

Changes in plasma prolactin and growth hormone concentrations during freshwater adaptation of juvenile chum salmon (*Oncorhynchus keta*) reared in seawater for a prolonged period

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Abstract

Freshwater adaptability of chum salmon was examined in juvenile fish reared in seawater for 4 months. The fish, weighing about 40g, were transferred directly to fresh water in October, when their cohorts are migrating in the North Pacific Ocean. Plasma sodium concentration decreased from 167 mM in seawater to about 130 mM during the first 24h, and increased gradually during 2–7 days after the transfer. No immunoreactive prolactin (PRL) was detected in the plasma of the seawater-adapted fish nor during the first 24h in fresh water. Significant levels of PRL were detected after 2–3 days. The maximal level (2.6 ng/ml) was observed after 5 days and became undetectable again after 7 days; no significant correlation was seen between the changes in plasma sodium and PRL levels during the transfer. Plasma growth hormone levels were relatively constant, except for a significant decrease 12h after the transfer. Although plasma thyroxine levels were highly variable during the experiment, a significant decrease and an increase were observed 12h and 5 days after the transfer, respectively. The present study indicates that juvenile chum salmon retain hyperosmoregulatory ability even after prolonged rearing in seawater. Examination of turnover rates, rather than changes in plasma levels, seems to be essential to clarify the osmoregulatory roles of the hormones.

Introduction

Most populations of Pacific salmon are anadromous, and have an extended period of freshwater residence as juveniles, usually for more than a year, as in the case of coho (*Oncorhynchus kisutch*), sockeye (*O. nerka*), masu (*O. masou*) and stream-type chinook (*O. tshawytscha*) salmon. They are unable to adapt to seawater as underyearlings, but develop hypoosmoregulatory ability or seawater adaptability after parr-smolt transformation (see Folmar and Dickhoff 1980; Wedemeyer *et al.*

1980). In contrast, chum (*O. keta*) and pink (*O. gorbuscha*) salmon do not have a distinct smolt stage, and migrate downstream to the ocean soon after completion of yolk absorption and emergence from the gravel (see Weisbart 1968; Iwata *et al.* 1982). Although pink salmon have been introduced successfully into the North American Great Lakes (Kwain 1982), no freshwater population of chum salmon has been reported. Recently, Hasegawa *et al.* (1987) have shown that juvenile chum salmon reared in fresh water for more than a year retained a high degree of euryhalinity. How-

ever, there seems to be no report on freshwater adaptability of the juvenile salmon during migratory period in the ocean.

It has become increasingly clear that prolactin (PRL) plays important osmoregulatory roles in euryhaline teleosts in fresh water. On the other hand, recent studies indicate osmoregulatory roles of growth hormone (GH) in seawater adaptation of salmonids (see Hirano 1986; Hirano *et al.* 1987). In the present study freshwater adaptability of chum salmon was examined in October, using juvenile fish reared in seawater for 4 months. Changes in plasma levels of PRL, GH and thyroxine (T_4) were also followed to clarify their osmoregulatory roles.

Materials and methods

Eyed embryos of chum salmon, *Oncorhynchus keta*, were transported from Hokkaido Salmon Hatchery at Chitose to Inland of the National Research Institute of Aquaculture. They hatched in January 1985, and were reared in running freshwater (15°C) tanks. Fry and juvenile were fed suitable particle size of commercial diet for trout (Nip-pun Shiryō). Experiments were performed under artificial lighting (12L;12D). In May, 200 fish, weighing 1–2g, were transported to the Marine station, and transferred directly to the aquarium containing 1000 l of seawater (15°C) and maintained for about 4 months. The food was restricted slightly because of the size of the aquarium. A small number of juvenile fish died in seawater. In October, the fish weighing 39.0 ± 0.6 g ($n = 131$) were transferred to fresh water. Blood was collected from the caudal vessels of 7–12 fish with 23-gauge syringe, 0, 6, 12, 24h and 2, 3, 5, 7 days after the transfer. Plasma was separated by centrifugation at 10,000 rpm for 5 min. No mortality was observed during the transfer experiment. Plasma sodium concentration was measured by atomic absorption spectrophotometry (Hitachi 180–50). Plasma levels of PRL, GH and T_4 were measured by radio-immunoassays as described previously (Hirano *et al.* 1985; Bolton *et al.* 1986; Tagawa and Hirano 1987).

Statistical analysis of plasma ion and hormone

concentrations were conducted by Duncan's new multiple range test. Since the plasma level of PRL in the fish in seawater was below the detectable range of the assay (0.1 ng/ml), the analyses were made by assuming the level as 0.1 ng/ml).

