NOTE

Changes in Circulating L-Thyroxine and L-Triiodothyronine of the masu salmon, *Oncorhynchus masou* accompanying the smoltification, Measured by Radioimmunoassay

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Synopsis

Serum levels of L-thyroxine (T₄) and L-triiodothyronine (T₃) of the masu salmon, *Oncorhynchus masou*, were measured by radioimmunoassay during the months from March to September. Serum levels of T₄ and T₃ varied from 0.06 to 1.44 µg and from 0.06 to 0.35 µg per 100 ml serum respectively, although T₄ content could not be detected in some specimens.

T₄ level showed a seasonal fluctuation in which a higher value was observed in April when parr-smolt transformation was proceeding. T₄ level in spring, particularly in April and June, was significantly higher in smolt than in parr.

T₃ content showed a small variation during the observed months and did not increase at smoltification, although the content tended to decrease to a minimum level in September when T₄ level showed also the lowest level.

In teleost fishes, seasonal changes in thyroid activity have been described for many species, and thyroid hormones were thought to be involved in some physiological phenomena such as smoltification, migration and gonadal maturation in these fishes (Pickford and Atz, 1957; Swift, 1960; Dodd and Matty, 1964; Sage, 1973). Some workers indicated histologically and radiochemically that, in salmonid fishes, the thyroid gland displayed a seasonal change in its activity in connection with gonadal maturation and down or up-stream migration (Swift, 1960; Fontaine et Leloup, 1962; Eales, 1963, 1965). Recently annual variations in circulating levels of thyroid hormones in *Salvelinus fontinalis* were studied by radioimmunoassay (RIA) by White and Henderson (1977), who showed

the maximal and minimum levels occurring in midspring and at the time of spawning, respectively. Furthermore they reported that thyroxine (T₄) concentration was lower than triiodothyronine (T₃) throughout a year.

In the present study, we described variations of serum thyroid hormones in the masu salmon, *Oncorhynchus masou*, during the period from March to September, with remarks on a participation of these hormones in parr-smolt transformation and reproduction of the fish.

Materials and Methods

Fish

One year-old masu salmon, (*Oncorhynchus masou*), were obtained from the Mori Branch, Hokkaido Fish Hatchery, monthly from March to September. In Hokkaido, the masu salmon in nature transforms from the parr into smolt stage in spring to begin down-stream migration. The fish used in this study

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were, however, kept in outdoor ponds with running fresh water under natural conditions of light and temperature even after their parr-smolt transformation. From such a cultured stock, males and females, 4 to 10 fish at a time, were sampled for examinations. Blood samples were withdrawn from caudal vessels by cutting the caudal peduncle of the fish. Sera were separated by centrifugation after clotting and stocked at -20°C until use.

Preparation procedure of sample

Serum (usually 0.1 ml) was added to a siliconized tube containing 1.0 ml of ethanol and vortexed for 1 min. The supernatant was separated by centrifugation at 3,000 rpm at 4°C for 20 min, transferred to another siliconized tube, and evaporated. Extraction efficiency was determined by using ^{125}I - T_4 or T_3 (about 6,000 cpm) in a sample from the same serum. The efficiencies were in the ranges of 65–83% for T_4 and 74–87% for T_3 respectively. Dry residue was dissolved with an appropriate mixture of human stripped serum (HSS) and buffer solutions. Assay data were corrected with the extraction efficiency. The assay was done usually using duplicate samples and triplicate standards.

T_4 RIA

T_4 RIA was performed after the method of Larsen *et al.* (1973). The antiserum was diluted 1:2,000 in a solution of 0.2 M glycine-0.12 M sodium acetate (pH 8.6), containing sodium salicylate (20 mg/ml) and bovine serum albumin (BSA, Sigma, 0.2 mg/ml). Just prior to use, ^{125}I - T_4 (440 mCi/ml, Daiichi Radioisotope Lab.) was added to the antibody solution to a final dilution of 50 pg/ml. To the evaporated tubes, 10 μl HSS and 90 μl buffer were added and vortexed for 1 min. Under these conditions, the antiserum bound 50% of ^{125}I - T_4 . After separation of bound T_4 , free ^{125}I - T_4 absorbed in the charcoal precipitate was measured in a Beckman Gamma Counter (Biogamma Counting System). In the present method, the minimal detectable level was 0.06 $\mu\text{g}/100\text{ ml}$ serum.

T_3 RIA

The immunoassay method of Larsen (1972) was used to measure T_3 in the fish serum. The antiserum for T_3 was made by immunization of rabbits with 1 mg L- T_3 (Sigma) conjugated to BSA, which was injected intradermally bi-weekly for 10 weeks. The diluent was the same glycine-sodium salicylate-BSA buffer as that used for T_4 RIA with an exception that sodium salicylate was added at the concentration of 10 mg/ml. Data were expressed as mean $\pm$ S.E. and results were statistically analyzed using a Student's *t* test. The minimal detectable level was 0.02 $\mu\text{g}/100\text{ ml}$ serum.

Results and Discussion

The serum level of T_4 in the masu salmon, *Oncorhynchus masou*, varied from 0.06 to 1.44 $\mu\text{g}/100\text{ ml}$, though in some specimens, T_4 content was not detectable. T_3 level varied from 0.06 to 0.35 $\mu\text{g}/100\text{ ml}$ (Fig. 1). Compared with these results, serum contents of T_4 and T_3 determined in the masu salmon in the present study were in similar ranges so far reported in fresh water fishes (Higgs and Eales, 1973; Drongowski *et al.*, 1975; Brown and Eales, 1977).

