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Carotenoids in Salmonidae and Their Relation to Reproduction of These Fish

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A.A. Yarzhombek

Carotenoid pigmentation of various tissues of Salmonidae (234) has often attracted attention of many investigators. They have studied qualitative composition of carotenoid pigments in individual species (33, 35, 37, 38, 39), the pigment contents in some tissues of Salmonidae (8, 41, 43), and utilization and metabolism of carotenoids (47, 42). It is believed that vitality of the offspring depends on the amount of carotenoid pigments in the fish eggs (43, 46) and that carotenoids have an important role in the life of fish (23, 28). However, this problem has not yet been studied sufficiently.

A strong carotenoid pigmentation of such important tissues as the ovaries with their membranes, muscles and skin of Salmonidae, which differentiates them from other groups evidently reflects some special forms of relations between Salmonidae and the environment or appears to be due to some peculiarities in the metabolism of Salmonidae. In each instance this is taken as evidence that special physiological mechanisms exist in these fish.

This paper presents results of a study of qualitative composition and dynamics of carotenoids in Salmonidae.

Material and Methods

Qualitative composition and the amount of pigments were studied in eggs of coho salmon (Oncorhynchus kisutch Walbaum), sockeye or red salmon (O. nerka Walbaum) from the Paratunka River basin (Kamchatka), chum salmon (O. keta Walbaum) from the Av'yavayam River (Kamchatka), pink salmon (O. gorbuscha Walbaum) from the island of Sakhalin, Siberian char (Salvelinus leucomaenis Pallas) from Lake Dal'nee (Kamchatka), landlocked and anadromous Dolly Varden char (S. malma Walbaum) from the Dal'naya River (Kamchatka), cutthroat trout * (Salmo clarkii Richardson) from Chernaya Reka farm (Black Sea coast of Caucasus)**, rainbow trout (S. irideus Gibbons) from "Ropshi" (Leningrad region), steelhead salmon (S. gairdneri Richardson) from North America, Sevan trout (S. ischchan Kessler), salmon S. trutta caspius Kessler from the Kura River, Baltic salmon and Atlantic salmon (S. salar Linné) from Kola Peninsula.

* In the Russian text referred to as "rainbow trout" similarly to S. irideus Gibbons. Translator's note.

** On appropriate feeding a red stripe appeared on the lower jaw of males of this trout; it is characteristic of only S. clarkii.

Expenditure of carotenoids during the embryonal period was determined in eggs of Kamchatka coho salmon, Baltic salmon and Sakhalin pink salmon. Eggs of coho salmon developed in Williamson's frame apparatus with spring water supply, eggs of pink salmon developed in flow-through cells and Baltic salmon eggs in cells with standing water which was periodically changed (once each 2 days). (235)

Sakhalin pink salmon at the stage of alevins was studied in the Aquarium Division of VNIRO (All-Union Scientific-Research Institute of Maritime Fisheries and Oceanography). Alevins were kept in flow-through 200-liter tanks at 10°C.

The material for studies of marine period of life was collected in summer (June through August) 1963 in the Pacific Ocean. Samples were taken of skin, muscles, eggs and liver of chum salmon, pink salmon, sockeye, coho, spring salmon (O. tschawytscha Walbaum), Kamchatka salmon (S. penshinensis Pallas), Dolly Varden char and also Siberian char from littoral regions of islands Shikotan and Iturup. Skin of fish in breeding dress was also studied. Skin of sockeye or red salmon, chum salmon, pink salmon and coho was transported from Kamchatka and skin of spawned-out O. masu Brevoont from Sakhalin.

Methods described by Kanemitsu and Aoe (44) were used in the investigation of carotenoid pigments. Carotenoids were identified by absorption spectra which were determined by means of a SF-10 spectrophotometer. Pigment mixtures were separated by column chromatography on calcium oxide (used for isolation of carotene) and aluminum oxide (used for separation of xanthophylls) and by paper chromatography by Sapozhnikov's method (19)

for separating astaxanthin from other xanthophylls.

A part of the material was processed while fresh and another part, from marine and spawning periods of life, was preserved in glycerol and transported to the site of processing. Lipids from the tissue were extracted with acetone. Soft tissues such as muscles, eggs and liver were triturated with anhydrous sodium sulfate but the skin was extracted without comminution.

Results of Investigations

Pigments of mature eggs

Spectra of acetone extracts of Salmonidae eggs (Fig. 1, 2 and 3) can be divided into two groups:

Absorption spectra in the form of smooth curves with a single absorption maximum at wavelength of approximately 480 mμ (Fig. 1),

absorption spectra with more than one inflection on the absorption curve (Fig. 2 and 3).

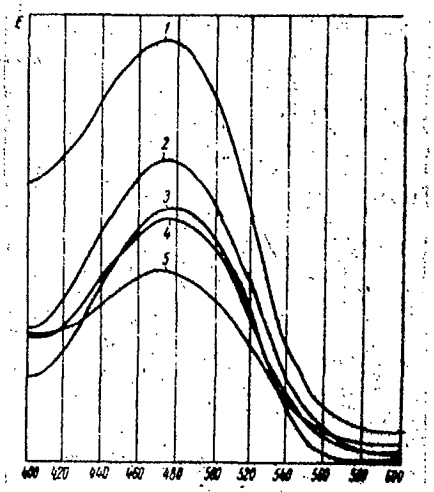
Only one carotenoid pigment is known to have a single absorption maximum at 480 mμ in acetone. It is astaxanthin, found by many investigators in muscles of salmon, trout eggs and shells of many crustaceans. Thus astaxanthin (diketo-dioxy-carotene) appears to be the basic carotenoid pigment (carotenoid) in the eggs of sockeye or red, coho, chum, pink, steelhead and Atlantic salmon.

Absorption spectra with curves which have more than one peak can also be divided into 2 groups:

Spectra with shoulders at 420-425, 445-450 and 475-480 mμ;

spectra with shoulders at 425-430, 450-455 and 480-485 mμ; (236)

The former is characteristic of lutein-dioxy-α-carotene,



Wavelength in mμ

Fig. 1. Absorption spectra of acetone extracts of eggs of various salmonidae:
1 - sockeye or red salmon; 2 - Atlantic salmon from Kola Peninsula; 3 - coho; 4 - chum; 5 - pink salmon.

which has been found by Steven (47) in muscles, eggs and skin of brook trout and the latter of zeaxanthin (dioxy- carotene) as the structural isomer of lutein. Thus, the eggs of Caspian salmon, Baltic salmon and rainbow trout contain lutein but the eggs of char and Sevan trout contain zeaxanthin.

The next stage of the study of qualitative composition of carotenoid pigments in eggs included determination of the presence of other carotenoid components. In many instances, the nature of absorption spectra alone shows evidence of the presence of other components. Thus the presence of astaxanthin could definitively be suspected in the eggs of Baltic and Caspian salmon and in rainbow trout. Paper chromatography according to Professor Sapozhnikova (19) allowed the detection of astaxanthin in eggs of Baltic and Caspian salmon, rainbow trout and Siberian char.

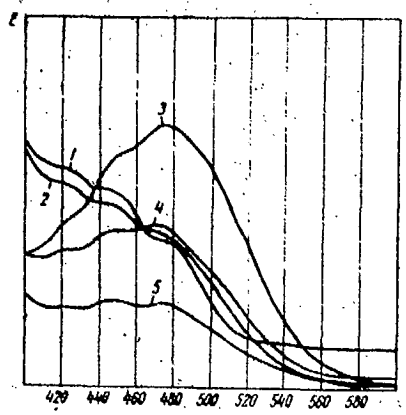
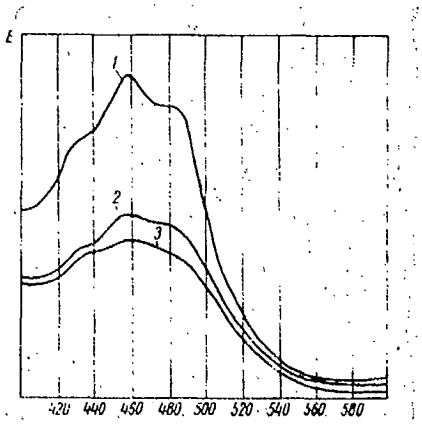


Fig. 2. Absorption spectra of acetone extracts of rainbow trout eggs (1), Caspian salmon eggs (2) and Baltic salmon eggs (3, 4, 5).

When petroleum ether was used to develop the chromatogram, astaxanthin rapidly separated from other carotenoids as a pink band.

Column chromatography was used to determine carotene in fish eggs and to test the homogeneity of xanthophylls. Carotene was not found in any instance. Eggs of the Far-Eastern salmon of the Oncorhynchus genus contained only astaxanthin which sometimes resolved into 2 fractions. Bailey (35) assumed that they were different stereoisomers of astaxanthin. Eggs of Salmonidae contained considerable amounts of steroids in addition to carotenoid pigments. Their amount was especially large in eggs of Caspian salmon, landlocked Dolly Varden char and in trout.



Portions of eggs from various females of Baltic salmon contained astaxanthin and lutein in various ratios. Three portions of the Baltic salmon eggs were studied. They could be called "yellow", "orange" and "red".

Fig. 3. Absorption spectra of acetone extracts of eggs of Dolly Varden char (1), Siberian char (2) and Sevan trout (3).

Table 1

Pigments of mature fish eggs

Species of fish	A*	L*	Z*	Pigment contents mg%
Sockeye				
migratory	+	—	—	8,9—10,4 (9,6)
dwarf	+	—	—	6,65
Coho	+	—	—	6,3—9,2 (7,2)
Chum	+	—	—	1,60—1,76
Pink salmon	+	—	—	0,96—1,23 (1,08)
Atlantic salmon	+	—	—	2,67
Steelhead salmon	+	—	—	2,50
Rainbow trout	+	+	—	0,598
Clark trout	+	+	—	0,768
Salmon				
Baltic	+	+	—	0,426—1,855 (1,03)
Caspian	+	+	—	0,145—0,187
Sevan trout	+	—	+	1,25
Landlocked Siberian char	+	—	+	0,547
Anadromous Dolly Varden char	+	—	+	0,775
Ruff	—	—	+	1,14
Perch	—	—	+	0,05
Sturgeon	—	—	+	0,554
Starred sturgeon	—	—	+	0,024

* A - astaxanthin, L - lutein, Z - zeaxanthin.

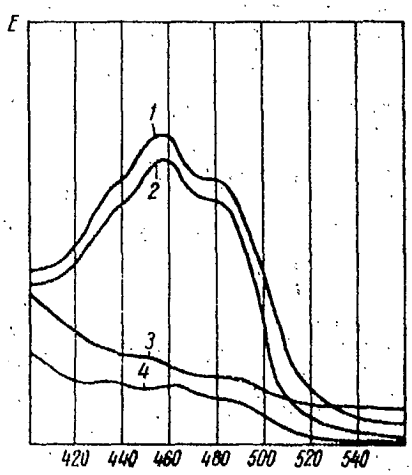


Fig. 4. Absorption spectra of acetone extracts of eggs of ruff (1), sturgeon (2), river perch (3) and starred sturgeon (4)

The red one contained little lutein, the yellow one had about the same contents of each of both pigments and the orange one was intermediate between the yellow and red portions as regards the pigments.

Table 1 presents data on quantitative composition of pigments in mature eggs of fish under study. (237)

A study of eggs of fish of other systematic groups (verbatim - translator's note) proved that

accumulation of carotenoids in fish eggs does not appear to be typical of Salmonidae. Eggs of ruff (Acerina cernua), perch (Perka fluviatilis), sturgeon (Acipenser güldenstädti) and starred sturgeon (A. stellatus) contained the carotenoid zeaxanthin as well (Fig. 4).

Embryonal period

It is known that animals cannot synthesize carotenoids (8). The accumulation of carotenoids in the fish egg cells at the time of ovulation is not completed during embryogenesis. It can only decrease due to utilization or, remain unchanged if the pigments are not consumed or, if they do not break down spontaneously. Therefore, the quantity of the compounds utilized can be the first external evidence of whether the compound participated in the process.

Several experiments were carried out with fish eggs of various Salmonidae. Eggs of Baltic salmon (Daugava River) developed for more than a month in enameled vessels at 5 and 10°C. The experiment showed (Fig. 2) that the consumption of pigments was negligible, only 0.14% a month in eggs incubated at 5°C, but considerably higher at 10°C, that is 3.55% a month. The eggs were incubated at 10°C under various conditions: in darkness and under (237) illumination with 300 or 3000 lux (luminescent lamp BS 30w). Expenditure of pigments was the same in dark and under illumination with 300 lux (3.55% per month) but, several times higher at 3000 lux, that is 20.1% of the initial amount in a month. It is interesting that at stronger illumination, in addition to increased expenditure of carotenoids, hatching of alevins was increased.

Table 2

Expenditure of carotenoids during the embryonal period of development in eggs of salmonidae

Fish	T°C	Illumination in lux	Days of expt.	E_i	E_f	Expenditure of carotenoids *
Baltic salmon	5	0	35	0,634	0,633	0,14
	10	0	35	0,634	0,612	3,55
	10	300	35	0,634	0,612	3,55
	10	3000	35	0,634	0,504	20,10
Pink salmon	10	0	35	0,331	0,307	7,50
Coho salmon	3,5—4,0	0	140	—	—	2,30

E_i - initial absorbance, E_f - final absorbance

* within a month, as percentage of initial amount.

Eggs of pink salmon, obtained as early as at the stage of "byes", were incubated at 10°C in complete darkness. Expenditure of carotenoids was 7.5% per month (Cf. Table 2).

Increased consumption of pigments due to the effects of heat and light on developing eggs suggested that these were non-specific effects of all kinds of radiation energy on carotenoids.

Table 3

Absorbance (E) of carotenoids in pink salmon eggs under the effect of a radioactive element (^{90}Sr)

	Experiment (curie/liter)		
Control	$5 \cdot 10^{-7}$	$5 \cdot 10^{-9}$	$5 \cdot 10^{-11}$
0,730	0,620	0,690	0,650

An experiment was set up in which pink salmon eggs were held in water containing a radioactive element ^{90}Sr. After incubation for 1.5 month, in running water which contained the radioactive compound, the pigment content was lower in control eggs (pure running water) than in experimental hatches (Table 3)*.

