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# RESEARCH NOTE

# Synergistic Effects of Thyroxine and Cortisol on the Seawater Tolerance of Sockeye Salmon (*Oncorhynchus nerka*)

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*Abstract*.— Thyroxine (T4) and cortisol (F) were investigated for their synergistic effects on the seawater tolerance of sockeye salmon (*Oncorhynchus nerka*) at Chitose Hatchery, Hokkaido, Japan. Juvenile sockeye salmon were separated into four groups (an untreated control, F, T4, and F and T4 in combination). Each treatment group received hormone orally via diet supplementation at a dose of 10 μg per body weight (1 g) for two weeks. The untreated control group was fed only the commercial diet. Seawater tolerance after hormonal treatment was estimated by gill $Na^+$,$K^+$-ATPase activity (gill-ATPase) and plasma sodium concentration (plasma-Na) following seawater transfer for 24 h. Furthermore, chloride cells observed in the primary gill lamellae were assessed immunohistochemically. The F-treated group showed a two-fold higher level of gill-ATPase and a significantly lower plasma-Na than those of T4-treated and control groups. A treatment with F and T4 combination had larger effects compared with a single treatment of either F or T4. The number of chloride cells observed in the F-treated group increased about two-fold more than in T4-treated and control groups. These results suggest that cortisol promotes seawater tolerance including activation of $Na^+$,$K^+$-ATPase accompanied with differentiation of chloride cells. F and T4 have a synergistic effect in promoting seawater tolerance in sockeye salmon.

**Key words:** sockeye salmon, seawater tolerance, gill $Na^+$,$K^+$-ATPase, chloride cell, cortisol, thyroxine

## Introduction

Anadromous salmonids generally undergo a smolt transformation (smoltification) in advance of their seaward migration. During smoltification, juvenile salmon acquire seawater tolerance including the development of chloride cells and $Na^+$,$K^+$-ATPase in gill tissues under hormonal control (Wedemeyer et al. 1980; Hoar 1988). Thyroxine (T4) and cortisol (F) have long been known as major hormones associated with smoltification, and their practical functions have been investigated on this process. For example, in masu salmon (*Oncorhynchus masou*) and amago salmon (*O. masou rhodurus*), administration of T4 induces morphological changes such as body silvering, fin margin blackening or reduced condition factor (Miwa and Inui 1983; Ikuta et al. 1985; Soyano et al. 1988). Fujioka et al. (1990) suggested that T4 is involved directly or indirectly in downstream movement in Biwa salmon (*O. masou rhodurus*). On the other hand, F has an important role on development of seawater tolerance (McCormick 1996). In coho salmon (*O. kisutch*) and steelhead trout (*O. mykiss*), adaptability to seawater is enhanced with administration of F (Richman III and Zaugg 1987; McLeese et al. 1994). Seidelin et al. (1999) reported an effect of F on the progression of gill $Na^+$,$K^+$-ATPase and chloride cell formation in brown trout (*Salmo trutta*). Uchida et al. (1998) also showed the involvement of F in the functional differentiation of chloride cells in chum salmon (*O. keta*). Ban (2002, 2004) conducted similar treatments for sockeye salmon (*O. nerka*) and confirmed that T4 induced fin margin blackening and F increased $Na^+$,$K^+$-ATPase activity and seawater tolerance, although his-

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tological study of chloride cells was not performed. Although T4 and F may have several different roles on smoltification as mentioned above, reciprocal actions of these hormones must be evaluated, because plasma levels of T4 and F elevate at similar periods (Folmar and Dickhoff 1981; Specker 1982; Thorpe et al. 1987; Ban et al. unpublished data). In this paper, reciprocal effects of T4 and F on smoltification in sockeye salmon were investigated. A further goal was to conduct an immuno-histochemical analysis of gill tissue accompanying the administration of F and T4.

