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## Changes in Fatty Acid Composition of Masu Salmon, *Oncorhynchus masou*, Reared in Sea Water\*

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### Abstract

The changes in the lipid contents and the fatty acid composition of masu salmon, *Oncorhynchus masou*, during the rearing in sea water for five months from June to October were investigated.

The growth of fish was more prominent in the females than in the males.

The lipid contents of female fish flesh increased from 1.4% in the fresh-water stage to 5.8% in the end of experiment, whereas that of the male fish tended to decrease in September and October.

Linoleic acid in the flesh lipids decreased, on the other hand, the ratio of total  $\omega 3$  acids to  $\omega 6$  acids increased. This tendency was more distinct in female lipids than in male lipids.

The fatty acid pattern in female fish lipids in the latter stage of experiment became similar to that of wild masu salmon caught from sea water.

It was considered that the different variations in the lipids between female and male fish during the rearing in sea water were closely related to the biological characteristics of masu salmon occurring in nature.

### Introduction

In previous reports, the lipid compositions and the fatty acid compositions of wild masu salmon during migration and early stage of sea water life had been investigated<sup>1)2)</sup>.

There are a few studies on the variations of the lipid components of salmonoid fish in the stage of early sea water life<sup>3)</sup>. Saddler et al.<sup>4)</sup> studied on the lipids of chum salmon fry (*Oncorhynchus keta*) during migration and early sea water growth and reported that the lipid contents and the total monounsaturated acids decreased. These results agreed well with the data on the lipids of juvenile masu salmon<sup>2)</sup>.

The nutritional requirement of the fish for various types of fatty acids, which function as a source of energy, have already been investigated by several authors<sup>5)6)7)</sup>. Yu and Shinnhuber<sup>8)9)</sup> and Watanabe et al.<sup>10)11)</sup> studied the effects of dietary  $\omega 6$  and  $\omega 3$  fatty acids on the growth of rainbow trout (*Salmo gairdneri*) and revealed that  $\omega 3$  fatty acids had high potency on fish growth as compared with  $\omega 6$  fatty acids.

\* Lipids of Masu Salmon -V.

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In recent years, much attention is being denoted to the mariculture of the fish. Detailed study is necessary to clarify the relation between biological changes and chemical components of the fish in order to lead to their appropriate growth during the culture.

The present investigation was undertaken to determine primarily the changes of the lipid contents and the fatty acid composition of masu salmon artificially reared in sea water and the data obtained were compared with the lipids of wild masu salmon.

### Materials and Methods

One-year-old masu salmon were used in this experiment. After they were kept in a tank supplied with fresh sea water and allowed to acclimate to sea water for two weeks, the fish (n=101) were then transferred into net enclosures (5m×5m, 4m in depth) anchored in Oshoro Bay, Hokkaido, and reared during five months from June to October.

Diets given to fish were a mixture of commercial food (Nippon Haigoshiryo Co., Ltd.) and freezed mysis (*Euphausia pacifica*) (6:4 w/w) (Diet A), and minced fish (Sand lance, *Ammodytes personatus*) (Diet B). The amount of diet was calculated according to the feeding table on quantity of basal diet by Leitritz<sup>12</sup>). Water temperature at 4 m in depth varied ranging from 15.2 to 21.3°C, the maximum occurring in early August<sup>13</sup>).

Four to six fish were taken from the net randomly every month. After their biological properties were measured, the flesh lipids were extracted by the method

Table 1. *Body length, body weight and lipid content of masu salmon.*

Date	Habitat	Sex*	Body length (cm)	Body weight (g)	G.I.**	Lipid content in flesh (%)
June 7	Fresh-water	F	17.1	57	0.4	1.4
		M	14.9	53	1.5	3.8
June 22	Acclimating stage to sea water	F	16.2	38	0.8	1.2
		M	15.9	55	1.8	4.9
June 29	Sea water	M	16.4	69	1.6	3.5
July 28		F	19.1	86	0.3	2.0
		M	17.2	71	1.0	3.9
Aug. 27		F	21.0	127	0.4	4.1
		M	18.8	101	3.5	4.8
Sept. 30		F	21.1	201	0.3	2.7
		M	18.6	70	5.0	1.4
Oct. 29		F	28.5	381	0.9	5.8
		M	18.1	99	3.0	2.8

\* F-Female, M-Male

\*\* Gonadosomatic index (Gonad weight × 100/Body weight)

of Bligh and Dyer<sup>14</sup>). The lipid contents of Diets A and B were 4.0 and 9.6%, respectively.

