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# Changes in Seawater Adaptability, $Na^+$, $K^+$-ATPase Activity and Circulating Thyroxine Levels in the Underyearling Broodstock Masu Salmon (*Oncorhynchus masou*) during and after Smoltification

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## Abstract

Changes in seawater adaptability, gill and kidney $Na^+$, $K^+$-ATPase activities, and serum thyroxine levels of underyearling broodstock masu salmon, *Oncorhynchus masou*, smolts in Mori Branch, Hokkaido Fish Hatchery, were investigated during and after smoltification. The occurrence of smolts judged from the external appearance (body silvering and pigmentation of dorsal fin margin) started in May, peaked in July, and decreased thereafter. High $Na^+$, $K^+$-ATPase activities in the gill were observed between early June and early August, while the activities in the kidney were highest in late July. Serum sodium concentrations of the fish transferred directly into seawater (30‰) showed the lowest levels in late July, which coincided with the highest activities of $Na^+$, $K^+$-ATPase in the both gill and kidney. The peaked concentrations of serum thyroxine were observed in late May and mid-June. These results suggest that gill and kidney might be involved in ion regulation during smoltification, and that thyroxine might play a role on the physiological characteristics in the smolting underyearling broodstock masu salmon.

## Introduction

Smoltification (parr-smolt transformation) in anadromous salmonids is a complex phenomenon comprising various morphological, physiological and behavioral changes, and is regulated by endocrine mechanisms (Hoar, 1976, 1988). During smoltification, circulating levels of many hormones are known to increase: growth hormone (Sweeting et al., 1985; Prunet et al., 1989; Young et al., 1989), prolactin (Richman, 1985), cortisol (Schreck, 1982; Specker and Schreck, 1982; Virtanen and Soivio, 1985; Young et al., 1989), somatomedin (Lindahl et al., 1985), insulin (Plisetskaya et al., 1988), and sex steroids (estradiol-17$\beta$: Sower et al., 1984, Yamada et al., 1989; testosterone: Yamada et al., 1989). In addition to these hormones, it is reported that the concentration of thyroxine show a sharp peak at the time of smoltification (Folmar and Dickhoff, 1979; Nishikawa et al., 1979; Nagahama et al., 1982; Lindahl et al., 1983; Sower et al., 1984; Yamauchi et al., 1984, 1985; Boeuf and Prunet, 1985; Patino and Schreck, 1986; Prunet et al., 1989; Young et al., 1989). However, the exact roles of these hormones on smoltification phenomena are still unclear.

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Masu salmon, *Oncorhynchus masou*, are distributed throughout Japan except in Kyushu, Shikoku and the Pacific side of central Southern Honshu (Kubo, 1980). In general, masu salmon develops into smolts as yearling fish in the spring of the second year after hatching (Kubo, 1980; Yamauchi et al., 1984, 1985). However, the life cycle of the broodstock masu salmon in Mori Branch, Hokkaido Fish Hatchery, is quite different. In the hatchery, a number of parrs from the broodstock smoltify as underyearling fish, followed by maturation the next year. Therefore, it is apparent that there is a major advantage in using such broodstock fish for the production of masu salmon because the life cycle is one year shorter than that of wild masu salmon. Until now, there is little information about the physiological characteristics of underyearling smolts in the masu salmon. This paper describes the changes in seawater adaptability, gill and kidney $Na^+$, $K^+$-ATPase activities and serum thyroxine levels during and after smoltification in the underyearling broodstock masu salmon in Mori Branch, Hokkaido Fish Hatchery.

## Material and methods

***Fish*** : Underyearling masu salmon, *Oncorhynchus masou*, used in this study were reared at Mori Branch, Hokkaido Fish Hatchery, in outdoor concrete ponds supplied with continuous flow of artesian well water and river water under ambient temperature and photoperiod conditions (Fig. 1). Fish samples were collected according to a lunar cycle; the fish were weighed (body weight and body length of the fish at sampling are shown in Fig. 2), and blood were obtained from dorsal vein using a 2.5 ml syringe after anesthetizing by 0.015% ethyl *p*-aminobenzoate. After the sacrifice, gill arches and kidney were taken out in order to investigate the $Na^+$, $K^+$-ATPase activity. Serum obtained by centrifugation at 12,000 rpm for 5 min

