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# RESEARCH NOTE

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

Masatoshi Ban

*Research Division, National Salmon Resources Center,*
*2-2 Nakanoshima, Toyohira-ku, Sapporo 062-0922, Japan*
*(dukeban @salmon.affrc.go.jp)*

*Abstract*. – In order to determine hormonal effects on the seawater tolerance of sockeye salmon (*Oncorhynchus nerka*), cortisol or growth hormone (GH) were administered via intraperitoneal injection at 1 $\mu$g per body weight for two weeks in March and May in the laboratory. In March, the gill $Na^+$, $K^+$-ATPase activity of the cortisol group was 1.5-fold higher than that of the control group injected only with saline. Plasma sodium concentrations 24 h after transfer to seawater for the cortisol and GH groups declined to 164.3 mM and 153.5 mM, respectively, while the control group had 191.1 mM. In May, however, the gill $Na^+$,$K^+$-ATPase activity in all these treatments elevated to 16.8 - 17.1 $\mu$mols Pi/mg pro/h and the plasma sodium concentrations decreased to 148.0 - 154.5 mM. Administration of cortisol and GH had no effect on seawater tolerance in May. These results indicate that cortisol and GH are important hormones promoting seawater tolerance in sockeye salmon, while effects of these hormones may vary with administration time.

**Key words** : sockeye salmon, seawater tolerance, gill $Na^+$, $K^+$-ATPase, cortisol, growth hormone

## Introduction

Juvenile salmonids generally show parr-smolt transformation (smoltification) as a pre-adaptation to ocean life. Smoltification is a complex phenomenon composed of morphological, physiological and behavioral changes (Wedemeyer 1980). The major physiological processes of smoltification include an increase in hypo osmoregulatory ability (seawater tolerance) and activation of gill $Na^+$, $K^+$-ATPase activity (Hoar 1988). Gill $Na^+$, $K^+$-ATPase has an important role for ion transport in seawater. These changes during smoltification are controlled by the endocrine system.

McLeay (1975) found activation of the pituitary-interrenal axis during smoltification in Atlantic salmon (*Salmo salar*). Specker and Schreck (1982) and McLeese et al. (1994) observed changes in the plasma corticosteroids with smoltification of coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*O. mykiss*). Elevation of corticosteroids during smoltification has also been shown to correlate with an increase in gill $Na^+$, $K^+$-ATPase activity in salmon (Specker et al. 1982; Shrimpton et al. 1994). Besides cortisol, evidence of the involvement of pituitary growth hormone (GH) in the smolting process was observed in both physiological and histological studies (Parry 1958; Saunders and Henderson 1970; Komourdjian et al. 1976a; McCormick 1996). These results indicate that cortisol and GH are important endocrinological factors for seawater tolerance in salmonids.

However, the roles of cortisol and GH involved with seawater tolerance in the sockeye salmon (*O. nerka*) are still unclear. In this paper, effects of cortisol and GH on seawater tolerance of sockeye salmon were investigated as a preliminary study to elucidate the hormonal mechanisms of seawater tolerance.

## Materials and Methods

*Fish*

Sockeye salmon used for this experiment were

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from brood-year 1994 stocks, which had been reared in an outdoor concrete pond at the Chitose Hatchery, National Salmon Resources Center in Hokkaido. Yearling fish collected from the pond were divided into three groups composed of 30 individuals (each) on March 12, and May 7, 1996. Fish were maintained in 25 l plastic tanks with flow-through fresh water from a groundwater source, and held at 10℃ for one week in order to acclimate them to new conditions prior to hormone treatment.

At each sampling time, all fish were anesthetized with MS-222 (100 mg/l), weighed, and their fork lengths were recorded.

*Hormone treatment*

Hormone administrations were performed for two weeks from March 19 and May 13, respectively. Fish in both months were allocated to one of three treatment groups: control (saline, n=30), salmon growth hormone (GH, n=30) or cortisol (n=30). Fish from their respective groups received an intraperitoneal injection of saline, GH (Kyowa Hakko Kogyo Co. ltd, dissolved in distilled water), and cortisol (Sigma, dissolved in ethanol) on six alternating days.

*Seawater tolerance*

To determine the seawater tolerance of treated fish, seawater challenge tests were conducted at the end of treatment administrations on March 31 and May 25. Twenty fish from each of three treatment tanks were directly transferred into a 60 l tank filled with aerated artificial seawater (Aqua-Ocean, Japan Pet Drugs co., ltd.) at a salinity of 33 ppt and 10℃. After 24 h, fish were anesthetized and blood was taken from their caudal veins using a micro tube. Serum was obtained by centrifugation at 1,000 g for 15 min at 4℃ and stored at -40℃ until assays for plasma sodium were performed. Ten fish from each treatment group were placed in fresh water for 24 h. The gill filaments from each fish were removed to measure the gill $Na^+$, $K^+$-ATPase activity. The excised gill filaments were placed in ice-cold homogenizing solution (250 mM sucrose, 6 mM EDTA 2Na, 20 mM imidazole, pH 6.8) and stored at -40℃ until assays for enzyme activity were performed.

