

ビワマスの降河行動と血中チロキシン濃度

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Downstream Migratory Behavior and Plasma Thyroxine Levels of Biwa Salmon *Oncorhynchus rhodurus*^{*1}

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Downstream migratory behavior, plasma T₄ concentrations and histological changes in the thyroid gland of biwa salmon underyearlings were examined during late spring and early summer for three consecutive years. The downstream migration was observed specifically when the flux of the river increased following rainfall. A close correlation was seen between the number of migrants and the rate of flux increase of the river. Plasma T₄ levels of the fry caught in the river were variable, and no correlation was seen between the plasma T₄ levels in the fish remaining in the river and lunar phase, rainfall, water flux or water temperature. However, significantly higher T₄ concentrations were observed in the fish during downstream migration when compared with those in the fish remaining in the river and postmigrants in the lake. The mean epithelial height of the thyroid follicles was greatest in the underyearling in May, shortly before the onset of the migratory season. The thyroidal activity of the migrants in late May and June tended to decrease and postmigrants in the lake had inactive thyroid. These results suggest that the rainfall or the associated changes in turbidity and flux is one of the initiators of the migratory behavior in biwa salmon, and also that thyroid hormone is involved directly or indirectly in downstream movement.

Introduction

In our previous study, it has been revealed that biwa salmon *Oncorhynchus rhodurus* go downstream to Lake Biwa as underyearlings, mainly from May to June, except for a small number of precociously mature male.¹⁾ During the migratory season, they show several morphological changes such as body silvering and reduction of condition factor, as in smolts of the other salmonids.^{2,3)} However, they do not develop seawater adaptability even during the migratory season, coinciding with their lacustrine nature.⁴⁾

Hormonal factors such as thyroid hormones, prolactin, growth hormone, and corticosteroids are considered to be involved in the process of parr-smolt transformation or smoltification.⁵⁻⁷⁾ Especially, thyroid hormones are suggested to induce some of the morphological and behavioral changes associated with smoltification, and a marked increase in plasma thyroid hormones has been frequently observed during parr-smolt trans-

formation in many salmonid species.⁷⁾ In hatchery-reared biwa salmon, an increase in plasma thyroxine (T₄) was also observed during body silvering.⁸⁾ The present study was undertaken to examine the role of thyroid hormones in downstream migratory behavior of biwa salmon fry and also to analyze factors initiating the downstream migration.

Materials and Methods

Study Sites and Fish

Observation of downstream migratory behavior and plasma samplings of biwa salmon *Oncorhynchus rhodurus* fry were performed in the Shiotsuokawa River and in the mouth of the river, located in the north of Lake Biwa. The length of the river is about 10 km, and the width 5-12 m. Further description of the river is given in a previous paper.¹⁾ Since there has been little natural stock of biwa salmon recently in the Shiotsuokawa River, the mature salmon were

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caught in the other rivers discharging into the lake in November. The eggs were artificially fertilized and reared at Takashima Branch of Shiga Prefectural Fisheries Co-operative Association. One hundred thousand fry (wild stock; 2.4–3.1 cm in standard length) were released in the upstream of the river in late March or early April during 1984–1986.

Since biwa salmon fry go downstream mainly during the middle of May and the end of June, the migratory behavior was also examined for the fry kept in the pond (10 m²), until the migratory season. Ten thousand fry of the 1986 stock were reared in the running spring water (12±1°C) at Shiga Prefecture Samegai Trout Farm from March 24 to May 26, 1986. They were fed on dry diet for trout (Nihonnousan Kogyo) twice daily until satiation. After clipping the adipose fin in order to distinguish from the wild stock, they (cultured stock; 4.5–7.5 cm in standard length) were released into the middle portion of the Shiotsuokawa River on May 26. In addition, 100 fry from the same stock were transferred to a small artificial stream (length; 3.5 m, width; 0.5 m, depth; 0.1 m), supplied with spring fresh water (12±1°C) on May 23. The current velocity was kept constant (1 cm/sec) by the inlet flow (20 l/min). On June 10, the velocity was increased to 9 cm/sec, by increasing the inlet flow to 129 l/min and changes in plasma T₄ concentrations of the fry were examined during 23 hours after increasing the water flow.