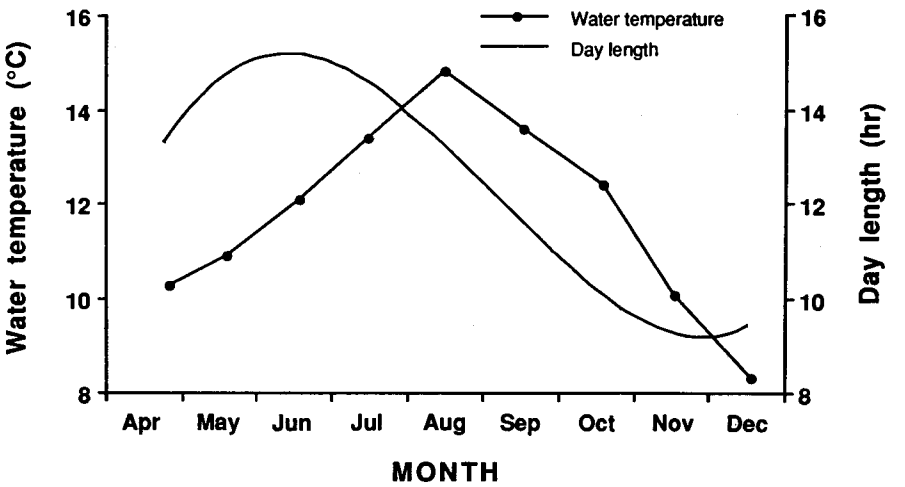


Fig. 1. Changes in rearing water temperature and day length in Mori Branch (Hokkaido Fish Hatchery) from April throughout December in 1986. Each point represents the mean± SEM.

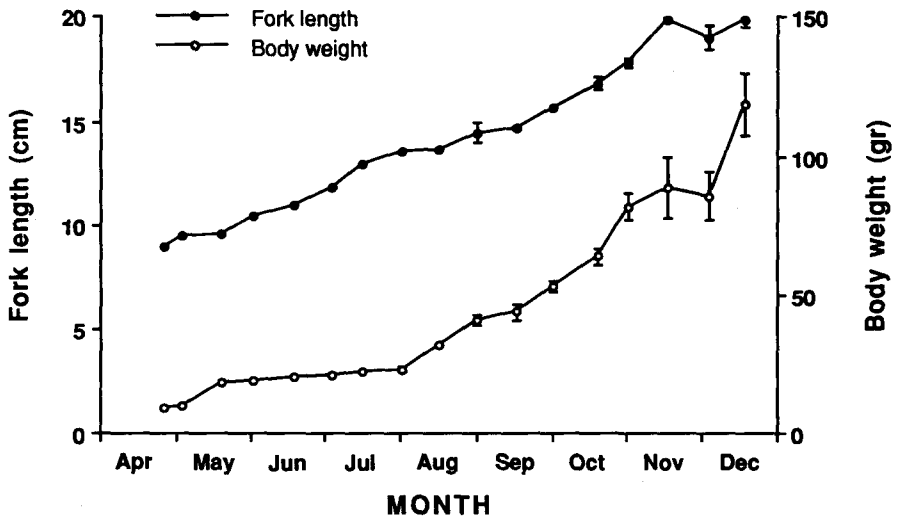


Fig. 2. Changes in fork length and body weight in the underyearling broodstock masu salmon during and after smoltification. The vertical bars represent±SEM.

were stored at −40°C until sodium assay and thyroxine radioimmunoassay. As a criteria of smolts, body silvering and pigmentation of dorsal fin margin were adopted (Yamauchi et al., 1984).

***Seawater challenge test*** : To determine seawater adaptability, seawater challenge tests were conducted (Clarke and Blackburn, 1978). Ten fishes from freshwater tank were directly transferred into 33‰ seawater. After 24 hrs, the fish were anesthetized, and blood were obtained by the same method described above. Serum obtained by centrifugation at 12,000 rpm for 5 min was stored at −40°C until the assay of serum sodium.

