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# Participation of Thyroxine in Smoltification of Sockeye Salmon (*Oncorhynchus nerka*)

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*Abstract*. – In order to describe effects of thyroxine on smoltification of sockeye salmon (*Oncorhynchus nerka*), seasonal patterns of smoltification were investigated. Factors assessed include seawater tolerance, fin margin blackening, condition factor and changes in plasma thyroxine concentration (plasma-T4). Juvenile sockeye salmon reared under natural photoperiod gradually developed seawater tolerance from January to May in two consecutive years (1991 and 1992). Fin margin blackening and decreases in condition factor were observed in May. Plasma-T4 showed significant elevations in concert with an increase in photoperiod from January to March, followed by rapid decreases. Temporary elevation of plasma-T4 was also induced by switchover of artificial photoperiod from short day (8/16 h light/dark cycle) to long day (16/8 h). With this hormonal surge, fish developed seawater tolerance and transformed to smolt out-of-season. However, a single administration of thyroxine supplemented in a commercial diet for twenty days did not induce seawater tolerance. These results suggest that thyroxine has a role as an enhancer of smoltification in sockeye salmon and operates at an early phase of this process indirectly.

**Key words** : sockeye salmon, smoltification, photoperiod, thyroxine

## Introduction

Smoltification in anadromous salmonids occurs during their freshwater phase and is a complex, developmental phenomenon (Hoar 1988). This transformation involves morphological, physiological and behavioral changes, which adapt them for seaward migration and the marine habitat (Wedemeyer et al. 1980). As smoltification progresses, an increased seawater tolerance is accompanied by activation of gill $Na^+$, $K^+$-ATPase as a physiological change, and fin margin blackening as well as reduction of condition factor as morphological changes.

Smoltification also occurs in association with the development of endocrine systems. Hoar (1939) was the first to report a more developed thyroid gland in smolt than in parr for Atlantic salmon (*Salmo salar*). Dickhoff et al. (1978) observed a distinct peak in plasma thyroxine (T4) levels during smoltification in coho salmon (*Oncorhynchus kisutch*). Ban et al. (1987) found hypertrophy of thyroid follicles in smolt of masu salmon (*O. masou*). There are various other studies supporting the relationship between smoltification and thyroid hormones (Alexander et al. 1998; Hutchison and Iwata 1998; Kelly and Wood 2001). These studies indicate that T4 plays important roles in smoltification in salmonid fish. However, seasonal change in plasma T4 concentration seems not to be uniformity among salmonid species. Masu salmon show a sharp and dramatic increase in plasma T4 in May, while amago salmon (*O. rhodurus*) have a plateau and blunt T4 peak from November to February (Yamauchi et al. 1984). Dickhoff et al. (1978) reported that duration and time of onset of the T4 surge varies in different stocks of coho salmon. Furthermore, serum T4 levels are correlated with the stream discharge rate (Youngson and Simpson 1984). Hence, secretion of T4 can be influenced by factors such as aquatic environments, strains or habitat.

Release trials for sockeye salmon (*O. nerka*) have been performed by the National Salmon Resources Center for a fisheries resource in northern Japan. In order to boost both the survival of released juveniles

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and the return of adults, data on the physiological characteristics of smoltification and improvement of stock enhancement techniques are really needed. This paper describes the seasonal pattern of smoltificaton including development of seawater tolerance and changes in plasma T4 concentration in juvenile sockeye salmon reared under natural photoperiod, effect of artificial photoperiodic control on smoltification and secretion of T4, and effect of T4 administration on smoltification.

## Materials and Methods

*Seasonal pattern (experiment-1)*

Juvenile sockeye salmon (brood year 1989) were reared in an indoor pond supplied with spring water from February to July in 1990 at the Chitose Hatchery, National Salmon Resources Center in Hokkaido. They were subsequently removed to an outdoor pond until May 1991 with spring and/or river water. Five hundred fishes were then transferred into an indoor fiberglass tank with 1.5 metric ton capacity and reared in spring and/or river water until July 1992. They were usually fed a commercial diet at 2% body weight/day, however this feeding ratio was switched to 1% body weight/day between November and the subsequent March. Changes in water temperature and photoperiod throughout the experiment are shown in Fig. 1.