The lipids were separated by silicic acid-celite 545 (2:1 w/w) column chromatography using chloroform and methanol into non-polar lipids and polar lipids. Each lipid fraction was then saponified with 1N alcoholic KOH solution. The recovered fatty acids were esterified with 14% BF<sub>3</sub>-methanol. The fatty acid methyl esters were analyzed by gas-liquid chromatography on 10% DEGS column at 185°C as described previously<sup>1</sup>).

### Results and Discussion

The body length, body weight, gonadosomatic index (gonad weight × 100/ body weight) and lipid contents of the flesh of masu salmon are shown in Table 1,

Table 2. Fatty acid composition of non-polar lipids of masu salmon flesh (as % of the total acids)\*<sup>1</sup>.

Fatty acid	June 7		June 22		June 29	July 28		Aug. 27		Sept. 30		Oct. 29	
	F**	M**	F	M	M	F	M	F	M	F	M	F	M
	Saturated acids												
14:0	2.7	3.9	2.3	3.7	4.5	7.9	5.4	7.7	8.4	6.7	3.9	6.6	5.0
16:0	22.5	20.5	30.8	20.5	20.6	28.4	24.7	26.7	26.3	23.0	19.0	21.7	21.1
17:0	0.3	0.5	0.3	0.3	0.9	1.0	0.7	0.9	0.8	1.2	1.2	1.1	1.0
18:0	2.5	2.9	2.7	3.0	3.2	3.4	3.3	3.7	2.5	3.1	3.5	3.4	3.5
	Monounsaturated acids												
16:1	6.3	7.8	5.2	8.3	7.8	8.1	8.8	9.5	9.0	9.1	5.7	10.2	9.2
17:1* <sup>3</sup>	0.3	0.4	0.2	0.4	0.6	0.8	0.6	0.9	0.8	1.3	0.8	0.8	0.8
18:1	29.4	28.5	18.9	33.1	25.9	19.3	28.3	21.6	20.5	18.5	17.9	20.9	21.9
20:1	3.9	7.3	3.4	5.6	5.9	4.9	6.8	4.6	5.1	4.1	6.0	3.8	5.0
22:1	1.6	2.6	1.0	1.4	2.6	2.9	3.0	3.3	3.3	2.8	3.6	1.7	2.1
	Polyunsaturated acids												
18:2 $\omega$ 6	10.0	11.3	7.0	10.5	7.8	5.0	8.0	1.6	4.5	1.6	4.2	1.1	5.5
18:3 $\omega$ 3	0.3	1.0	0.5	1.0	1.0	0.6	0.6	0.5	0.2	0.2	0.7	0.5	0.4
18:4 $\omega$ 3	0.6	1.0	0.5	1.0	1.0	0.9	1.1	0.9	1.0	1.9	1.0	1.7	1.2
20:4 $\omega$ 6	0.5	Tr* <sup>4</sup>	0.6	Tr	0.6	1.1	0.5	1.0	1.0	1.2	2.1	0.9	1.0
20:4 $\omega$ 3	Tr	Tr	Tr	0.1	0.6	0.3	0.5	Tr	Tr	1.7	1.1	0.7	0.7
20:5 $\omega$ 3	2.7	2.9	6.1	2.6	5.2	5.3	3.1	7.0	6.9	9.9	7.4	8.4	6.4
22:5 $\omega$ 3	Tr	Tr	Tr	Tr	0.4	0.5	Tr	1.7	0.6	1.6	2.3	3.0	1.7
22:6 $\omega$ 3	15.8	8.1	20.4	7.8	10.5	8.7	3.8	7.3	8.4	11.0	18.4	12.1	12.3
Total saturated acids	28.1 28.1		36.2 27.7		29.5	41.2	34.5	39.8	38.4	34.7	28.0	33.4	31.3
Total monounsaturated acids	41.6 46.8		28.7 48.9		43.1	36.4	47.9	40.2	39.0	36.2	34.5	38.2	39.5
Total polyunsaturated acids	30.3 25.1		35.1 23.4		27.4	22.4	17.6	20.0	22.6	29.1	37.5	28.4	29.2