Most experiments were set up with developing eggs of coho salmon (80 analyses). This permitted with reasonable assurance conclusions on the expenditure of carotenoids during the embryonal period of the development of coho salmon and the exclusion of fluctuations of molar absorbance of pigments which can occur in developing eggs, as has been mentioned before.* Expenditure of pigments after fertilization through hatching was higher than 10% of the initial amount. Very likely, the utilization of carotenoids in Salmonidae eggs can fluctuate to a considerable extent in various species depending, most of all, on temperature.

Stage of alevins

It is characteristic of the alevin stage that carotenoids pass from yolk into skin. As soon as alevins start active feeding, carotenoids present in the food enter their organisms.

The first lipophores can be detected in the fin plica of coho (239
and steelhead salmon as early as a week after hatching. However, massive transport of carotenoid pigments from the yolk sac into skin in Salmonidae starts at the stage of alevins. Here it is possible to observe phenomena which are important for understanding metabolism of carotenoids in Salmonidae.

First, there do exist specific differences in lipophoric pigmentation of the skin of alevins. There are only yellow cellular pigments called xanthophores in the skin of chum and

pink salmon, red cellular pigments called erythrophores can be found, in addition to xanthophores, in the skin of sockeye or red salmon, coho salmon, rainbow trout and steelhead and Baltic salmon alevins.

Lipophoric pigmentation of the integument was noted in alevins even before commencement of active feeding, and in larger fry when they were starved or were fed rations free of carotenoids. In such instance, there are no doubts about the origin of carotenoids: they come from the yolk sac. Fish, such as Far-Eastern and steelhead salmon with a simple composition of yolk pigments, were of special interest. Only astaxanthin, an orange-red pigment, is present in the yolk of these fish. Appearance of a yellow carotenoid in the skin of alevins of these fish indicated structural changes in the pigment molecule, as well as, biochemical enzymatic mechanisms, which caused such a reaction.

Changes in the quantitative composition of carotenoid pigments in the bodies of pink salmon alevins were observed, particularly, because the red pigment had been completely transformed into a yellow one after its migration into the skin of these fish. Immediately after hatching, alevins were placed into a large tank (200 liters) with running water and maintained at 10°C.

As the pigment accumulated in the skin of alevins, the maxima of absorption spectra at 422, 445 and 475 mμ were more clearly marked; this corresponded to the absorption spectrum of taraxanthin (8). From the relative heights of absorption maxima it can be concluded that the total amount of the pigment in alevins initially decreased, and increased again as alevins started

* Confusing formulations in the original text - translator's note.

to feed on larvae of chironomids. When pink salmon alevins started feeding on oligochaetes, the pigment from their skin finally disappeared. Lutein accumulated in skin of pink salmon alevins which were feeding on chironomids. After some time, lutein replaced the "alevin pigment" taraxanthin.

Alevins of Clark trout hatched in the Chernorechensk Farm (Caucasus) from eggs which were very poor in carotenoids (because of irregular feeding of the breed stock on foods devoid of carotenoids), rapidly acquired signs characteristic of this species, i.e. red edging on fins and the lower jaw, after they had been fed with cyclops.

Stage of fingerlings

The fish can be called fingerlings as long as they are several times smaller than sexually mature fish. The term "stage of fingerlings" has a more specific meaning for migratory salmon, because it partially coincides with the freshwater period of life of sexually immature fish. After their descent in the sea, the fingerlings, as a rule, are rarely available for observation; later on they appear in scientific and commercial catches, but by that time, because of the large size of the fish, the definition of "the fingerling" does not apply.

In this paper the term "the fingerling" will be used (240) when discussing migratory Salmonidae to designate fish, which did not, even once, descend in the sea and when discussing freshwater Salmonidae to designate fish, which did not, even once spawn but have their gonads in the stage of juveniles or "II".

Siberian and pink salmon fingerlings weighed no more than one gram at the time of descending in the sea in their first

year of life. Fingerlings of these species are called the young of the year (0+). Fingerlings of other species of migratory (anadromous) Salmonidae live in fresh water until stage 1+, 2+, 3+, or even 4+ and can achieve a weight of 100 g. Coho salmon fingerlings weighing 300, 400, 500 even 1300 g can be found in Lake Dal'nee. However, this is not typical of salmon. Usually the weight of anadromous salmon (smolt) is 20 to 50 g.

In some species the meaning of "fingerling" becomes complicated, because dwarf sires, which also were called neotenic sires by L.S. Berg (4) do exist. Neotenic forms are known in migratory salmon of the Salmo species (4), char of the Salvelinus species (22), sockeye or red salmon (11), coho (7) and masu salmon (O. masu)(4). Often, the dwarf sires do not differ in dimensions, from large smolt of migratory forms.

Qualitative composition and the amount of carotenoid pigments were studied in fingerlings of pink salmon (in tanks), coho, sockeye or red salmon and char from Lake Dal'nee (Kamchatka). In addition, later on few data will be presented on mature land-locked char - Dolly Varden and Siberian char, of which carotenoid composition of the muscular tissue, ovaries with their membranes and some other organs were studied.

Carotenoids in skin and fins of juveniles

Extracts of bright straw colour were obtained in all instances when fins and skin were extracted with acetone (fingerlings of pink and chum salmon, sockeye or red salmon, coho, Baltic salmon and char). It was impossible to detect a small amount of an orange pigment, astaxanthin, in such extracts only by means of chromatography. Erythrophores can be found easier in skin

by examining fresh skin or skin fixed in glycerol under microscope. Erythrophores in skin of Salmonidae always contain astaxanthin (48); the red pigment in the skin of other fish families can be different in nature, e.g. pterine (8).

Table 4

Erythrophores and xanthophores in skin of Salmonidae fingerlings

Fish species	Xantho-phores	Erythro-phores	Location of erythrophores
Dolly Varden char	+	+	fins, spots
Salmon			
Baltic	+	+	the same
Caspian	+	+	-"-
steelhead	+	+	fins, diffusively on sides
Rainbow trout	+	+	fins
Clark trout	+	+	fins, lower jaw
Coho	+	+	fins, diffusively on sides
Sockeye or red salmon	+	-	-------
Spring salmon	+	-	-------
Chum salmon	+	-	-------
Pink salmon	+	-	-------

Data on the results of studies of skin of fingerlings of various (241) Salmonidae are presented in Table 4.

Presence of astaxanthin in samples of skin which contained erythrophores was proved by paper chromatography. When analyzing absorption spectra of skin extracts, it was proved that the yellow pigment of xanthophores was partially composed of lutein and partially of zeaxanthin isomers. The possibility cannot be excluded that in a number of cases both the closely related pigments are present in skin, but as usual , one of them is predominating.

Table 5

Pigments in skin of juvenile Salmonidae

Fish species	Age	Asta-xanthin	Lutein	Zea-xanthin
Pink salmon	0+	-	+	-
Spring salmon	1+	-	+	-
Sockeye or red salmon . . .	1+ ,2+ ,3+	-	+	-
Coho salmon	0+ ,1+ ,2+	+	+	-
Rainbow trout	2+	+	-	+
Baltic salmon	1+	+	+	-
Dolly Varden char	1+ , 2+	+	-	+

Carotenoid contents were determined in skin of sockeye or red salmon, coho salmon and Dolly Varden char from the Dal'naya River (Kamchatka).

It follows from data in Table 6 that there is more pigments in the skin of young char than in the skin of young Far-Eastern salmon; in addition, there is more pigments in the skin of coho than in the skin of the young of sockeye or red salmon. At the age of 2+ the young of red salmon contained less pigments per square unit of skin than younger sockeye (1+), but, the total pigment (carotenoid) contents in the skin

Table 6

Carotenoid contents in skin of the young of sockeye and coho salmon and Dolly Varden char

Fish species	Age	Weight, g	Pigment concentration, mg / m^2	Total amount, mg	Number of fish under study
Sockeye or red salmon	1+	5,9 (3,3—7,9)	20 (15,0—27,0)	0,042 (0,026—0,055)	6
	2+	61 (49—96)	10,5 (8,0—12,5)	0,159 (0,135—0,192)	4
Coho salmon	1+	2,9 (1,5—5,9)	25,5 (20,0—34,0)	0,025 (0,018—0,037)	9
Dolly Varden char . .	—	5,0 (0,4—14,4)	151 (80—215)	0,259 (0,093—0,816)	6

of larger juveniles (2+) was larger than in small yearlings.

C a r o t e n o i d s i n m u s c l e s o f f i n g e r l i n g s were studied in Far-Eastern salmon (sockeye or red salmon, coho, spring and pink salmon), Dolly Varden char, Baltic salmon and rainbow trout. It must be noted that the meat of fingerlings of a majority of Salmonidae contains only traces of carotenoid pigments. Their meat is white. The origin of yellow coloration of concentrated extracts of fingerling muscles is mainly due to lipids, particularly steroids. Thus, when working with fingerlings, particularly with the small ones, and even when determining the presence of carotenoids, we should resort to column chromatography. Carotenoids are only in (242) traces in the muscles of sockeye or red salmon aged 0+ and 1+, coho 0+, 1+ and 2+, char up to 15 cm long (evidently, aged up to 2+) and rainbow trout weighing up to 250 g (from "Skhodnya" farm). In all fish mentioned the gonads were very small: less than 100 mg in females and several milligrams in males. The presence of carotenoid pigments was clearly marked in muscles: in fingerlings of sockeye or red salmon over 20 g; in rarely found fingerlings of coho weighing more than 100 g; in char weighing 100 g and in Baltic salmon fingerlings of about 20 g.

When studying absorption spectra of acetone extracts of fingerling muscles, it has clearly been established that muscles of sockeye or red salmon and coho contained astaxanthin and muscles of char (brook trout) and Baltic salmon contained yellow xanthophylls. Spectral characteristics of yellow xanthophyll present in muscles of char evidenced zeaxanthin while the xanthophyll with 3 absorption maxima found in salmon and rainbow trout

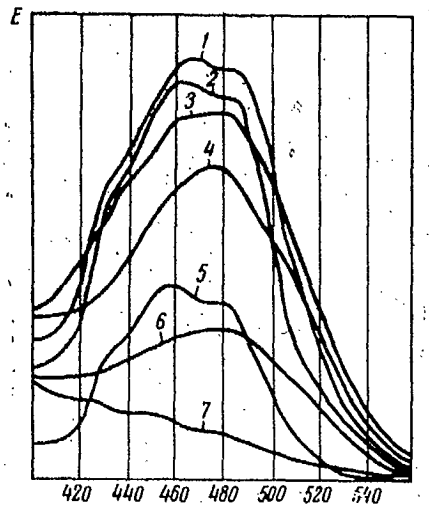


Fig. 5. Absorption spectra of acetone extracts of muscles of Sevan trout (1), Dolly Varden char (2), Siberian char (3), the young of sockeye or red salmon (4), Kamchatka trout (S. mykiss)(5), the young of coho (6), and the young of Baltic salmon (7).

fingerlings was lutein (Fig. 5).

Chromatographic investigation of pigments in coho and sockeye or red salmon indicated that muscles of these salmon did not contain any carotenoids other than astaxanthin. Astaxanthin was detected by gel filtration in extracts of muscles of Dolly Varden char, Baltic salmon and rainbow trout. In addition, analysis of muscles of freshwater Salmonidae such as Siberian char, Kamchatka trout (S. mykiss) and Sevan trout other than fingerling weight and age, revealed zeaxanthin and astaxanthin (Table 7).

Table 7

Qualitative composition of carotenoids in muscles of freshwater Salmonidae and juveniles of anadromous species

Fish species	Astaxanthin	Lutein	Zeaxanthin
Sockeye or red salmon	+	-	-
Coho salmon	+	-	-
Baltic salmon	+	+	-
Rainbow trout	+	+	-
Kamchatka trout	+	-	+
Sevan trout	+	-	+
Dolly Varden char	+	-	+
Siberian char	+	-	+

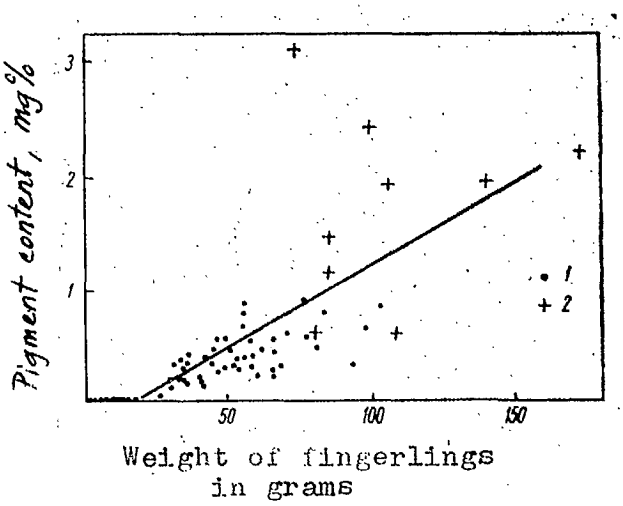


Fig. 6. Astaxanthin contents (concentration) in muscles of the young of sockeye (1) and of "dwarfs" of various weight (2)

Carotenoid contents in muscles of fingerlings were determined in sockeye or red salmon. Juveniles 1 year of age or more were studied. Forty nine fingerlings were analyzed. Astaxanthin contents in their muscles fluctuated from traces (0.000 mg%) in juveniles, weighing 3 to 17 g, to one mg% in large fish in their second or third years of life (80-150 g).

Statistical analysis revealed a linear correlation between the weight of fingerlings and astaxanthin concentration in their muscles. The correlation coefficient was 0.82. It is interesting to compare carotenoid contents in muscles of juveniles and dwarf sires or red salmon from Lake Dal'nee (Fig. 6). Until May of the year of spawning, the carotenoid contents in muscles of the "dwarfs" did not differ from the contents in juveniles of the same weight, but in May the amount of astaxanthin started to increase and in August to September reached up to 3 mg%. (243)

Also were analyzed 5 fingerlings of sockeye or red salmon from Lake Blizhnee (basin of the Paratunka River in Kamchatka), which weighed 3.7, 6.8, 6.9, 7.2 and 13.3 g. We did not succeed in determining the amount of pigment in fingerling muscles, because it was quite small. Nevertheless, we were able to detect by

chromatography the presence of traces of astaxanthin. Fingerlings of sockeye or red salmon from Lake Blizhnee very quickly attained weight of 20 g or more; at that time large amounts of the pigment started to accumulate in their muscles.

