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Author(s)	KOBAYASHI, Shinjiro; YUKI, Ryogo
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DIFFERENCES IN CATALASE ACTIVITY IN THE TISSUES  
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MASU, *ONCORHYNCHUS MASOU*

Shinjiro KOBAYASHI and Ryogo YUKI  
*Faculty of Fisheries, Hokkaido University*

The trigger mechanism for the smolt transformation of the salmonid fishes has not yet been demonstrated. However, Fontaine and his co-workers have reported a series of biochemical changes associated with the parr-smolt transformation. The literature of this problem has been reviewed in detail by Fontaine<sup>1)</sup> and Hoar.<sup>2,3)</sup> The latter has concluded that the parr-smolt transformation involves morphological, biochemical and ethological changes and is associated with increased endocrine activity, and that the precise patterns of fish migration are dependent upon a delicate interrelationship between the fish and the cyclical changes in its environment.<sup>3)</sup>

As far as the present authors are aware, there have been very few reports on enzymatic studies in relation to the parr-smolt transformation. Only the study of the serum lipase of the Atlantic salmon has been reported by Fontaine *et al.*<sup>4)</sup> Cyto-histological differences of the mitochondrial and Golgi materials of the chloride cells in the gill have been found between the smolt and parr of *C. masou*.<sup>5)</sup> It may be said that the finding is related to this field but indirectly.

In the previous paper,<sup>6)</sup> the authors reported that a high catalase activity of the kidney is commonly found in the salmonid fishes, and proposed that this specificity may be associated with their osmoregulatory potentiality though the physiological significance of the tissue catalase activity is not clear at present. However, the specificity just described may be of some genetic significance which is common to the salmonid fishes or to the fishes evolutionally new, as Spiegelman's concept in the microorganisms and in the mice.<sup>7,8)</sup> Also, the reason why both the smolt and parr show equally such a high catalase activity in the kidney is obscure. With such things in mind, the present experiment was undertaken to find in the smolt and parr of *O. masou* some differences in the seasonal changes of the catalase activity in the tissues and blood.

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MATERIALS AND METHODS

Fishes. Larvae of *O. masou* hatch out late in autumn in the freshwater area, and after having spent there more than one year the smolts of this salmon migrate

into the sea in the period from May to June in Hokkaido.<sup>9)</sup> The fishes used are those which were artificially fertilized and reared in a freshwater pond of the Faculty of Fisheries at Nanaye near Hakodate. At the end of March of the second year, the smolts begin to appear in the pond though the smoltification is yet faint in most of them. So, both the smolts and the parrs used in this month were carefully selected. Even if the smolts were retained in the freshwater pond for a prolonged period, they did not show any high mortality as in the case of the chum salmon fry.<sup>10)</sup> However, at the end of September no smolts were yet observed in the pond. Possibly, it may be reduced to the converse transformation of smolt-parr which occurred in September. In this experiment, 57 parrs and 36 smolts were sacrificed. The smolts used were all female, and the parrs were all male except the two parr-females used in August.

The fishes used in the period from June 30 to February 11 are those which were reared in the years 1952—'54, and those from March 29 to June 2 in 1953—'54. Although the fishes of the former group were relatively poor in growth when compared with those of the latter, the comparison of the enzymatic activity in both the smolt and parr sampled at the same time may be allowable. The growth of the fishes and the water temperature of the pond are given in Tables 1 and 2.

