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Citation	北海道大學水産學部研究彙報, 21(2), 123-127
Issue Date	1970-08
Doc URL	http://hdl.handle.net/2115/23419
Туре	bulletin (article)
File Information	21(2)_P123-127.pdf



# BIOCHEMICAL STUDIES ON THE SKIN OF FISH III

Seasonal Variation of Purine Contents in the Skin of Yamame (Freshwatertype masu salmon, Oncorhynchus masou)

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#### Introduction

Masu salmon spends one winter in freshwater after hatching. Most females migrate downriver to the sea in the following spring. A considerable proporition of the females remain in freshwater-type masu salmon is often called yamame. Yamame which do not migrate downstream turns into gold color during the following April and May, and becomes darker during the following summer and autumn.<sup>1)2)3)</sup>

In the previous report the author investigated the seasonal variation of purine contents in the skin of masu salmon during smoltification.<sup>4)</sup> The results showed that a remarkable increase of guanine (G) and hypoxanthine (Hx) was found at both occasion of change from parr to silvery parr and from silvery parr to smolt. Furthermore it has also been found that a large quantity of G was not necessarily correlative with the silvering of the smolt.

In this paper the author investigated the seasonal variation of G and Hx contents in the acid-soluble fraction of the skin, using the freshwater-type masu salmon, yamame.

#### Experiment

(Sample) Yamame were caught from the same river near Hakodate. In Table 1 are shown the age, the date of their capture, the numbers of fishes used in experiments.

(Experimental method) The skin of fishes was torn off immediately after killing by clubbing and extracted with 4 parts of 0.6 N HClO<sub>4</sub> by skin weight. Because the following experimental methods are the same as described in a previous reprot, details are omitted.

# **Experimental results**

G, Hx, HxR and GR were separated by using Dowex 1×2 column, and IMP,

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Table 1. The date of capture, the age, the numbers of fish, total length, body weight and skin weight of fish used in experiments.

Date	'69 7-20	'69 8-30	'68 10-30	'68 12-22	'69 3-1	'69 3-21	'69 <b>4-3</b> 0	'69 6-18	'68 8-7	'68 10-30	'69 3-20		
Age	One-year old (0+)						Two-year old (1+)				Three-year old (2+)		
No. of fish sampled	3	4	5	4	2	3	3	4	2	6	1		
T.L. (cm)	9.1	12.4	10.9	11.7	14.9	12.2	15.7	14.4	15.9	14.7	23.4		
B.W. '(g)	8.1	22.3	13.6	10.2	30.0	17.1	33.7	24.4	45.9	31.6	124.8		
Skin W. (g)	1.3	1.2	2.5	2.5	2.3	2.2	2.5	2.5	2.6	2.4	2.5		

Table 2. The analytical data of the acid-soluble fraction in the skin of yamame.

Date	'69	'69	'68	'68	'69	'69	'69	'69	'68	'68	'69
	7-20	8-30	10-30	12-22	3-1	3-21	4-30	6-18	8-7	10-30	3-21
Hx	7.00	5.87	6. 17	6.66	5.42	5. 96	7. 66	7. 23	2.84	6.30	5.3
$\mathbf{G}$	17. 16	18.94	14.96	13.37	20.09	18.72	8.37	23.40	7.52	12.69	16.89
$\mathbf{H}\mathbf{x} + \mathbf{G}$	24.16	24.81	21.13	20.03	25. 51	24.68	16.03	30.63	10.36	18.99	22.20
G/Hx	2. 75	3. 23	2.42	2.00	3. 72	3. 25	1.09	3.24	2.65 (GR)	2.01	3. 18
HxR	0.51	0.33	0.72	0.41	0.40	0.25	0.23	1.57	0.50	0.42	0.2
IMP	1.28	0.39	0.71	0.40	0.87	0.46	1.16		0.97	0. 22	0.68
ADP	+	+	+	+	+	+	+	+	+	+	+
Pteridine	+	+	+	+	+	+	+	+	+	+	+

µmoles/g

ADP and pteridine by Dowex  $1\times8$  column. Each component was identified by means of paper chromatography, UV spectra and the determination of the amount of phosphate and ribose.