The following sampling procedures were conducted monthly. At each sampling ten fish were caught randomly and measurements of fork length (FL, mm) and body weight (BW, g) were made after anesthesia with tricaine methanesulfonate (MS222, 100 mg/liter). A condition factor (CF) was calculated from BW x $10^3$/$FL^3$. Blood samples were collected from the severed caudal vein of each fish using a micro tube. Then, plasma was obtained after centrifugation of the blood at 2,000 g for 15 min and stored at -40°C until assays for thyroxine concentration (plasma-T4). The gill filaments were excised from the same fish and frozen with homogenizing solution (250 mM sucrose, 6 mM EDTA 2Na, 20 mM imidazole, pH 6.8) for later analysis of $Na^+$, $K^+$-ATPase activity (gill-ATPase).

To determine seawater tolerance, twenty fish were randomly taken from the tank and transferred directly into artificial seawater at 33 ppt. At the 24 h after this transfer, plasma samples were obtained via the method above and stored at -40°C until assay for sodium concentration (plasma-Na) could be conducted.

*Effects of photoperiod (experiment-2)*

Juvenile sockeye salmon (brood year 1996) were moved from an outdoor pond to an indoor fiberglass tank (1 metric ton capacity) at the Chitose Hatchery on August 5, 1997, and divided into two groups with 400 individuals each. Photoperiod was controlled by artificial light (2 x 40 w), and kept at a 8/16 h light/dark cycle from August 5 to October 1. One group (8L-group) was maintained at this constant short daylight cycle until November 27. The photoperiod of the other group (16L-group) was switched to a 16/8 h light/dark condition from Octo-

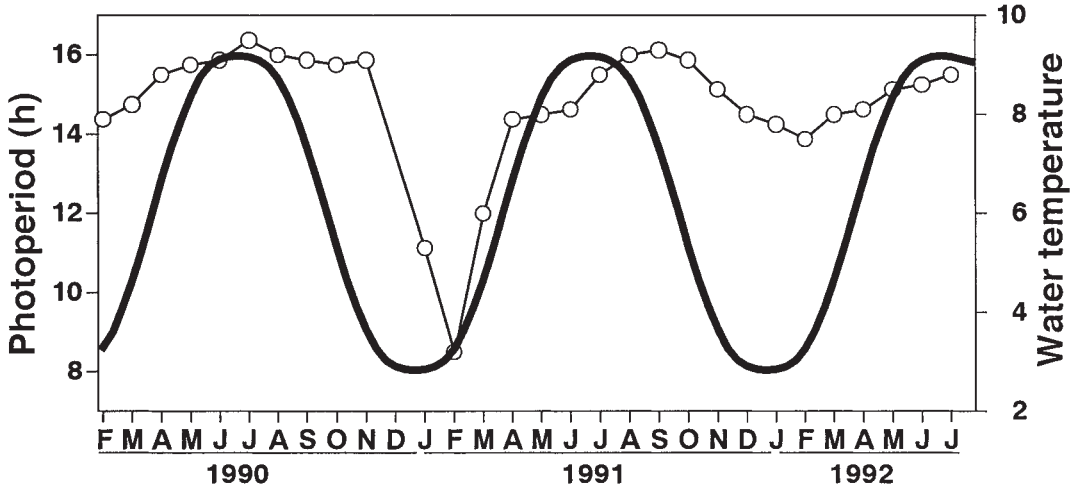


**Fig. 1.** Changes in natural photoperiod (solid line) and water temperature (circles) during Experiment-1 from February, 1990 to July, 1992.

ber 2 to November 27. The natural photoperiod at the beginning of this experiment (August 5) was 15/9 h. Both groups were fed a commercial diet at 2% body wt/day, and supplied recirculating water at 8°C throughout the experiment.

Samples (n=5 each) were collected at two-week intervals from October 2 to November 27. At each sampling, plasma and gill samples were acquired from ten fish of both groups for the assay of plasma-T4 and gill-ATPase. In addition, twenty additional fish were used for a seawater challenge test, and their blood was collected for assays of plasma-Na.

*Effects of T4 (experiment-3)*

Juvenile sockeye (brood year 1995) were transferred from the outdoor pond at the Chitose Hatchery to indoor plastic tanks with 30 l capacity on November 10, 1996, and divided into two groups with 30 individuals each. One group (T4 group) was given thyroxine (T4, Sigma chem. Co.) as a daily supplement to the commercial diet until November 30 for twenty days. The administered dosage of this hormone for each specimen was at 20 μg per day. The other group was given only the commercial diet (control group). The photoperiod was natural and tanks were supplied with well water at 10°C.