Observations of the Downstream Migration

The fry migrating downstream from the Shiotsuokawa River to the lake were caught by a trap set at 1 km above the mouth of the river during May 19 and June 23, 1986. Since the trap was destroyed by the flood on June 24, no further observation was possible. The width of the trap cage (4.1 m) covered about 40% of the width of the river. The number of the fry caught by a set net (Ami-Eri) at the mouth of the river was also counted. Usually, the number of fry trapped was counted at 8 a.m. every day. The water temperature and flux of the river were also measured. The rate of flux increase (the ratio before and after the rain fall) was also examined. Data for the rainfall along the river basin were obtained from Hikone Regional Meteorological Observatory.

Plasma Samplings and Analytical Techniques

Blood samples were collected from the fish caught by a small net, a trap cage in the river and a

set net in the mouth of the river during 1984–1986. In 1984, the fish caught in the river were transported to the laboratory, and blood samples taken after 1 hour. The other blood samples were collected in the field within 10 min. of removal from the net, trap cage or set net. Blood was collected by capillary from the caudal artery as in Iwata *et al.*⁹⁾ Blood samples and fish carcasses were placed on ice and transported to the laboratory within 1 hour. Capillary tubes were centrifuged for 5 min. at 10000 rpm and plasma were stored at –25°C until T₄ assay. Plasma T₄ concentration was measured by radioimmunoassay as in Tagawa and Hirano.¹⁰⁾ After measurement of standard length and body weight, the lower jaw was removed from some fish and fixed in Bouin's fluid for histological examination of thyroid follicles. The tissue was embedded in paraffin, and sections (5 μm) were stained with hematoxylin-eosin. An index of thyroid activity was obtained for each fish by measuring the epithelial cell height in ten randomly selected follicles.

Statistics

All data are presented as mean ± SEM. Significant differences in changes in plasma T₄ concentrations and height of the follicle epithelium were assessed by the Duncan's new multiple range test, Student's test or Cochran-Cox test, where appropriate.

Results

Observation of Downstream Migration

As shown in Fig. 1, there were three large peaks in the number of the wild stock trapped, which coincided with rainfall and an associated increase in the flux and/or turbidity. The number of fish caught by the trap in the river was correlated significantly ($P < 0.01$) with the rate of increased flux by the rainfall at the time of their capture (Fig. 2). The peaks of downstream migration in the river were well correlated with the increase in the number of the fish captured by set net at the mouth of the river. The downstream movements were seen both during the night (May 19) and day (May 30 and June 17) when flux and turbidity increased after the rain. The number of the cultured stock trapped showed four peaks, also coincided with the rainfall, except for the peak just after the release (May 27). The relationship between the changes in water temperature, lunar phase and the occurrence of downstream migratory behavior