C a r o t e n o i d c o n t e n t s i n f i n g e r l i n g o v a r i e s and their membranes were determined in Far-Eastern Salmonidae. Because of the low weight of the ovaries and their membranes in small fingerlings, no data were obtained on the qualitative composition and the amount of pigments present. A reliable quantitative analysis could be carried out with muscles weighing not less than 300 mg. It is necessary, therefore, to combine samples from several fingerlings which were caught at the same time, had the same length, and the same weight of the bodies and gonads. Extracts of ovaries plus their membranes of coho and sockeye or red salmon fingerlings contained only free astaxanthin, which was also found in the ovaries plus their membranes of Siberian char fingerlings. In ovaries and membranes of Dolly Varden and Siberian char fingerlings, zeaxanthin was found in addition (Table 8).

T a b l e 8

Qualitative composition of carotenoid pigments in ovaries plus membranes in juvenile Salmonidae

Fish species	Pigment
Coho salmon	Astaxanthin
Sockeye or red salmon	Astaxanthin
Siberian char	Astaxanthin, zeaxanthin
Dolly Varden char	Zeaxanthin

The study of biochemistry of gonadotropins in juveniles of sockeye or red salmon is connected with the study of problems of dwarf sires. A part of red salmon juveniles matures in some

water reservoirs (particularly, in Lake Dal'nee) without descending in the sea. At the late stages of sexual development the (244)
"dwarfs" can easily be recognized by the outer look of their gonads: they are larger than in juveniles of the same weight. However, the question as to how early the juveniles set themselves for the development in fresh water or for development which is characteristic of migratory fish, remains unsettled. Mature eggs of "dwarf" females are much smaller than mature eggs of migratory salmon. Since an essential point in establishing the stage of sexual maturity in females is the determination of oocyte dimensions, such criteria of maturity are poorly applicable to dwarf females. Differences in biochemical quality of the ovaries plus membranes in red salmon juveniles could be, therefore, expected if there were differences in biological qualities between dwarf fish and juveniles of migratory fish belonging to the same school. Astaxanthin contents were determined in ovaries plus their membranes of juveniles of sockeye or red salmon from Lake Dal'nee and from the Dal'naya River through

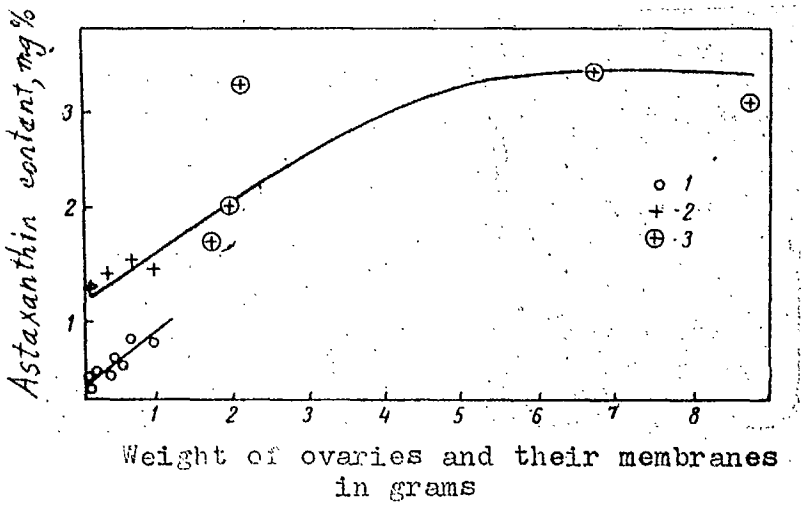


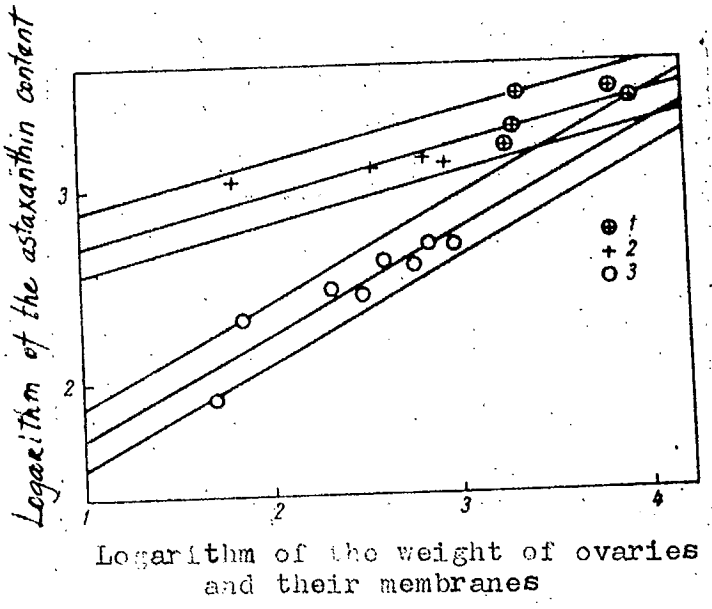
Fig. 7. Astaxanthin contents (concentration) in the ovaries and their membranes of sockeye juveniles:
1 - juveniles with low pigment contents in oocytes;
2 - juveniles with high pigment contents;
3 - dwarf sires.

which the juveniles descend from the lake. In addition, the total content of acetone-soluble lipids (so-called raw fat) was determined to calculate the carotenoid pigment contents in lipochondria, i.e. fatty inclusions in oocytes.

Fluctuations in the astaxanthin contents of red salmon ovaries plus membranes were very large, that is from 0.08 to 3.5 mg%. Figure 7 shows that the data can be divided into 2 groups based on qualitative differences, or more precisely, these data represent two clearly distinguishable groups. One group of such data characterizes a part of juveniles with a low weight of gonads which contain relatively little carotenoid pigments (the weight of gonads was less than 1 g and the pigment contents 0.08 to 0.52 mg%). The other group of fish contained much more astaxanthin in ovaries plus their membranes (1.2-5.5 mg%). Fish, both with small and large gonads, belonged to the latter group. This group appeared as though completed with dwarf sires (Fig. 7).

Plotting the data in logarithmic scales straightens the lines that show dependence of the carotenoid content on the weight of gonads (Fig. 8). This makes it possible to extrapolate the trend of the processes. Data in Figute 8 have proven that the same results in the development of gonads can be achieved in both groups of fish, but the differences are quite large at early stages of gonadogenesis.

To get an idea about the astaxanthin content in ovaries plus their membranes in coho juveniles from Lake Dal'nee, a sample of the ovaries plus membranes pooled from 11 fish, was (245) analyzed. The astaxanthin content in coho juvenile ovaries appeared to be much higher than in the ovaries of sockeye or



Logarithm of the weight of ovaries and their membranes

Fig. 8. Astaxanthin content in ovaries and their membranes of the young of the sockeye and in dwarfs (logarithmic scale): 1 - "dwarfs"; 2 - fingerlings with high pigment contents; 3 - fingerlings with low pigment contents.

Table 9

Astaxanthin content in the ovaries and membranes of coho fingerlings

Weight in grams of fingerlings	grams of gonads	Oocyte diameter	Astaxanthin content in mg%
15-23 (19.6)	0.152	0.3-0.7 (0.5)	3.9

red salmon juveniles (Table 9).

The pigment content (determined as zeaxanthin) and the sum of lipids were determined in Dolly Varden and Siberian char. All char individuals spawned the first times, weighed 280-305 g,

Table 10

Carotenoid contents in the ovaries and their membranes of Dolly Varden char

Weight of gonads, g	Oocyte diameter, mm	Pigment content, mg%	Fat content, %
2,8	0,8—1,3	0,32	2,53
3,4	1,5—1,8	0,46	2,39
3,4	1,8	0,81	2,68
2,9	1,2—1,5	0,08	3,55
2,5	1,1—1,5	0,42	2,13

Table 11

Carotenoid contents in the ovaries and their membranes and in muscles of Siberian char

Weight of		Oocyte diameter, mm	Contents of			Stage of maturity
fish, kg	gonads, g		pigments in ovaries, mg%	fat in ovaries, %	pigments in muscles, mg%	
0,5	6,6	1,0	1,49	4,47	0,59	II
0,2	0,9	0,8	0,50	3,36	0,65	II
0,4	5,0	1,2	0,60	1,77	0,60	II—III
0,7	21,0	1,8	0,35	8,17	0,70	II—III
1,9	45,0	3,0	1,26	7,50	0,73	III

their ovaries plus membranes weighed 2.45-3.40 g and the diameter of oocytes was 0.8-1.8 mm.

As evident from Table 10, the carotenoid content in ovaries and their membranes of Dolly Varden char can fluctuate regardless of the stage of maturity (all fish were at the stage II-III). (246) Later on, several tens of Dolly Varden char specimens were opened and in some of them very pale ovaries, which contained little carotenoids, were found. The colour of Dolly Varden char meat widely fluctuated from quite white to pink-orange.

Siberian char specimens subjected to analysis (Table 11) were of very different weight. Of five fish caught, only one could be called fingerling (it weighed 170 g), while the others weighed 400 to 2000 g. Ovaries contained eggs of various generations but the largest eggs predominated.

The material collected from trout is too small to be used for evaluation of the dynamics of carotenoids in trout

during the sexual cycle.

Carotenoids were not found in the liver of sockeye salmon fingerlings but were found in the liver of char and also in the air bladder of fingerlings.

Marine period of life

Majority of Salmonidae are migratory fish although freshwater species and forms do exist among them. All species of Far-Eastern salmon (Oncorhynchus), with the exception of two Japanese endemic species and the sockeye, coho and masu salmon descend in the sea and live there for one or more years under the conditions of marine salinity. The genus of noble salmon (Salmo) consists of both migratory and freshwater species. Majority of migratory also comprises freshwater forms. The same can be said about the genus Salvelinus.

Qualitative composition of carotenoid pigments

Liver. Acetone extracts were prepared with 4 g samples of liver from sockeye, chum and pink salmon. Spectrophotometry of yellow extracts indicated that large amounts of steroids, absorbing light in the region between ultraviolet and visible parts of the spectrum, were present. Column chromatography showed that carotenoid pigments seldom appeared in the liver of Far-Eastern salmon (traces of some yellow carotenoid were found in 2 of 23 instances).

Skin. Coloration of salmon of the genus Oncorhynchus and Atlantic salmon from Kamchatka (S. penshinensis) is typical of marine pelagic fish; the sides are silvery, the dorsum dark with dark blue tint, and the fins have a neutral gray colour. Differences can be found only in the character of melanization. A study of skin under miscoscope evidenced the presence of

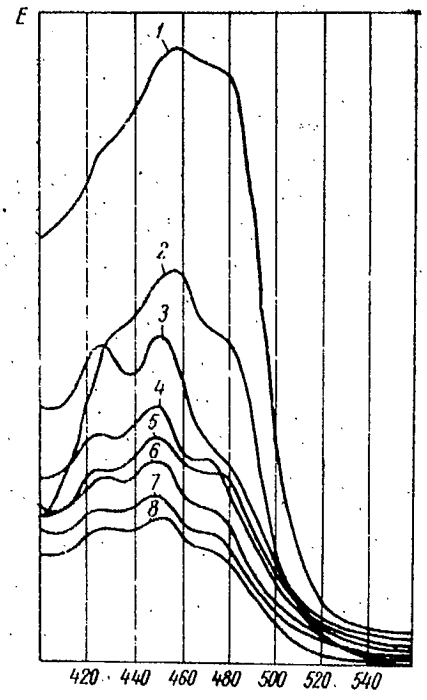


Fig. 9. Absorption spectra of acetone extracts of marine salmonidae skin:
1 -Siberian char; 2 - sexually immature Dolly Varden char; 3 - kelt of Dolly Varden char;
6 - Atlantic salmon from Kamchatka;
4, 5, 7, 8 - various representatives of the Oncorhynchus genus.

xanthophores suggesting that only yellow xanthophylls occurred and astaxanthin was absent in skin. Astaxanthin could (247) be found besides yellow xanthophylls in marine char skin, as it contained erythrophores in addition to xanthophores.

Absorption spectra of acetone extracts of marine salmon skin showed (Fig. 9) that yellow pigments in salmon skin are basically composed of lutein, probably, with a small admixture of violaxanthin. Zeaxanthin was the main carotenoid pigment in the skin of marine char. Large amounts of a pigment which had its absorption spectrum similar to that of mutatochrome (citraxanthin) was present in the skin of spawning char.

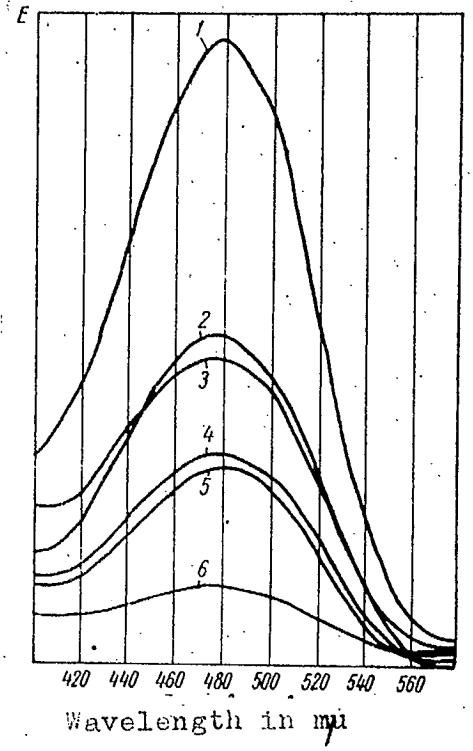


Fig. 10. Absorption spectra of acetone extracts of ovaries and their membranes of salmonidae in the sea:
1 - Atlantic salmon from Kola Peninsula;
2 - Atlantic salmon from Kamchatka;
3 - sockeye or red salmon; 4 - spring salmon; 5 - chum salmon; 6 - pink salmon.

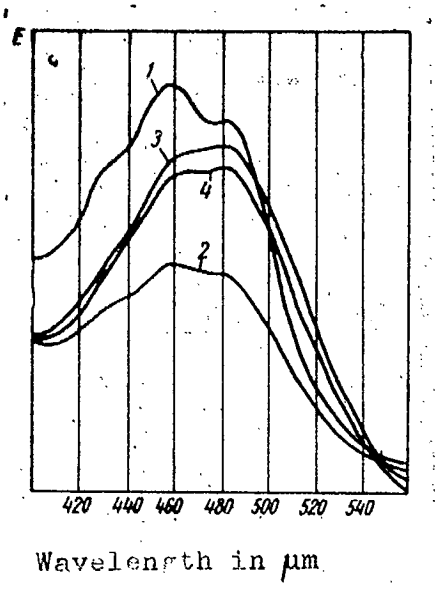


Fig. 11. Absorption spectra of extracts of muscles and ovaries with their membranes of char from the sea.
Ovaries and their membranes of:
1 - Dolly Varden char; 2 - Siberian char;
Muscles of:
3 - Dolly Varden char; 4 - Siberian char.