On December 1, gill and plasma samples were taken from ten fish for both groups, and another twenty fish from each group were transferred to artificial seawater at 33 ppt. At 24 h after transfer, plasma samples were obtained. Plasma-Na and gill-ATPase were measured.

*Assay of plasma-T4, plasma-Na and gill-ATPase*

Plasma-T4 (ng/ml) was measured by using a DELFIA thyroxine reagent kit with a DELFIA Fluorometer (Wallac) in experiment-1, and by radioimmunoassay according to the method of Suzuki and Suzuki (1981) in experiment-2.

Plasma-Na (mM) was measured by using an Atomic Absorption and Flame Emission Spectrometer (Shimadzu, AA-640-13). At the time of analysis, defrosted serum was diluted 1,000 times with distilled water.

Gill-ATPase was determined according to the method of Ban and Yamauchi (1991). Defrosted gill filaments were homogenized in the homogenizing solution and centrifuged at 2,000 g for 5 min at 4°C. The supernatant was incubated for 20 min at 37°C. Phosphorus (Pi) produced from the incubation was measured according to the method of Goldenberg and Fernandez (1966). Protein concentrations were determined by the method of Lowry et al. (1951). The enzyme activity was expressed as micromoles Pi per milligram protein per h (μ mols Pi/mg pro /h).

*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 T4 and plasma Na, and gill-ATPase. A probability level of less than 0.05 was considered significant.

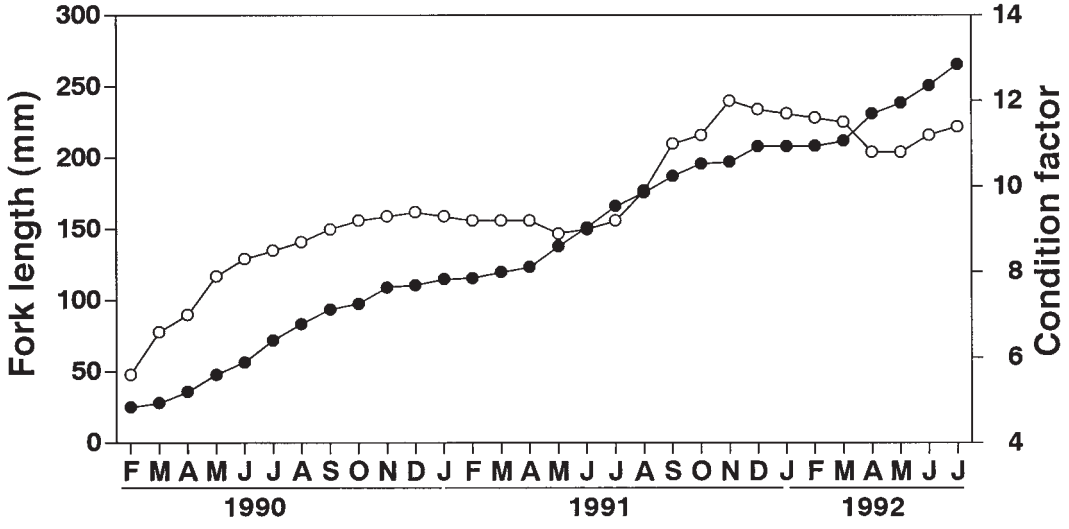


**Fig. 2.** Changes in fork length (solid circles) and condition factor (open circles) for juvenile sockeye salmon reared in Experiment-1 from February, 1990 to July, 1992.