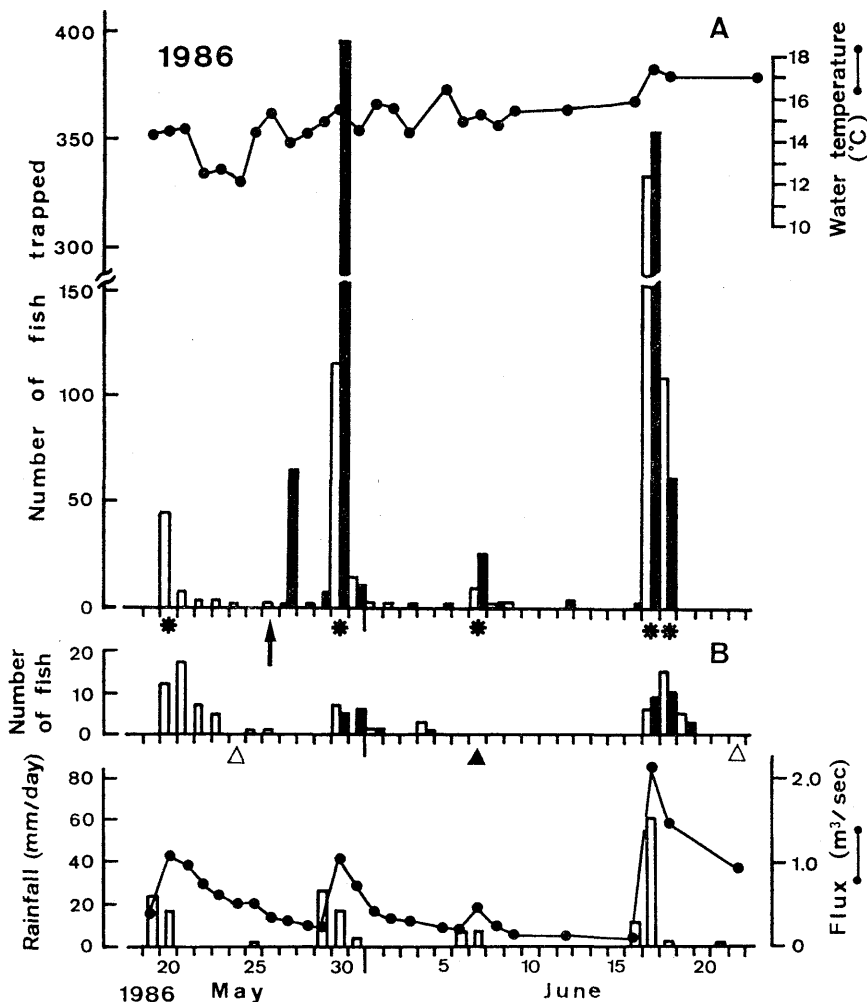


Fig. 1. Number of biwa salmon trapped in the river (A) and at the mouth of the river (B). White and black columns indicate the numbers of the fry of the wild stock and of the cultured stock, respectively. Asterisks show turbidity seen in the river. Arrow indicates the day when the cultured stock were released to the river. ▲, new moon; △, full moon.

was not clear.

Changes in the Plasma T_4 Concentrations

Fig. 3 shows the plasma T_4 concentration of the biwa salmon fry caught in the river, at the mouth of the river or during downstream migration during April and June in three consecutive years. In 1984, plasma T_4 concentrations of the fry in the river in May and early June were below 3 ng/ml. No significant difference was seen between the fish in the river and those at the mouth of the river except for a significantly ($P < 0.01$) lower level in the river fish caught on June 15. Plasma T_4 levels of the fish in the river ranged from 2.8 to 5.6 ng/ml in 1985, but no significant difference

was seen between these levels. A similar level was observed in the fish caught at the mouth of the river on May 23. The plasma T_4 level of the fish caught during downstream movement (June 8) was significantly ($P < 0.01$) higher than those in the river. In 1986, the T_4 concentration was lowest (0.5 ± 0.1 ng/ml) on April 21, and increased gradually until May 19 (11.7 ± 1.8 ng/ml). The hormone level decreased significantly ($P < 0.01$) on May 28, and then increased significantly after 10 days. Higher T_4 levels were seen in the fish during downstream migration, but they were not significantly different from the level of fish remaining in the river (May 30). In any of the three years examined, no correlation was seen

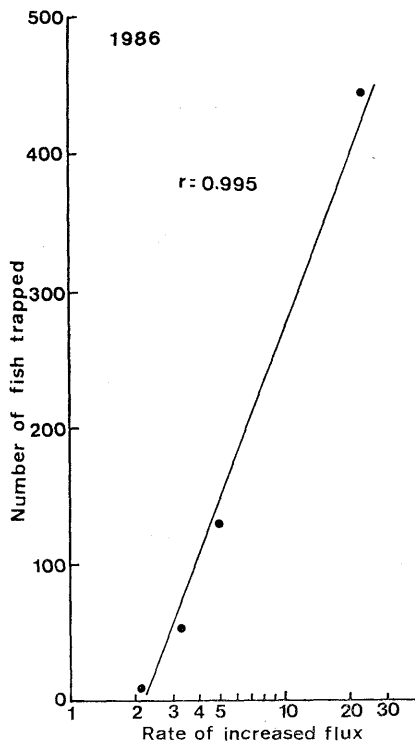


Fig. 2. Relationship between the number of the wild stock trapped in the river and the rate of increased flux by rainfall pertaining at the time of their capture. The number of fish trapped is the total for 2 days when the flux increased.

between the plasma T_4 levels in the fish remaining in the river and lunar phase, water flux or water temperature.

