

Seawater Tolerance of Lacustrine Sockeye Salmon (*Oncorhynchus nerka*) from Lake Toya

Masatoshi Ban^{*1}, Hiroyuki Haruna^{*2}, and Hiroshi Ueda^{*2}

^{*1} Research Division, National Salmon Resources Center, Fisheries Agency of Japan,
2-2 Nakanoshima, Toyohira-ku, Sapporo 062-0922, Japan
(dukeban@salmon.affrc.go.jp)

^{*2} Toya Lake Station for Environmental Biology, Faculty of Fisheries, Hokkaido University,
122 Tsukiura, Abuta-cho, Abuta-gun, 049-5723, Japan

Abstract.— External changes and seawater tolerance related to smoltification of yearling lacustrine sockeye salmon (*Oncorhynchus nerka*) originating from Lake Toya (LSS) were investigated monthly from March to July 1993. Ocean-run sockeye salmon (OSS) originating from the Bibi River were examined for comparison. As an external index of smolting, dorsal and caudal fin margin blackening was observed in more than 80% of LSS and OSS during May and June, while it was below 55% during other months. The serum sodium concentration of LSS and OSS 24 h after transfer to seawater decreased below 165 mEq/L from April to June and from April to May, respectively. The gill Na⁺, K⁺-ATPase activity of both groups in fresh water showed a rapid increase from March and reached the maximum level of about 18 μ mol Pi/mg pro/h in May, followed by a gradual decrease. These results indicate that yearling LSS smolt, like OSS, are maximally seawater tolerant in May.

Key words : sockeye salmon, seawater tolerance, smoltification

Introduction

Smoltification in salmonids is a very complex phenomenon that includes morphological, physiological, and behavioral changes (Hoar 1976). External body silverying and fin margin blackening are the most dramatic characteristics used to distinguish smolts from parr (Kubo 1980; Gorbman et al. 1982; Yamauchi et al. 1984). The major physiological process of smoltifying salmonids is the increase in the gill Na⁺, K⁺-ATPase activity and gill chloride cell number concurrent with increased seawater tolerance preceding life in seawater (McCormick et al. 1987). Salmonid smolts decrease aggressive behavior and tend to form aggregates or schools. There have also been observations that migrating smolts will actively swim downstream to reach seawater (Solomon 1978). Most salmonids smolt in the natal river or lake prior to seaward migration.

Sockeye salmon (*Oncorhynchus nerka*) exhibit a greater variety of life history patterns than chum (*O.*

keta) or pink salmon (*O. gorbuscha*). The sockeye salmon is typically anadromous and usually migrates to the ocean after smolting. The kokanee is a form of the sockeye salmon that remains in fresh water to mature and reproduce. They show the seawater tolerance cycle typical of sockeye salmon, despite not having experienced an anadromous life history for as much as 10,000 years (Foote et al. 1994). In Japan, lacustrine sockeye salmon occur, but not native anadromous sockeye salmon. Lacustrine sockeye salmon living in Lake Toya (LSS), which are a very important fishery resource of this lake, have reproduced for many generations without oceanic migration. They were probably planted approximately 100 years ago into Lake Toya from Lake Akan, however, there are no detailed records on the origin of LSS. The life cycle of LSS is similar to that of kokanee, however, there have been few investigations about their physiological condition, especially in relation to smoltification. Recently, releases of LSS into rivers have been performed to produce a new fishery resource of anadromous sockeye salmon in Japan. In this study, changes in seawater tolerance related to smoltification of yearling LSS were examined and compared with ocean-run sockeye salmon.

Materials and Methods

Fish sampling

Yearling LSS and ocean-run sockeye salmon (OSS) from Lake Toya and Bibi River, respectively, were used for this experiment. LSS were hatched and reared at Toya Lake Station for Environmental Biology, Faculty of Fisheries, Hokkaido University. OSS were hatched and reared at the Chitose Branch of the National Salmon Resources Center. In June 1992, 1,000 individuals of LSS from stock the group and OSS transferred from the Chitose Branch were placed in several fiberglass tanks (1 ton capacity) at the Toya Lake Station as underyearlings. Mean fork length of LSS and OSS in June was 55.8 mm and 40.1 mm, respectively. Both groups were fed commercial pellet at a ration of 3% body weight per day. The photoperiod was natural and the temperature of supplying well water was 9-10°C throughout the experiment. Samplings were conducted monthly from March to July, 1993.