***$Na^+$, $K^+$-ATPase activity*** : Gill arches and kidney removed were immediately placed and rinsed in ice-cold homogenizing solution (250 mM imidazole, 337.5 mM $CaCl_2$, 162.5 mM KCl, 50 mM $MgCl_2$). The tissues were homogenized in 1 ml of the solution, and 100 $\mu$l of the homogenates were incubated in either 400 $\mu$l of reaction mixture A (250 mM imidazole, 12.5 mM ATP2Na, 337.5 mM NaCl, 162.5 mM KCl, 50 mM $MgCl_2$) or 400 $\mu$l of reaction mixture B (reaction mixture A+2.5 mM oaubain) were incubated for 20 min at 37°C in the shaking incubator. The reaction was stopped by adding 10 ml of iron TCA solution (50 gr TCA, 5 gr thiourea, 15 gr frerrous ammonium sulfate in 1 liter). Free Pi was measured according to the method of Goldenberg and Fermandoz (1966) and protein concentrations were determined by the method of Bradford (1976).

***Serum sodium concentrations*** : Concentrations of serum sodium in freshwater and seawater fish were measured using an atomicabsorption spectrophotometer (Hitachi 518).

***Serum thyroxine concentrations*** : Serum thyroxine concentrations were measured by radioimmunoassay according to the method of Suzuki and Suzuki (1981).

## Results

***Rate of smolts*** : Smolts first appeared in early June (39.6%), and thereafter the number of smolts increased. The smolt rate peaked in late July (89.0%), which decreased afterwards.

***Serum sodium concentration*** : Serum sodium concentrations of the freshwater fish during the sampling period were between 120 and 150 mEq/l, indicating no significant changes (Fig. 3). In contrast, the concentration of serum sodium of the fish kept in seawater for 24 hrs was 195±3 mEq/l in late April, which decreased gradually afterwards, and showed the lowest value (154±4 mEq/l) in mid-July, indicating the fish had developed seawater adaptability. The values were higher than that in fishes kept in freshwater. The low concentration was maintained until late July, which increased again, and in October almost the same level (183±4 and 186±5 mEq/l) as that observed in late April was achieved.

***$Na^+$, $K^+$-ATPase activity*** : In the freshwater fish, gill $Na^+$, $K^+$-ATPase activity showed 1.5±0.05 μmole Pi/mg protein/hr in late April, which gradually increased, and reached 7.3±0.1 μmole Pi/mg protein/hr in June and 7.1±0.1 μmole Pi/mg protein/hr in July, respectively (Fig. 4). Thereafter, the activity gradually decreased. In contrast, kidney $Na^+$, $K^+$-ATPase activities showed 9.3±0.8–13.2±1.6 μmole Pi/mg protein/hr between April and early July, and peaked dramatically (17.6±0.7 μmole Pi/mg protein/hr) in mid-July. In early August, the activity dropped to 12.4±0.3 μmole Pi/mg protein/hr, followed by decreasing gradually until October. After that the activity again peaked (12.6±0.5 μmole Pi/mg protein/hr) in early December.

***Serum thyroxine concentration*** : Changes in serum thyroxine concentrations were measured from April to August (Fig. 5). The concentrations indicated low levels of

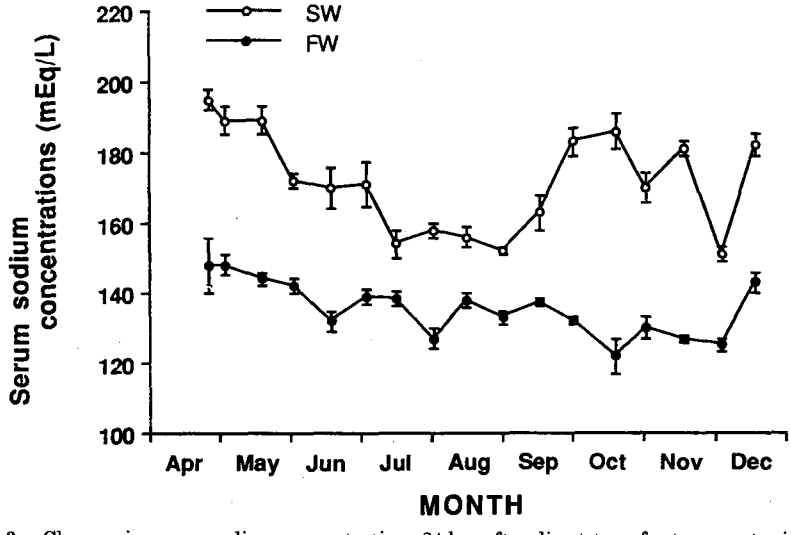


Fig. 3. Changes in serum sodium concentrations 24 hrs after direct transfer to seawater in the underyearling broodstock masu salmon. The vertical bars represent±SEM.