Muscles of all species under study from the sea had clear orange coloration. Absorption spectra of extracts of muscles from all species of the Oncorhynchus genus and from Kamchatka salmon evidenced presence of astaxanthin (Fig. 10). Zeaxanthin was the main carotenoid pigment in char muscles. Column chromatography showed that astaxanthin was the only carotenoid present in muscles of sockeye, pink, chum, coho, spring and Kamchatka salmon. Astaxanthin was found, in addition to zeaxanthin, in muscles of Dolly Varden and Siberian char.

Ovaries of Salmonidae were rich in carotenoid pigments. It was established by means of spectrophotometric (Fig. 11) and chromatographic methods that the ovaries of Far-Eastern and Kamchatka salmon contained only astaxanthin, the ovaries of Siberian char contained astaxanthin and zeaxanthin, and the ovaries of Dolly Varden char contained zeaxanthin.

Testes of Salmonidae contained small amounts of astaxanthin which was, evidently, located in the linings (tunica vaginalis testis? - translator's note). (248)

C a r o t e n o i d c o n t e n t was studied in muscles of sockeye or red salmon (41 fish), chum salmon (48 fish) and pink salmon, and it was found that astaxanthin content in the muscles of sockeye salmon increased proportionally to the growth of fish which were at the maturity stage II. Astaxanthin content in muscles remained at the same level (as the fish developed - added by translator) from stage II-III to the stage III. In muscles of pink and chum salmon no increase of astaxanthin concentration (contents) was found (Fig. 12).

Carotenoid content in the ovaries and their membranes of marine Salmonidae changed proportionally to maturation of oocytes.

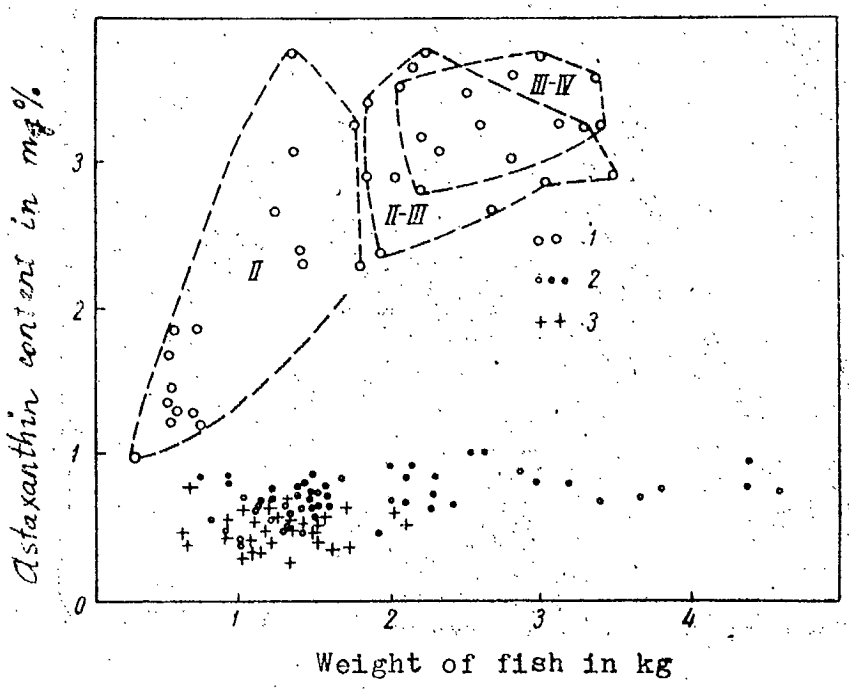


Fig. 12. Astaxanthin content in muscles of Far-Eastern salmonidae during their marine period of life:

1 - sockeye or red salmon; 2 - chum salmon; 3 - pink salmon. (Roman letters denote stages of maturity in the sockeye or red salmon, using a six-point scale).

Table 12

Astaxanthin content in the ovaries and their membranes of coho salmon during their marine period of life

Index	Ovary number:					
	1	2	3	4	5	6
Oocyte diameter in mm	2,4	3,3	3,5	4,2	4,2	4,9
Pigment content in mg%	4,1	3,6	2,5	3,4	2,5	2,1

In pink, chum and sockeye salmon a marked increase of the pigment concentration, depending on the growth of oocytes, was observed (Fig. 13). A decrease of the pigment concentration proportional to the growth of oocytes was observed in the ovaries and their membranes of coho salmon (Table 12). (249)

Carotenoid content in natural food of Salmonidae

Carotenoid which accumulate in Salmonidae, originate exclusively from their food. Qualitative composition and pigment contents were, therefore, studied in some invertebrates on which Salmonidae feed.

Qualitative composition of pigments was studied in zooplankton from several Kamchatka lakes. Paper chromatography of lipids in plankton,

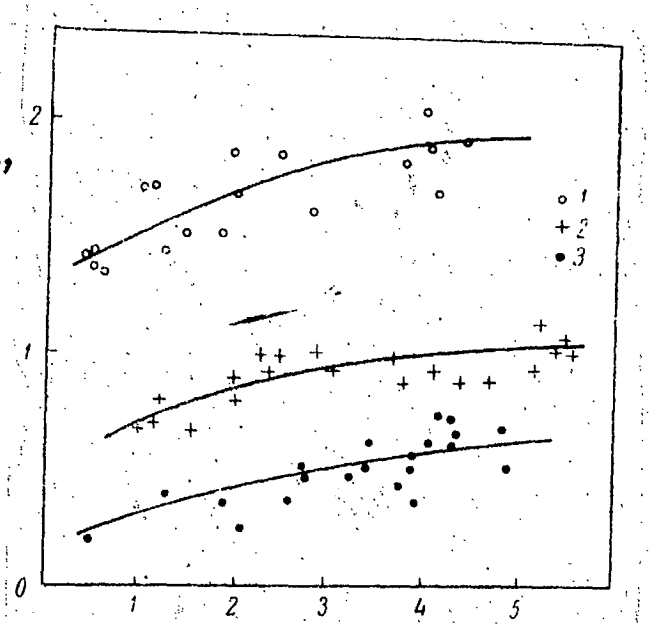


Fig. 13. Astaxanthin content in oocytes of Far-Eastern salmonidae in the sea depending on the diameter of oocytes:
1 - sockeye or red salmon; 2 - chum salmon; 3 - pink salmon.

consisting of Cyclops, Diaptomus and Daphnia species produced three coloured zones; two red zones corresponded to free and esterified astaxanthin and a yellow zone to lutein.

Opossum shrimp (Neomysis intermedia) from areas located directly above the mouths of Kamchatka rivers contained lutein, and various Calanus copepods from the Behring Sea contained only astaxanthin. The latter pigment is also present in euphasiid crustaceans.

Freshwater molluscs of the Linnea species contained lutein (250) and, larvae of chironomids (a mixture of Chironomus plumosus and Ch. tummii) zeaxanthin. Oligochaetes (Enchitreus albidus), which are often used for feeding the young of Salmonidae in hatcheries, contained a very small amount of an unknown pigment when grown on white bread. Several analyses were also carried out with the stomach contents of chum, pink, and sockeye salmon from the Pacific Ocean. Food chips contained plankton crustaceans and pteropod molluscs (quantitative data presented in Table 13).

Table 13

Carotenoid pigment contents in various natural foods of Salmonidae

Material	Qualitative composition	Concentration, mg%	Note
Zooplankton	Astaxanthin, lutein		Lake Dal'nee, 1960:
		3.0	August,
		3.2	September,
		5.0	February,
		11.0	April
Zooplankton	Astaxanthin	128	Lake Zelenoe, Sept. 1960
Zooplankton	Astaxanthin, lutein	35.2	L.Topolovoe, Sept. 1960
Larvae of chironomids	Zeaxanthin	5.87	Districts near Moscow, April 1963
Limnea species	Lutein	1.33	L.Dal'nee, July 1963
Gastric contents in marine salmon	Astaxanthin	4.8	Chum salmon
		3.9	Chum salmon
		5.1	Pink salmon
		6.2	Sockeye salmon
		4.6	Sockeye salmon

Period of spawning

At the time of spawning some Salmonidae undergo considerable changes with regard to carotenoid metabolism. Especially noticeable is the accumulation of carotenoids in the skin of some Salmonidae species. In Far-Eastern Salmonidae the skin becomes heavily colored with carotenoids. Breeding colour caused by carotenoids was also evident in other Salmonidae (genera Salmo and Salvelinus). Freshwater representatives of the genus Salmo do not possess brightly marked breeding dress but, more precisely, are quite brightly colored throughout their entire period of life.

Changes during breeding period of the pigment contents in muscles and ovaries plus their membranes were studied with sockeye or red salmon from Lake Dal'nee as an example. A month after entering the spawning water reservoir until the commencement of spawning, muscular tissue in both males and females decolorized to such extent that the muscles did not contain any carotenoid pigment at all. Ovaries with their membranes became brightly colored at this time and their growth more than doubled. Hence, it followed that a process of mobilizing carotenoid pigments in oocytes had taken place. No accumulation of carotenoids occurred in the testicular fluid.

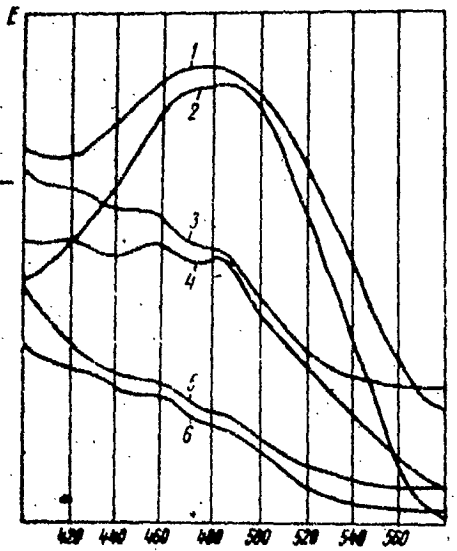
Analyses were carried out on tissues of sockeye salmon caught in the Dal'naya River at the time of upstream migration into Lake Dal'nee, and at the time of spawning which began, on an average, a month after entering the lake. Data on the pigment contents of muscles, ovaries with their membranes, and skin are presented in Table 14.

spectra of the Salmonidae skin (Fig. 14) indicate that astaxanthin (or more exactly its ester, as was shown by a phase assay) is the basic pigment or red parts of the skin. Yellow parts of skin are coloured with one or several pigments which have three absorption maxima ("bands" in the original Russian text - translator's note).

It was proved by absorption spectra of skin extracts that astaxanthin was present in red parts of the skin of anadromous sockeye or red, coho and chum salmon. Yellow parts of chum salmon skin contained lutein. Skin of pink salmon, "dwarf" sockeye and skin on the head of sockeye salmon contained a mixture of yellow pigments (Fig. 15-18).

The following results on epiphasic lipid fractions from the skin of Salmonidae were obtained by chromatography on aluminum oxide columns.

I. Skin of sockeye salmon: a. astaxanthin; on the column the pigment gave several bands and each fraction had the same



Wavelength in mμ

Fig. 14. Absorption spectra of whole skin of salmonidae at spawning:

1 - body skin of the sockeye or red salmon; 2 - red parts of the skin of chum salmon; 3 - "dwarf" sockeye; 4 - coho salmon; 5 - head skin of the sockeye; 6 - yellow parts of the chum salmon skin.

Table 14

Carotenoid contents in the sockeye salmon tissues during spawning period

No.	Muscles, mg%		Skin, mg / m^2		Eggs, mg%
	males	females	males	females	
At the time of heading to spawn					
1	3,40	4,20	14,6	12,2	7,0
2	3,40	4,10	10,3	11,6	6,40
3	3,30	4,00	12,4	9,3	7,0
4	3,24	3,94	11,92	10,2	7,4
5	3,18	3,88	12,6	16,5	7,2
Mean	3,30	4,01	12,0	11,4	7,2
At the time of spawning					
1	Traces	Traces	320	170	10,40
2	»	»	316	168	10,20
3	»	»	224	1444	8,90
4	»	»	280	140	8,94
5	»	»	256	126	9,06
Mean	0,00	0,00	279	149	9,50

It follows from Table 14 that considerable changes in the pigment contents in various tissues occurred at the time when the fish prepared for spawning.

Changes in the pigment contents in tissues of char were of the same nature as in Far-Eastern Salmonidae: the pigment content considerably increased in the skin and was manifested by more intensive coloration of red zones on the body surface and on fins while carotenoids were absent in muscles. However, the picture was not uniform in char: in a part of Dolly Varden char and in Siberian char with sexual products in fluid form, meat of some was completely or partially decolorized. Females

with nondecolorized meat were more frequently found than males.

Studies were conducted on carotenoid pigments in the skin of freshwater salmon such as the sockeye from Lake Dal'nee, coho from the Dal'naya River, chum salmon from the Av'yavayam River, pink salmon from the Ozernaya River, Kamchatka river salmon (Salmo mykiss Walbaum) from the Plotnikovaya River, and char from Lake Dal'nee (Kamchatka). In addition, skin of Sevan trout and Atlantic salmon from Kola Peninsula was analyzed. The variety of breeding coloration in Salmonidae is being explained by the influence of the colour composition of chromatophores. Thus, an olive colour is produced when black and brown melanophores come close to yellow xanthophores. Mixing of melanophores with erythrophores can give various tints of red, scarlet and violet colours. If a considerable contraction of melanophores appears, thus breaking off access of air to the surface of skin, two quite pure colours remain - red and yellow. Red parts of the skin always contain erythrophores and xanthophores, while xanthophores predominate in yellow parts of the body. At the time of spawning the amount of lipophores in skin of Salmonidae is so large, and distributed so thick that one can speak about them as a compact lipophoric layer. Such layer was 5 μ thick in (252
the skin of sockeye and coho salmon. In general, melanophores lie under the lipophoric layer, being similar to a black or brown screen, on the background of which the colour tint of lipophores can vary to a considerable extent.

It is quite clear that two carotenoid pigments, a yellow and a red one, participate to a lesser extent in the formation formation of breeding coloration in Salmonidae. Even absorption

spectra of the Salmonidae skin (Fig. 14) indicate that astaxanthin (or more exactly its ester, as was shown by a phase assay) is the basic pigment or red parts of the skin. Yellow parts of skin are coloured with one or several pigments which have three absorption maxima ("bands" in the original Russian text - translator's note).