\*<sup>1</sup> Other acids, including 12:0, 15:0, Iso 16:0, 14:1, 24:1, 20:2 $\omega$ 6, 20:3 $\omega$ 6 and 21:5 $\omega$ 2, were detected as minor components.

\*\* F-Female, M-Male \*\* Includes 16:2 $\omega$ 4. \*<sup>4</sup> Trace (less than 0.1%)

where the values are mean of determinations on an individual fish. The lipid contents of females obtained from fresh-water pond was 1.4%, showing a level lower than that of males. In the procedure of the rearing in sea water, the lipid contents of the females increased gradually and reached 5.8% at the end of the experiment, whereas there were reductions in those of the males in September and October. Table 1 also shows that the growth of fish is more prominent in females than in males and the gonadosomatic index of males tends to increase during their rearing in sea water, with a maximum occurring in late September. It is thought that the decrease of lipid contents of males is closely correlated with sexual maturation as described previously<sup>15</sup>).

The fatty acid compositions of non-polar lipids and polar lipids of masu salmon flesh are shown in Tables 2 and 3, where several of minor components of less than 1% of the total fatty acids were omitted. From the comparison of fatty acid compositions between male and female lipids in the stages of fresh-water and of

Table 3. *Fatty acid composition of polar lipids of masu salmon flesh (as % of the total acids)\*1.*

Fatty acid	June 7		June 22		June 29	July 28		Aug. 27		Sept. 30		Oct. 29	
	F**2	M**2	F	M	M	F	M	F	M	F	M	F	M
	Saturated acids												
14:0	0.9	1.5	1.0	1.7	2.3	3.9	4.0	4.5	3.7	3.4	3.0	3.5	4.1
16:0	29.2	30.2	31.7	30.6	30.2	46.0	45.8	36.0	31.9	43.2	31.3	33.0	39.0
17:0	0.3	0.2	0.6	0.4	0.5	1.0	0.3	0.5	0.6	1.1	0.9	1.7	0.9
18:0	8.4	8.2	7.6	8.9	6.1	5.1	7.7	5.9	5.1	7.9	7.6	5.3	4.5
	Monounsaturated acids												
16:1	2.3	3.6	2.6	3.8	4.2	4.6	4.3	4.1	4.5	4.2	7.0	4.9	5.0
17:1**3	Tr**4	Tr	0.4	0.3	0.5	0.4	0.2	0.5	0.5	1.2	0.8	1.2	0.5
18:1	16.1	20.3	14.1	21.3	16.0	17.1	17.8	15.6	15.6	11.6	14.0	14.6	18.0
20:1	1.0	2.7	0.6	2.8	1.7	1.9	2.1	2.4	2.3	0.7	2.2	1.7	2.0
22:1	Tr	Tr	Tr	Tr	0.6	0.4	1.0	0.5	1.0	1.1	0.8	Tr	Tr
	Polyunsaturated acids												
18:2 $\omega$ 6	3.6	6.0	4.3	6.0	3.9	2.3	3.1	0.6	2.0	1.3	2.3	0.8	1.6
18:3 $\omega$ 3	Tr	0.4	0.1	0.6	0.4	Tr	Tr	0.3	0.4	Tr	Tr	0.5	Tr
18:4 $\omega$ 3	Tr	0.3	Tr	0.5	0.4	—	Tr	Tr	Tr	Tr	—	Tr	Tr
20:4 $\omega$ 6	0.4	0.4	2.1	0.9	1.4	1.1	1.0	2.4	2.7	1.2	3.2	1.9	1.7
20:4 $\omega$ 3	Tr	Tr	—	Tr	0.5	Tr	—	Tr	Tr	Tr	Tr	0.6	—
20:5 $\omega$ 3	1.3	2.1	6.2	4.2	6.1	4.0	2.1	7.1	7.6	5.3	6.5	10.6	6.0
22:5 $\omega$ 3	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.8	Tr	1.0	2.0	0.8
22:6 $\omega$ 3	35.4	23.6	27.6	16.1	24.2	11.5	9.8	18.2	20.4	15.5	18.9	16.8	15.5
Total saturated acids	39.9 40.4		42.1 42.8		40.1	56.7	58.6	48.3	42.2	57.8	43.3	44.4	49.0
Total monounsaturated acids	19.4 26.6		17.7 28.2		23.0	24.4	25.4	23.1	23.9	18.8	24.8	22.4	25.5
Total polyunsaturated acids	40.7 32.9		40.3 29.0		36.9	18.9	16.0	28.5	33.8	23.3	31.9	33.2	25.6

