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Lipids of masu salmon - IV. Changes of lipid composition and fatty acid composition in flesh lipids of juvenile masu salmon in the early stage of sea water life

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Lipids of Masu Salmon-IV.

Changes of lipid composition and fatty acid composition
in flesh lipids of juvenile masu salmon in the
early stage of sea water life

Tōru ŌTA*

Abstract

The lipid class distribution and the fatty acid composition in each lipid class of the flesh lipids of the juvenile masu salmon (*Oncorhynchus masou*) in the early stage of sea water life were investigated, and were compared with the previous results with regard to the flesh lipids of river-caught juvenile masu salmon.

Squalene was a major component of hydrocarbons in the flesh lipids, but pristane which was not detected in the flesh lipids of river-caught masu salmon was present as a minor component.

Triglycerides in the total lipids decreased at the early stage of sea water life and increased at the end of June. The content of phospholipids in the flesh did not change much during early life in sea water (about 800 mg/100 g of flesh). In the fatty acids of triglycerides, the ratio of (18:2+18:3) to the polyenoic acids, C₁₆ and C₁₈ acids decreased, while the ratio of total ω3 to ω6 fatty acids, C₂₀ and C₂₂ acids increased.

The fatty acid composition in sterol esters contained more 20:5ω3 at the early stage in sea water life than that in other lipid classes. Palmitoleic and oleic acids in sterol esters increased; on the contrary, 20:5ω3 and 22:6ω3 progressively decreased with the growth of the fish in sea water.

It was considered that the fatty acid composition in the flesh lipids of the juvenile masu salmon in the early stage of sea water life was rapidly converted from fresh-water fish type to marine fish type by the effects of marine environmental factors and that sterol esters played an important role in lipid metabolism.

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Introduction

Saddler et al.¹⁾ and Parker et al.²⁾ have studied the changes that occur in the lipid content of juvenile chum salmon (Oncorhynchus keta)* when they have descended to the sea, demonstrating that the lipid content of the fishes' bodies, and the total monoenoic acids in the fatty acids that compose the lipids, both decline during early sea water life. However, no investigation has been made of how other lipid components change during this period.

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The author and a colleague have previously reported³⁻⁵⁾ research on the topic of how fish physiology and environmental factors are related to changes in body lipid components as fishes grow and mature. These studies used masu salmon (Oncorhynchus masou).**

We expect that juvenile masu salmon undergo the same kind of rapid change in their body lipid components as chum salmon when their living environment is also altered in the spring, as they leave the rivers they were spawned in and descend to the sea. In order to clarify this point, the present author conducted an investigation of the lipid composition and the fatty acid composition of the flesh of juvenile masu salmon during the early stage of their sea life. Furthermore, the author compared these results with the lipid composition⁵⁾ of the flesh of river-caught juvenile masu salmon, studying the changes that occur in composition during early sea life.

*Translator's note: The Japanese name for this fish is sake or sakemasu. Reference 2, according to the title, is concerned with pink salmon and not chum salmon.

**Translator's note: The Japanese name for this fish is sakuramasu. I have followed the author's supplied translation - masu salmon (Oncorhynchus masou) - although a leading Jap.-Eng. fisheries dictionary lists this fish as pink salmon (Oncorhynchus gorbusha).

Experimental Methods

Test Fish and Lipid Extraction: The masu salmon used in the experiment were all captured in 1972, some being caught in middle and late May off the coast of Kamiiso in Hokkaido, and some in late June off the coast of Samani in the Hidaka district of Hokkaido. After measuring the body length and body weight of each fish, lipids were extracted from the flesh using the procedure of Bligh and Dyer⁶⁾.