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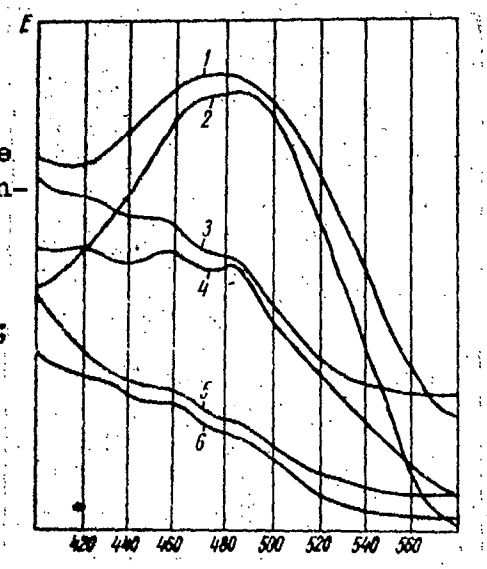


Fig. 14. Absorption spectra of whole skin of salmonidae at spawning:

1 - body skin of the sockeye or red salmon; 2 - red parts of the skin of chum salmon; 3 - "dwarf" sockeye; 4 - coho salmon; 5 - head skin of the sockeye; 6 - yellow parts of the chum salmon skin.

absorption maximum. Most likely, they were various esters of astaxanthin; b. a yellow, rapidly moving fraction; a pigment, which was isolated in a much smaller quantity than astaxanthin, evidently, was lutein. (253

II. Skin of dwarf sockeye contained two fractions in addition to astaxanthin: a. lutein (a rapid fraction) and b. zeaxanthin (a slow fraction, Fig. 15).

III. Skin on the head of sockeye salmon contained two pigments: a. lutein (a rapid fraction, Fig. 15); b. a pigment with absorption spectrum similar to aurochrome (slow fraction).

IV. Skin of the coho salmon. Two orange fractions were obtained (Fig. 16) and, evidently, neither appeared to be a single pigment solution.

V. Chum salmon. Fractions isolated by chromatography of extracts of the red parts of skin were not pure (Fig. 17).

VI. In addition to some astaxanthin, lutein and aurochrome were present in the pink salmon skin.

VII. Zeaxanthin and lutein (Fig. 18) were found in the skin of

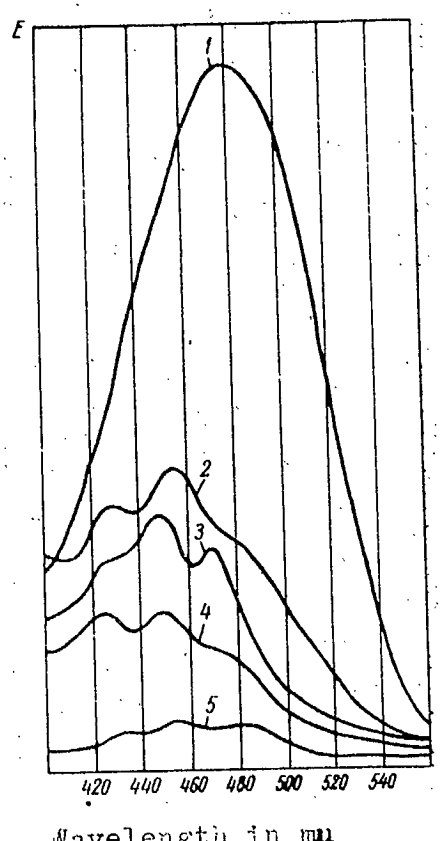


Fig. 15. Absorption spectra of pigments from the skin of spawning sockeye or red salmon:
1 - whole extract of body skin; 2 - whole extract of the skin of head; 3 - lutein from the skin of head; 4 - so-called "aurochrome" of the skin of head; 5 - lutein from the skin of body.

the Kamchatka river salmon.

VIII. In addition to zeaxanthin, astaxanthin was found in the skin of spawning Siberian and Dolly Varden char.

IX. Zeaxanthin and astaxanthin were found in Sevan trout skin.

X. Astaxanthin, lutein and the pigment with a spectrum similar to aurochrome were present in the skin of Atlantic salmon from Kola Peninsula.

Table 15

Qualitative composition of carotenoids in the skin of spawning Salmonidae

Number	Fish species	Pigments
1	Sockeye or red salmon;	Astaxanthin, lutein, aurochrome
2	Sockeye or red salmon dwarfs;	Astaxanthin, lutein, zeaxanthin
3	Coho;	Astaxanthin, neurosporin
4	Chum;	Lutein, astaxanthin, neurosporin
5	Pink salmon;	Lutein, aurochrome, astaxanthin
6	Dolly Varden char;	Zeaxanthin, astaxanthin
7	Siberian char;	Zeaxanthin, astaxanthin
8	Kamchatka trout (S. mykiss);	Lutein, zeaxanthin, astaxanthin
9	Sevan trout;	Zeaxanthin, astaxanthin
10	Atlantic salmon from Kola Peninsula	Astaxanthin, lutein, aurochrome

Since separation of pigments from the chum and coho salmon skin was unsatisfactory, an attempt was made to separate such (254)
pigments by paper chromatography. As a result two fractions were obtained - a yellow and a red one. The red fraction (slow fraction), as anticipated, proved to be astaxanthin, and the yellow one was a pigment not found before in Salmonidae; the first peak of its absorption spectrum was reduced (in size - added

by translator) and the two other were of the same magnitude. Similar absorption spectra are characteristic of carotenoids in fruit - perhydrolycopenes. Evidently, this pigment has pseudo-rings with one or more double bonds hydrogenated (8).

Data on qualitative composition of carotenoids in the skin of Salmonidae at the time of spawning are presented in Table 15.

Discussion of results

Qualitative composition of carotenoid pigmentation in Salmonidae

Astaxanthin, lutein and

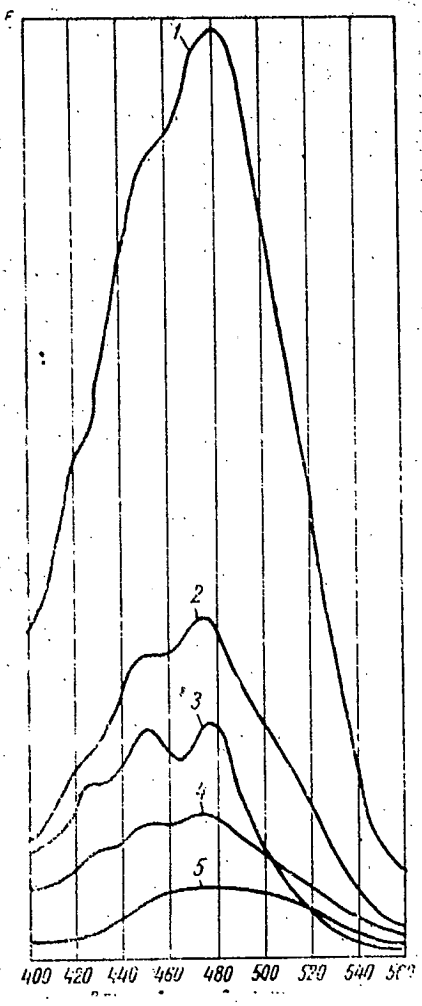


Fig. 16. Absorption spectra of the pigments from the spawning coho salmon skin: 1 - whole extract of body skin; 2, 4 - impure fractions obtained by chromatography on aluminum oxide; 3 - so-called "neurosporin" obtained by paper chromatography; 5 - astaxanthin.

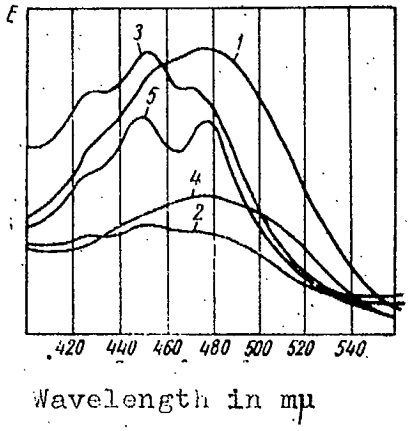


Fig. 17. Absorption spectra of the skin of spawning chum salmon: 1 - whole extract of red parts of the skin; 2 - impure orange fraction obtained by partition on aluminum oxide; 3 - pigments from yellow parts of skin; 4 - astaxanthin; 5 - a "neurosporin"like pigment.

zeaxanthin are basic pigments in Salmonidae; two of them are always present in the organism of each member of Salmonidae. Taraxanthin was found in the skin of alevins and early fingerlings of pink, Baltic and steelhead salmon. Evidently, taraxanthin appears in the skin of alevins of all Salmonidae whose eggs contain astaxanthin. At the breeding time, epoxyderivatives of pigments and pigments similar to hydrogenated lycopenes appeared in the skin.

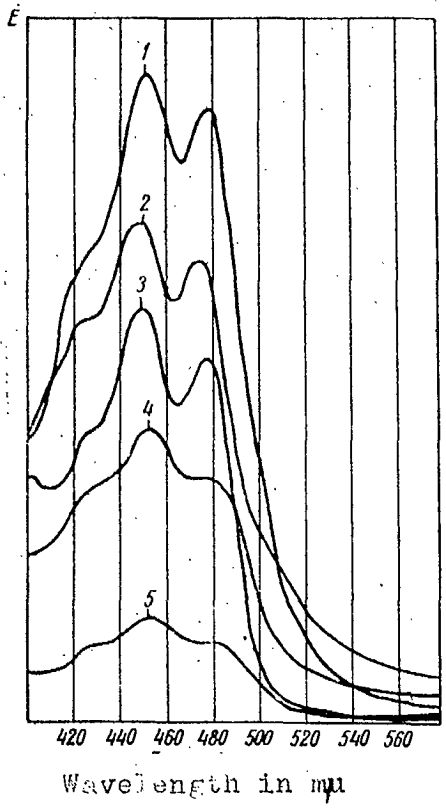


Fig. 18. Absorption spectra of the skin of the Kamchatka trout (S. mykiss) and "dwarf" sires of the sockeye or red salmon:
1 - whole extract of the "dwarf" skin; 2 - lutein from the "dwarf" skin; whole extract of the Kamchatka trout skin; 4, 5 - zeaxanthin from the skin of the Kamchatka trout and "dwarfs".

D.M. Steven (47, 48) and (255)
H. Hartmann (41) emphasized that they found carotene, though in small amounts, in eggs of brown (Salmo trutta trutta) and rainbow trout. Authors of this paper did not find carotene even once in tissues of Salmonidae. Also other researchers (44, 46) did not find carotene in Salmonidae. Obviously, some artifacts, resulting from severe treatment of the pigment during saponification, were considered by Steven and Hartmann to be carotene.

Table 16 presents data on qualitative composition of xanthophylls in Salmonidae under study.

Table 16

Qualitative composition of xanthophylls in salmonidae

Species	Muscles	Eggs	Skin	Skin (during the breeding period)
Salvelinus malma	AZ	Z	ZA	CZA
S. alpinus	A (Z?)			
S. leucomaenis	AZ	ZA	ZA	ZA
Salmo trutta	AL	AL	AL	AL
S. trutta caspius	AL	AL	AL	
S. salar balticus		A L		
S. salar (Scandin.)	A	A		CLA
S. ischchan	AZ	AZ		ZA
S. gairdneri	A	A	A+L	
S. irideus	AL	AL	ZA	
S. clarkii		LA	L	
S. penshynensis	A	A	VL	
S. mykiss	ZA			LZA
Oncorhynchus nerka	A	A	A+LV	ALVZ++
O. keta	A	A	LV	ALN
O. kisutch	A	A	A+LV	ALN
O. gorbuscha	A	A	T+LV	LCA
O. tschawytscha	A	A	LV	A
O. masu	A	A	L	AZ

Note: A - astaxanthin, L - lutein, Z -zeaxanthin, T - taraxanthin, N - a neurosporin-like pigment, C - a pigment similar to citroxanthin (aurochrome), V - violaxanthin.
+ - in the skin of alevins, + + - in the "dwarf" form.

Astaxanthin was found in eggs of all species under study except Dolly Varden char. In addition to astaxanthin, lutein was found in eggs of rainbow and brown trout and Baltic and Caspian salmon, and zeaxanthin in eggs of Dolly Varden and Siberian char and Sevan trout.

Astaxanthin was found in muscles of all species under study. Lutein and zeaxanthin were present together with astaxanthin in muscles of those fish which contained these pigments in eggs.

Astaxanthin was not found in the skin of fish at the time when they had typical coloration of pelagic fish and lead a pelagic way of life (that is in Salmonidae of the genera Oncorhynchus and Salmo at their marine period of life), in the young of sockeye salmon inhabitating the pelagic parts of lakes, and in the young of spring salmon. These fish contain only a yellow pigment in their skin; basically it appears to be lutein (fingerling and marine stages of life in the sockeye, coho, chum, pink and Caspian salmon, fingerlings of Baltic salmon and marine Atlantic salmon from Kamchatka). In char, rainbow trout and (256)
Sevan trout the yellow pigment in skin was zeaxanthin.

Both lutein and zeaxanthin were found together in the skin of dwarf sockeye and also in the Kamchatka river salmon (S. mykiss) at the breeding time.

Astaxanthin was found in skin of benthic and brook fish (Dolly Varden char, brown and rainbow trout, Kamchatka river salmon, Sevan trout), in the young of coho salmon, in alevins and fry of sockeye salmon, as well as in skin of all Salmonidae at the spawning time.

In addition to zeaxanthin, a pigment similar to aurochrome (which was found in the skin of head in sockeye and in the skin of spawning pink salmon), participated in forming breeding coloration in Salmonidae *. Moreover, this pigment was also found in the skin of Dolly Varden kelt (a fish descending in the sea after spawning). Evidently, this pigment - a furanoid - is formed by oxidation of lutein, zeaxanthin or astaxanthin at the time when the breeding coloration fades.

*It is not clear from the original text whether it was the pigment under study or aurochrome, which was found in the fish mentioned - translator's note.

The yellow pigment, present in red parts of skin in spawning coho and chum salmon, appeared to be a product of partial break-down of lutein or astaxanthin. Absorption spectrum of this pigment was the same as that of hydrolycopenes ("gidrorolikopinov" in the original text evidently misspelled - translator's note).

Carotenoids are always present in the skin in the form of esters of fatty acids, i.e. they appear always as oxycarotenoids and xanthophylls).