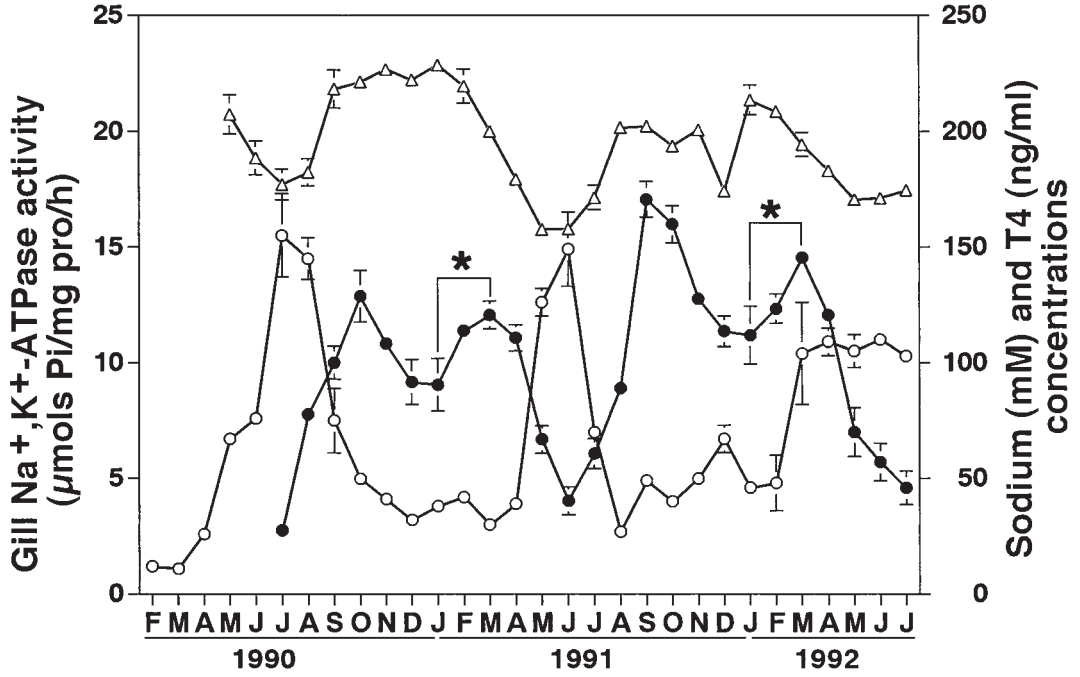


**Fig. 3.** Changes in gill $Na^+$, $K^+$-ATPase activity (open circles) and plasma thyroxine concentration (solid circles), and plasma sodium concentration (triangles) 24 h after transfer into seawater for juvenile sockeye salmon observed in Experiment-1 from February, 1990 to July, 1992. Vertical bars indicate SE. Some SE are hidden by symbols. Asterisks above points show significant differences ($p<0.05$).

## Results

*Seasonal pattern*

Fork length of juvenile sockeye salmon increased from 25.1 mm in February 1990 to 280.5 mm in July 1992 (Fig. 2). Mean monthly elongation of fork length ranged between 2.7 - 3.7 mm from November to the subsequent March, and 9.3 - 13.4 mm in other months. The CF changed from 5.6 in February 1990 to 11.4 in July 1992 (Fig. 2). Remarkable gains in CF were recognized from February to June in 1990 and from July to November in 1991. In April and May in 1991 and March and April in 1992, CF showed a temporary reduction. Fin margin blackening was observed in July and September in 1990, May and July in 1991, and May and July in 1992.

Plasma-T4 increased significantly ($p<0.05$) in January and March in 1991 and 1992 (Fig. 3). Dramatic elevations of plasma-T4 were also observed from July to October in 1990 and from June to September in 1991. The levels of plasma-T4 decreased and reached their lowest levels during June - July every year. For February to June 1990 samples, analyses of this hormone were not conducted because plasma volumes were insufficient for an assay.

Plasma-Na showed significant ($p<0.05$) declines from May to July in 1990 and from January to May in both 1991 and 1992, but the lowest concentrations (approximately 157 mM) were obtained during May and June in 1991 (Fig. 3). In other months, plasma-Na was higher levels more than 190 mM. There were no data from February to April in 1990, because all of the fish transferred to seawater have died within 24 h.

Gill-ATPase displayed a dramatic increase and reached at approximate 15 $\mu$ moles Pi/mg pro/h in July - August 1990, and in May - June 1991, following rapid decreases (Fig. 3). This enzyme activity also showed a rapid increase in March 1992, and subsequently reaching a plateau at a level of approximate 11 $\mu$ mols Pi/mg pro/h until July. In almost all other months, gill-ATPase was lower than 5 $\mu$ mols Pi/mg pro/h.

*Effects of photoperiod*

There was no significant difference in fork length and condition factor of fish between the 8L and 16L groups (Table 1). Plasma-T4 in the 16L-group showed a temporary increase in plasma-T4 on October 30 and reached 6.1 ng/ml (Fig. 4). Other than

**Table 1.** Changes in fork length and condition factor of 8L- and 16L-groups at the start and end of Experiment-2.

| Date | Group | Fork length (mm) | Condition factor |
|---|---|---|---|
| August 5 | 8L | 74.3±0.9* | 8.5±0.1 |
| (start) | 16L | 76.5±1.4 | 8.6±0.1 |
| November 27 | 8L | 96.6±1.6 | 10.7±0.1 |
| (end) | 16L | 95.5±2.0 | 10.1±0.1 |

* Mean±SE

**Table 2.** The mean fork length (FL, mm), condition factor (CF), gill $Na^+$, $K^+$-ATPase activity (gill-ATPase, $\mu$ mols Pi/mg pro/h) and plasma sodium concentration 24 h after seawater challenge of control group and T4 treated group in sockeye salmon at the end of the Experiment-3 (December 1, 1996).

| Group | FL | CF | gill-ATPase | plasma-Na |
|---|---|---|---|---|
| control | 101.3±1.9* | 8.6±0.3 | 4.9±0.4 | 201.5±6.1 |
| T4 | 101.2±2.4 | 8.8±0.3 | 5.0±0.3 | 194.5±3.0 |

* Mean±SE

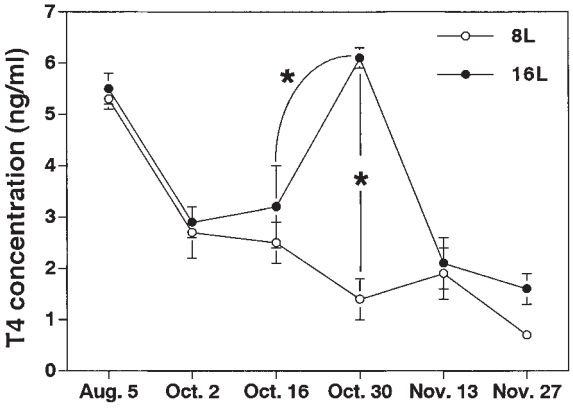


**Fig. 4.** Changes in plasma thyroxine concentration for 8L-group (open circles) and 16L-group (solid circles) fish in Experiment-2. Vertical bars indicate SE. Some SE are hidden by symbols. Asterisks in the graph show significant differences ($p<0.05$).

this increase, both groups showed similar reductions in plasma-T4 from 5.3 - 5.5 ng/ml on August 5 to 0.7 - 1.6 ng/ml on November 27. Differences in plasma-T4 between October 16 and October 30, and the 8L-group and the 16L-group on October 30 were significant. Plasma-Na of both groups maintained higher levels of 200 mM from August 5 to October 16, followed by decrease to 174.2 mM in the 16L-group and 190.9 mM in the 8L-group on November 27 (Fig. 5). Significant differences ($p < 0.05$) in plasma-Na between the two groups were observed from

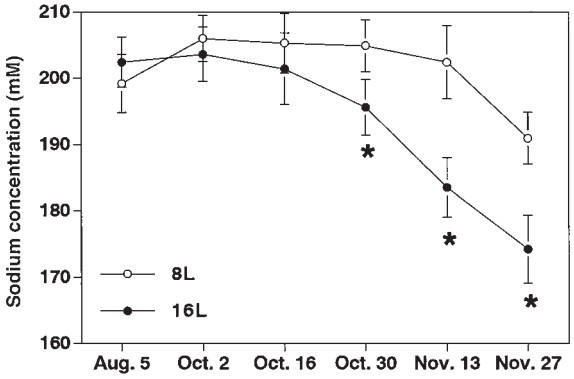


**Fig. 5.** Changes in plasma sodium concentration 24 h after transfer to seawater for 8L-group (open circles) and 16L-group (solid circles) fish in Experiment-2. Vertical bars indicate SE. Asterisks in the graph show significant differences between the 8L- and 16L-groups ($p<0.05$).

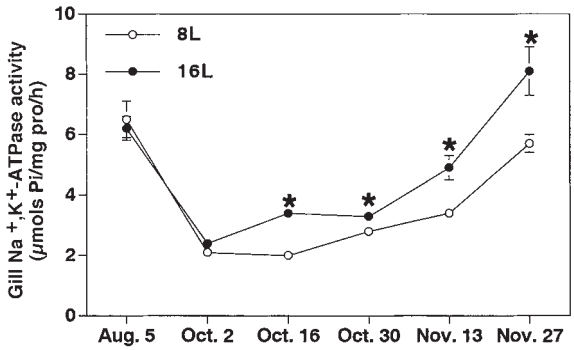


**Fig. 6.** Changes in gill $Na^+$, $K^+$-ATPase activity for 8L-group (open circles) and 16L-group (solid circles) fish in Experiment-2. Vertical bars indicate SE. Some SE are hidden by symbols. Asterisks above the symbols show significant differences between the 8L- and 16L-groups ($p<0.05$).

October 30 to November 27. Gill-ATPase of both two groups declined from 6 $\mu$ mols Pi/mg pro/h on August 5 to 2 $\mu$ mols Pi/mg pro/h on October 2. Subsequently, gill-ATPase increased to 8 $\mu$ mols Pi/mg pro/h in the 16L-goup and 5 $\mu$ mols Pi/mg pro/h in the 8L-group on November 27 (Fig. 6). Gill-ATPase activity for the 16L-group was significantly higher than that of the 8L-group from October 16 to November 27.

*Effects of T4*

There was no significant difference in these smoltification characters (FL, CF, gill-ATPase, and plasma-Na) between two groups (Table 2). However, fin margin blackening was observed only in the T4-group.

## Discussion

Sockeye salmon in their first year (underyearling) and second year (yearling) after hatching transform to smolt in early summer and spring, respectively (Ban and Yamauchi 1991). Shrimpton et al. (2000) also reported the similar smoltification occurring in two consecutive years on Atlantic salmon. In the present experiment, three age-classes (underyearlings, yearlings and 2-year olds) of sockeye salmon displayed obvious smolting characteristics. Anadromous salmonids likely repeat smoltification regularly if they are continually kept in fresh water. However, the smoltification, which occurred at each age showed different patterns (Fig. 3). Seawater tolerance, as indicated by plasma-Na and gill-ATPase, reached a peak during July - August in underyealings, May - June in yearlings and May - July in 2-year olds. Peaks in the gill-ATPase of underyearlings and yearlings were sharp, though that of 2-year olds was lower and at a plateau. Yearling smolt showed lower plasma-Na than fish in the other two classes. The CF of underyearlings continuously increased, while CF in yearlings and 2-year olds fish decreased during smoltification. Fin margin blackening was observed in every age class' smolt. Thus the quality of smolt was not definite during each year. Yearly variations in smoltification seem to be modulated by growth, threshold size, metabolic and endocrine conditions, and/or environmental factors as pointed out by Hoar (1988). Since physiological quality of juvenile fish is assumed to affect on early life mortality in nature, we should correctly assess the quality of smolt at the time of their release. In order to improve the stock enhancement programs for sockeye salmon, accurate estimations of effects of smolt quality on long term survival (i.e., after seaward migration) is also necessary.

Given that smoltification occurs seasonally, this phenomenon can be correlated with periodical changes in the endocrine systems. Thyroxine is one of the major hormones involved in smoltification (Hoar 1939; Dickhoff et al. 1978; Youngson and Simpson 1984). In the present study, long-term changes in plasma-T4 of juvenile sockeye salmon reared under natural photoperiod were examined in order to define the role of thyroxine on smoltification. The data shows that plasma-T4 has a repeated semi-annual variation over two years (Fig. 3). The level of plasma-T4 uniformly reached peaks in March and September, October, and bottomed out in June, July and December. These months essentially correspond to the switchover time of natural photoperiod (equinox) in Japan. Such changes in plasma-T4 according to photoperiod also reappeared under artificial light conditions in the present experiment. Decreasing photoperiod from 15 h to 8 h reduced the plasma-T4, while lengthening photoperiod to a constant 16 h elevated plasma-T4 levels. These phenomena are very close to the plasma-T4 profile under a natural photoperiod from October to May the following year in experiment-1. From these results, it can be predicted that a change in photoperiod from maximum or minimum daylight stimulates secretion of T4. Effects of water temperature and rearing conditions on T4 secretion, however, were not recognized as of significant importance in comparison with the photoperiod. On the other hand, it is known that plasma-T4 levels surge during new moon in coho salmon (Grau et al. 1981). Similar changes in T4 levels coinciding with the new moon have also been demonstrated in masu salmon (Yamauchi et al. 1984) and amago salmon (Fujioka et al. 1990). Although, the correspondence of plasma-T4 increased with the lunar cycle occurred during a limited period at the peak of smoltification. The present study suggests that the photoperiod is the most important environmental factor controlling the long-term annual cycle of T4 secretion in sockeye salmon.

