, Kelly PA (1995) Expression cloning of a cDNA encoding a fish prolactin receptor. Proc Natl Acad Sci USA 92: 6037-6041

Singh H, Griffith RW, Takahashi A, Kawauchi H, Thomas P, Stegeman JJ (1988) Regulation of gonadal steroidogenesis in *Fundulus heteroclitus* by recombinant salmon growth hormone and purified salmon prolactin. Gen Comp Endocrinol 72: 144-153

Specker JL, King DS, Nishioka RS, Shirahata K, Yamaguchi K, Bern HA (1985) Isolation and partial characterization of a pair of prolactin released *in vitro* by the pituitary of cichlid fish, *Oreochromis mossambicus*. Proc Natl Acad Sci USA 82: 7490-7494

Suzuki R, Kishida M, Ogasawara T, Hasegawa S, Hirano T (1987) Prolactin and growth hormone secretion during long-term incubation of the pituitary pars distalis of mature chum salmon, *Oncorhynchus keta*. Gen Comp Endocrinol 68: 76-81

Tan CH, Wong LY, Pang MK, Lam TJ (1988) Tilapia prolactin stimulates estradiol-17β synthesis *in vitro* in vitellogenic oocytes of the guppy *Poecilia reticulata*. J Exp Zool 248: 361-364

(Received February 1, 1996 / Accepted March 28, 1996)