Qualitative composition of pigments in muscles and ovaries with their membranes (eggs) does not change in Salmonidae during their entire length of life. The same qualitative composition can be found even in such groups of fish that lead a different way of life, as long as they belong to the same species. Thus, the same composition of pigments in muscles and oocytes occurs in migrating and freshwater forms of Dolly Varden and Siberian char, in migrating and freshwater (dwarf) sockeye, in brook trout and anadromous Caspian salmon.

Relations between carotenoids in food and body

It was demonstrated long ago that carotenoids in fish originate from food (8). Fish lose pigments after some time , if their food does not contain carotenoids. Feeding fish, which have lost their pigments, with food rich in carotenoids, restored the characteristic pigmentation. Steven (47, 48) in his experiments, using qualitative data, showed that carotenoid composition of food affected the ratio of lutein to astaxanthin in the bodies of brown trout. Trout maintained on a diet free of carotenoids were almost not pigmented. When such trout were fed food rich in

astaxanthin, normal amounts of astaxanthin and lutein accumulated in their bodies within 35 days. Thus, the reaction

astaxanthin ⟶ lutein

proceeded without difficulties in Salmonidae.

Supplementation of food with carotene did not result in the restoration of normal contents of xanthophylls, i.e., there does not exist any mechanism in the organisms of Salmonidae accomplishing the reaction

carotene ⟶ xanthophylls.

Supplementation of food with lutein caused its increase in the bodies, but did not induce accumulation of astaxanthin. This means that the reaction

lutein ⟶ astaxanthin

is not possible in the bodies of Salmonidae. Supplementation of food with astacin did not cause accumulation of lutein and astaxanthin. Hirao (42) reported that synthetic apo-2-carotenal cannot replace carotenoids in the food of rainbow trout.

Results of experiments presented in this paper give (257)
evidence that lutein accumulated in pink salmon fingerlings which were supplied with food that contained zeaxanthin (larvae of chironomids). This means that in Salmonidae, the reaction

zeaxanthin ⟶ lutein

proceeds without difficulties. The reaction is based on displacement of a double bond in the ionone group. Accumulation of taraxanthin in the skin of pink and steelhead salmon alevins, while utilizing the yolk sac that contained exclusively astaxanthin, indicated that a biochemical mechanism catalyzing the

reaction

astaxanthin ⟶ taraxanthin

does exist.

For this reason, the reactions of converting astaxanthin into lutein, taraxanthin, furanoid derivatives and pigments with pseudo-rings can be assumed as possible in Salmonidae. Lutein and zeaxanthin can be substituted mutually but they cannot be transformed into astaxanthin, i.e. the displacement of carboxyl and carbonyl groups in the ionone sections of carotenoids is not possible in Salmonidae; only the cleavage of a carbonyl group from the molecule is possible. The foregoing statements indicate that astaxanthin is an "essential" compound for Salmonidae.

The main source of astaxanthin for fish are crustaceans. Astaxanthin was found in many freshwater as well as marine crustacenas (8, 37, 45). Astaxanthin was also detected in some insects, particularly in locusts (8). It is remarkable that Kamchatka river salmon (S. mykiss), feeding mainly on benthos (molluscs and insect larvae) and fish (Gasterosteus, Pungitius and Salvelinus species), contain very little astaxanthin, but the sockeye salmon, feeding mainly on crustaceans, accumulate mainly astaxanthin. Generally, presence of one of the yellow pigments, that is zeaxanthin or lutein, in muscles and ovaries with their membranes is characteristic of river fish such as trout and char. This indicates that the species adapt to accumulate the pigment that is most available.

Deficit of carotenoids in food, e.g. when artificially

rearing fish, leads to their decreased contents in various organs and tissues. S. Murayama and M. Yanase (46) showed that rainbow trout from fish farms contained little carotenoid pigments, particularly astaxanthin, in their eggs. Evidently, the food of trout in these farms contained little astaxanthin. Obviously, a deficiency of astaxanthin in food in farms that rear trout, char and salmon, leads not only to a formation of sexual products of unsatisfactory value (as will be shown later) but even to incomplete exploitation of the growth potential by fish. Supplementing food with (carotenoid - added by translator) ingredients favorably affected the Salmonidae. Good results were obtained when shrimp flour (36) was added to the food for bull trout (Salvelinus lepechini). Weight indices in trout improved when fed with crustaceans (a food rich in astaxanthin) as compared with control trout which were fed fish and meat.

Feeding of Salmonidae in fresh water has been studied quite well (7, 8, 11, 13, 14). In agreement with published data, char feed on benthos (larvae of insects and molluscs), aerial insects and fish. They consume quite large amounts of fish eggs at the time when Salmonidae and stickleback (Gasterosteidae) are spawning. Also crustaceans present in stomachs of swallowed can serve as a source of astaxanthin for char, in addition to plankton and benthic crustaceans, which appear seldom in char rations. A study of deficiency of yellow pigments in char was unnecessary, because such pigments are present in sufficient amounts in molluscs and larvae of insects.

The food of salmon fingerlings in rivers practically does not contain astaxanthin but only interchangeable yellow

pigments; however, salmon fingerlings in rivers do not usually reach such dimensions at which astaxanthin starts to accumulate in their muscles and ovaries with membranes. Freshwater forms of fish and large fingerlings appear, particularly, in species of Far-Eastern salmon, which spend their fingerling stage in lakes rich in zooplankton. Perhaps, protracted residence of these species (sockeye, coho and masu salmon) in fresh water is possible, particularly, because their food is rich in astaxanthin.

Carotenoid dynamics in the bodies of Salmonidae during ontogenesis

In contrast to statements made by M. Glover et al.(39), our experiments indicated that during the embryonal period of development, utilization of carotenoids amounted to several percent of their initial contents in the eggs. Moreover, it was found that the consumption of carotenoids in eggs depended on the conditions of their development. (Increased - added by translator) temperature, illumination and radioactivity increased the consumption of carotenoids in a time unit. This phenomenon was connected with an accelerated rate of the development of embryos. Nonspecific action of various kinds of radiation energy on egg pigments gave evidence that similar, if not even the same, effects (of radiation energy - added by translator) may influence the eggs. Yu. B. Kudryamov et al. (12) explained the breakdown of carotenoids irradiated in a lipid solution by indirect effects of free radicals formed in the lipids. Evidently, the same mechanisms affect the acceleration of many developmental processes and, consequently, the rate of embryonal development in general. It is not yet clear whether carotenoid play a role of "extinguishers"

of free radicals or, whether they are the source for their formation.

Utilization of carotenoids continued after the hatch of alevins. It is not known whether consumption of carotenoids has any physiological significance at that time or, whether it appears to be a natural breakdown of relatively unstable compounds due to the effects of light.

It was possible to establish that the young of fish preserved a certain, sufficiently stable, concentration of carotenoids in skin provided that their food contained sufficient amounts of carotenoids. Steroids, which also are yellow but of another tint, started to accumulate in xanthophores, if the food contained quite a small amount of carotenoids. The colour of pink salmon fingerlings which had fed on carotenoid-deficient food (oligochaetes), was unnatural, that is lettuce-green.

Viable, carotenoid-free trout obtained by D.M. Steven (48) cannot be considered fully valuable for life under natural conditions, because it is known that unnaturally coloured organisms fall prey to predators first (49). No serious attention has been paid to carotenoid diet in the young fish in fish hatcheries. Only traces of carotenoids were found in fingerlings of the Caspian salmon from the Chimkent fish farm, and in trout fingerlings from the fish farm in Chernaya Reka. However, the unnaturally coloured young of fish are not fully valuable. Fish usually have various coloration irrespective various periods of life. The smallest fingerlings of the sockeye (below 0.5 g) contain astaxanthin in their skin, larger fingerlings (yearlings) contain less pigments in their skin than the young of the year. This is

connected with the fact that the young of the sockeye live in the littoral parts of lakes for some time after leaving the redd, and later become pelagic fish. The young of the coho salmon are more firmly bound to littoral areas and brooks and have a typical parr coloration; this is not true for the lake sockeye. Char, whose skin contains even more carotenoids, are bound to the water-reservoir bottom even firmer than the young of the coho. (259)

Carotenoid contents in skin of Salmonidae at their marine period of life are about the same as in the pelagic sockeye. Hence, it follows that the functional role of pigments in skin is the same, that is, to produce masking coloration. Hirao et al. (42) studied carotenoid pigments in the skin of 26 species of marine fish and found that absorption spectra of skin pigments were identical with transmission spectra of sea water. This gives pelagic fish an impression of natural gray coloration, when viewed under water.

Appearance of carotenoid pigments in muscles, their accumulation up to the maximum and disappearance at the time of spawning, were investigated in several species of Salmonidae. Astaxanthin started to accumulate in the muscles of sockeye juveniles when they reached about 20 g of weight and continued to accumulate proportionally to the weight increase until the maturity stage II-III of their gonads. Later on, astaxanthin content (concentration) remained constant in themuscular tissue until the beginning of the spawning period, when carotenoids from muscles were mobilized into ovaries and their membranes and skin. There are no reasons to assume that different conformity with the dynamics of carotenoids in fish muscles could be

found in other species of Salmonidae. Unfortunately, it was not possible to catch and analyze chum, pink and coho salmon weighing between 20 and 500 g, when astaxanthin concentration increased in their muscles. Individuals of these fish species caught already possessed maximum concentration of pigments in their muscles.

The dynamics of carotenoids in muscles of other fish species were studied in less details. Insufficient material on Dolly Varden and Siberian char allow us to judge only the maximum pigment concentration in muscles, but, because there is very little pigments in muscles of char juveniles, it can be assumed that the nature of their accumulation is the same as in the sockeye salmon muscles. This has also been shown by Steven's data on the dynamics of carotenoids in trout muscles (47).

When the fish prepared for spawning, carotenoids in many Salmonidae were transported partially or completely into ovaries and their membranes and in skin. Without exception, complete discoloration of meat was observed in all representatives of the Oncorhynchus genus. In char the transport of carotenoids from the muscles into skin and eggs, evidently, proceeded to the extent required.

Accumulation of carotenoids in oocytes of the species under study proceeded to various extents. Xanthophyll content in mature oocytes of various species fluctuated from a fraction of a mg% to 10 mg%. Dynamics of the accumulation process was different in various groups. In freshwater species such as the Siberain and Dolly Varden char and brown trout, no large fluctuations in the pigment concentration in oocytes were observed; even if some differences

did occur , it was impossible to explain them in terms of the degree of maturity of sexual products.

The pigment accumulation in oocytes of migratory Far-Eastern Salmonidae is quite a complicated problem. The general feature of all species under study (sockeye, coho, chum and pink salmon), is an increased concentration of astaxanthin during the breeding period. There also are common features in the nature of the pigment accumulation in the early stages of gonad development.

An increase of pigment concentration in the ovaries and their membranes was observed in sockeye, chum and pink salmon during their marine period of life; in the coho, on the other hand, assessing within the limitations of small amount of material, (260) the pigment concentration rather decreased at that time.

Balance of carotenoid accumulation in bodies of the sockeye salmon during ontogenesis from fertilized eggs to spawning (Fig. 19) can be calculated from the data obtained. The calculation was carried out for hypothetic fish which had lived for three years in fresh water and for three years in the sea. Sockeye salmon with such a life cycle appear very seldom, because fish, which had lived for three years in fresh water, spend a year in the sea, while fish descending in the sea at the age of 1 to 2 years, live in the sea for 3 years. However, such an assumption permitted to show the relations between pigments in the bodies of fingerlings of various growth groups and to separate the marine fish into groups according to maturity stages (Fig. 19). The sockeye salmon usually passed through stages II and II-III during the first 1 to 2 years of life in the sea and the stage

III and higher , in the last year of their life. Fig. 19 illustrates the role of muscles as "a repository" of pigments. The total amount of carotenoids in the sockeye salmon bodies

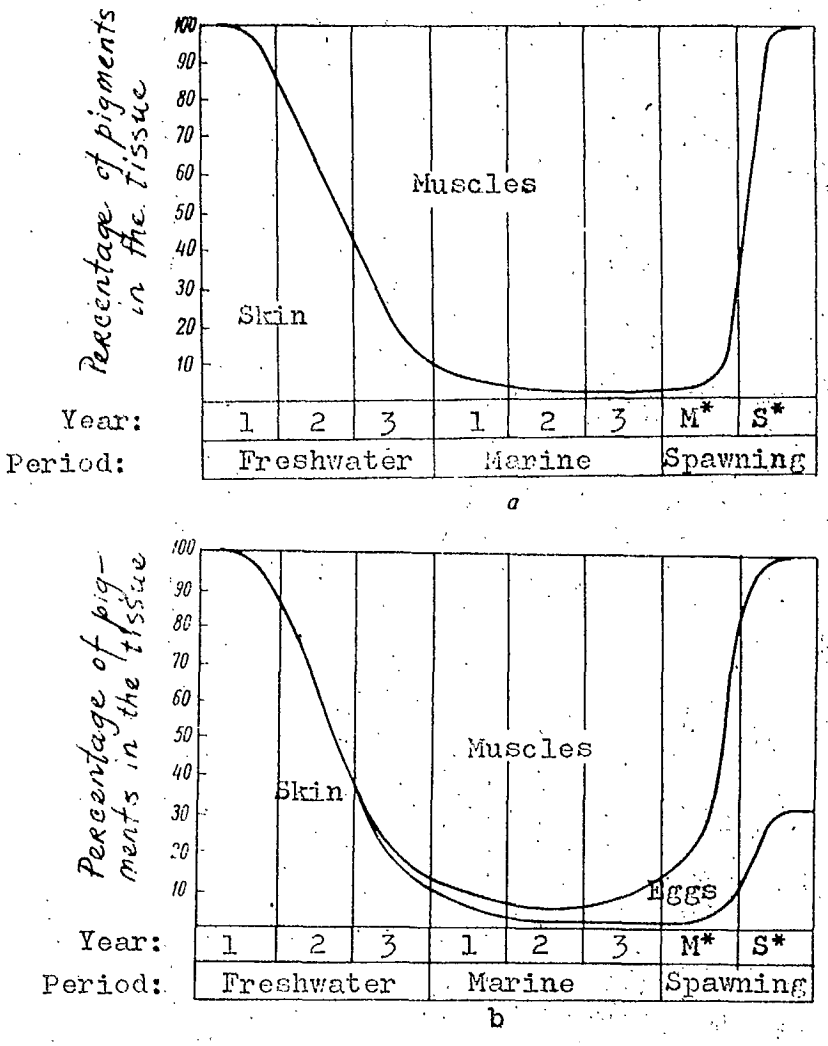


Fig. 19. Distribution of carotenoids in the bodies of males (a) and females (b) of sockeye or red salmon during their lifetime.
M* - "upstream migration",
S* - spawning.

increased several thousand times in the course of life as compared with the carotenoid contents in the eggs. After the absorption of egg yolk, all carotenoids concentrated in the skin. When the fish attained 20 g of weight, the carotenoid pigments started to accumulate in muscles. The portion of carotenoids (261)

in muscles increased until the end of the marine period of life and, at the time of entering rivers, it reached 0.9. in females (probably printer's error - translator's note) and as much as 98% in males. Carotenoid contents (portion) in the ovaries and their membranes appeared considerably high in the last year of life in the sea, when sexual organs intensively grew, and reached a maximum of 68% at the time of spawning. This gives rise to an impression that the residues (truly , they are quite considerable) of pigments, that did not participate in the composition of eggs in females, were transported into skin. In male salmon carotenoids were transported into skin completely.