In the masu salmon, serum T_4 level showed a remarkable change during the period from March to September (Fig. 1). In spring when parr-smolt transformation was proceeding, smolt showed higher T_4 level in particular in April ($0.997 \pm 0.092\text{ }\mu\text{g/dl}$) and June ($0.573 \pm 0.066\text{ }\mu\text{g/dl}$) than the parr levels ($0.253 \pm 0.160\text{ }\mu\text{g/dl}$ and $0.088 \pm 0.020\text{ }\mu\text{g/dl}$) which included some specimens with no detectable T_4 content at the levels of 2 and 0.1% respectively. In smolt, T_4 content then tended to decrease to hold a plateau level in summer when the gonad began to grow (Table 1), while in parr, T_4 level remained in a lower level than in smolt. Besides the seasonal changes, average T_4 level in smolt ($0.45 \pm 0.053\text{ }\mu\text{g/dl}$) throughout the experimental term was significantly higher than that in parr ($0.205 \pm 0.046\text{ }\mu\text{g/dl}$) at the level of 1%. Before the spawning time in September, T_4 content of smolt and parr tended to decrease to the minimum level. On the other hand T_3 level did not show a remarkable fluctuation.

In contrast to these findings obtained in the masu salmon, T_3 level determined by RIA in *Salvelinus fontinalis* was reported to be higher than T_4 level for a year, and fluctuated seasonally in parallel with the change in T_4 level (White and Henderson, 1977). The difference between these two observations might be ascribed

Fig. 1. Seasonal changes in the serum levels of thyroxine (T₄) and triiodothyronine (T₃) in masu salmon. Each point indicates the concentration of T₄ and T₃ in the serum, in female smolt (●), male smolt (▲), female parr (○) and male parr (△). A solid line indicates the mean of smolt, and a broken line indicates the mean of parr. In some specimens, T₄ was not detectable. These specimens were regarded as 0 µg when the average was calculated.

to the differences of the monodeiodination rate of T₄ to T₃ and/or of the disposal rate of T₄ and T₃.

In the masu salmon in Hokkaido, parr-smolt transformation occurs in spring and smolts begin down-stream migration in nature. It is well known that smolt which are continuously kept in the fresh water pond lose the characteristics of smolt and eventually regress to parr condition. Kubo (1974) called these fish the "regressive

smolt." Eales (1973) reported that thyroid activity measured with histological observations in juvenile salmon, *Oncorhynchus nerka* and *O. kisutch*, increased in connection with smoltification and declined rapidly in post-migration when held in the fresh water pond. Furthermore, Eales (1965) found that juvenile *Salmo gairdneri* had two peaks in thyroid activity, the first at the period of parr-smolt transformation and the second in late summer when water

Table 1. Change in gonadosomatic index (GSI)* of the masu salmon in March to August. Values are given as means $\pm$ S.E. Range is given in parenthesis.

Month	Male		Female	
	parr	smolt	parr	smolt
March		0.125 (0.11–0.14)		0.84 $\pm$ 0.04 (0.76–0.98)
April	0.22 (0.15–0.30)	0.20	0.26 (0.25–0.28)	0.91 $\pm$ 0.28 (0.06–0.133)
May	0.47	0.22		0.995 (0.89–1.10)
June	1.87 $\pm$ 0.63 (0.62–4.17)			3.02 $\pm$ 0.66 (0.42–5.82)
July		5.2 (5.1–5.3)		6.66 $\pm$ 1.66 (4.9–10.0)
August		12.17 $\pm$ 0.13 (11.8–12.8)	2.76 $\pm$ 1.71 (0.4–6.1)	12.72 $\pm$ 3.05 (5.4–16.5)
Sept.		8.46 $\pm$ 1.16 (7.3–10.81)	0.55 (0.5–0.6)	23.66 $\pm$ 1.89 (20.0–24.7)

$$* \text{ GSI} = \frac{\text{gonad weight}}{\text{body weight}} \times 100$$

temperature became higher. In the masu salmon, the high content in circulating T_4 in spring may have a possible relation to smoltification, and the lower content of T_4 in smolt in the subsequent months seems to reflect the state of the "regressive smolt." An evident plateau level of T_4 observed in summer may be influenced by rising water temperature.

In September when gonads reached the ripe stage, the circulating levels of T_4 and T_3 fell in the masu salmon. It was shown that the circulating thyroid hormones have a tendency to decrease prior to spawning in *Salmo salar* (Fontaine et Leloup, 1962) and in *Salvelinus fontinalis* (White and Henderson, 1977). On the other hand, an increase in the activity of the thyroid gland has been observed in accordance with gonadal development and spawning in various fishes (Pickford and Atz, 1957; Matty, 1960; Hickman, 1962). Sage (1973) suggested that an early role of thyroid hormones was associated with reproduction in fishes. Furthermore, Drongowski *et al.* (1975) reported that hypothyroid conditions evoked from iodine deficiency was related

to lower fertility and fecundity in *Onchorhynchus kisutch*. It seems likely that the maintenance of thyroid hormones in a suitable level is required for gonadal maturation.

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