Results

Figure 1 shows the changes in plasma sodium and hormone concentrations of juvenile chum salmon reared in seawater for 4 months after direct transfer to fresh water. Plasma sodium levels were 167 mM in seawater, decreased significantly 6h after the transfer, and the minimum level (132 mM) was observed after 24h. There was a tendency toward an increase during 2–7 days after the transfer. Plasma PRL levels in seawater fish were below the detectable range (0.1 ng/ml) of the assay. No PRL immunoreactivity was detected during the first 24h after the transfer. Significant levels of PRL (0.9–1.0 ng/ml) were observed after 2–3 days, increased to maximal level (2.6 ng/ml) after 5 days and then decreased to undetectable level after 7 days. Plasma levels of GH were relatively constant throughout the transfer experiment (6–8 ng/ml), except for a significantly ($p < 0.05$) lower level observed 12h after transfer as compared with the initial level in seawater. T_4 levels were highly variable; a significant decrease was seen after 6h and an increase after 5 days as compared with initial control.

Discussion

It has been recognized that smoltification is a reversible process. In most species of Pacific salmon, the parr-smolt transformation occurs in spring. Smolts retained in fresh water undergo a decrease in hypoosmoregulatory capacity, the decrease being termed desmoltification (Folmar *et al.* 1982). According to Hasegawa *et al.* (1987), juvenile chum salmon reared in fresh water for more than a year retain a high degree of seawater adaptability, when tested during May to December. In the present study, juvenile chum salmon reared in seawater for

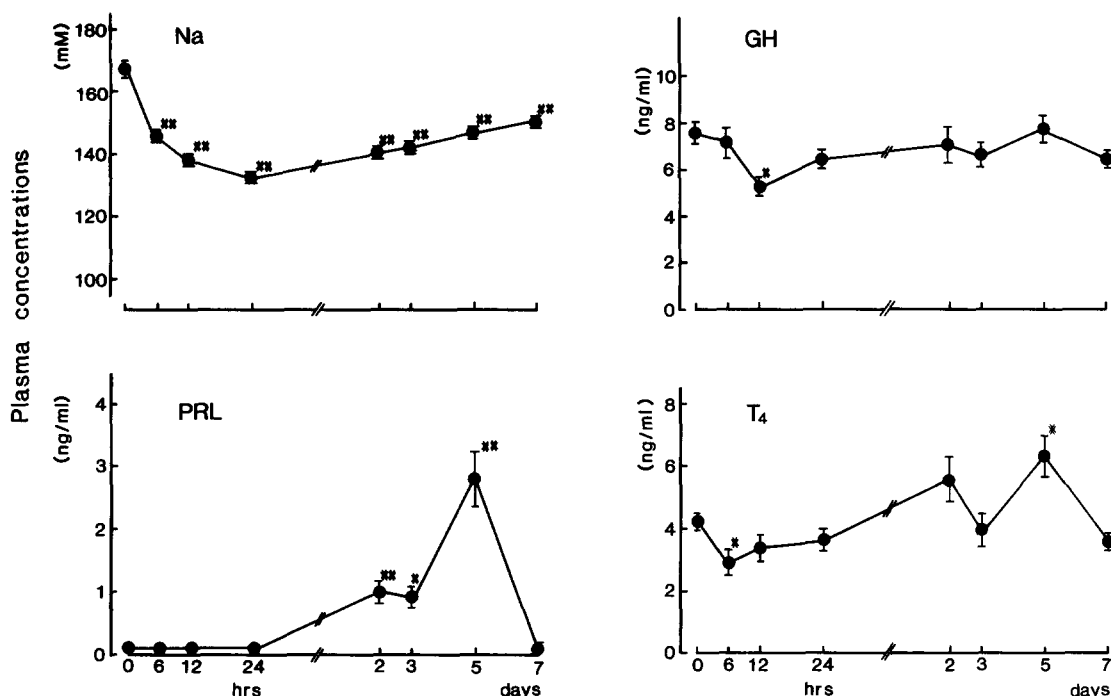


Fig. 1. Changes in plasma sodium, prolactin (PRL), growth hormone (GH) and thyroxine, (T_4) concentrations of juvenile chum salmon reared in seawater for 4 months after direct transfer to fresh water. Vertical bars represent standard errors of the mean ($n = 15$ for sodium, $n = 8$ for PRL, GH and T_4). *, ** Significantly different from the value at 0h, $p < 0.05$ and $p < 0.01$, respectively.