When the fry reared in the pond (the cultured stock) were released into the river, the plasma T_4 level of the fish caught in the river 2 days after release was significantly ($P < 0.05$) higher than the level in the fish in the pond. A further increase ($P < 0.05$) in the hormone level was seen in the downstream migrants caught 2 days after the release (Fig. 4). As shown in Fig. 5, no significant change in the plasma T_4 concentration was observed when the current velocity and flux were increased in a small artificial stream.

Histological Changes in the Thyroid Glands

The thyroid follicles of the fish caught in the river on April 13 appeared inactive. The follicle epithelium was relatively low cuboidal with smaller nuclei (Fig. 6A). The cells became more active in the fish remaining in the river on May 9 (Fig. 6B). Thyroid glands of migrants caught on June 17 appeared slightly less active, and least active

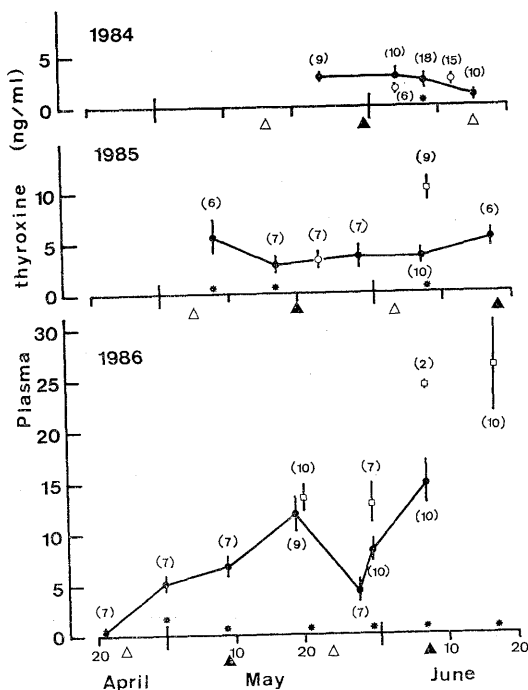


Fig. 3. Plasma thyroxine concentrations of biwa salmon caught in the river (●), at the mouth of the river (○) and during downstream migration (□). Asterisks show the time when turbidity was seen in the river. The number of animals is shown in parenthesis. The vertical bars represent the mean \pm SEM. ▲, new moon; △, full moon.

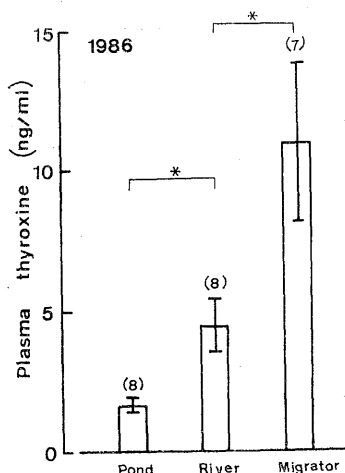


Fig. 4. Plasma thyroxine concentrations of biwa salmon kept in the pond on May 28, 1986 (Pond), 2 days after the release into the river on May 28 (River) and during downstream migration on May 30 (Migrator). The number of animals is shown in parentheses. The vertical bars represent the mean \pm SEM. *Significantly different at $P < 0.05$.

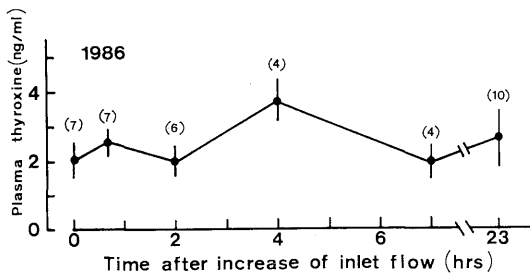


Fig. 5. Influence of an increase of inlet flow (20 l/min to 129 l/min) on plasma thyroxine levels in biwa salmon. The number of animals is shown in parentheses. The vertical bars represent the mean \pm SEM.

with flattened epithelial cells and small nuclei in the fish caught in the lake on June 30 (Fig. 6C, D).

As shown in Fig. 7, the mean height of the follicular epithelium of the fish caught in the river increased significantly ($P < 0.01$) from mid April ($7.8 \pm 0.6 \mu\text{m}$) to early May ($10.3 \pm 0.6 \mu\text{m}$). Similarly activated level was seen in the migrants on May 20. However, the epithelial height of the migrating fish collected on May 30 was less than that of the fish remaining in the river. The mean cell height in the fish caught in the lake was even less both in June and October.

Discussion

Although there are many investigations on various aspects of salmonid smoltification, little has been studied on the environmental signal to initiate downstream migration. In chum and pink salmon, one of the releasers for downstream migration seems to be the dark condition.^{11,12)} The downstream movement of coho salmon shows a correlation with lunar cycle.¹³⁾ Grau *et al.*¹⁴⁾ suggested that the initiation of steelhead trout migrations is also correlated with lunar or semilunar periodicity. On the other hand, Solomon¹⁵⁾ suggested that increased turbidity and discharge following heavy rain initiate migratory movement in Atlantic salmon and sea trout. In masu and coho salmon, the new moon and rainfall are reported to initiate their migration.¹⁶⁻¹⁸⁾

In the present study, the onset of the downstream migration of biwa salmon fry was closely correlated with rainfall or increased discharge following rainfall. According to Solomon,¹⁵⁾ the pattern of migratory movement in Atlantic salmon and sea trout differed from river to river, possibly due to differences in the conditions of rivers such as discharge, water temperature and turbidity. Youngson *et al.*¹⁹⁾ reported that the rate at which migrants of Atlantic salmon leaves the river is

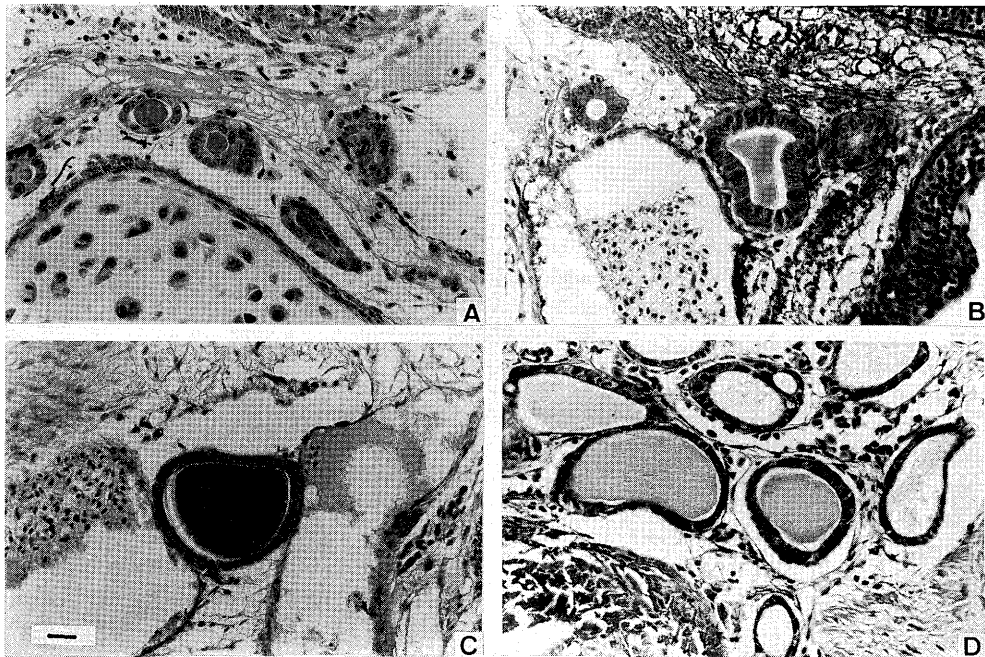


Fig. 6. Thyroid follicles of biwa salmon caught in the river on April 13 (A), May 9 (B), during downstream migration on June 17 (C), and in the lake on June 30 (D). The bar indicates 20 μm .

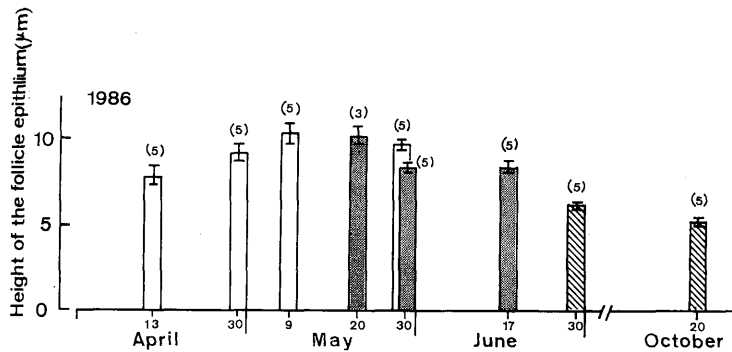


Fig. 7. Changes in height of the follicle epithelium of thyroid gland in biwa salmon. The number of animals is shown in parentheses. The vertical bars represent the mean \pm SEM. White, dotted and shaded columns indicate the fish caught in the river, during downstream migration, and in the lake, respectively.

dependent on the stream discharge rate. In this study, the flux of the Shiotsukawa River increased approximately 2–23 times from the basal level, and the water became turbid whenever it rained during the experiment. A close correlation was also observed between the number of migrants and the rate of flux increase. Thus, rainfall and/or increased discharge or turbidity may be the primary cues to initiate downstream migration in biwa salmon.

In some salmonid species, an increase in plasma T_4 levels has been observed during the migratory season, particularly coinciding with new moon.^{14,16,20} The T_4 surge may act in conjunction with environmental cues to synchronize salmon migration.^{14,20} In the present study, no correlation was seen between the plasma T_4 levels and lunar cycles in the biwa salmon remaining in the river during the three consecutive years examined. Significantly higher concentrations of the plasma T_4 , however, were observed in the fry caught during downstream migration, as compared with the fish remaining in the river and those in the lake. In chum salmon, T_4 concentration in the whole body also increased in the fry migrating downstream.* Subtle changes in environmental water seem to stimulate thyroid hormone secretion in salmonids.^{21–25} Nishioka *et al.*²³ suggested that “novel water” stimuli may act at some levels of the hypothalamo-hypophysio-thyroid axis to permit enhanced responsiveness of the thyroid gland to environmental and endocrine cues. In the present study, however, no significant increase in plasma T_4 levels was seen in the fry

caught in the river when the river was turbid by rainfall (Fig. 3, June 8, 1984 and May 17, 1985). Furthermore, the T_4 concentrations of the fry migrating downstream were higher than those of the fish remaining in the turbid water (Fig. 3, May 30, 1986). A correlation between serum T_4 levels and the stream discharge rate was reported in the migrators of Atlantic salmon.²² In the biwa salmon fry, however, plasma T_4 concentration was not affected by an increased water velocity of the artificial stream. At any rate, the higher concentration of the plasma T_4 in migrating biwa salmon seems to indicate that the hormone is involved in some aspects of downstream migratory movement.

As assessed by histological observations, the thyroid gland of biwa salmon appeared most active in the underyearling in May, shortly before the onset of migration and body silvering. The thyroid activity of the fish during migration in late May and June tended to decline, and postmigrants in the lake had inactive thyroid. Similar observations have been made on the thyroid activity during smoltification and downstream migration in several salmonids.²⁶ Hoar and Bell²⁷ has suggested that the increased thyroid activity in migrating salmon is related to the increased metabolic work for seawater adaptation and a response to different ambient iodide levels between fresh water and seawater. This hypothesis would not be applicable to biwa salmon, since they go downstream to “freshwater” Lake Biwa. According to Hoar *et al.*,^{28,29} an increase in swimming activity was observed in chum, coho and

* M. Iwata, M. Tagawa, T. Yamanome, H. Ida, K. Suzuki, and T. Hirano: *Otsuchi Mar. Res. Cent. Rept.*, 15, 1–9 (1989).

sockeye salmon, when treated with T_4 . In contrast, yearling Atlantic salmon injected with T_4 reduced swimming activity and upstream orientation.³⁰⁾ Recently, Iwata *et al.*³¹⁾ reported that chum salmon fry treated with T_4 move to the open water where it may be advantageous for the fish to form school and migrate seaward in daytime. Thus, thyroid hormones seem to be involved directly or indirectly in the induction of some "still unknown" physiological conditions required for the salmon to initiate downstream migration.

Acknowledgments

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