The results of quantitative analysis are shown in Table 2. And the seasonal variation of the sum of G and Hx values and their ratios (G/Hx) are shown in Fig. 1.

In Fig. 1, the sum of G and Hx indicates a small peak in March for the two-year-old fish (1+) and a remarkable peak in June for the two-year-old. In the latter peak G+Hx was  $30.63 \,\mu$ moles/g skin, G value Was  $23.40 \,\mu$ moles/g skin and G/Hx was 3.24. And at this time the body color indicated a somewhat strong brightness. No distinct seasonal variation of IMP, HxR and ADP was found.

## Discussion

Generally the variation of the sum of G and Hx and body color were less in yamame than the variation during the smoltification reproted in a previous paper.

Abbreviation G; guanine, Hx; hypoxanthine, HxR; inosine, GR; guanosine, IMP; inosine monophosphate, ADP; adenosine diphosphate.

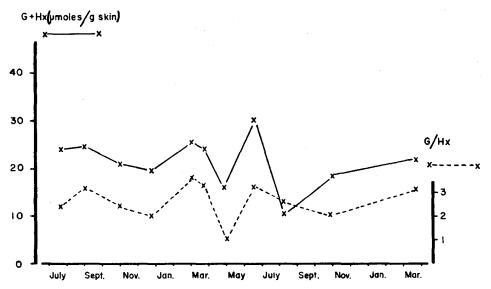


Fig. 1. The seasonal variation of the sum of G and Hx and their ratio (G/Hx).

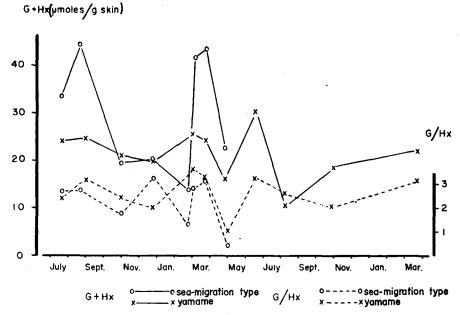


Fig. 2. The comparison of yamame with the sea-migration type masu salmon in the G+Hx value and the ratio (G/Hx).

In yamame a remarkable increase of G+Hx was found in June in the two-year-old. At this time the sum of G and Hx was 30.63  $\mu$ moles/g skin and G value was 23.40  $\mu$ moles/g skin. But these values were smaller than those indicated during smoltification. Namely during smoltification G+Hx increased up to 41.79–44.49  $\mu$ moles/g skin and G value up to 30.99–33.15  $\mu$ moles/g skin. The comparison of yamame with the sea-migration type in the G+Hx value and the ratio (G/Hx) is shown in Fig. 2. In June the body color of yamame did not indicate silvery as the smolt did, but the brightness of the skin became a somewhat strong. In yamame the variation of G+Hx was correlative with the ratio (G/Hx), as the same correlation was also found during smoltification. These facts suggest an interesting problem with respect to the purine metabolism.

Up to the present the physiological meaning concerning the deposition of G and Hx in the skin of some fishes has not been precised. But some factors to make these two purines in the skin increase are already known. For exmaple Johnston et al. indicated that the quantity of these purines during smoltification, is effected by water temperature, photoperiod, intensity of incident and background illumintion, feeding and increase in salinity<sup>5</sup>). It is also assumed in yamame that these purines are influenced by the above factors. But it is assumed that the sensitivity to these factors is fairly small in yamame compared with the sea-migration type of masu salmon. Although the difference between yamame and the sea-migration type may be considered to have a bearing on the sensitivity to the above factors and the difference of hormonal effects,<sup>6</sup>) further detailed biochemical studies on these problems are expected to reveal.

#### Summary

A remarkable increase of the contents of G+Hx was found in June in two-year -old yamame. But the seasonal variation of these purines in the skin of yamame is rather less than that of the sea-migration type. It was found that the sum of G and Hx was correlative with their ratio (G/Hx).

# Acknowledgement

The auther would like to express his grateful acknowledgement to Professor T. Saito, Mr. T. Kubo and Mr. J. Kosaka for their cordial advice.

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