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RESEARCH NOTE

Effects of Handling Stress on Osmoregulation of Juvenile Sockeye Salmon (*Oncorhynchus nerka*) in Seawater

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Abstract. — Effects of handling stress on osmoregulatory ability of juvenile sockeye salmon (*Oncorhynchus nerka*) were investigated to assess responses of fish sampled for physiological studies. Yearling juveniles were initially reared in either fresh water (May) or natural seawater (August), and subsequently separated into three treatment groups. For each treatment, fish were placed in artificial seawater (33 ppt) using one of three handling methods. Fish were either carefully transferred to the seawater (Group 1), hung on a fishing hook and suspended in the seawater (Group 2), or confined in a small net and dipped into the seawater (Group 3). Serum sodium concentration and gill Na^+,K^+-ATPase activity were measured 4 hrs after transfer of fish. In May and August, the serum sodium concentrations of Groups 2 and 3 fish were significantly higher than that of Group 1 fish, whereas there was no significant difference in the gill Na^+,K -ATPase activity among three groups. These results indicate that handling stress may disturb osmoregulatory ability of fish in seawater without activating enzyme in the gills and that the serum sodium concentration increases in a short period.

Key words: sockeye salmon, handling stress, osmoregulatory ability

Introduction

It has been reported that salmonids respond physiologically to exogenous stresses. Chronic stresses cause stunt, disease or ultimately death (Fagerlund et al. 1995), while acute stresses drastically increase the plasma levels of catecholamines and cortisol, and stress-related hormones, as well as heart rate (Wedemeyer 1969; Pickering et al. 1982; Sumpter et al. 1986). Stresses also affect reproductive activity and metabolic changes (Strange et al. 1979; Nikinmaa et al. 1983; Redding and Schreck 1983; Kubokawa et al. 1999). It has been noted that confinement stress decreases plasma levels of sex steroid hormones during the breeding season in rainbow trout *Oncorhynchus mykiss* (Kubokawa et al. 1999) and gill Na^+,K^+-ATPase activity, a key enzyme of osmoregulation in seawater, in rainbow trout (Strange et al. 1979). Hauling stress also induces metabolic changes in an osmoregulatory compensation (Nikinmaa et al. 1983; Redding and Schreck 1983).

When physiological studies are carried out using wild salmonids, collection of samples is always accompanied with stresses. Fishing methods such as the long-line fishing and the set net are considered to impose acute and extreme stresses to the caught fish. To precisely estimate data from fish, it is indispensable that affects of stress are taken into consideration. However little information is known regarding effects of fishing stress on the osmoregulatory ability of wild salmonids. The aim of the present study is to investigate effects of handling stress upon the osmoregulatory ability of yearling sockeye salmon (*Oncorhynchus nerka*) in seawater, in order to assess responses of fish caught for physiological studies to fishing implements. Here, long-line and set net fishing conditions were simulated to examine potential stresses.

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Materials and Methods

Thirty yearling sockeye salmon smolts were randomly collected from a freshwater rearing pond at the Chitose Hatchery, National Salmon Resources Center on May 5, 1996. Smolts were divided into three treatment groups of ten fish. Each group experienced a different transfer method into a 60 l tank filled with artificial seawater (33 ppt). Smolts were either carefully transferred to the tank (Group 1), hung on a fishing hook suspended in the tank (Group 2) or confined in a small net (30 cm × 30 cm) and dipped in the tank 10 cm below the water surface (Group 3). Groups 2 and 3 were designed as experimental simulations of long-line fishing and set net, respectively. Four hours after each treatment, blood samples were collected in microtubes from cut caudal fins and centrifuged at 3,000 g for 15 min to separate serum. The resultant sera were stored at -40°C until assay. The gill filaments were also sampled and immersed in homogenizing solution (250 mM sucrose, 6 mM EDTA 2Na, 20 mM imidazole, pH 6.8), and frozen at -80°C. Serum sodium concentration (mEq/l) was measured using an Atomic Absorption and Flame Emission Spectrometer (Shimadzu, AA-640-13). Gill Na^+,K -ATPase activity was measured using the method of Ban and Yamauchi (1991).

On May 7, 1996, one hundred of yearling sockeye salmon smolts were transferred from a fresh water pond into a fiberglass tank (3 tons). They were acclimated gradually from a salinity of 20 to 33 ppt for two weeks and kept in full-strength seawater (33 ppt) until early August. In a preliminary study (unpublished data), I found that the osmoregulatory function of yearling sockeye salmon reared in seawater became stable in August. The aerated and circulated seawater was controlled at 8°C. Fish were fed at 3% body weight per day with a commercial dry diet (Oriental Yeast, Co, Ld.). On August 4, 30 sockeye salmon were randomly collected from the tank. These fish were subsequently divided into three groups and subjected to the same transfer treatments as indicated above.

The data were subjected to a one-way analysis of variance (ANOVA) followed by Student t-test to determine significant differences in serum sodium concentration 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 and Discussion

For both sampling dates (May 5 and August 4), there were no significant differences in the fork length of yearling sockeye salmon among three treatment groups (Table 1). The mean serum sodium concentration of Group 1 fish was 154.4 mEq/l in May and 165.6 mEq/l in August (Fig. 1). Wedemeyer et al. (1980) reported that a serum sodium concentration of less than 170.0 mEq/l in seawater was optimal range for smolt. Therefore, Group 1 fish must have attained a high seawater tolerance in both months. However, serum sodium concentration of Groups 2 and 3 fish were 177.8 and 176.7 mEq/l (May), and 216.9 and 218.1 mEq/l (August), respectively. These values were significantly ($p<0.05$) higher than that of Group 1 fish. Waring et al. (1996) reported a similar elevation in serum sodium

Table 1. Mean fork length (cm) ± S. E. of yearling sockeye salmon in Groups 1, 2, and 3 sampled on May 5 and August 4, 1996.

Date sampled	Group 1	Group 2	Group 3
May 5	12.6 ± 0.3	13.1 ± 0.4	12.5 ± 0.3
Aug. 4	19.5 ± 0.8	18.2 ± 0.8	18.0 ± 1.4

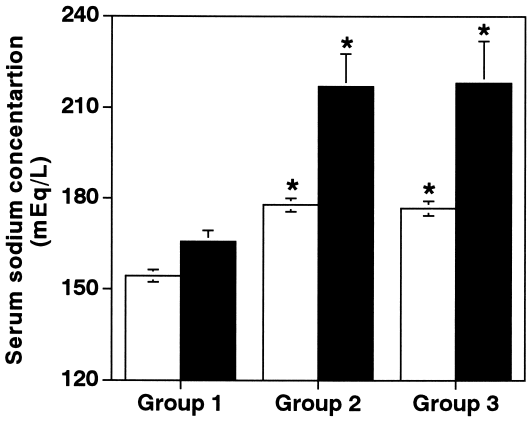

Fig. 1. Mean serum sodium concentration of yearling sockeye salmon 4 hrs after transfer from fresh water to artificial seawater in May (white column) and from natural seawater to artificial seawater in August (black column) for different treatment groups. Group 1 fish were transferred carefully and slowly as control. Group 2 fish were hung on a fishing hook and suspended in seawater. Group 3 fish were confined in a small net and dipped in seawater. Bars indicate SE. Asterisks above columns show significant difference ($p<0.05$) between Group 1 and Groups 2 and 3 in the same period.

concentration in accordance with confinement stress in turbot (*Scophthalmus maximus*). These results indicate that acute stresses such as hanging or confinement hinder the normal sodium regulatory functions of yearling sockeye salmon in seawater.

The mean gill Na^+,K^+-ATPase activity of the three group fish ranged from 18.0 to 19.4 µmols Pi/mg pro./h in May and from 13.2 to 14.1 µmols Pi/mg pro./h in August (Fig. 2). These enzyme activities are similar to those of yearling sockeye salmon smolt reported by Ban and Yamauchi (1991). Reductions in the mean enzyme activity observed during May and August may be due to a seasonal change, but not an effect of body size. This is indicated by lack of correlation between body size and enzyme activity in both months. Furthermore, I similarly found a slight reduction in the enzyme activity of normal yearling sockeye salmon reared in seawater during spring and summer seasons in a preliminary study (unpublished data). For both months, a significant difference in this activity was not observed between the control and stressed groups. It is well known that gill Na^+,K^+-ATPase plays a valuable role in ionoregulation of marine animals (Towle 1981). Specifically, this enzyme is activated during excretion of superfluous sodium ions by salmonids in seawater (Wedemeyer et al. 1980; Ban and Yamauchi 1991). Since little change in gill Na^+,K^+-ATPase activity occurred with the handling stresses, it appears that elevations of serum sodium concentration in the stressed groups are not due to decreased sodium excretion ability of gill Na^+,K^+-ATPase. Fletcher (1992), however, noted that handling stress increased net water efflux via the gill filaments and disturbed the serum ion balance of a marine flatfish (*Pleuronectes platessa*). Therefore, it seems that a serum change due to increased water efflux via the gill filaments and subsequent serum water loss in accordance with handling stress could explain the elevated serum sodium concentrations of stressed yearling sockeye salmon in the present study.

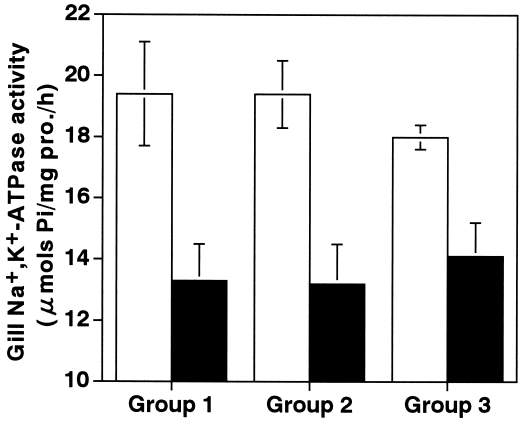

Fig. 2. The mean gill Na^+,K^+-ATPase activity of yearling sockeye salmon 4 hrs after transfer from fresh water to artificial seawater in May (white column) and from natural seawater to artificial seawater in August (black column) for different treatment groups. Group 1 fish were transferred carefully and slowly as control. Group 2 fish were hung on a fishing hook and suspended in seawater. Group 3 fish were confined in a small net and dipped in seawater. Bars indicate SE. There was no significant difference among groups in the same period.

There are several reports on physiological responses to stresses in salmonids reared in fresh water (Utida et al. 1972; Strange and Schreck 1978; Graham et al. 1982; Sumpter et al. 1986). After strenuous exercise, the plasma sodium levels increased in rainbow trout (Graham et al. 1982). This phenomenon is explained in that increases in intracellular lactate accompanied with exercise stress activate an inflow of water and sodium into the body. Plasma cortisol levels also increase under the influence of acute stress in chinook salmon (*O. tshawytscha*) and brown trout (*Salmo trutta*) (Strange and Schreck 1978; Sumpter et al. 1986). Utida et al. (1972) reported that the injection of cortisol increased the amount of water and sodium transported through the intestine of Japanese eel (*Anguilla japonica*) in fresh water. In this study, changes in the internal water movement or plasma cortisol concentrations after acute stresses were not measured. However, I might expect that an acute stress induces elevation in the plasma cortisol levels of yearling sockeye salmon in a short period, and that increased cortisol stimulates sodium inflow passage from the intestine, even in seawater. It is possible that increases of serum sodium concentration (Fig. 1) cause an extra inflow of sodium ion from organs other than the gills. This inflow may exceed the excretion capacity of sodium ion via Na^+,K^+-ATPase in the gills.

A drastic change in the salinity of ambient water also imposes stress on fish (Wedemeyer et al. 1990). The experimental fish in May were transferred from fresh water to seawater, whereas those in August were moved from natural seawater to artificial seawater. It is possible that the fish in May, but not in August, received an intensive stress. However, serum sodium concentrations of the stressed fish in

May were lower than those in August. One reason for this is that gill Na^+,K^+-ATPase activity of fish in May was higher than in August, whose fish were already acclimated to seawater. This phenomenon may be an excessive preparation by smolt in advance of the drastic changes in ambient salinity. Furthermore, Barton et al. (1985) reported that coho salmon (*O. kisutch*) smolt responded more distinctly to acute handling stress than pre-smolt fish. The present results show that sockeye salmon smolt in fresh water acquires tolerance not only for extreme environmental changes in salinity that accompany seaward migration, but also for some unpredictable stresses.

The present study warns investigators to consider the effects of handling stress on physiological condition when examining osmoregulation of wild salmonids.

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ハンドリングがベニザケ幼魚の海水中における浸透圧調節機能に与える影響

伴　真俊

生理学的研究に供試する魚が漁具により受ける影響を把握するため，種々のハンドリングストレスがベニザケ1年魚の浸透圧調節機能に与える影響を調べた．淡水飼育されたスモルトを5月に，また天然海水飼育された幼魚を8月に，10尾ずつの3群に分けて，塩分33の人工海水へ移行した．人工海水へ安静に移した群をG-1，釣針に掛けて海水中に吊した群をG-2，小さな網袋に入れて海水中に漬けた群をG-3とした．各処置後4時間目の血中ナトリウム(Na)濃度と鰓のNa^+,K^+-ATPase活性を測定した．両月とも，G-2とG-3の血中Na濃度はG-1に比べて有意に高まったが，鰓のNa^+,K^+-ATPase活性には有意差が認められなかった．この結果は，ハンドリングによるストレスが，海水中の魚における鰓以外の浸透圧調節機能を阻害し，比較的短時間に血中ナトリウム濃度を上昇させることを示している．