Table 1. Growth of the smolt and parr of *Oncorhynchus masou* reared in a freshwater pond

Date		March 29, '54	April 26, '54	June 2, '54	June 30- July 9, '53	July 29, '53	Aug. 25, '53	Sept. 25, '53	Nov. 27, '53	Feb. 11, '54
Body	No. of fishes	8	1	6	10	6	8	8	4	6
Parr	L* ±σ	115±11	116	154±10	141±18	148±12	167±27	166±26	163±17	173±21
	Wt±σ	24±7	22	53±16	49±14	56±15	87±45	84±40	61±19	76±28
	No. of fishes	8	6	6	8	—	8	—	—	—
Smolt	L* ±σ	125±2	131±7	151±9	129±7	—	148±12	—	—	—
	Wt±σ	25±2	44±6	44±6	23±4	—	49±11	—	—	—

\* Total length

Table 2. Monthly average of the water temperature of the pond

Year	1953							1954					
Month	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Water temp. C°	12.2	15.0	15.9	14.2	11.0	5.0	3.2	2.2	3.1	4.7	8.7	11.6	12.4

The catalase activity of the liver, kidney, gill, stomach and white skeletal muscle, and of the blood was examined. The method of the estimation of them is the same as that described in the previous paper.<sup>6)</sup>

## RESULTS

### Differences in the catalase activity in the tissues and blood between the smolt and parr

As already described, the smolts were found in the pond in the period from March to August. In this period, the catalase activity of the liver, kidney, gill, stomach, muscle and blood of the smolt was compared with that of the parr at intervals of about a month. The significance of the differences between them is indicated in Table 3. The seasonal changes of the catalase activity in the two forms are illustrated in Figs. 1 and 2.

Table 3. Comparison of the catalase activity of the tissues and blood in the smolt and parr

Date of exp.	Smolt or parr probability	No. of fishes	Catalase activity $\pm$ Standard error of mean *					
			Liver	Kidney	Blood	Gill	Stomach	Muscle
March 29, '54	Smolt Parr P**	8(1)	40 $\pm$ 2.2	12 $\pm$ 0.7	15 $\pm$ 0.7	1.1 $\pm$ 0.36	0.87 $\pm$ 0.18	0.09 $\pm$ 0.01
		8(1)	47 $\pm$ 2.0 <0.05	11 $\pm$ 1.1 0.53	20 $\pm$ 1.7 <0.02	1.3 $\pm$ 0.16 0.63	1.3 $\pm$ 0.45 0.40	0.09 $\pm$ 0.04 —
April 26, '54	Smolt Parr P	6 1	46 $\pm$ 2.4 46	11 $\pm$ 1.1 13	17 $\pm$ 1.3 21	1.0 $\pm$ 0.17 1.5	1.0 $\pm$ 0.24 0.82	0.10 $\pm$ 0.01 0.11
June 2, '54	Smolt Parr P	6	53 $\pm$ 2.3	14 $\pm$ 1.1	16 $\pm$ 1.4	1.5 $\pm$ 0.22	0.71 $\pm$ 0.16	0.08 $\pm$ 0.04
		6	40 $\pm$ 1.5 <0.001	10 $\pm$ 1.1 <0.05	19 $\pm$ 1.6 0.21	1.4 $\pm$ 0.15 0.72	1.2 $\pm$ 0.38 0.27	0.12 $\pm$ 0.02 0.41
June 30 - July 9, '53	Smolt Parr P	8	58 $\pm$ 1.7	18 $\pm$ 0.7	18 $\pm$ 0.7	1.9 $\pm$ 0.17	1.2 $\pm$ 0.12	0.34 $\pm$ 0.12
		10	64 $\pm$ 2.4 0.06	18 $\pm$ 1.5 —	13 $\pm$ 0.2 <0.001	2.3 $\pm$ 0.54 0.49	0.93 $\pm$ 0.10 0.10	0.20 $\pm$ 0.04 0.29
Aug. 25, '53	Smolt Parr*** P	8(2)	65 $\pm$ 1.6	13 $\pm$ 0.9	19 $\pm$ 1.4	2.1 $\pm$ 0.29	0.64 $\pm$ 0.16	0.31 $\pm$ 0.13
		8	63 $\pm$ 1.7 0.40	9.8 $\pm$ 1.12 <0.05	23 $\pm$ 3.1 0.34	3.0 $\pm$ 0.48 0.15	0.74 $\pm$ 0.12 0.49	0.52 $\pm$ 0.18 0.37

\* Standard error of mean =  $\sqrt{\sum d^2/n(n-1)}$

\*\* Probability was calculated from the table of Student's *t*, using the equations:

$$t = \frac{m_1 - m_2}{\sqrt{SE_1^2 - SE_2^2}} \text{ and } n = (n_1 - 1) + (n_2 - 1),$$

where  $m_1$  and  $m_2$  represent the two means,  $SE_1$  and  $SE_2$  the two standard errors, and  $n_1$  and  $n_2$  are the number of fishes from which the respective means are obtained.

\*\*\* Of the eight parrs examined, two were female.

(1) Eight fishes were examined only on the blood and 5 on the other tissues; (2) 8 on the liver, kidney and blood, and 5 on the gill, stomach and muscle.

*Liver.* Except the case of June 2, the liver catalase activity of the parr is nearly equal to, or rather higher than, that of the smolt. For example in March, the activity of the parr is higher than that of the smolt, and the difference between them is probably significant ( $P < 0.05$ ). Then the activity of the parr declines suddenly at the beginning of June and becomes significantly lower than that of the

smolt ( $P < 0.001$ ). However, at the beginning of July it returns to the level nearly equal to, or rather higher than that of the smolt. Thus, the first peak of the activity in the parr is found at the end of July. In August, the activity of the parr shows a slight depression. Possibly, the peak of the activity in the smolt may also be found in July if examinations were made.

Generally, the liver catalase activity of the smolt increases progressively and linearly accompanying with the growth of the fish body, while that of the parr shows a complicated, up-and-down fluctuation, at least in the comparable period in the two forms.

*Kidney.* The kidney catalase activity in both the smolt and parr resembles each other in their levels and also in their seasonal fluctuations. However, only at the beginning of June is the activity higher in the smolt than in the parr the same as in the case of the liver; the difference between them is probably significant ( $P < 0.05$ ). It is noticeable that in spite of the marked growth of the body in the parrs of this stage the kidney catalase activity does not increase at all. The peaks of the activity in the two forms were found at the same month, the beginning of July. At the end of August, both forms show a sudden fall of the activity, respectively. Then, the fall of the parr is more marked than that of the smolt, and the difference between them is also probably significant ( $P < 0.05$ ).

*Blood.* At the end of March, the blood catalase activity of the parr is higher than that of the smolt; the difference between them is probably significant ( $P < 0.02$ ). Thereafter, the activity of the parr progresses to become nearly equal to, or rather higher than that of the smolt. However, at the beginning of July the activity of the parr falls off suddenly, showing conversely to be significantly lower than that of the smolt ( $P < 0.001$ ). That is, the sudden fall of the blood catalase activity in the parr was found to be delayed about a month, when compared with the similar depression of the activity in the liver and with the standstill of the activity in the kidney. However, such a depression of the activity in the parr returns to the normal level in the next month.

*Gill, stomach and muscle.* No significant differences of the catalase activity in any of these tissues were found between the parr and smolt, as indicated in Table 3 and Fig. 2.

#### **Seasonal changes of the catalase activity of the tissues and blood in the parr**

The changes of the catalase activity of the tissues and blood of the parr in the period between March and August have been described in the preceding chapter. The changes subsequent to this period will be stated below. Those changes are illustrated in Figs. 1 and 2.

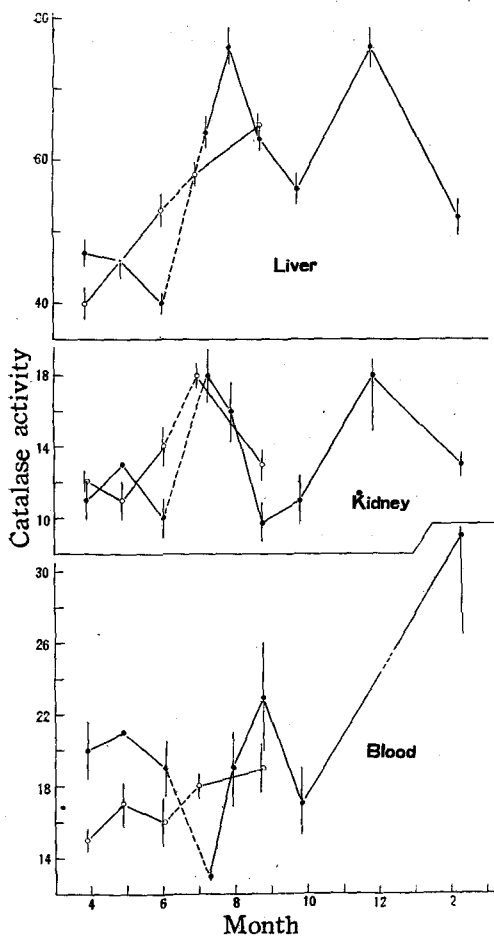


Fig. 1. Seasonal changes in the catalase activity of the liver, kidney and blood in the smolt and parr; each bar indicates the standard error of the mean.

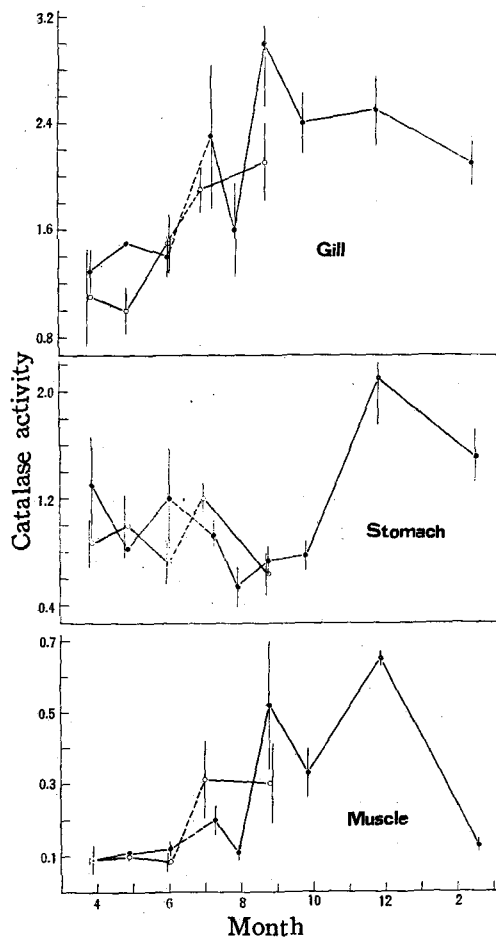


Fig. 2. Seasonal changes in the catalase activity of the gill, stomach and white skeletal muscle in the smolt and parr; each bar indicates the standard error of the mean.

*Liver, kidney and muscle.* The seasonal changes of the catalase activity in both the liver and kidney progress in a similar manner. That is, the activity of both tissues shows a sudden fall early in autumn, respectively. In November, the activity of both tissues increases promptly to the level equal to that in summer, showing respective second peaks. In February, the activity of both tissues falls again, but slightly. Although the muscle catalase activity is very low, its seasonal change goes on similarly to those of the liver and kidney.

*Blood.* In September, a slight fall was also found in the catalase activity of the blood. After this fall, the activity appeared to increase progressively till February.

*Gill.* Of the various tissues examined, only the catalase activity of the gill showed a somewhat different progress of the seasonal change. After a slight fall of the activity in July, it increased rapidly up to the peak within only a month. Thereafter, it progressed without any marked fluctuations till February.

*Stomach.* In the three months from July to September, the stomach catalase activity progresses at a low level. However, it increases promptly in November, showing the peak of the annual fluctuation. In February it decreases slightly.

## DISCUSSION

In the previous paper,<sup>6)</sup> the authors reported the existence of the specificity of the kidney catalase activity in the salmonid fishes, sweetfish and lamprey, and discussed a probable correlation between the high enzymatic activity of this tissue and the high potentiality of osmoregulation in these diadromous fishes. However, as the physiological significance of tissue catalase activity is not clear at present, such a correlation is only a presumption and must be substantiated by further studies. Still it is of much interest that a high catalase activity of the kidney was also reported by Fukuda and Higuchi<sup>11)</sup> in gray-mullet which is an euryhaline fish but not diadromous.

By the present experiment, not only in the kidney but also in both the liver and blood, the differences of the catalase activity were found between the smolt and parr of *O. masou* at the time when the former form in the natural habitat migrate into the sea. Besides such differences, the progress of the seasonal changes in the catalase activity of the tissues and blood of both forms was found to be different from each other.

Except for the case of the catalase activity in the gill, the activity in the tissues and blood in the parr is depressed in the months of the maturation of the male gonad, August and September. The male gonad is not yet at all mature at the end of July. But, at the end of August it has ripened, at least in a small number of the fishes, it being possible to make the fishes emit the semen with a slight pressure on the abdomen. At the end of September the male gonads of all fishes have completely ripened. From this fact, it may be possible to presume that there is a close correlation between the catalase activity of the tissues and blood and the maturation of the male gonad in the parr. However, this presumption is contrary to the experimental results of Adams<sup>12)</sup> in mice where it was found that the marked and rapid fall or rise in the liver catalase level follow castration or administration of testosterone. He has also reported that adrenalectomy in the mice produces a greater fall in the catalase level of the male liver than castration does. Consequently, the uniform depressions in the catalase activity of the tissues and blood in the parr might be caused either by some reciprocal interferences of the effective hormones or by some physiological changes different from those in mammals.

Further, in *O. masou*, not all the smolts are female and also not all the parrs are male, though in the case of all the fishes used throughout this experiment, except the two parrs examined in August, the smolts were female and parrs male. The exceptional two parrs were female. These fishes were probably parrs which transformed conversely from the smolt, because August is the final month in which the smolts were found in the pond. Although eighteen parrs were examined after September, they were all male. The female fishes in this pond may have been exhausted by the experiments up to this month. As the authors unfortunately could not have a chance to examine the catalase activity on many fishes of the female-parr form, it is not clear at present whether the results obtained in this experiment are based on the differences between the smolt and parr or on the sex difference. This question will be clarified by the studies of sex hormonal effects on the enzymatic activities in the tissues and blood of both forms.

Marked rise of the catalase activity was found in most of the tissues in November. It may possibly be reduced to the absorption of the male sexual materials into these tissues and also to the effect of the favourable conditions<sup>13)</sup> in the environment of the fishes such as the moderately cold water temperature.

The parallel increase of the tissue catalase activity in both the smolt and the parr in summer may be caused by the high metabolic rate of the fishes which appears in company with the marked growth of the body in this season.

#### SUMMARY

To find some enzymatic differences between the smolt and the parr of *Oncorhynchus masou*, the seasonal changes in the catalase activity of the liver, kidney, gill, stomach and white skeletal muscle, and of the blood were examined on fishes reared in a freshwater pond. After more than one year from the hatching of the larvae, the smolts began to appear at the end of March and disappeared at the end of September in the pond.

1) In the catalase activity of the gill, stomach and muscle, no significant differences were found between the smolt and the parr.

2) Generally, the catalase activity of the liver, kidney and blood in the parr are nearly equal to, or rather higher than, that of the smolt in level. However, at the beginning of June which time corresponds to the active season of the seaward migration of smolt in the natural habitat, the catalase activity in both the liver and kidney of the parr becomes conversely lower than that of the smolt. A similar fall of the blood catalase activity of the parr was found to be delayed about a month. These declines in the catalase activity in the parr return to the normal level in the next month.

3) The catalase activity of the tissues and blood in the parr decreases in September, at which time the male gonad has completely ripened (except the two



female-parrs examined in August, all the parrs were male and all the smolts were female throughout the present experiment).

4) In November, the catalase activity in most of the tissues in the parr increases equally to, or rather higher than, the level in summer. Thereafter, the activity of those tissues falls off slightly, except that of the blood which showed the highest value in February.

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