Biological measurements and collection of gill filaments

At each sampling time, fork length (FL, mm), body weight (BW, g) and gonad weight (GW, g) were measured for 30 fish in fresh water, after anesthetization by ethyl 3-aminobenzoate methanesulfonic acid salt (Acros Co., 100 mg/L). Gill filaments were dissected from 10 of the 30 fish mentioned above, rinsed in ice-cold homogenizing buffer (250 mM sucrose, 6 mM EDTA 2Na, 20 mM imidazole, pH 6.8) and kept frozen in buffer (20 times weight/volume) at -40°C for the analysis of Na⁺,K⁺-ATPase activity.

External smolt characters

External smolt characters were estimated visually, based on the appearance of distinct blackening on the dorsal and caudal fin margins, for the 30 fish used for biological measurement. A smolt ratio (%) was calculated as the proportion of externally smolted fish among 30 fish.

Seawater tolerance

To estimate seawater tolerance, 20 fish were transferred directly into artificial seawater (salinity 33‰, 10.1 ± 1.0°C) for 24 h and serum sodium concentration was measured in survivors. For this assay, serum was separated from blood samples obtained from the caudal vessels using a micro test tube with centrifugation at 2,000 g for 15 min and was stored at -40°C. After dilution of the thawed

serum with distilled water (1:1000), serum sodium concentration was measured using an Atomic Absorption and Flame Emission Spectrometer (Shimadzu, AA-640-13).

The Na⁺,K⁺-ATPase activity of the gill filaments was determined using the method of Folmar and Dickhoff (1979), modified by Ban and Yamauchi (1991). The enzyme activity was expressed as amount of inorganic phosphate transformed (Pi) per milligram protein per hour (μ mol Pi/mg pro/h).

Precocious males

A gonad somatic index was calculated as $GSI = 100 \times GW/BW$. Males which showed a GSI over 0.1% were classified as precocious males; immature males showed GSI under 0.1% throughout the experiment. Data for precocious males were removed from the mean serum sodium concentration and gill Na⁺, K⁺-ATPase activity analysis.

Statistical analysis

The data presented were expressed as mean $\pm$ SEM. The data were subjected to one-way analysis of variance (ANOVA) followed by Student t-test or Cochran-cox test to determine significant differences between LSS and OSS, and monthly changes within each group. A probability level of less than 0.05 was considered significant in all tests.

Results

Mean fork length of LSS and OSS increased from 114.8 mm and 105.3 mm in March to 158.0 mm and 144.2 mm in July, respectively (Fig. 1). The fin margin blackening in both groups began in April and the smolt ratio was greater than 80% in May and June (Fig. 2). Most unsmolted fish during this period were precocious males. The rate of occurrence of precocious males in LSS was 20% in May and 10% in June, and in OSS it was 6.7% in both months. Mean fork lengths of the precocious males and immature males of LSS were 153.8 mm and 140.8 mm in May and 161.7 mm and 154.1 mm in June, respectively. In the case of OSS, mean fork lengths of precocious and immature males were 137 mm and 129.6 mm in May and 145.3 mm and 138.6 mm in June, respectively. A significant difference ($p < 0.05$) in mean fork length was observed only between precocious and immature males of LSS in May. In July, the smolt ratio of LSS and OSS reduced to 55% and 10%, respectively, due to fading of fin margin blackening.

All fish transferred to seawater survived for 24 h. Serum sodium concentrations of LSS and OSS 24 h

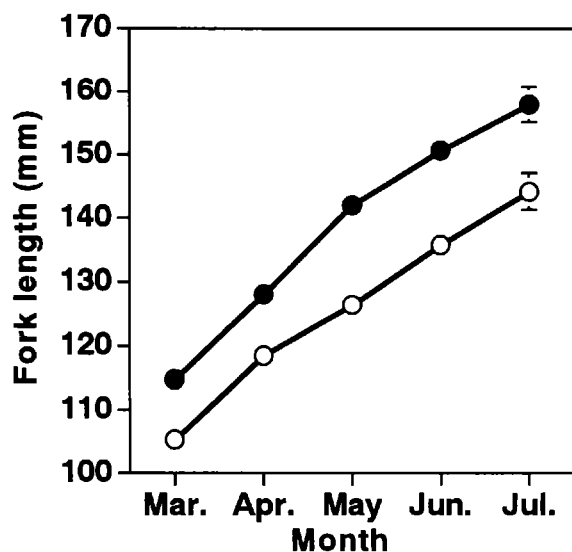


Fig. 1. Changes in mean fork length of yearling LSS (closed circles) and OSS (open circles). The vertical bars show standard errors. Some standard errors are hidden by symbols.

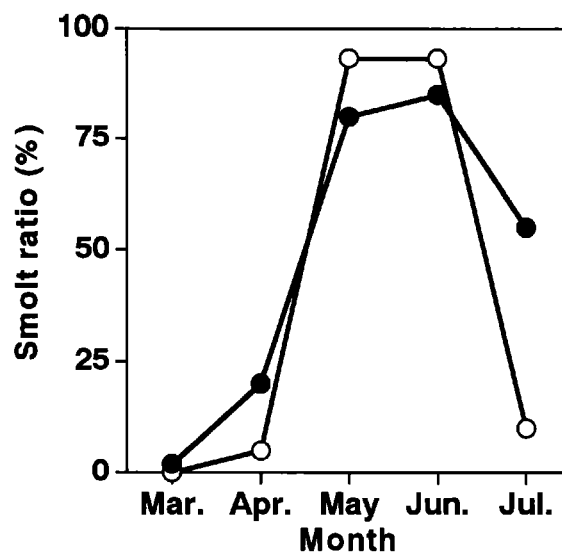


Fig. 2. Changes in smolt ratio of yearling LSS (closed circles) and OSS (open circles).

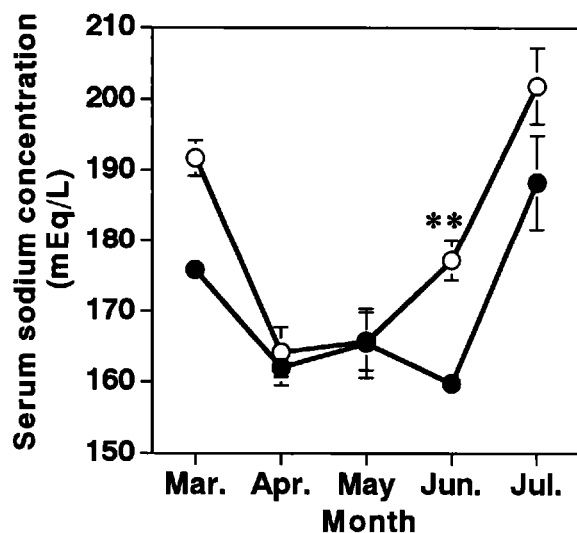


Fig. 3. Changes in mean serum sodium concentration 24h after transfer to seawater of yearling LSS (closed circles) and OSS (open circles). The vertical bars show standard errors. Some standard errors are hidden by symbols. Asterisks above point show significant difference (** $p < 0.001$) between LSS and OSS.

after transfer to seawater in March were 175.8 mEq/L and 191.7 mEq/L, respectively (Fig. 3). Both groups decreased significantly ($p < 0.001$) below 165.0 mEq/L in April. LSS maintained this low level until June, followed by a significant increase ($p < 0.001$) to 188.3 mEq/L in July. The serum sodium

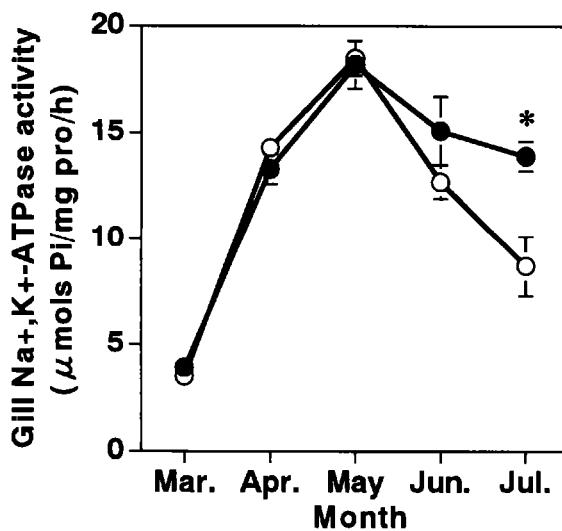


Fig. 4. Changes in mean gill Na⁺,K⁺-ATPase activity of yearling LSS (closed circles) and OSS (open circles). The vertical bars show standard errors. Some standard errors are hidden by symbols. An asterisk above point shows significant difference (* $p < 0.01$) between LSS and OSS.

um concentration of OSS was elevated significantly ($p < 0.001$) after June, averaging 201.9 mEq/L in July. The difference in serum sodium concentration between the groups in June was significant ($p < 0.001$). The gill Na⁺, K⁺-ATPase activity of both groups increased from about 4.0 μ mol Pi/mg pro/h

in March to about $18.0 \mu\text{mol Pi/mg pro/h}$ in May (Fig. 4). The enzyme activity of LSS and OSS was reduced to $13.9 \mu\text{mol Pi/mg pro/h}$ and $8.7 \mu\text{mol Pi/mg pro/h}$ in July, respectively. The difference in enzyme activity between LSS and OSS in July was significant ($p < 0.01$).

Discussion

Yearling ocean-run sockeye salmon display distinct pigmentation on the dorsal and caudal fin margin (Ban and Yamauchi 1991). In this study, more than 80% of LSS showed clear fin margin blackening from May to June and the degree of this coloration was similar to that of OSS. This result demonstrates that LSS externally smolted during May and June. However, the smolt ratio of LSS during this period was slightly lower than that of OSS as a result of a difference in appearance rate of precocious males. Larger males usually mature earlier than smaller ones, and this sexual maturation inhibits smoltification (Saunders et al. 1982; Aida et al. 1984; Foote et al. 1991). We also found that precocious males were larger than immature males, and that precocious males did not smolt. Therefore, we suggest that high growth rate of juveniles may obstruct smoltification of male LSS.

Clarke and Blackburn (1978) suggested that the optimal serum sodium concentration of smolts after a 24 h transfer to seawater was actually in the range of 150-160 mEq/L. To regulate serum sodium concentration of fish in seawater, the gill Na^+ , K^+ -ATPase system is involved in excretion of sodium ions directly from the blood to the external environment (Kamiya 1972). Some salmonids increase their gill Na^+ , K^+ -ATPase activity during smoltification (Ban and Yamauchi 1991; Ewing and Rodgers 1998; Pirhonen and Forsman 1998). In the present study, LSS transferred to seawater showed serum sodium concentrations below 165 mEq/L from April to June. Furthermore, the gill Na^+ , K^+ -ATPase activity of LSS increased rapidly from March and peaked in May, like OSS. These results indicate that LSS maintains the capacity to develop seawater tolerance in the spring. The serum sodium excretion ability in the gills of LSS was highest in May. This capacity has been preserved for many generations with or without an oceanic migration.

Johnston and Eales (1970) found that, although temperature is a key factor for external changes during smoltification in Atlantic salmon (*Salmo salar*), its effect is highly dependent on the size of fish considered. Smolting occurs sooner at higher temperatures, and changing temperature seems to be more

stimulatory than constant temperature to Atlantic salmon and steelhead trout (*O. mykiss*) (Wagner 1974; Jonsson and Ruud-Hansen 1985). In the present study, LSS showed dramatic changes in fin margin blackening and seawater tolerance, despite their having been reared under stable food conditions and ambient temperature from the underyearling stage. Thus, it is likely that the trigger for smoltification in LSS was stimulated by environmental factors other than water temperature or growth pattern. Photoperiod may be particularly important as a regulating factor (McCormick et al. 1987; Bjornsson et al. 1998; Duncan and Bromage 1998).

If smolts are retained in fresh water past the normal downstream migration period, external characters and seawater tolerance usually decrease (Zaug and McLain 1970; Lasserre et al. 1978). This decrease has been termed desmoltting. Stefansson et al. (1998) reported that temperature significantly affects desmoltification. In this study, LSS and OSS demonstrated fading in fin margin blackening and decreased seawater tolerance, despite they were reared under stable water temperature conditions. This indicates that desmoltification in LSS is not initiated by changing water temperatures. Mortensen and Damsgard (1998) suggested that Atlantic salmon kept at salinities of 15‰ or higher could maintain a high and stable hypoosmoregulatory capacity. A moderate influx of sodium ion into the body may be necessary to keep seawater tolerance at a high level. Thus, desmoltification seems to be a fresh water adaptation process occurring in smolts whose seaward migration is delayed or prevented.

The magnitude of desmoltification in LSS was less than OSS in the present study. These more gentle reversions may be a feature of the lacustrine stock which is adapted to a more stable environment (lake) than the pelagic ocean. Unfortunately, we have done this comparison only in one year. To ascertain if this observation is significant, further investigations are required.

In conclusion, yearling LSS originating from Lake Toya smolt and develop maximum seawater tolerance in May. This capacity to smolt may be an inherited character unrelated to their lack of experience of ocean life

Acknowledgements

We wish to express our appreciation to the staff of the Chitose Branch, National Salmon Resources Center for their effort supplying the experimental fish. Thanks also to Dr. Jill B. K. Leonard for revision of this manuscript.

References

- Aida, K., T. Kato, and M. Awaji. 1984. Effects of castration on the smoltification of precocious male masu salmon *Oncorhynchus masou*. Bull. Jap. Soc. Sci. Fish., 50: 565-571.
- Ban, M., and K. Yamauchi. 1991. Seasonal changes in seawater adaptability of the hatchery reared juvenile sockeye salmon, *Oncorhynchus nerka*. Sci. Rep. Hokkaido Salmon Hatchery, 45: 25-33.
- Björnsson, B. T., G. V. Stefansson, Å. I. Berge, T. Hansen, and S. O. Stefansson. 1998. Circulating growth hormone levels in Atlantic salmon smolts following seawater transfer: effects of photoperiod regime, salinity, duration of exposure and season. Aquaculture, 168: 121-137.
- Clarke, W. C., and J. Blackburn. 1978. Seawater challenge tests performed on hatchery stocks of chinook and coho salmon in 1977. Can. Fish. Mar. Serv. Tech. Rep., 761: 19 p.
- Duncan, N., and N. Bromage. 1998. The effect of different periods of constant short days on smoltification in juvenile Atlantic salmon (*Salmo salar*). Aquaculture, 168: 369-386.
- Ewing, R. D., and J. D. Rodgers. 1998. Changes in physiological indices of smolting during seaward migration of wild coho salmon, *Oncorhynchus kisutch*. Aquaculture, 168: 69-83.
- Folmar, L. C., and W. W. Dickhoff. 1979. Plasma thyroxine and gill Na⁺-K⁺ ATPase changes during seawater acclimation of coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol., 63A: 329-332.
- Foote, C. J., W. C. Clarke, and J. Blackburn. 1991. Inhibition of smolting in precocious male chinook salmon, *Oncorhynchus tshawytscha*. Can. J. Zool., 69: 1848-1852.
- Foote, C. J., I. Mayer, C. C. Wood, W. C. Clarke, and J. Blackburn. 1994. On the developmental pathway to nonanadromy in sockeye salmon, *Oncorhynchus nerka*. Can. J. Zool., 72: 397-405.
- Gorbman, A., W. W. Dickhoff, J. L. Mighell, E. F. Prentice, and F. W. Waknitz. 1982. Morphological indices of developmental progress in the parr-smolt coho salmon, *Oncorhynchus kisutch*. Aquaculture, 28: 1-19.
- Hoar, W. S. 1976. Smolt transformation: evolution, behavior, and physiology. J. Fish. Res. Board Can., 33: 1233-1252.
- Johnston, C. E., and J. G. Eales. 1970. Influence of body size on silvering of Atlantic salmon (*Salmo salar*) at parr-smolt transformation. J. Fish. Res. Board Can., 27: 983-987.
- Jonsson, B., and J. Ruud-Hansen. 1985. Water temperature as the primary influence on timing of seaward migrations of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquatic. Sci., 42: 593-595.
- Kamiya, M. 1972. Hormonal effect on Na-K-ATPase activity in the gill of Japanese eel, *Anguilla japonica*, with special reference to seawater adaptation. Endocrinol. Jpn., 19: 489-493.
- Kubo, T. 1980. Studies on the life history of "Masu" salmon (*Oncorhynchus masou*) in Hokkaido. Sci. Rep. Hokkaido Salmon Hatchery, 34: 1-95. (In Japanese with English summary.)
- Lasserre, P., G. Boeuf, and Y. Harache. 1978. Osmotic adaptation of *Oncorhynchus kisutch* Walbaum. I: Seasonal variations of gill Na⁺-K⁺ ATPase activity in coho salmon, 0⁺-age and yearling, reared in freshwater. Aquaculture, 14: 365-382.
- McCormick, S. D., R. L. Saunders, E. B. Henderson, and P. R. Harmon. 1987. Photoperiod control of parr-smolt transformation in Atlantic salmon (*Salmo salar*): changes in salinity tolerance, gill Na⁺,K⁺-ATPase activity, and plasma thyroid hormones. Can. J. Fish. Aquatic. Sci., 44: 1462-1468.
- Mortensen, A., and B. Damsgård. 1998. The effect of salinity on desmoltification in Atlantic salmon. Aquaculture, 168: 407-411.
- Pirhonen, J., and L. Forsman. 1998. Relationship between Na⁺,K⁺-ATPase activity and migration behaviour of brown trout and sea trout (*Salmo trutta* L.) during the smolting period. Aquaculture, 168: 41-47.
- Saunders, R. L., E. B. Henderson, and B. D. Glebe. 1982. Precocious sexual maturation and smoltification in male Atlantic salmon (*Salmo salar*). Aquaculture, 28: 211-229.
- Solomon, D. J. 1978. Some observations on salmon smolt migration in a chalk stream. J. Fish. Biol., 12: 571-574.
- Stefansson, S. O., Å. I. Berge, and G. S. Gunnarsson. 1998. Changes in seawater tolerance and gill Na⁺,K⁺-ATPase activity during desmoltification in Atlantic salmon kept in freshwater at different temperatures. Aquaculture, 168: 271-277.
- Wagner, H. H. 1974. Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). Can. J. Zool., 52: 219-234.
- Yamauchi, K., N. Koide, S. Adachi, and Y. Nagahama. 1984. Changes in seawater adaptability and blood thyroxine concentrations during smoltification of the masu salmon, *Oncorhynchus masou*, and amago salmon, *Oncorhynchus rhodurus*. Aquaculture, 42: 256-274.
- Zaugg, W. S., and L. R. McLain. 1970. Adenosinet-

riphosphatase activity in gills of salmonids: seasonal variations and salt water influence in coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol., 35: 587-596.

洞爺湖産ベニザケの海水適応能

伴 真俊・春名寛幸・上田 宏

洞爺湖産ベニザケ（ヒメマス）1年魚を飼育し、スモルト化にともなう外見的变化と海水適応能の変化を、3月より7月までの間毎月1回調べた。対

照群として、美々川産の降海型ベニザケを用いた。外見的变化として、背鰭と尾鰭先端のつま黒の状態を観察した結果、5月から6月にかけて両群の80%以上の個体に発現が認められた。海水適応能を調べるために、海水移行24時間後の血清ナトリウム（Na）濃度と、鰓のNa⁺, K⁺-ATPase活性を調べた。ヒメマスと降海型ベニザケのNa濃度は、各々4-6月および4-5月にかけて低値を示し、鰓の酵素活性は両群とも5月に最高値に達した。これらの結果から、洞爺湖産ヒメマス1年魚は降海型ベニザケと同様にスモルト化すること、その際、海水適応能は5月に最も高まることが分かった。