## Materials and Methods

*Fish and rearing conditions*

Juvenile sockeye salmon of brood-year 1991 were collected at random from an outdoor pond at the Chitose Hatchery, National Salmon Resources Center (Hokkaido), and divided into four groups on January 12, 1993. All groups were composed of 30 individuals and were held in 25-l plastic tanks with flow-through groundwater at 10℃. The fork lengths and body weights of individuals in the four groups ranged from 116.7 - 121.4 mm and 14.3 - 16.5 g at the beginning of this experiment.

*Hormone treatment and samplings*

Hormonal treatments for the three treatment groups included thyroxine (T4; Wako Pure Chemical Industries, Ltd), cortisol (F; Wako Pure Chemical Industries, Ltd) and thyroxine and cortisol in combination. All treatments were performed from January 16 to February 1 in 1993. Each hormone dissolved in 70% ethanol was diluted with distilled water and dripped on commercial diet, and administered orally at a dose of 10 μg per body weight (1g) every day. The untreated control was fed the commercial diet only.

At the end of these treatments, 10 fish were collected from each group and anesthetized with tricaine methanesulfonate (MS222, 100 mg/l). Gill filaments were excised and frozen with homogenizing solution (250 mM sucrose, 6 mM EDTA 2Na, 20 mM imidazole, pH 6.8) at -40℃ for later analysis of $Na^+$,$K^+$-ATPase activity (gill-ATPase). Additional gill tissues from the same fish were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 48 h and embedded in paraffin for later immunohistochemical study. Twenty fish from each group were transferred directly into artificial seawater (Reisea Salt G, REI-SEA Co.) at 33 parts per thousand to determine seawater tolerance. After 24 h in seawater, blood samples were collected from the caudal vein of anesthetized fish using a micro test tube. Plasma was obtained after centrifugation of the blood at $10^4$ g for 10 min and stored at -40°C for sodium concentration (plasma-Na) assays.

*Gill-ATPase, plasma-Na and Immuno-histochemical study*

Gill-ATPase was determined according to the method of Folmar and Dickhoff (1979), modified by Ban and Yamauchi (1991). The enzyme activity was expressed as micromoles Pi per milligram protein per h (μmols Pi/mg pro/h). Plasma-Na (mM) was measured by using an Atomic Absorption and Flame Emission Spectrometer (Shimadzu, AA-640-13). Immuno-histochemical study followed the method of Ura et al. (1996). The primary antiserum of the α-subunit of $Na^+$,$K^+$-ATPase produced by Ura was provided by Professor K. Yamauchi of the Department Fisheries, Hokkaido University.

*Statistical analysis*

Data were subjected to a one-way analysis of variance (ANOVA) followed by a Student's t-test to determine significant differences in plasma-Na and gill-ATPase among hormonal treatment groups. A probability level of less than 0.05 was considered significant.

## Results and Discussion

The gill-ATPase and plasma-Na of the control and T4-treated groups after hormonal treatment were about 3.5 μmols Pi/mg pro/h and 200 mM, respectively. There were no significant differences between these two groups in both parameters (Figs. 1 and 2). However, the F-treated group showed significantly higher gill-ATPase and lower plasma-Na than those of control and T4-treated groups (6.8 μmols Pi/mg pro/h and 168.5 mM, respectively). These results indicate that F affects the development of seawater tolerance in sockeye salmon by activation of gill $Na^+$,$K^+$-ATPase, while T4 have no significant effects on this process. This ineffectiveness of T4 on hypo-osmoregulatory ability was reported in masu salmon (Ikuta et al. 1985), chum salmon (Iwata et al. 1987), and sockeye salmon (Ban 2004).

Furthermore, the significance of F on seawater tolerance was also demonstrated in coho salmon (Richman III and Zaugg 1987) and sockeye salmon (Ban 2002). Present results correspond with those previous findings.

The combined administration of T4 and F induced a significantly greater seawater tolerance compared with a single treatment of F, and the gill-ATPase and plasma-Na reached levels of 7.8 μmols Pi/mg pro/h and 155.7 mM, respectively (Figs. 1 and 2). In these phenomena, F likely plays a principal role as mentioned above, and T4 probably enhances the effect of F. Synergistic effects of several hormones on seawater tolerance have been reported elsewhere. Miwa and Inui (1985) found a significant elevation of gill-ATPase in T4-treated amago salmon combined with growth hormone. McCormick (1996) reported that growth hormone (GH) and F could increase salinity tolerance and gill-ATPase of Atlantic salmon (*S. salar*) and act together in synergy. The present data indicate that F and T4 also have synergistic effects on the development of seawater tolerance in sockeye salmon. Because it is well known that T4, F, GH and other hormones increase abruptly in accordance with smoltification, researchers and managers must consider interrelationships among these hormones when performing endocrinological investigations concerned with the development of seawater tolerance in anadromous fishes.

Immuno-histochemical staining of gill sections

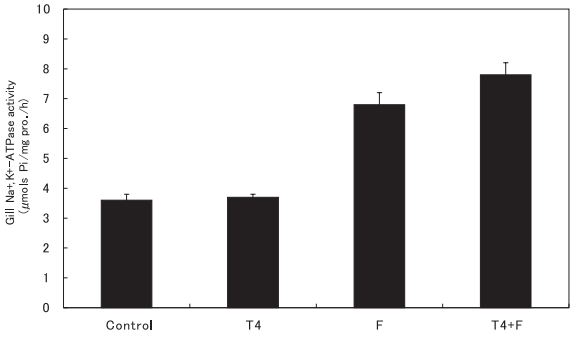


**Fig. 1**. Gill $Na^+,K^+$-ATPase activity of sockeye salmon administrated with thyroxine (T4), cortisol (F), and T4 and F in combination (T4 + F). Control group was fed only commercial diet. Bars indicate SE.

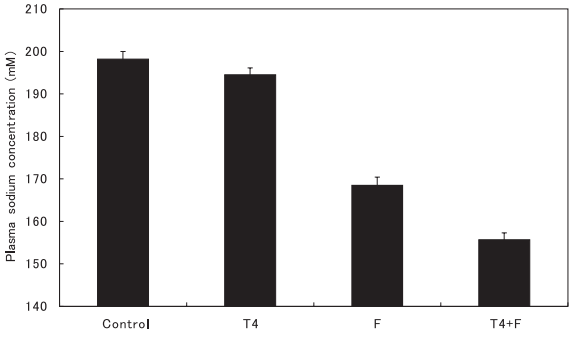


**Fig. 2**. Plasma sodium concentration of sockeye salmon administrated with thyroxine (T4), cortisol (F), and T4 and F in combination (T4 + F). Control group was fed only commercial diet. Bars indicate SE.

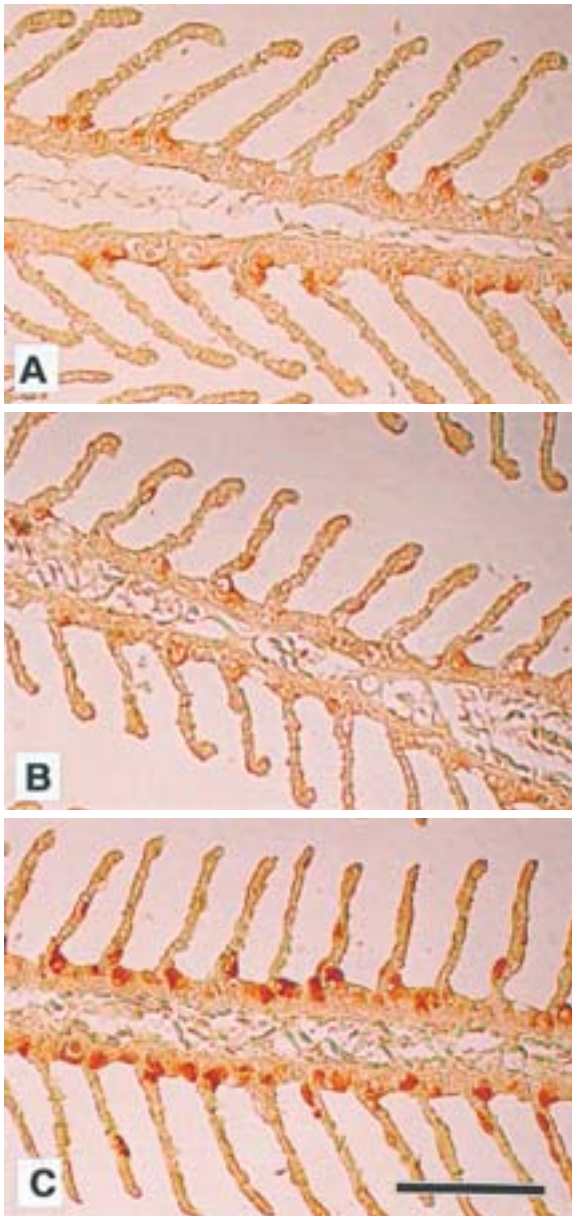


**Fig. 3**. Adjacent sections of sockeye salmon gill stained with the antibody to $Na^+,K^+$-ATPase α-subunit after administration of a commercial diet only (A), thyroxine (B), and cortisol (C). Bar is 200 μm.

are shown in Fig. 3. Most immunoreactivity was observed in large cells on the proximal end of the secondary lamellae and on the surface of the primary lamellae of three groups. These large cells specifically bind with the antibody of $Na^+,K^+$-ATPase α-subunit and are identified as chloride cells from their immuno-histochemical and structural features (Ura et al. 1996). The mean number of chloride cells observed in the primary lamellae of control and T4-treated fish was 22.0±1.5/mm and 20.6±1.2/mm, respectively. However, F-treated fish showed a significantly greater number of chloride cells (46.2± 1.7/mm). Furthermore, the stain of chloride cells of the F-treated group was more concentrated than the other two treatment groups. These results indicate that F affects the differentiation of chloride cells and increases the amount of $Na^+,K^+$-ATPase in gill tissue. The association of F with stimulation of chloride cells in the gill has been suggested by Seidelin et al. (1999) in brown trout. Moreover, Richman III and Zaugg (1987) have shown an effect of F on the proliferation or differentiation of chloride cells in coho salmon. Although these reports support the present data, ultrastructual and molecular studies are needed to clarify the precise role of F on seawater tolerance in sockeye salmon.

The present results suggest that F develops seawater tolerance including the activation of $Na^+,K^+$-ATPase activity accompanied with differentiation of chloride cells. T4 has a synergistic effect with F in promoting seawater tolerance in sockeye salmon. This work underscores the critical roles played by specific hormones in anadromous salmonids and their importance for seawater tolerance during the smoltification process.

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## ベニザケの海水適応能に与えるチロキシンとコーチゾルの相乗効果

伴　真俊

ベニザケの海水適応能に与えるチロキシン(T4)とコーチゾル(F) の影響を調べるため，幼魚を4群（対照群，T4群，F群，T4+F群）に分け，体重1 g当たり10 μgのホルモンを2週間に亘って経口投与した．海水適応能は，鰓の$Na^+$,$K^+$-ATPase活性(gill-ATPase)と海水移行24時間後の血中ナトリウム濃度(plasma-Na)を基に調べた．また，鰓に分布する塩類細胞を免疫組織学的に調べた．F群のgill-ATPaseは対照群とT4群に比べて約2倍高く，plasma-Naは有意に低かった．T4+F群はF群より高い海水適応能を示した．F群の塩類細胞数は，対照群ならびにT4群の約2倍だった．これらの結果は，Fが塩類細胞の分化とgill-ATPaseの活性化を促してベニザケの海水適応能を高めること，またその過程でT4と相乗効果を示すことを示唆している．