*Gill $Na^+$, $K^+$-ATPase activity and plasma sodium concentrations*

Gill $Na^+$, $K^+$-ATPase activity was determined according to the method of Ban and Yamauchi (1991). Defrosted gill tissues were homogenized in a homogenizing solution and centrifuged at 1,000 g for 5 min at 4℃. The supernatant was incubated for 20 min at 37℃. Phosphorus (Pi) produced from the incubation was measured according to the method of Goldenberg and Fernandez (1966). Protein determination was made according to the method of Lowry et al. (1951). $Na^+$, $K^+$-ATPase activity was expressed as micromoles Pi per milligram protein per hour ( $\mu$ mols Pi/mg pro/h).

Plasma sodium concentrations (mM) were measured using an Atomic Absorption and Flame Emission Spectrometer (Shimadzu, AA-640-13). Defrosted plasma was diluted 1,000 x with distilled water for analysis.

*Statistical analysis*

The data were subjected to a one-way analysis of variance (ANOVA) followed by a Student's t-test to determine significant differences in plasma sodium concentrations and gill $Na^+$, $K^+$-ATPase activity among the three groups in each collection time. A probability level of less than 0.05 was considered significant.

## Results

Fork length and body weight of all three groups increased during the experimental period (Table 1). There was no significant difference in body size among the three groups in each month.

The gill $Na^+$, $K^+$-ATPase activities of GH and cortisol groups were higher than that of the control group in March. In particular, the enzyme activity of the cortisol group significantly differed from the control group and reached 6.0 $\mu$mols Pi/mg pro/h

**Table 1.** The mean fork length and body weight of sockeye salmon injected saline (control), growth hormone (GH) or cortisol in March and May 1996.

| Date | Treatment | Fork length (cm) | Body weight (g) |
|---|---|---|---|
| March 31 | Control | 10.4±0.1* | 11.1±0.5 |
| | GH | 10.6±0.2 | 12.1±0.7 |
| | Cortisol | 10.4±0.3 | 11.5±0.9 |
| May 25 | Control | 11.9±0.2 | 16.9±1.5 |
| | GH | 12.5±0.4 | 17.9±1.8 |
| | Cortisol | 12.4±0.2 | 17.8±2.0 |

* Mean±SE

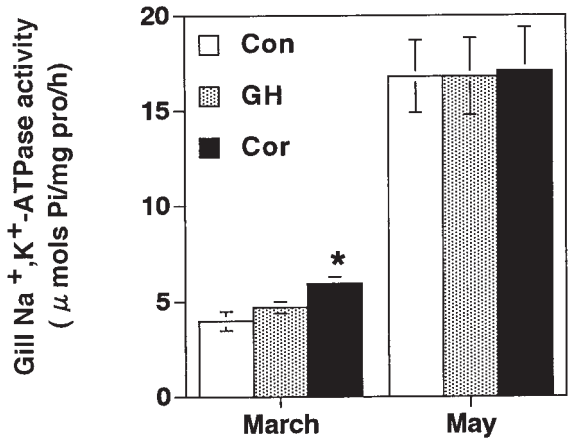


**Fig. 1.** Gill $Na^+$, $K^+$-ATPase activity of sockeye salmon injected with saline (Con), cortisol (Cor) or growth hormone (GH) in March and May. Bars indicate SE. Asterisks above columns indicate a significant difference ($p<0.05$) among experimental groups in March and May.

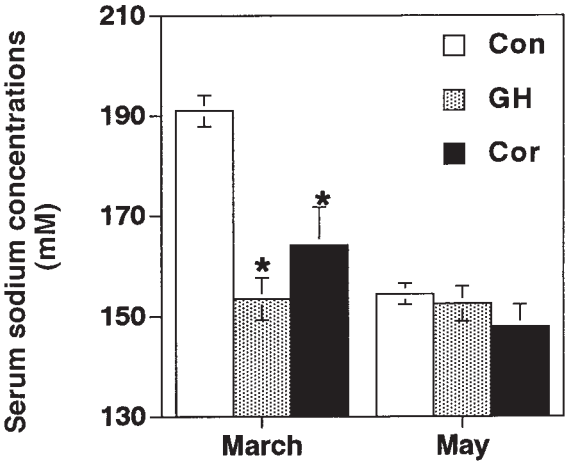


**Fig. 2.** Serum sodium concentrations of sockeye salmon injected with saline (Con), cortisol (Cor), and growth hormone (GH) in March and May. Bars indicate SE. Asterisks above columns indicate a significant difference ($p<0.05$) among experimental groups in March and May.

(Fig. 1). The plasma sodium concentrations of GH and cortisol groups in March were 153.5 mM and 164.3 mM, respectively (Fig. 2). These levels were significantly lower than that of the control group (191.1 mM).