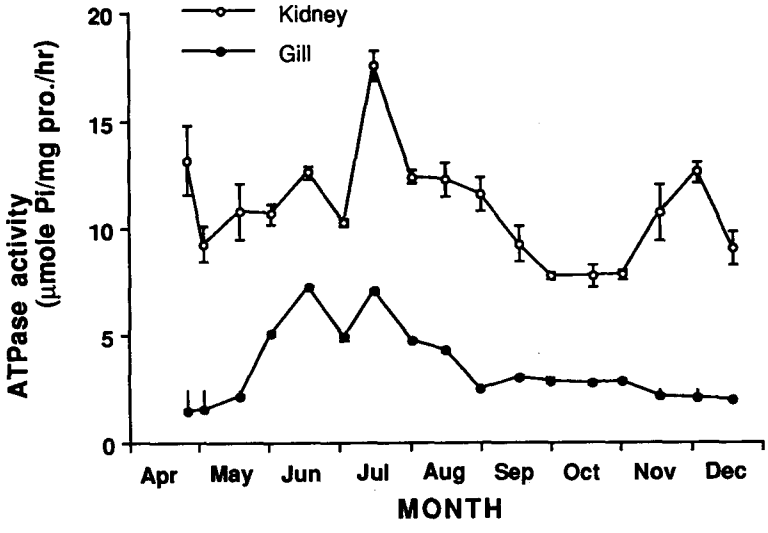


Fig. 4. Changes in kidney and gill $Na^+$, $K^+$-ATPase activities in the underyearling broodstock masu salmon during and after smoltification. The vertical bars represent±SEM.

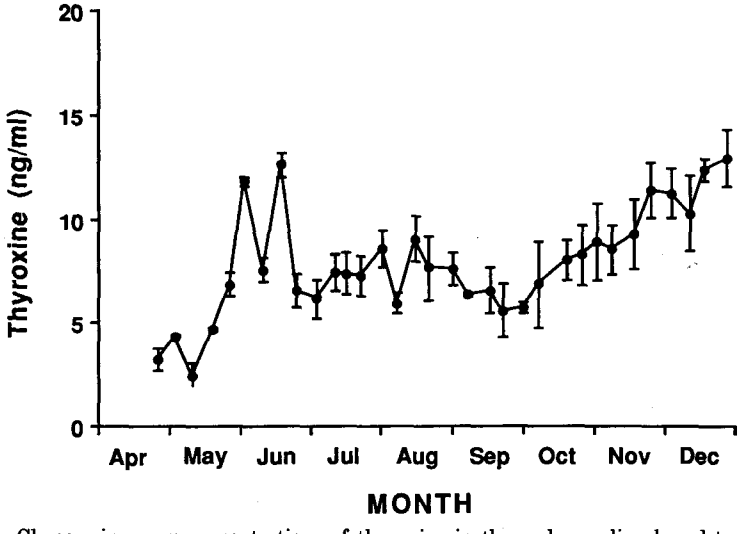


Fig. 5. Changes in serum concentrations of thyroxine in the underyearling broodstock masu salmon during and after smoltification. The vertical bars represent±SEM.

2-4 ng/ml between late April and mid-May, and thereafter the levels increased, followed by peaking in early and mid-June (10.6 and 11.9 ng/ml, respectively). After that, the levels decreased, and attained to the low values of 6.1±0.5 ng/ml. The concentrations of thyroxine fluctuated between 6.3±1.5-9.3±0.9 ng/ml, which increased gradually to 12.8±1.2 ng/ml in late December.

## Discussion

In general, the wild masu salmon in natural rivers of Japan smoltify as a yearling fish in the spring of the second year after hatching. However, some of the broodstock masu salmon reared in Mori Branch, Hokkaido Fish Hatchery, develop into smolts as an underyearling fish in early summer (Ban et al., 1987a; Yamauchi et al., 1984, 1985; Kasahara et al., 1989). It seems likely that the occurrence of underyearling smolts is caused by the warm water temperature during winter (Atoda, 1974; Ban et al., 1987a; Kasahara et al., 1989). In this hatchery, mixture of artesian well water and river water was used as rearing water, and the water temperature was maintained around 6–8°C throughout winter. Since environmental factors such as photoperiod, water temperature and rearing conditions are known to stimulate smoltification in salmonids (Hoar, 1976, 1988). The higher water temperature in the rearing ponds than that in the river water during winter may have caused underyearling smolts.