Chromatography: Silicic acid column chromatography (CC) was used for lipid fractionation and thin-layer chromatography (TLC) for preparative purification of lipid components, following the methods previously reported⁵⁾. To identify lipid components, TLC was carried out using a thin-layer plate having Wakogel B-5 as adsorbent (thickness and size of the plate were 0.25 mm and 20 x 20 cm respectively). Lipid components were activated for 60 minutes at 110°C and then developed with n-hexane-ethyl ether-acetic acid (90:10:1 or 80:20:1 v/v). The reagent used for detection was a 10% phosphomolybdic acid ethanol solution. Gas chromatographical analysis (GLC) of hydrocarbons, sterols and fatty acids employed a 10% DEGS column and a 2% OV-17 column. Sterols were converted to acetates and fatty acids were methyl esterified, after which analysis was carried out under the same conditions reported previously⁵⁾.

Results and Discussion

Change in Lipid Content: Table 1 indicates the body length, body weight and flesh lipid content of the fish used in the experiment. As a previously published report⁵⁾ demonstrated, the lipid content in the flesh of masu salmon that are still in a river but will descend to the sea, is

about 1/2 that of the type of masu salmon that will eventually remain in the river - 1.9% as compared with 4.2%. The present experiment indicates that the lipid content in the flesh of the sea-going type of masu salmon tends to decline even further in the early period of their sea life, as contents of 1.5% and 1.4% were found for fish captured in mid-May and late May respectively. Moreover, in late June, before the fish begin their migration to deeper offshore waters⁷⁾⁸⁾, body length became longer and the lipid content in the flesh increased to 2.7%. This kind of decline in the lipid content during the early stage of sea water life has also been observed in chum salmon¹⁾ and pink salmon^{*2)}. The explanation for this change is thought to be that lipid metabolism picks up in response to the fish's new living environment and its qualitatively and quantitatively altered diet, with the result that lipids are utilized as an energy source.

Changes in Lipid Composition: The lipid compositions of the flesh of masu salmon caught at three different times for this experiment are shown in Table 2. In addition to the lipid components shown in Table 2, wax esters, diacyl glyceryl ethers and fatty alcohols were also found to be present in trace amounts, according to the results of TLC analysis (Fig. 1).

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The main components of the flesh lipids were found to be triglycerides and phospholipids; sterols, both in ester form and free form, were somewhat lower in content than^h those found in masu salmon during their river period⁵⁾. The quantity of phospholipids per 100 g of flesh was approximately 800 mg, practically unchanged from that found in river masu salmon; in contrast,

*Translator's note: The Japanese name for this fish is karafutomasu; it differs from the sakuramasu here referred to as masu salmon but elsewhere called pink salmon.

the amount of triglycerides had declined following the descent to sea water. Consequently, the diminished lipid content in the flesh that was described above is mainly accounted for by a decline in triglyceride components, as the quantity of phospholipids shows little variation.

As can be seen from Fig. 2, GLC analysis of hydrocarbons and sterols /33 indicated that squalene was the major component of hydrocarbons, but that pristane, which was not detected in the flesh lipids of river-caught masu salmon, was also present. Moreover, a trend was observed for the amount of pristane to increase as the number of days the fishes have spent in sea water increases. As zooplankton form the chief part of the diet of masu salmon in their early sea life, it is thought that the pristane detected as a component of hydrocarbons comes from plankton lipids⁹⁾¹⁰⁾. The major component of sterols was found to be cholesterol.

Changes in Fatty Acid Composition: Table 3 shows the fatty acid compositions of flesh lipid classes. The fatty acid compositions of triglycerides and diglycerides were rather similar, having a higher content of monoenoic acids than the other lipid classes. In contrast, free fatty acids and phospholipids were high in acid 16:0, and phospholipids were found to have particularly high quantities of 22:6 ω 3. As for the fatty acid compositions of sterol esters, the content of acid 16:1 resembled that /34 found in triglycerides and diglycerides, while the amounts of 16:0, 18:1 and some other acids were similar to those detected in phospholipids. Acid 20:5 ω 3 comprised 17% of sterol esters in mid-May, a higher proportion than any other sterol ester component during the fishes' early stage of sea life.

If we examine how triglyceride component fatty acids change when masu salmon migrate from their river environment to the sea, we find from

the various fatty acid ratios¹¹⁾ in Table 4 that the content of acid 16:0 does not vary much between fresh-water and sea water, remaining constant in comprising around 65% of total saturated acids. Since acids 16:1 and 18:1 showed a tendency to decline when the fishes begin sea life, the ratios 16:0/16:1 and 16:0/(16:1+18:1) increased. In contrast, acid 20:1 did not decline but increased when the fishes reached the sea, and acids 22:1 and 24:1 were also detected, with the result that the ratios of (20:1+22:1+24:1) to the total monoenoic acid content showed higher values than those found under fresh-water conditions. The polyenoic acids 18:2 ω 6 and 18:3 ω 3, which have high contents in flesh lipid triglycerides of fresh-water masu salmon, declined in quantity as soon as the fish reached the sea, whereas 20:5 ω 3 and 22:6 ω 3 increased. These variations in polyenoic acid content also were exhibited by the (18:2+18:3/total polyenoic acid) ratios and the values for $\Sigma\omega$ 3/ $\Sigma\omega$ 6. Saddler et al.¹⁾ showed that the proportion of monoenoic acids found in the lipid component fatty acids of chum salmon diminishes, while the ratio of polyenoic acids increases, during the early period of their life in the sea. A similar trend was observed in this study of masu salmon.

As for the differences between fresh-water and marine fishes in the fatty acid compositions of their lipids, it is already known that acids 16:1, 18:2 ω 6 and 18:3 ω 3 are found in greater quantities in fresh-water fishes, while 20:1, 22:1, 20:5 ω 3 and 22:6 ω 3 are more abundant in marine fishes^{9,11,12)}. It has been observed that acids C₁₆ and C₁₈ comprise a smaller proportion of the lipid component fatty acids when masu salmon migrate from the river to the sea, whereas acids C₂₀ and C₂₂ increase in amount. It is believed that the flesh lipid components of masu salmon rapidly change under the influence of dietary lipids and other factors after the fishes are in sea water; the shifts in fatty acid ratios shown in Table 4 illustrate well

how the fatty acid compositions of lipids vary from fresh-water conditions to marine conditions.

As described above, there is a large amount of acid 20:5 ω 3 in the fatty acids comprising sterol esters during the early stage of the masu salmon's life in the sea. This tendency is similar to that pertaining to the flesh lipids of river-caught masu salmon. However, the content was found to be higher in the type of masu salmon that descends to the sea, 9.3%, compared to a value of 7.6% observed for the type that remains in the river.⁵⁾ Addison et al.¹³⁾ investigated the lipid components of cod /35 (Gadus morhua) flesh and reported that sterol esters had a very high content of acid 20:5 ω 3 - from 25.9% to 27.5% - in contrast to the 7.3 - 8.9% levels found in triglycerides. Glomset¹⁴⁾ has reported that plasma cholesterol is formed from cholesterol and the position-2 fatty acid of lecithin by means of lecithin-cholesterol acyltransferase (LCAT); this indicates that the large amounts of 20:5 ω 3 found in the fatty acids that compose sterol esters may be due to phospholipid fatty acids. If we look at the changes in the fatty acids that compose sterol esters, we find that acids 20:5 ω 3 and 22:6 ω 3, which display high contents in the early period of sea life, decline as time passes in a sea water environment, whereas in contrast to this acids such as 16:1 and 18:1 show increased contents. This change is opposite to that which occurs in triglycerides with respect to the above-named fatty acids. Reflecting on these variations in fatty acid composition, and bearing in mind that esterification of sterols accelerates with an increasing degree of unsaturation of fatty acids¹⁵⁾, it can be inferred that lipid metabolism becomes more active in the early period of sea life, and that sterol esters are intimately involved in lipid metabolism in the masu salmon's body during this time.¹⁶⁾¹⁷⁾¹⁸⁾

Summary

The flesh lipid composition and fatty acid compositions of masu salmon during their early stage of sea water life were investigated and the variations found for lipid components were studied. The results can be summarized as follows:

- 1) The main component of hydrocarbons was squalene. Pristane, which could not be detected in the flesh lipids of river-caught masu salmon, was also present.
- 2) The quantity of triglycerides detected declined in the early stage of sea life, resembling the variation in lipid content; however, triglycerides increased in amount at the time when masu salmon begin their migration to offshore waters. On the other hand, the amount of phospholipids showed no marked variation, staying at around 800 mg per 100 g of flesh.
- 3) Among the fatty acids comprising triglycerides, C_{16} and C_{18} decreased in quantity after the masu salmon reached sea water, as did the (18:2+18:3/total polyenoic acids) ratio. Contrary to this, the amounts of C_{20} and C_{22} increased, as did the $\Sigma\omega3/\Sigma\omega6$ ratio.
- 4) The fatty acids comprising sterol esters showed a different trend from that seen for other lipid components, having high amounts of 20:5 $\omega3$ during the early sea life period. Acids 16:1 and 18:1 increased in quantity as time passed in sea water, while acids 20:5 $\omega3$ and 22:6 $\omega3$ declined. This tendency was opposite to that found for these fatty acids in triglycerides.
- 5) It is thought that lipid metabolism is very active in the body of masu salmon during the early period of their life in the sea, and that the fatty acids composing flesh lipids are rapidly converted from the fresh-water type to the marine type of composition under the influence of the fishes'

new diet and other changes in their external environment. It is further supposed that sterol esters play an important role in lipid metabolism during this period.

In conclusion, the author wishes to thank Professor Tetsu Takagi of the Faculty of Fisheries at Hokkaido University for reviewing this paper in manuscript.

Table 1. *Body length, body weight and lipid content in the flesh of the juvenile masu salmon.*

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Date of catch Locality	May 16, '72 Coast of Kamiiso	May 30, '72 Coast of Kamiiso	June 22, '72 Coast of Samani
Number of fish	9	7	2
Sex	Female	Female	Female
Body length (cm)	16.1	14.4	23.9
Body weight (g)	56	41	191
Lipid content in flesh (%)	1.5	1.4	2.7

Table 2. *Lipid class distribution in the flesh of the juvenile masu salmon (as per cent of total lipids).*

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Lipid class	May 16	May 30	June 22
Hydrocarbons	0.1	0.2	0.2
Sterol esters*	0.6	0.5	0.4
Triglycerides	24.9	19.0	50.9
Diglycerides	2.2	1.8	3.6
Free fatty acids	3.8	10.3	8.3
Sterols**	4.6	5.1	3.6
Phospholipids	57.5	60.2	28.9
Unknowns***	6.2	2.9	4.0

* Includes small amounts of wax esters and diacyl glyceryl ethers.

** Includes small amounts of fatty alcohols.

*** Monoglycerides may be present.

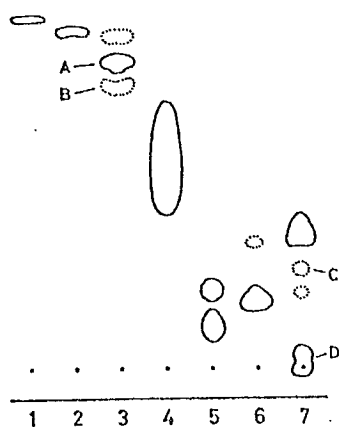


Fig. 1. Thin-layer chromatograms of each fraction in neutral lipids separated by the silicic acid column chromatography.

1: Hydrocarbon fr., 2,3: Sterol ester fr., 4: Triglyceride fr., 5: Diglyceride fr., 6: Sterol fr., 7: Free fatty acid fr. A-Wax esters, B-Diacylglyceryl ethers, C-Fatty alcohols, D-Unknowns. WAKOGEL B-5 (0.25 mm), Activation: 110°C, 60 min. Solvent: n-Hexane-Ethyl ether-Acetic acid (No. 1-3 90:10:1 and No. 4-7 80:20:1 v/v)

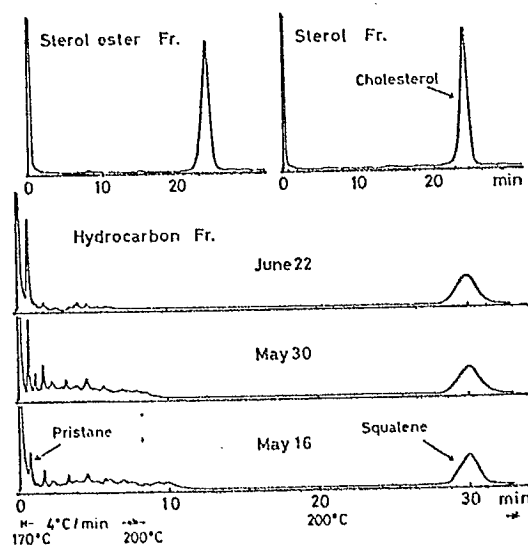


Fig. 2. Gas liquid chromatograms of hydrocarbons and sterol acetates in flesh lipids of the juvenile masu salmon.

Table 3. Fatty acid composition of lipid classes in flesh lipids of the juvenile masu salmon (%).

Fatty Acid	Sterol esters			Triglycerides			Diglycerides			Free fatty acids			Phospholipids		
	A*	B*	C*	A	B	C	A	B	C	A	B	C	A	B	C
Saturated acid															
12:0	1.1	2.7	1.4	0.5	0.3	0.2	1.1	0.2	0.3	0.3	Tr	Tr	Tr	0.5	Tr
14:0	1.6	1.7	6.6	5.2	4.9	5.7	4.4	4.3	5.7	2.7	3.1	4.0	1.9	1.5	2.2
15:0	0.7	0.9	2.0	0.6	0.6	0.5	0.5	0.7	0.7	0.5	0.3	0.3	0.4	0.4	0.2
16:0	30.1	36.5	24.6	19.9	24.0	20.0	28.8	25.8	17.9	43.5	37.1	35.0	30.1	32.5	27.0
17:0	2.0	2.5	1.4	1.1	1.4	0.7	1.2	1.9	1.0	0.9	1.1	0.8	1.1	1.2	0.7
18:0	2.4	2.2	5.3	4.6	5.3	3.9	3.6	4.3	3.4	3.8	3.4	4.3	4.0	3.4	5.9
Total	37.9	46.5	41.3	31.9	36.5	31.0	39.6	37.2	29.0	51.7	45.0	44.4	37.5	39.5	36.0
Monoenoic acid															
14:1	0.9	1.4	2.6	0.5	0.4	0.4	0.3	0.4	0.6	0.2	0.2	0.3	0.2	Tr	Tr
15:1	0.4	0.7	0.9	0.1	0.1	0.1	Tr**	0.2	0.2	0.1	Tr	Tr	1.0	0.4	0.2
16:1	7.4	10.0	11.2	11.6	7.2	8.7	8.9	7.3	9.8	5.1	4.5	5.3	4.5	2.8	2.9
17:1***	0.9	1.0	1.3	1.1	0.7	0.8	0.5	0.6	0.9	0.5	0.4	0.6	0.5	0.4	0.3
18:1	10.9	11.5	14.2	23.3	20.2	21.7	18.1	22.6	24.8	12.8	12.0	15.9	11.2	10.3	13.5
19:1	0.3	Tr	Tr	0.4	0.4	Tr	0.4	0.3	0.3	0.2	Tr	0.5	0.2	Tr	Tr
20:1	1.1	1.0	1.0	3.0	2.5	6.7	2.4	2.3	6.9	1.7	1.2	4.3	0.6	0.8	1.8
22:1	0.8	Tr	Tr	1.9	1.2	4.2	1.2	1.4	3.7	1.1	Tr	1.9	—	—	0.5
24:1	Tr	Tr	3.1	1.1	0.6	0.5	Tr	1.0	0.8	Tr	Tr	Tr	0.4	Tr	Tr
Total	22.7	25.6	34.3	43.0	33.3	43.1	31.8	36.1	48.0	21.7	18.3	28.8	18.6	14.7	19.2
Polyenoic acid															
18:2 ω 6	1.2	1.4	2.2	3.9	2.3	1.7	2.3	2.0	1.7	1.5	1.9	1.3	1.3	1.3	0.6
18:3 ω 3	0.8	1.2	0.9	3.0	1.6	1.3	2.0	1.2	1.4	1.7	1.7	1.3	1.2	1.3	0.5
18:4 ω 3	0.8	0.8	1.1	1.7	2.1	2.3	1.4	1.3	1.9	0.9	0.9	1.5	0.4	1.1	0.5
20:2 ω 6	Tr	Tr	0.6	0.4	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
20:3 ω 6	Tr	Tr	Tr	0.4	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
20:4 ω 6	2.1	1.5	1.5	1.0	1.0	0.6	1.2	1.3	0.7	1.7	1.5	0.7	2.3	2.6	0.9
20:4 ω 3	2.1	1.8	5.4	1.5	1.7	1.2	1.0	1.2	1.9	1.5	1.6	1.4	1.3	1.7	0.9
20:5 ω 3	17.0	11.3	6.3	4.2	6.0	7.0	5.6	4.3	5.3	8.7	11.6	9.0	8.5	8.8	7.6
21:5 ω 2?	Tr	Tr	Tr	0.5	Tr	0.4	Tr	Tr	Tr	Tr	Tr	Tr	0.4	—	Tr
22:5 ω 3	1.2	1.3	1.0	1.8	2.0	2.4	1.9	1.7	1.7	1.3	2.0	1.7	2.1	2.9	2.2
22:6 ω 3	14.2	8.6	5.4	6.7	13.5	9.0	13.2	13.7	8.4	9.3	15.5	9.9	26.3	26.1	31.6
Total	39.4	27.9	24.4	25.1	30.2	25.9	28.6	26.7	23.0	26.6	36.7	26.8	43.8	45.8	44.8

* A-May 16, B-May 30, C-June 22 ** Trace *** Includes 16:2 ω 4

Table 4. Fatty acid chain length composition and several ratios of fatty acids in triglycerides of flesh lipids of the juvenile masu salmon.

	Fresh-water*	Sea water		
	Apr. 30	May 16	May 30	June 22
Fatty acid chain lengths (%)				
C ₁₂	0.7	0.5	0.3	0.2
C ₁₄	4.9	5.7	5.3	6.1
C ₁₅	0.9	0.7	0.7	0.6
C ₁₆	35.6	31.5	31.2	28.7
C ₁₇	2.5	2.2	2.1	1.5
C ₁₈	45.7	36.5	31.5	30.9
C ₁₉	0.3	0.4	0.4	Tr**
C ₂₀	6.8	10.5	11.2	15.5
C ₂₁	—	0.5	Tr	0.4
C ₂₂	2.6	10.4	16.7	15.6
C ₂₄	—	1.1	0.6	0.5
Ratios				
16:0 as % of saturated acids	65.3	62.4	65.8	64.5
16:0/16:1	1.5	1.7	3.3	3.0
16:0/(16:1+18:1)	0.54	0.57	0.88	0.66
(20:1+22:1+24:1)/Monoenoic acids	0.02	0.14	0.13	0.26
(18:2+18:3)/Polyenoic acids	0.6	0.3	0.1	0.1
$\Sigma\omega 3/\Sigma\omega 6$	1.9	3.3	8.2	10.1

* Data quoted from the previous paper⁵¹ about river-caught masu salmon.

** Trace

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