## Discussion

There are many reports concerned with effects of cortisol and/or GH on seawater tolerance and gill $Na^+$, $K^+$-ATPase activity. In coho salmon, plasma sodium levels after transfer to seawater are lowered by cortisol treatment (Richman and Zaugg 1987). Also, oral administration of cortisol has a positive effect on the salinity tolerance of masu salmon (*O. masou*) parr (Ouchi 1985) and cortisol implants activate gill $Na^+$, $K^+$-ATPase in coho salmon (Specker et al. 1994). Furthermore, the gill $Na^+$, $K^+$-ATPase activity of Atlantic salmon, amago salmon (*O. rhodurus*) and coho salmon are activated by GH treatment (Komourdjian et al. 1976b; Miwa and Inui 1985; Richman et al. 1987). Bolton et al. (1987) also demonstrated that juvenile rainbow trout (*O. mykiss*) given injections of chum salmon (*O. keta*) GH or ovine GH have lower increases in plasma sodium concentrations than control groups injected with saline only. These results show that cortisol and GH are important hormones promoting an adaptation to seawater in salmonid fishes. In the present study, the experimental groups treated with cortisol or GH had decreased plasma sodium concentrations after transfer to seawater and increased gill $Na^+$, $K^+$-ATPase activity. These results indicate that cortisol and GH also have an important function in improving the seawater tolerance of sockeye salmon by stimulating their gill $Na^+$, $K^+$-ATPase activity.

Effects of cortisol and GH on seawater tolerance were not the same in March fish. The plasma sodium concentration of the GH group was lower than that of the cortisol group, while the gill $Na^+$, $K^+$-ATPase activity of the cortisol group was higher than that of the GH group. Richman et al. (1987) reported the similar result that implants of bovine growth hormone in hypophysectomized coho salmon did not increase the gill $Na^+$, $K^+$-ATPase activity, and 48 hrs after fish transfer to seawater these implants reduced plasma sodium concentrations. An explanation for these results may be due to differences in the optimal dosage of each hormone or the administration period. However, McCormick (1996) and Pelis and McCormick (2001) observed a more positive effect on the development of seawater tolerance using a combination of cortisol and GH, compared to separate cortisol and GH treatments. These findings suggest that cortisol and GH have different roles in the developmental process of gill $Na^+$, $K^+$-ATPase or sodium excretion ability in seawater, though both hormones cooperate to develop seawater tolerance during smoltification. We need more research to understand the interaction between cortisol and GH.

In May fish, the GH and cortisol groups showed dramatic increases in the gill $Na^+$, $K^+$-ATPase activi-

ty and it reached 16.8 - 17.1 μmols Pi/mg pro/h (Fig. 1). Their plasma sodium concentrations after transfer to seawater synchronously decreased to 148.0 - 152.5 mM (Fig. 2). These values were equal to levels observed in sockeye salmon smolts reared at the Chitose Hatchery (Ban and Yamauchi 1991). However, the gill $Na^+$, $Ki^+$-ATPase activity and plasma sodium concentrations of the control group were at the same levels as in the GH and cortisol groups and there was no significant difference among the three groups. Exogenous cortisol and GH had no effect on seawater adaptation in May as compared with the results in March. A reason for this result may be due to endogenous cortisol and GH. In the present experiment, endogenous concentrations of cortisol and GH were not measured. Under normal rearing conditions, yearling sockeye salmon show temporal surges in endogenous cortisol and GH during March and April (Ban, unpublished data). Following these surges, the fish have a peak of seawater tolerance in May. Therefore, it seems that sockeye salmon used in this experiment in May had already developed seawater tolerance via their endogenous cortisol and GH prior to the hormonal treatments.

The present study suggests that cortisol and GH are involved with the development of seawater tolerance in sockeye salmon. More detailed research is necessary to understand the respective roles of each hormone in seawater tolerance.

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## コーチゾルと成長ホルモンがベニザケの海水適応能に与える影響

伴　真俊

コーチゾルと成長ホルモン(GH)が，ベニザケの海水適応能に与える影響を調べるため，体重当り1 $\mu$gの濃度に調整した各ホルモンを，腹腔内へ2週間に亘って注射した．同様の実験を3月と5月の2回行った．3月のコーチゾル群における鰓の$Na^+$，$K^+$-ATPase活性は，生理食塩水を投与した対照群に比べて約1.5倍高い値を示した．海水移行24時間後の血中ナトリウム (Na) 濃度は，対照群が191.1 mMだったのに対して，コーチゾル群とGH群は各々164.3 mMと153.5 mMを示した．しかし，5月には3群とも共通して鰓の$Na^+$，$K^+$-ATPase活性が16.8-17.1 $\mu$mols Pi/mg pro/hまで上昇するとともに，海水移行後の血中Na濃度は148.0-154.4 mMまで低下し，ホルモンの投与効果が認められなかった．これらの結果は，コーチゾルとGHが，ベニザケの海水適応能獲得過程に重要な役割を果たすことを示している．しかし，ホルモンの効果は投与時期により異なることが予想される．