4 months exhibited an efficient freshwater adaptability in October, when their cohorts are migrating in the ocean. These results indicate that juvenile chum salmon retain euryhalinity in spite of their obligatory anadromous nature. During the 1987 cruise of R/V Oshoro-Marui of Hokkaido University of the northern North Pacific Ocean (June 6–August 18), coho and sockeye salmon were caught at various stages of sexual maturity. Healthy-looking fish were transferred directly to a freshwater tank on board, and some of them survived for at least 4 days (T. Ogasawara, unpublished observation). Thus, Pacific salmon or salmonids in general may retain hyperosmoregulatory ability throughout their entire life history. Mature chum salmon, on the other hand, are known to lose seawater adaptability after completion of ovulation or spermeation (Hirano *et al.* 1985; Bolton *et al.* 1987).

In the present study, plasma PRL levels increased significantly 2–5 days after transfer to fresh water and decreased to an undetectable level after 7 days.

Similar changes in PRL have been observed in larger fish transferred from seawater to fresh water (Hasegawa *et al.* 1987). In the immature rainbow trout, Prunet *et al.* (1985) reported a significant increase in plasma PRL, lasting for 20 days after transfer to fresh water. The changes in plasma prolactin levels observed in these studies had no significant correlation with the changes in the plasma electrolyte levels; the increase in the plasma hormone levels was generally seen after the ion concentrations were approaching a freshwater acclimated level. Based on the high variation in serum PRL levels among individual tilapia, especially among those kept in fresh water, Nicoll *et al.* (1981) suggested pulsatile release of the hormone. In the present study, the peak levels of plasma PRL observed 5 days after transfer to fresh water were also highly variable; the individual values ranged from 0.7–5.8 ng/ml. At any rate, plasma concentrations of the hormone are results of equilibrium between the rates of secretion and those of consumption of the hormone. Examination of secretion

rate as well as metabolic clearance of PRL is called for to clarify mode of action in freshwater adaptation.

GH has been implicated in seawater adaptation of some salmonid species (see Hirano 1986; Hirano *et al.* 1987). Recently, Richman and Zaugg (1987) reported an increase in Na,K-ATPase activity in the gills of coho salmon after GH treatment for 2 weeks. Long term administration of GH usually results in body growth. Several species of juvenile salmonids have been shown to become progressively tolerant to seawater as they grow older and larger: for a given age, the larger fish are more tolerant (see Clarke and Hirano 1988). However, short term administration of GH into chum salmon fry and rainbow trout has clearly improved their seawater adaptability, reducing peak level of plasma sodium upon transfer from fresh water to seawater (Hirano *et al.* 1987; Bolton *et al.* 1987). Hasegawa *et al.* (1987) observed an increase in plasma GH levels after transfer of juvenile chum salmon to seawater, and a decrease when transferred back to fresh water. A significant decrease in plasma GH observed 12h after transfer to fresh water in the present study may also indicate an important role of GH in their seawater adaptation. As in the case of PRL, kinetic studies of GH secretion and clearance seem to be essential to elucidate its osmoregulatory roles.

There are conflicting reports regarding the ability of thyroid hormones to improve seawater tolerance in salmonids. However, they do not appear to control the development of hypoosmoregulatory capacity, although they probably affect behaviour, growth and morphological development such as body silvering (Milne and Leatherland 1980; Miwa and Inui 1985; Ikuta *et al.* 1985; Iwata *et al.* 1987). Kinetic analyses of T_4 secretion rate and other parameters of T_4 metabolism were made in juvenile coho salmon by Specker *et al.* (1984). However, changes in environmental salinity had relatively minor effects on T_4 kinetics. Hasegawa *et al.* (1987) observed no consistent change in plasma T_4 levels following transfer of juvenile chum salmon to seawater or to fresh water. Although mean levels of plasma T_4 in the present study were also variable, the levels showed a significant increase 5 days

after transfer to fresh water when plasma PRL increased also significantly. T_4 and GH have some roles in hyperosmoregulation of juvenile chum salmon. Miwa and Inui (1985) reported that administration of a combination of T_4 and ovine GH into juvenile amago salmon (*O. rhodurus*) stimulated growth, body silvering and gill Na,K-ATPase activity as compared with the fish treated with T_4 or GH alone. Thus, synergistic or antagonistic actions of various hormones are also to be studied to clarify their osmoregulatory roles, in addition to the kinetic studies of each hormone during the processes of adaptation to different salinities.

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