There is considerable disparity between the size of wild and hatchery-reared fish (Piggins and Mills, 1985). Elson (1957) suggested that to develop into smolts, the fish must reach a certain size. Similarly, Hoar (1976) and Uto (1977) have pointed out that the occurrence of smolts may be associated with body size at the time of initiation of smoltification in salmonids. It seems that in Hokkaido Fish Hatchery, high water temperature of rearing water in winter accelerated the growth of the masu salmon, resulted in the occurrence of underyearling smolts as Kasahara et al. (1989) have reported. Similarly, in coho salmon, *Oncorhynchus kisutch*, Brannon et al. (1982) have reported that under a rearing regime initiated by higher temperatures, the hatchery stock can be induced to underyearling smolts.

Seawater challenge test (Clarke and Blackburn, 1978) is useful for the assessment of the development of seawater adaptability in anadromous salmonids (see Hoar, 1988). According to the test, broodstock underyearling masu salmon showed poor hypoosmoregulatory ability when introduced into seawater for 24 hrs in April, but such ability improved in July and August. The performance then declined to low levels in April. These changes coincided with the peak of smoltification which can be assessed by changes in the external appearance, confirming observation of Ban et al. (1987a). The ability in July and August, however, was lower than that of the yearling fish (Yamauchi et al., 1984; Ban et al., 1987a). In addition, the peak of smoltification of underyearling fish appeared about two month later than that of yearling fish. In order to elucidate these differences observed between underyearling and yearling fish, further studies are required.

$Na^+$, $K^+$-ATPase activity is also used as an seawater adaptability in salmonids (Hoar, 1976, 1988). In the present study, gill $Na^+$, $K^+$-ATPase activity gradually increased as smoltification developed, and decreasing afterwards, confirming the data from Ban et al. (1987a, b). These results were coincided with many other observations reported in salmonids (see Hoar, 1988). The kidney $Na^+$, $K^+$-ATPase activity also increased at the time of smoltification. In the Atlantic salmon, *Salmo salar*, Virtanen and Soivio (1985) reported that the enzyme activity in kidney was synchronous with the changes in plasma sodium concentrations. It seems that $Na^+$, $K^+$-ATPase in kidney as well as gill are involved in the regulation of circulating ion at the time of smoltification in the masu salmon.

Since Hoar (1939) observed histologically the high activity of the thyroid gland in the Atlantic salmon at the time of smoltification, there have been many papers about thyroid hormone(s) being involved in the events occurring during smoltification (Hoar, 1976, 1988). Recent studies using radioimmunoassay showed that circulating thyroxine increased, coinciding with the development of smoltification in coho salmon (Folmar and Dickhoff, 1979; Dickhoff et al., 1982; Nishioka et al., 1985; Patino and Schreck, 1986; Young et al., 1989), Atlantic salmon (Lindahl et al., 1983; Boeuf and Prunet, 1985) and amago salmon (Nagahama et al., 1982; Yamauchi et al., 1984). Similarly, in masu salmon, a peak of circulating thyroxine levels has been observed at the time of smoltification (Nishikawa et al., 1979; Yamauchi et al., 1984, 1985). In addition to yearling masu salmon, in the underyearling fish, increases in the concentrations of thyroxine were shown in the present study. However, the maximum levels in the underyearling fish were lower than that of the yearling fish (Yamauchi et al., 1984, 1985), and the peak was observed to be earlier than the peak of smoltification (Yamauchi et al., 1984; Ban et al., 1987a). These differences in the thyroxine profiles observed between underyearling and yearling fish may be due to the difference in seawater adaptability between both fish. The treatment of parr with thyroid hormone creates pseudo-smolts (Eales, 1979). Moreover, although recent studies have no suggestive evidences on the role of the thyroid hormone in seawater adaptability (Eales, 1979; Hoar, 1988), Miwa and Inui (1985) reported that in amago salmon thyroxine increases seawater tolerance and induces a significant elevation in the gill $Na^+$, $K^+$-ATPase activity in combination with growth hormone, but if alone did not improve seawater adaptability. It seems therefore, that the thyroid hormone plays an important role in enhancing smoltification which is regulated endogenously by other hormonal factors (Hoar, 1988).

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