Functional importance of carotenoid pigments

Since carotenoids accumulated in muscles and were transferred into eggs and skin when the fish prepared for spawning, it follows that the muscles were the place where these compounds were deposited. It was not yet proven whether carotenoids play any metabolic role in muscles. The muscles do not function as the "repository" of carotenoids in all fish. This role is played by fish liver of the Perciformes order. In some Gobriidae very large amounts or carotenoids accumulate in the liver, coloring this organ brightly orange. It was also noted (45) that in lumpfish (Cyclopterus lumpus) carotenoids are transferred from the liver into other tissues. In some Salmonidae the liver, in addition to muscles, is the place where carotenoids concentrate. Carotenoids were found by the author of this paper in the liver of char, by B. von Euler (38) in the liver of noble salmon, and by Steven in the liver of trout (48). Liver of all Far-Eastern Salmonidae very seldom contain even traces of carotenoids and do

not appear to be the site of their regular accumulation.

Carotenoids are found in muscles in the form of stable lipoprotein complexes. They are transported into other organs and tissues by blood in the form of lipoid micelles, stabilized with β-globulin.

For the greater part of life of fish the function of carotenoids in skin is, obviously, to participate in the formation of protective coloration, i.e. the carotenoids have a physical function. The question of the function in coloration during breeding is more complicated. A.I. Smirnov (24) showed that the antikeratinizing role of carotenoids promotes regeneration of skin during the breeding period. Skin becomes loosened, scales partially resorbed and sunken in the depth of skin. Such a state of skin accelerates gas exchange in skin, particularly respiration. Smirnov's assumption (24) that carotenoids in the skin of spawning salmon stimulate respiration (oxygen uptake) by activating molecular oxygen through the mechanism of peroxides was not proven because no peroxide compounds were found in the skin of spawning salmon (33).

Concerning the role of carotenoids in oocytes, T. Gudvin (8) postulated that the presence of carotenoids in a free, unesterified forms appears illusive, especially from the viewpoint of metabolic and not physical functions in developing eggs. The process of accumulating carotenoids in immature oocytes sets in gradually and assumes some metabolic roles in developing oocytes during maturation process in ovaries. It has been proven (34) that the peaks of astaxanthin accumulation in functional elements of ovaries, that is in lipochondria, coincided with (262)

the time of enhanced nitrogen metabolism, and, consequently, with loading of the internal medium of the organism with nitrogen-containing metabolites. A break on the curve, expressing carotenoid accumulation in oocyte lipochondria, indicates that there was

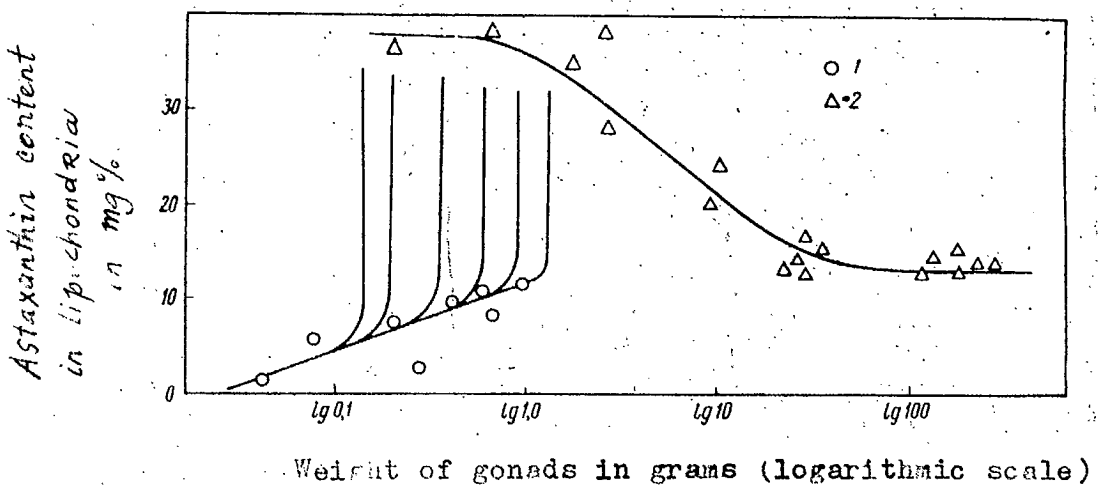


Fig. 20. Astaxanthin content in lipochondria of oocytes in sockeye or red salmon during the freshwater (1) (fingerling) and marine (2) periods of life.

a sharp rise in the astaxanthin accumulation in lipochondria immediately after the descent of fish in the sea (Fig. 20). Metabolic changes due to effects of fluctuating salinity occurred in fish (1). Accumulation of toxic nitrogen-containing metabolites was also observed in the organisms after entering fresh water for spawning (16); at this time a pre-spawning rise in astaxanthin accumulation in oocytes was observed.

The peak of carotenoid concentration in eggs at spawning was present in all migratory salmon species under study. This leads to a hypothesis that the role of carotenoids in oocytes is connected with an increased resistance of some important oocyte structures to poisoning due to nitrogen-containing

metabolites. This is also evidenced by the results of experiments with developing salmon eggs (33). It was proven (32) that the coho eggs, being richer in pigments, were more resistant to the effect of endogeneous ammonia than the rainbow trout eggs (Fig. 21). It can be assumed that free xanthophylls take part in an emergency cycle of ammonia detoxication at the time when basic systems (e.g. the ornithine cycle) are overloaded.

The fluidity stage V is characterized by the presence of oocytes in the body cavity, unconnected to the blood circulatory system. Evidently, this is responsible for pre-spawning poisoning in completely mature eggs, and can lead to their irreversible overmaturation (5). It is, particularly, the poisoning of eggs that explains the striking decrease of the level of cellular respiration in oocytes before discharge (egg deposition).

Before deposition of eggs, the maximum of carotenoid concentration was observed both in oocytes and in oocyte lipochondria. Obviously, the role of carotenoids lies in diminishing intoxication of oocytes at stage V. This alone can explain a higher percentage of fertilized eggs in rainbow trout which contain more carotenoid pigments (46).

The amount of carotenoid pigments correlates quite (263)
closely with the flow rate of water in speawning grounds of Salmonidae, i.e. also in their redds. Water flow rate in spawning grounds of several Salmonidae species was determined by Krobkin and Krogius (11a), and Vasil'ev and Yurovitskii (6). Combining their data, and comparing them with data on the astaxanthin contents in eggs, which were characteristic of certain

species made possible to pick a line of pink, chum, coho and sockeye salmon with inverse relationship between the pigment contents and water flow rates on spawning grounds (Table 17)

T a b l e 17

Dependence of pigmentation of Salmonidae eggs on the water flow rate on spawning grounds

Species	Flow rate (m sec.)	Astaxanthin content (mg%)
Pink salmon	0.3 - 1.2	1.2
Chum salmon	0.1 - 0.9	1.65
Coho salmon	0.2 - 0.3	6
Sockeye or red salmon . . .	0.1	9

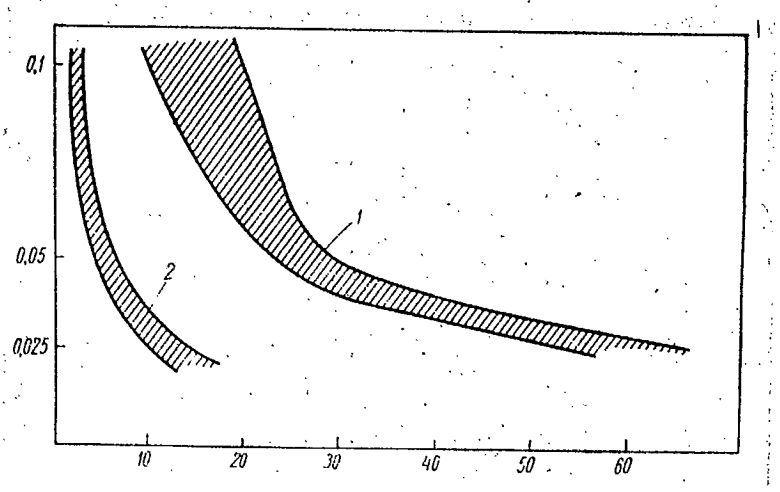


Fig. 21. Dependence of the time of occurrence of death to embryos of coho (1) and rainbow trout (2) on ammonia concentration in water.

An interesting viewpoint concerning functional importance of carotenoid pigments was published by Smirnov (23) and Soin (25, 26, 27) who noted that the intensity of carotenoid pigmentation of various fish correlated with the conditions under which their eggs developed. The main emphasis was laid

on the availability of oxygen. The authors concluded that the carotenoids in eggs seemed to have a function to stimulate consumption of oxygen at its low level in water. In this connection, Smirnov (23) introduced the following chemical schemes:

carotenoid + O_2 —— peroxide —— carotenoid + 2 O (1)

carotene + O_2 —— xanthophyll (2)

However, presence of carotenoids of hydrocarbon nature in fish eggs is quite questionable (in Russian: carotenoids hydrocarbons - translator's note) and, in addition, at the present time, equation (2) is considered impossible. In agreement with (264) recent studies (20), hydroxyls and carbonyls of xanthophylls are not capable of participating in the metabolism of oxygen. At the present time the following systems (18), in which carotenoids participate in oxidation, are considered possible:

violaxanthin —— anteraxanthin,

zeaxanthin —— anteraxanthin —— lutein,

violaxanthin —— anteraxanthin —— zeaxanthin.

A carotenoid epoxide appears to be an indispensable member of each system, mentioned above. Only oxygen of the epoxide and furanoid groups can be liberated and, thus, participate in oxidation processes. Nevertheless , carotenoid epoxides have not been found in eggs of Salmonidae.

Also, several objections could be raised to Model 1, suggested by Smirnov. It is known that peroxides of polyenes decompose with simultaneous breakdown of the carbon chain of the compound. In such event, if carotenoids would participate in oxidation, their expenditure should considerably be higher than observed. Carotenoids would be rapidly spent because the breakdown

reaction of carotenoids is irreversible in living organisms. In addition, also, other objections (33) were raised against a "peroxide" scheme of carotenoid participation in the respiration of eggs. Basically, the objections are as follows: decomposition of peroxide derivatives of polyenes is catalyzed by peroxidases which were found only in protoplasmic structures of developing eggs, where carotenoids are not present; while, peroxidases are absent in yolk which contains carotenoids. Carotenoids alone do not indicate to possess peroxidase activity in living organisms.

Results of Hartmann's experiments (41), which were set up to prove that astaxanthin in trout eggs stimulates taxis and mobility of spermatozoids, though promising, were refuted by Goodwing (40).

Antihistamine properties of carotenoids, perhaps, are important in numbing (deadening) the sensitivity of pain-sensing and tactile receptors in skin at the time of difficult migration to the spawning grounds, preparation of redd, and impetuous spawning period.

Carotenoid pigments, probably, play some role in the counteraction against developing fungal flora on skin, since general and unpigmented fish, if injured, became covered with Saprolegnia much more than the fish in breeding dress.

As regards the role of carotenoid pigments as an element of breeding coloration, one even cannot completely exclude its function of protective coloration. Although Salmonidae are coloured very brightly at the time of spawning, it should not be forgotten that they spawn in fall, close to the banks covered with grass and trees which also are brightly coloured. Reflection of

fall flora in turbulent water makes brightly coloured fish difficult to distinguish. Furthermore, some fish, e.g. the coho, usually hide under the banks when chased.

Besides, it is not known how red and yellow colours are perceived by the eyes of spawning salmon. It is quite possible that the red coloration simply replaces black melanophoric pigmentation.

The assumption, which has existed for a long time, that harmful metabolites are accumulated in the skin of spawning salmon, is not baseless. When studying the skin of spawning salmon, foul smelling compounds were noted upon boiling alcoholic extracts of skin. Such phenomenon was not observed in experiments with the skin of fish free of breeding changes. It can be assumed that, at the spawning time, the skin is the place where degradation (265) products of protein metabolism are bound; carotenoids play a role in absorbing such products.

Conclusions

1. Qualitative composition of carotenoid pigments in muscles and ovaries of Salmonidae is constant throughout their life.

2. Qualitative composition of carotenoids in the skin, changes during the life cycle and is different at the stages of alevins, fingerlings and the marine and spawning periods.

3. Various species (of Salmonidae - added by translator) often differ in their qualitative composition of carotenoid pigments in muscles, ovaries and skin.

4. Three xanthophylls were found in muscles and ovaries: astaxanthin, lutein and zeaxanthin. Astaxanthin alone or in combinations with lutein and zeaxanthin can be present in muscles and ovaries of Salmonidae.

5. Besides lutein, zeaxanthin and astaxanthin, the following pigment derivatives appeared in the skin of Salmonidae at certain periods of life: taraxanthin, violaxanthin, a pigment similar to aurochrome and neurosporin.

6. Muscles were the site where carotenoids were deposited in all Salmonidae, and the liver, in addition, were such site in representatives of the Salmo and Salvelinus genera.

7. Presence of carotenoids in fine structures such as the air bladder and tunica vaginalis testis (testicular lining) can be connected with antikeratinizing properties of carotenoids.

8. Presence of carotenoids in oocytes is most probably connected with their resistance to harmful effects of endogeneous and exogeneous amines.

9. Presence of carotenoids in skin is connected with phototropic reactions (coloration) of the organism and with antikeratinizing properties of carotenoids, participating in pre-spawning changes in skin.

10. Astaxanthin appears to be an "essential" compound for Salmonidae. It cannot be synthesized in the organism from other compounds, even of quite similar composition, and must be administered with food. Other xanthophylls present in the organisms of Salmonidae, can be synthesized from astaxanthin and, to a small extent, by interconversion from each other.

11. Eggs which contain more carotenoid pigments, give a higher percentage of fertilization and are more resistant to deficient water flow rates, and hence, to the resulting increase of the metabolite contents in water.

12. Carotenoid pigments must be present in the rations of the young of Salmonidae. Food rich in xanthophylls consists of marine and many freshwater crustaceans, larvae of chironomids, and eggs of benthic fish.

13. In fish farms raising trout, great attention should be paid to the carotenoid contents in the rations of the breeding herd. Food for females must contain astaxanthin.

Bibliography

1. A k u l i n , V.N., B a k s h t a n s k i i , E.L., Y a r - z h o m b e k , A.A. Spetsificheskoe deistvie na obmen veshchestv u lososei izmeneniya solenosti vody. Sbornik nauchno-tekhnicheskoi informatsii VNIRO, No. 11, 1964. (Specific effects of changed salinity of water on the metabolism in Salmonidae. Collection of scientific and technical information, All-Union Scientific Research Institute of Maritime Fisheries and Oceanography).

2. A n d r i e v s k a y a , L.D. Pitanie tikhookeanskikh lososei v severo-zapadnoi chasti Tikhogo okeana. Materialy po biologii morskogo perioda zhizni dal'nevostochnykh lososei. Izd-vo AN SSSR, 1958. (Nutrition of Pacific Salmonidae in the North-Western part of the Pacific Ocean. Materials on Biology of the Marine Period of Life in Far-Eastern Salmonidae. Publishing House of the Academy of Sciences of the USSR).

3. B a l a k h o v s k i i , S.D. Antigistaminy i ikh terapevticheskoe znachenie. "Vrachebnoe delo", 1958, No. 6 (Antihistamines and their therapeutic significance. "Medical Affairs", 1958, No. 6). (266)

4. B e r g , L.S. O proiskhozhdenii forelei i drugikh presnovodnykh lososevykh. Sb. Pamyati S.A. Zernova. Izd-vo AN SSSR, 1948. (On the origin of trout and other freshwater Salmonidae. Collection of papers in Commemoration of S.A. Zernov. Publishing House of the Academy of Sciences of the USSR, 1948).

5. B r a s h e , Zh. Biokhimicheskaya embriologiya, IL (Izdatel'stvo innostrannoi literatury), 1961. (Biochemical embryology, Publishing House of Foreign Literature, 1961).

6. V a s i l ' e v , I.S., Y u r o v i t s k i i , Yu.G. Kislorodnye usloviya razvitiya amurskoi letnei kety i gorbushi. "Zoologicheskii zhurnal", T. 33, Vyp. 6, 1954. (Oxygen conditions under which chum and pink salmon develop in summer in the Amur River. "Zoological Journal", vol. 33, No. 6, 1954).

7. G r i b a n o v , V.I. Kizhuch. Biologicheskii ocherk. Izvestiya TINRO (Tikhookeanskii nauchno-issledovatel'skii institut morskogo rybnogo khozyaistva i okeanografii), T. 28, 1948. (The coho. A biological review. Transactions of the Pacific Scientific Research Institute of Maritime Fisheries and Oceanography, vol. 28, 1948).

8. G u d v i n , T. Sravnitel'naya biokhimiya karotinoidov . IL (cf. Reference 5.) 1954. (Comparative biochemistry of carotenoids).

9. K a z a r n o v s k i i , M. Ya. Pitanie pokatnoi molodi gorbushi i mal'my v rekakh Sakhalina. "Rybnoe khozyaistvo", 1965, No. 6 . (Feeding of anadromous fingerlings of pink salmon and Dolly Varden char in Sakhalin rivers. "Fish farming", 1965, No. 6).

10. K o z l o v a , L.L., Y a r z h o m b e k , A.A. Ammiak - produkt zhiznedeyatel'nosti ikry foreli. Sbornik nauchno-tekhnicheskoi informatsii VNIRO (cf. Reference 1.),No. 5, 1965.(Ammonia - a metabolic product in trout eggs).

11. K r o k h i n , E.M., K r o g i u s , F.V. Ozernaya forma krasnoi iz Kronotskogo ozera na Kamchatke. DAN SSSR, T. 4, No. 2, 1936. (Lake form of the sockeye from Lake Kronotskoe in Kamchatka. Doklady of the Academy of Sciences of the USSR, vol. 4, No. 2, 1936).

11a. K r o k h i n , E.M., K r o g i u s , F.V. Ocherk basseina r. Bol'shoi i nerestilishch lososevykh, raspolozhennykh v nem. Izvestiya TINRO (cf. Reference 7.), T. 28, 1937. (An essay on the basin of the Bol'shaya River and spawning grounds of Salmonidae located in that basin. vol. 28, 1937).

12. K u d r y a s h o v , Yu.B., B e l b e r d y z , L e D y k - L ' e u . O vozmozhnosti nepryamogo effekta ioniziruyushchei radiatsii v lipoidnykh rastvorakh. Radioliz karotina v oleinovoi kislote. DAN SSSR (cf. Reference 11.), T. 156, No. 1, 1964. (On a possibility of indirect effects of ionizing radiation in lipid solutions. Radiolysis of carotene in oleic acid. Vol. 156, No. 1, 1964).

13. K u r e n k o v , I.I. Kormovaya baza molodi lososei vo

vnutrennykh vodoemakh Kamchatki. Lososevoe khozyaistvo Dal'nego Vostoka. Izd-vo AN SSSR (cf. Reference 2.), 1964. (Food base of the young of Salmonidae in the Kamchatka inland water reservoirs. Salmon fisheries of the Far East).

14. L e v a n i d o v , V.D., L e v a n i d o v a , I.M. Pitanie molodi kety i gorbushi v protokakh Amura. Izvestiya TINRO (cf. Reference 7.), T. 45, 1957. (Feeding of the young of chum and pink salmon in channels of the Amur River. Vol. 45, 1957).

15. L u z h i n , B.P., B a r a m z i n , N.A. O znachenii protochnosti vody pri inkubatsii ikry foreli gegarkuni. Izvestiya AN (Akademii Nauk) Kirgizskoi SSR. Seriya biologicheskaya. T. 3. Vyp. 1, 1961. (On the importance of water flow-rate on incubation of "gegarkuni" eggs. Transactions of the Academy of Sciences of Kirghiz SSR. Biological series, vol. 3, No. 1, 1961).

16. L y s a y a , I.M. Ob izmenenii sostava krovi lososei v period nerestovykh migratsii. Izvestiya TINRO (cf. Reference 7.), T. 35, 1951. (On the changes of composition of blood in Salmonidae during spawning migration. Vol. 35, 1951).

17. P r i v o l ' n e v , T.I. Vliyanie povyshennogo partsial'nogo davleniya kisloroda na dykhanie embrionov ryb. Izvestiya VNIORKh (Vsesoyuznyii nauchno-issledovatel'skii institut ozernogo i rechnogo rybnogo khozyaistva) T. 23, 1949. (Effect of increased partial pressure of oxygen on the respiration in fish embryos. Transactions of the All-Union Scientific Research Institute of Lake and River Fish Farming, vol. 23, 1949).

18. S a a k o v , V.S. O roli karotinoidov i mekhanizme perenosa kisloroda fotosinteza. DAN SSSR (cf. Reference 11.) T. 155, No. 5, 1964.(On the role of carotenoids in oxygen transport during photosynthesis, vol. 155, no. 5, 1964).

19. S a p o z h n i k o v , D.I., B r o n s h t e i n , I.A., P o p o v a , O.F. Primenenie metoda bumazhnoi khromatografii dlya analizov pigmentov zelenogo lista. "Fiziologiya rastenii", T. 20. Vyp. 3, 1956.

*A fast-growing form of Sevan trout (Salmo ishchchan). Translator's note.

(Application of a paper chromatographic method on the analysis of pigments in green leaves."Plant Physiology", vol. 20, No. 3, 1956).

20. S a p o z h n i k o v , D.I., A l k h a z o v , D.G., E i d e l ' m a n , Z.M., B o z h a n o v a , N.V., L e m b e r g , I.N., M a s l o v a , T.G., G i r s h i n , A.B., P o p o v a , I.A., S a a k o v , V.S., P o p o v a , O.F., S h i r y a e v , G.A. Ob uchastii ksantofillov v perenose kisloroda v protsesse fotosinteza. DAN SSSR (cf. Reference 11.), T. 154, No. 4, 1964. (On participation of xanthophylls in the transport of oxygen in photosynthetic processes. Vol. 154, No. 4, (1964).

21. S e m k o , R.S. O biotsenoticheskikh vzaimootnosheniyakh tikhookeanskikh lososei i gol'tsov v nerestovo-vyrostnykh uchastkakh r. Bol'shoi. "Zoologicheskii zhurnal". T. 27. Vyp. 1, 1948. (On biocenological relations in Pacific Salmonidae and Salvelinus trouts in sections of the Bol'shaya River where the fish spawn and grow. "Zoological journal", vol. 27, No. 1, 1948).

22. S a v v a i t o v a , K.A. K sistematike gol'tsov roda Salvelinus (Salmonidae) iz basseina Vostochno-Sibirskogo morya. Nauchnye doklady Vysshei shkoly. Biologicheskie nauki. No. 2, 1961. (On taxonomy of trouts of the genus Salvelinus (Salmonidae) from the basin of the East-Siberian Sea. University scientific reports. Biological sciences. No. 2, 1961).

23. S m i r n o v , A.I. Znachenie karotinoidnoi pigmentatsii embrional'noi i lichinochnoi stadii karpovykh ryb. DAN SSSR (cf. Reference 11.). T. 73, No. 3, 1950. (Significance of carotenoid pigmentation during embryonal stage and in fry of Cyprinidae. Vol. 73, No. 3, 1950).

24. S m i r n o v , A.I. Funktsional'noe znachenie predneresto-vykh izmenenii pokrovov lososei. "Zoologicheskii zhurnal". T. 33. Vyp. 5, 1959. (Functional significance of pre-spawning changes in the coating of Salmonidae. "Zoological

Journal", vol. 33, No. 5, 1959).

25. S o i n , S.G. O dykhatel'nom znachenii karotinoidnogo pigmenta v ikre lososevykh ryb i drugikh predstavitelei otryada Clupeiformes. "Zoologicheskii zhurnal". T. 35. Vyp. 9, 1956. (On respiratory significance of carotenoid pigments in eggs of Salmonidae and other representatives of the order Clupeiformes. "Zoological Journal", vol. 35, No. 9, 1956).

26. S o i n , S.G. Embrional'nye prisposobleniya k dykhaniyu u ryb i osobennosti ikh razvitiya u baikal'skikh podkamenshchikov. "Voprosy ikhtiologii". T. 2. Vyp. 1, 1960. (Embryonal adaptations to respiration in fish and their peculiarities in the development of Baikal bullheads (Cottidae). "Problems of Ichthiology", vol. 2, No. 1, 1960).

27. S o i n , S.G. Embrional'no-lichinochnye prisposobleniya morskikh igl i morskikh sobachek. Trudy Novorossiiskoi biologicheskoi stantsii. T. 73, 1961. (Embryonal and larval adaptations in marine pipefish (Syngnathus) and blennies (Blenniidae). Transactions of the Biological Station in Novorossiisk. Vol. 73, 1961).

28. S o i n , S.G. Adaptivnye osobennosti stroeniya i razvitiya ikry i zarodyshei ryb, sposobstvuyushchie uluchsheniyu dykhaniya. "Vestnik MGU"(Moskovskii Gosudarstvennyi Universitet), Seriya VI, No. 1, 1964.(Adaptational peculiarities of the formation and development of eggs and embryos in fish, which improve respiration. "Bulletin of the Moscow State University", Series VI, No. 1, 1964).

29. S y n k o v a , M.I. O pitanii tikhookeanskikh lososei v kamchatskikh vodakh. Izvestiya TINRO (cf. Reference 7.), T. 34, 1951. (On feeding of Pacific Salmonidae in waters off Kamchatka. Vol. 34, 1951).

30. T r o i t s k i i , T.B. Elektroforeticheskoe izuchenie fiziko-khimicheskoi svyazi belkov syvorotki krovi s vitaminom A i karotinom. "Biokhimiya". T. 15. Vyp. 6, 1950. (Electrophoretic studies of relations between blood serum proteins, vitamin A and carotene. "Biochemistry". Vol. 15, No. 6, 1950).

31. T r o i t s k i i , T.V. Produkty avtooksidatsii vitamina A. "Biokhimiya". T. 15. Vyp. 6, 1950. (Products of vitamin A autooxidation. "Biochemistry", vol. 15, No. 6, 1950).

32. Y a r z h o m b e k , A.A. Razlichnaya stepen' toksichnosti ammiaka dlya embrionov kizhucha i raduzhnoi foreli. Sbornik nauchno-tekhnicheskoi informatsii VNIRO (cf. Reference 1.), No. 5, 1964. (Various degrees of ammonia toxicity against coho and rainbow trout embryos).

33. Y a r z h o m b e k , A.A., G r a c h e v , L.E. Materialy k poznaniyu funktsional'nogo znacheniya karotinoidov lososevykh ryb. "Voprosy ikhtiologii". T. 4. Vyp. 2 (31), 1964. (Contribution to the knowledge of functional importance of carotenoids for Salmonidae. "Problems of Ichthiology". Vol. 4, No. 2(31), 1964). (267

34. Y a r z h o m b e k , A.A. Dinamika zhira i astaksantina v gonadakh lososei. "Voprosy ikhtiologii". T. 6. Vyp. 1, 1966. (Dynamics of fat and astaxanthin in gonads of Salmonidae. "Problems of Ichthiology", vol. 6, No. 1, 1966).

35. B a i l e y B. E. The Pigments of Salmon. J. Biol. Bd, Canada, № 3, 1937.
36. B o u t h i l l i e r L. P. The Influence of Various Diets on Trout Growth and Pigmentation. Progr. Fish-Cult. v. 23, № 4, 1961.
37. E m m e r i e A. M., M. v a n E c k e l e n, J o s e f y B., W o l f L. Salmon Acid, a Carotenoid from Salmon. Acta. Br. neerl. № 4, 1934.
38. E u l e r B., E u l e r H. Liver Oil from Fish and Bird. