Behavior of Carotenoids in the Chum Salmon *Oncorhynchus keta* During Development*¹

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As part of the life historical study of carotenoids in the chum salmon, the behavior of carotenoids was investigated during development.

The carotenoids which were stored in the eggs during the anadromous migration period, stayed in a constant quantitative (0.677-0.687 mg/100 g) and composition ratio, with a 76-80% astaxanthin rate, until hatching. After hatching, the amount of carotenoids decreased and astaxanthin rate also decreased. In the post-larva stage when the yolk was absorbed, it was reduced to 24%. In the stage of the fry transferred to a river, it was reduced to 6%.

Most (80%) of the carotenoids of the pro-larva just after hatching were in the yolk. As the yolk was absorbed, they were gradually transported to the embryo and stored in the skin of the larva after esterification. When the fry were transferred to a river, the carotenoids were not found in its muscle.

In the post-larva stage, antheraxanthin, lutein and salmoxanthin-like pigment appeared newly. The composition ratio of those carotenoid increased with the growth of the fry.

During the anadromous migration for spawning, salmon transfer a large amount of carotenoids, which consist mainly of astaxanthin, from the muscle to the ovary.¹⁾ The development process of salmon eggs after fertilization is considered to be the change in the half-closing system.²⁾ Although there is transport of water and inorganic salts across the egg membrane, there is no transport of carotenoids.^{3,4)} Therefore it is a very interesting problem in biochemistry to pursue the change of carotenoids accumulated in eggs.

Since, with salmonids, artificial hatching is common and materials on any developmental stage are available, much study has been reported on the carotenoids.⁵⁻⁸⁾ However, detailed study has not yet been reported on the changes in the relative proportions of carotenoids during the chum salmon's development.

The author has investigated carotenoid behavior during development of the chum salmon as part of the life historical study of carotenoids in that one.

Development of the chum salmon were collected at the Hokkaido Salmon Hatchery (Chitose) in 1977.

Analysis of Carotenoids

Carotenoids were extracted with acetone and separated on a MgO column (MgO: celite 545, 1:1) using petroleum ether as developing solvent. Co-chromatography was carried out with silica gel (Wakogel C-200). Thin layer chromatography was carried out with silica gel F plates (Merck).

The identification of carotenoids was carried out by the following methods: behavior of the chromatogram on column and thin layer chromatography, absorption spectra, λ max in various solvents, partition coefficient, detection of epoxide and allylic-hydroxy group, reduction with sodium borohydride, and comparison with authentic standards on TLC.

The amounts of carotenoids were calculated from the E value of absorption maximum. The $E_{1\%}^{1cm}$ value was postulated as 2200. All carotenoids were counted as astaxanthin.

Results and Discussion

The Amount of Carotenoids in Each Stage from Egg to Fry

The amount of carotenoids was examined in

Materials and Methods

Materials

The samples used in the research during de-

*¹ Carotenoid pigments in chum salmon—II.

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Table 1. Changes in the carotenoid contents in egg, embryo and larva of chum salmon

Stage	After fertilization days	Average wt. (g)	Carotenoid (mg/100 g)
Unfertilized egg		0.20	0.687
Fertilized egg	0	0.22	0.681
	15	0.22	0.697
Eyed period	30	0.22	0.704
Just before hatching	57	0.25	0.682
Just after hatching	65	0.26	0.677
Pro-larva			
15 days after hatching	75	0.27	0.457
Just before emergence	100	0.28	0.310
Emergence-larva	115	0.29	0.270
Post-larva			
no feeding	115-130	0.32	0.272
feeding	130-150	0.53	0.208
Fry (transferred to a river)	180-230	1.02	0.183

each stage during development⁹⁾: unfertilized egg, fertilized egg, eye period~hatching, pro-larva, emergence-larva, post-larva, fry, and so forth. The results are shown in Table 1. The amount of carotenoids in the pro-larva of the yolk-absorption stage was measured including the yolk sac. With the post-larva after emergence, the amount of carotenoids of the whole body was measured. The salmon eggs used in this study hatched in 60 days and there was no quantitative change in carotenoid contents observed until the hatching. Since in this period there is no supply of carotenoids from outside, the carotenoids in the egg do not seem to take part in development; they stay unchanged. The behavior of the carotenoids in this period is similar to that of lipids.¹⁰⁾

At the pro-larva stage, 15 days after hatching, the amount of carotenoids was reduced to about 3/5 of that in the fertilized egg. It was reduced to about half just before emergence, 40 days after hatching. It was reduced further as development advanced.

The Amount of Carotenoids during Post-Embryonic Development

Table 2 shows the change in the amount of carotenoids during post-embryonic development in which the yolk was gradually absorbed after hatching. As development advanced, the amount of carotenoids in the pro-larva gradually decreased. The carotenoids which were in the yolk at the beginning were gradually transferred into the embryo as it grew. Since the rate of growth of the embryo is faster than the rate of increase of the carotenoids in the embryo, the process of transferring carotenoids is considered to be the following. First, the chromatophore, the tissue containing the carotenoids, develops in the embryo. Then, the carotenoids are transferred from the yolk to the embryo.

Changes in Absorption Spectra

Fig. 1 shows the change in absorption spectra (in benzene) of crude carotenoids in each stage from the fertilized egg to the fry transferred to a

Table 2. Changes in the carotenoid contents in larva of chum salmon at the period from hatching to emergence

Stage	After fertilization (days)	Carotenoid contents of larva (mg/100 g)	Weight of embryo/larva (%)	Carotenoid contents of embryo/larva (%)
Just before hatching	57	0.682	9.9	8.9
Just after hatching	65	0.677	18.0	19.3
Pro-larva				
15 days after hatching	75	0.457	43.8	38.5
Just before emergence	100	0.310	86.5	77.4
Emergence larva	115	0.270	100.0	100.0

Fig. 1. Changes in the absorption spectra of crude carotenoids in larva of chum salmon in five stages (in benzene).

(1) Yolk of larva newly hatching; (2) Yolk of pro-larva, 15 days after hatching; (3) Embryo of pro-larva, 15 days after hatching; (4) Emergence-larva; (5) Post-larva (no feeding); (6) Post-larva (feeding)

river. The spectra from the fertilized egg to the yolk and embryo just after hatching show a symmetrical bell-shape similar to that of astaxanthin. With the pro-larva, 15 days after hatching, the spectra of both the yolk and embryo changed to have two λ max's (460, 480 nm), the latter (480 nm) had a higher absorbance with the yolk, and the former (460 nm) had a higher absorbance with the embryo. This suggests that carotenoid composition started to differ between the two. With the after-feeding post-larva, an absorption peak appeared at 420 nm as well as at the above two figures. This absorption spectrum is similar to that of salmoxanthin which is a main component of skin carotenoids in the adult salmon.

Changes in the Carotenoids Composition

Since the qualitative and quantitative changes in carotenoids at the time of hatching were ex-

pected, the carotenoids in each stage from fertilized egg to fry were separated by column chromatography and the change in their composition was investigated. The results are shown in Table 3 and Table 4.

1. From the fertilized egg to the eye period

In the 30 days between fertilization and eye period, no qualitative or quantitative change in carotenoids was observed. The ratio of astaxanthin, the main carotenoid component, was constant at around 80%; there was no significant change in other low-quantity carotenoids such as β -doradoxanthin, zeaxanthin, or canthaxanthin.

2. From the hatching to just before emergence

Carotenoid composition in the yolk of pro-larva in this stage was the same as that of the egg, but the ratio of main component, astaxanthin, was gradually reduced as the yolk was absorbed. At the larva stage just before emergence (40 days

Table 3. Changes in the composition of carotenoids in eggs and embryo of chum salmon at the period from unfertilized egg to eyed period

Stage Composition	Unfertilized egg		Fertilized egg	15 days after fertilization	Eyed period
	immature	mature			
Y-pigment	6.6	7.2	9.9	6.9	6.8
Canthaxanthin	0.5	0.9	1.0	1.2	1.0
Zeaxanthin	4.1	2.0	2.7	2.2	3.4
Diatoxanthin	2.7	1.9	2.8	1.6	1.4
Cynthiaxanthin	1.9	1.4	2.1	1.2	0.9
Astaxanthin	77.4	80.5	80.0	80.4	80.6
β -Doradoxanthin	7.1	5.6	6.0	6.1	5.9
Unidentified		0.4	1.9	0.4	0.3
Carotenoid contents (mg/100 g)		0.687	0.681	0.695	0.704

Table 4. Changes in the composition of carotenoids in larva and fry of chum salmon at the period from hatching to fry

Composition	Hatching		Pro-larva		Emergence-larva	Post-larva		Transferred fry to a river
	Yolk sac.	Embryo	Yolk sac.	Embryo		no feeding	feeding	
Y-pigment	6.0		9.9					
Canthaxanthin	1.2	trace	2.3	trace	trace	trace	trace	0.9
Zeaxanthin	3.1	4.6	3.6	8.8	14.4	14.2	10.2	19.9
Diatoxanthin	1.3	2.5	2.9	7.0	14.8	31.6	23.4	23.6
Cynthiaxanthin	1.3	0.9	1.4	3.6	14.8	31.6	23.4	23.6
Astaxanthin	76.6	75.9	72.7	64.5	55.3	23.7	11.3	6.1
β -Doradexanthin	9.9	5.1	7.4	6.7	5.6	6.3	19.8	10.3
Salmoxanthin						3.5	4.6	9.2
Antheraxanthin						5.2	7.3	13.9
Lutein								
Unidentified	trace	trace	trace	8.4	9.3	15.5	23.4	16.0
Carotenoid contents (mg/100 g)	0.680		0.310		0.270	0.272	0.208	0.183

after hatching), 70–80% of the yolk was absorbed and the astaxanthin rate was reduced to about 70%. In the embryo, Y-pigment¹¹⁾ which was found in the egg and yolk was not present. Carotenoid composition in the embryo was the same as in the yolk except for Y-pigment, but astaxanthin rate was lower. As absorption of the yolk advanced, this tendency of astaxanthin reduction became distinct and increase in other xanthophylls, zeaxanthin, diatoxanthin, and cynthiaxanthin, was observed.

3. From the emergence larva to the fry transferred to a river

In the emergence stage, 55 days after hatching, the yolk was completely absorbed and astaxanthin rate was reduced to 55%. Next, in the post-larva stage, 55–77 days after hatching, it was reduced to 24%. When the fry were transferred

to a river, astaxanthin rate was about 6%.

In the post-larva, salmoxanthin-like pigment newly appeared (about 3.5%). This rate gradually increased as larva grew. There were no carotenoids stored in the muscle even with the fry transferred to a river, 120–170 days after hatching, the last stage examined in this study. As the carotenoids transferred from yolk to embryo accumulated not in the muscle but in the skin of the fry, it is worth noting that salmoxanthin-like pigment appears in the skin in the post-larva stage. Antheraxanthin and a small amount of lutein appear in the skin of the post-larva about the same time as salmoxanthin-like pigment. Therefore, the original form of the carotenoid composition in salmon skin during the marine period is considered to be already prepared in the post-larva and fry stages.

Fig. 2. Behavior of the carotenoid in egg, larva and fry of chum salmon during development.

▨: Astaxanthin, □: Other xanthophylls, ■: Y-pigment

Fig. 2 shows rearranged experimental results. According to these results, the amount of carotenoids and the rate of astaxanthin seem to decrease during development in a similar manner. Therefore, it is concluded that the decrease in carotenoids is attributed to the decrease in astaxanthin.

Changes in the Partition Rate

It was clear that chum salmon carotenoids change remarkably both in quantity and quality during development around the time of hatching. Thus, changes of the partition rate of larva carotenoids were examined as part of examination in the qualitative changes during development. The results are shown in Table 5. The carotenoids in the period from fertilized egg to just before hatching were hypophasic and was considered to be in the free state. After hatching, hypophasic degree decreased slightly but the carotenoids stayed in the free state. In the emergence larva stage, 40–55 days after hatching, the yolk has been gradually absorbed, and the carotenoids were eitherphasic. It became epiphasic in the post-larva stage, 55–70 days after hatching, when the yolk was completely absorbed.

From these results, it seems that around hatching time salmon carotenoids are transferred from the yolk to embryo, and that they are stored in the skin of larva after esterification.

Fig. 3 shows the changes in absorption spectra of each phase separated in the partition test. With the hypophasic (1) and (3) around time of hatching, the spectra were similar to that of astaxanthin. With the epiphasic (2) and (4) astaxanthin-like spectra were not observed since amount of ester-type carotenoid was not signi-

Fig. 3. Absorption spectra of carotenoids in partition both phases in larva of chum salmon (in benzene).

(1), (2) Newly hatched larva; (3), (4) Pro-larva, 15 days after hatching; (5), (6) Just before emergence; (7), (8) Just after emergence; (9), (10) Post-larva (no feeding); (11), (12) Post-larva (feeding). Odd-numbered groups: hypophasic; Even-numbered groups: epiphasic

Table 5. Changes in the partition rate of carotenoids in larva of chum salmon

Stage	Epiphasic (%)	Hypophasic (%)	
Embryo			
Just before hatching	8.7	91.3	h
Newly hatched	20.3	79.7	h
Emergence larva			
Just before emergence	56.0	44.0	i
Just after emergence	73.3	26.7	e
Larva			
no feeding	80.4	19.6	e
feeding	80.9	19.1	e

Partition test (PE: 90%MeOH)

h; Hypophasic, i; Eitherphasic, e: Epiphasic

ficant in this stage. With the hypophasic (5) and (7) of the emergence larva, λ max shifted by 5–10 nm toward shorter wavelengths than those of (1) and (3). This suggests that rate of astaxanthin was largely reduced. With the epiphasic (6) and (8), the spectra indicate clearer keto-carotenoid than (2) and (4). In the post-larva stage, the hypophasic (9) of the no-feeding case still shows the spectrum of keto-carotenoid a little, but with (11) of the feeding case, it has disappeared. With the epiphasic (10) and (12), the spectra changed to have two λ max at 450 and 470 nm.

From these changes in the absorption spectra of each phase obtained in the partition test, the author might infer that during development, astaxanthin which was in the free state at the beginning was gradually reduced, and was converted into the other xanthophylls of ester-type.

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References

- 1) K. NISHINO, K. SAITO, and T. KITAHARA: *Sci. Rep., Hokkaido Salmon Hatchery*, **23**, 15-22 (1969).
- 2) J. ISHIDA: *Hasseiseikagaku*, Shokaboh, Tokyo, 1968, pp. 92-100.
- 3) M. GLOVER, R. A. MORTON, and D. G. ROSEN: *Biochem. J.*, **50**, 425-429 (1952).
- 4) Y. YAMAMURA and S. MUTO: *Bull. Tohoku Reg. Fish. Res. Lab.*, **19**, 171-179 (1961).
- 5) M. HARTMANN, F. G. MEDEM, R. KUHN, and H. J. BIELIG: *Z. Naturforsch.*, **2**, 330-343 (1947).
- 6) D. M. STEVEN: *J. Exp. Biol.*, **26**, 295-303 (1948).
- 7) T. W. GOODWIN: *The Comparative Biochemistry of the Carotenoids*, Chapman and Hall LTD., London, 1952, pp. 194-196.
- 8) M. JITARIU, E. CHERA, E. DUCA, G. LINCK, P. ROTIMBERG, and I. SZILAGYI: *Rev. Roum. Biol.*, **20**, 269-274 (1975).
- 9) K. YAMAMOTO, K. OTA, K. TAKANO, and T. ISHIKAWA: *Bull. Japan. Soc. Sci. Fish.*, **31**, 123-132 (1965).
- 10) K. ANDO: *J. Tokyo Univ. Fish.*, **54**, 61-98 (1968).
- 11) T. KITAHARA: *Comp. Biochem. Physiol.*, **76B**, 97-101 (1983).