\*1 As in Table 2. \*\*2 F-Female, M-Male \*\*3 Includes 16:2 $\omega$ 4.

\*\*4 Trace (less than 0.1%)

Table 4. *Compositions and ratios of fatty acids of dietary lipids (as % of the total acids)\*<sup>1</sup>.*

Fatty acid	Diet A		Diet B	
	NL* <sup>2</sup>	PL* <sup>2</sup>	NL	PL
Saturated acids				
14:0	2.8	1.5	11.4	4.0
16:0	18.8	20.9	15.6	32.3
17:0	1.4	1.0	1.8	3.0
18:0	2.6	3.7	2.0	7.1
Monounsaturated acids				
16:1	4.8	3.1	8.5	4.1
17:1* <sup>3</sup>	0.6	0.6	1.2	0.6
18:1	21.0	18.5	9.4	12.7
20:1	5.0	3.8	7.1	3.3
22:1	2.3	1.4	7.7	0.9
Polyunsaturated acids				
18:2 $\omega$ 6	20.6	11.2	0.7	0.6
18:3 $\omega$ 3	1.9	1.3	0.3	0.3
18:4 $\omega$ 3	1.4	0.6	2.5	2.2
20:4 $\omega$ 6	1.0	1.2	3.2	2.8
20:4 $\omega$ 3	0.9	Tr* <sup>4</sup>	Tr	0.8
20:5 $\omega$ 3	8.4	9.1	15.4	6.1
22:5 $\omega$ 3	Tr	0.7	0.6	0.1
22:6 $\omega$ 3	5.8	20.8	9.4	18.7
Total saturated acids	26.1	27.2	31.8	46.7
Total monounsaturated acids	33.9	28.0	35.4	21.6
Total polyunsaturated acids	40.0	44.9	32.8	31.6
Ratio				
20:1+22:1+24:1/Total mono-unsaturated acids	0.22		0.43	
18:2 $\omega$ 6+18:3 $\omega$ 3/Total poly-unsaturated acids	0.56		0.03	
$\Sigma\omega$ 3/ $\Sigma\omega$ 6	0.9		6.9	

\*<sup>1</sup> As in Table 2.\*<sup>2</sup> NL-Non-polar lipids, PL-Polar lipids\*<sup>3</sup> Includes 16:2 $\omega$ 4.\*<sup>4</sup> Trace (less than 0.1%)

acclimation to sea water, it is clear that both non-polar lipids and polar lipids of the latter contain less monounsaturated acids except for 18:1 of non-polar lipids in fresh-water stage, and much more 22:6 $\omega$ 3 than in the former. These results were approximately similar to those obtained by one of the authors in a study of wild masu salmon lipids in the period of seaward migration<sup>1</sup>).

Linoleic acid which was originally rich in non-polar lipids of masu salmon reared in fresh-water decreased during the rearing in sea water in spite of the supply of diets containing a high amount of 18:2 $\omega$ 6 (Table 4), while 20:5 $\omega$ 3 tended to increased. Especially, there was a high reduction of 18:2 $\omega$ 6 in female lipids.

The variations of several ratios of fatty acids in non-polar lipids are shown in Fig. 1. In these ratios, the ratio of the sum of long-chain monounsaturated acids (20:1, 22:1 and 24:1) to the total monounsaturated acids was about 0.2

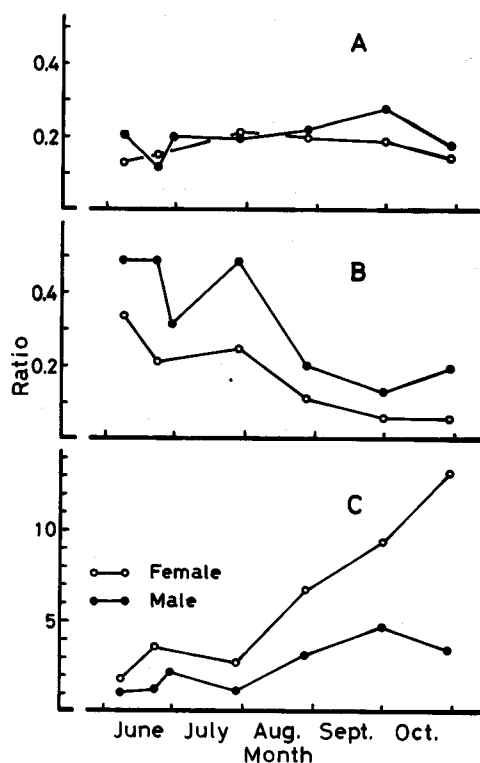


Fig. 1. Variations of ratios of fatty acids in non-polar lipids of masu salmon flesh.  
 A - Ratio of the sum of long-chain monounsaturated acids (20:1, 22:1 and 24:1) to total monounsaturated acids  
 B - Ratio of the sum of 18:2 $\omega$ 6 and 18:3 $\omega$ 3 to total polyunsaturated acids  
 C - Ratio of total  $\omega$ 3 acids to  $\omega$ 6 acids

and did not change markedly in both male and female lipids throughout the experiment. It is well known that marine lipids have high amounts of 20:1, 22:1, 20:5 $\omega$ 3 and 22:6 $\omega$ 3, inversely low contents of 18:2 $\omega$ 6 and 18:3 $\omega$ 3 as compared with fresh-water fish lipids<sup>16)17)18)</sup>. Ackman<sup>19)</sup> states that the ratio of total  $\omega$ 3 acids to  $\omega$ 6 acids is higher in marine lipids than in fresh-water fish lipids. Since the contents of long-chain monounsaturated acids in wild masu salmon living in stream were low<sup>15)</sup>, the relatively high level of the ratio of the sum of long-chain monounsaturated acids to the total monounsaturated acids in the early stage of the experiment probably indicates the effect of pre-experimental dietary history of the fish. On the other hand, the ratio of the sum of 18:2 $\omega$ 6 and 18:3 $\omega$ 3 to the total polyunsaturated acids tended to decrease, but this value was constantly larger in males than in females. The ratio of total  $\omega$ 3 acids to  $\omega$ 6 acids increased gradually with a prolonged rearing of masu salmon in sea water. Especially this tendency was more conspicuous in female lipids than in male lipids.

The fatty acid compositions and the ratios of fatty acids in non-polar lipids of wild masu salmon flesh caught from sea water of South Hokkaido are shown in

Table 5. *Compositions and ratios of fatty acids in non-polar lipids of wild masu salmon (as % of the total acids)\*1.*

Date of catch	Jan. 28	Feb. 27	Mar. 21	Apr. 24	May 19	Oct. 6	June 22 <sup>2)</sup>
Sex	Female						
Body length (cm)	41	34	30	37	49	50	24
Body weight (g)	950	750	460	800	2340	2000	191
Gonadosomatic index	0.5	1.0	0.6	1.3	18.7	23.2	—
Lipid content in flesh (%)	5.2	4.1	5.7	2.1	0.6	1.2	2.7
Fatty acid	Saturated acids						
14:0	5.5	4.1	4.5	6.1	7.2	2.8	5.7
16:0	17.7	22.9	20.8	19.5	13.7	13.9	20.0
17:0	1.1	1.3	0.9	1.5	1.4	0.5	0.7
18:0	5.6	4.1	3.6	3.4	3.6	3.2	3.9
	Monounsaturated acids						
16:1	7.1	8.5	8.2	7.9	8.2	5.4	8.7
17:1 <sup>2)</sup>	1.0	0.8	0.7	1.1	1.4	0.4	0.8
18:1	23.9	28.3	27.5	24.6	16.5	18.8	21.7
20:1	11.2	6.0	7.3	5.9	8.0	16.1	6.7
22:1	4.4	2.0	4.1 <sup>3)</sup>	3.3	9.1	7.5 <sup>3)</sup>	4.2
	Polyunsaturated acids						
18:2 $\omega$ 6	1.1	0.8	1.4	1.0	1.7	1.0	1.7
18:3 $\omega$ 3	0.3	0.7	0.5	0.6	1.7	1.4	1.3
18:4 $\omega$ 3	1.4	1.2	Tr <sup>4)</sup>	1.4	2.2	1.1	2.3
20:4 $\omega$ 6	1.0	0.7	1.2	1.9	1.2	2.1	0.6
20:4 $\omega$ 3	1.0	1.4	—	1.0	1.5	—	1.2
20:5 $\omega$ 3	5.9	4.9	4.9	10.0	8.6	8.1	7.0
22:5 $\omega$ 3	1.3	1.2	3.1	2.0	3.4	7.7	2.4
22:6 $\omega$ 3	7.8	9.5	9.9	5.2	9.0	9.8	9.0
Total saturated acids	30.9	33.0	30.7	31.4	26.8	20.7	31.1
Total monounsaturated acids	49.1	46.3	48.2	44.5	43.9	48.2	43.0
Total polyunsaturated acids	20.0	20.8	21.0	24.1	29.3	31.2	25.9
Ratio							
20:1+22:1+24:1/Total mono-unsaturated acids	0.33	0.17	0.24	0.23	0.39	0.49	0.26
18:2 $\omega$ 6+18:3 $\omega$ 3/Total poly-unsaturated acids	0.07	0.07	0.09	0.07	0.12	0.08	0.12
$\Sigma\omega$ 3/ $\Sigma\omega$ 6	7.7	9.9	7.1	7.0	9.1	9.1	10.1

\*1 As in Table 2. \*2 Includes 16:2 $\omega$ 4. \*3 Includes 20:4 $\omega$ 3. \*4 Trace less than 0.1%

Table 5. A comparison of the compositions and the ratios of fatty acids of non-polar lipids of the experimental fish with those of wild fish shows that the values in female lipids are relatively similar to those of wild fish with a mean value of about 9 in the ratio of total  $\omega$ 3 acids to  $\omega$ 6 acids as compared with those of male lipids.

It is well known that juvenile masu salmon during their growth in fresh-water are generally divided into two groups<sup>20)</sup>, namely seaward migration type and residual type; female masu salmon belong mainly to the former and migrate into sea water in spring after smolt transformation, whereas many of the male remain in stream (residual type) and in part mature. Such biological character-



istics may be closely related to the sexual differences in fatty acid composition as mentioned above. The similarity of fatty acid composition in female lipids to that of wild masu salmon suggests that female masu salmon are liable to adapt to marine environments as compared with male masu salmon. On the other hand, the slow degrees of growth rate and of conversion into marine lipid type in male masu salmon during the rearing in sea water may represent to a certain extent the biological characteristic of male masu salmon occurring in nature as mentioned above.

Several investigators have advocated the effectiveness of  $\omega$ 3 acids for the growth of fish. Considerable attention must be paid to the biological characteristics of the fish, the environmental factors (water temperature and size of net enclosures) as described by Terao et al.<sup>13)</sup>, and furthermore the properties of diets, for example, the quality and quantity of fatty acids contained in dietary lipids in order to stimulate the growth of fish in mariculture.

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