Periodic changes in plasma-T4 and the annual cycle of smoltification were not parallel (Fig. 3). However, significant increases in plasma T4 and decreases in plasma-Na were synchronized during January and March, accompanied by a lengthening daylight period. This phenomenon was repeated in both 1991 and 1992. The decline of plasma-Na commenced with a temporal elevation in plasma-T4 which was induced by the lengthened photoperiod in the present experiment. Positive effects of longer photoperiod on seawater tolerance have already been reported in Atlantic salmon (Sigholt et al. 1998; Berrill et al. 2003). The present results further indicate that T4 is strongly involved with the progress of seawater tolerance in sockeye salmon, although plasma-T4 was reduced to its lowest levels when seawater tolerance reached peaks in both 1990 and 1991. Furthermore, an increase plasma-T4 was observed only at the beginning of the developmental stage of

seawater tolerance in Experiment-2. From these results, it appears that T4 acts at an early phase of smoltification as an enhancer of this process.

On the other hand, increases in plasma T4 during June - October did not cause smoltification. Moreover, administration of T4 had no significant effect on the plasma-Na and gill-ATPase in Experiment-3. These results indicate that T4 alone cannot lead to seawater tolerance. Recently, evidence of the effect of growth hormone (GH) and cortisol on hypo-osmoregulation in salmonids has been accumulating. Plasma GH and cortisol levels increase during smoltification (Specker and Schreck 1982; Sweeting et al. 1985; Stefansson et al. 1991). Administration of GH and/or cortisol develops seawater tolerance in Atlantic salmon and sockeye salmon (McCormick 1996; Ban 2002). Furthermore, lengthening the photoperiod induces smoltification, accompanied with increases in plasma GH and cortisol in both Atlantic salmon and sockeye salmon (Björnsson et al. 2000; Ban unpublished data). In experiment-3, fin margin blackening was observed in the T4-group but not in the control group. This feature is more marked when T4 is combined with GH (Miwa and Inui 1983, 1985). These findings suggest that T4 promotes smoltification in cooperation with GH and/or cortisol, which also show a periodic cycle with photoperiod. Future endocrine researches with the aim of enhancing our knowledge of the relationship between photoperiod and smoltification in sockeye salmon are highly needed.

Duncan and Bromage (1998) observed smoltification in juvenile Atlantic salmon under a constant, short photoperiod condition. In Experiment-2, the 8L-group also gradually developed seawater tolerance, while the levels of plasma-Na and gill-ATPase were significant lower than that of the 16L-group. The constant short daylight period may have similar effect as a lengthened photoperiod, as a result of the accumulation of daylight. There may be a threshold in the daytime as a trigger for smoltification. Therefore, a transformation to smolt probably commences and progress faster in 16L-group than in 8L-group.

In conclusion, the present study reveals a periodic pattern for smoltification and changes in plasma-T4. Smoltification commences in accordance with the elevation of plasma T4, which is induced by a lengthening of the photoperiod. However, T4 alone does not lead to seawater tolerance. The present findings suggest that T4 acts as an enhancer of smoltification in sockeye salmon and that it has a significant impact, albeit indirectly, at an early phase of this process.

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## チロキシンがベニザケのスモルト化に果たす役割

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チロキシン（T4）がベニザケのスモルト化に果たす役割を明らかにするため，海水適応能，つま黒，肥満度から判断されるスモルト化と血中T4濃度の周年変化を調べた．自然日長下で飼育されたベニザケ幼魚は，1月から5月にかけて徐々に海水適応能を高めた．つま黒の発現と肥満度の減少は5

月に認められた．血中T4濃度は1月から3月にかけて有意な上昇を示した．一方，人為的に日長を短日（LD 8：16）から長日（LD 16：8）へ切り換えることで，一時的な血中T4濃度の上昇と，それに続くスモルト化を誘起することができた．しかし，T4の単独投与はスモルト化を促さなかった．これらの結果は，日長の増加にともなって上昇するT4が，ベニザケのスモルト化を間接的に誘起することを示唆している．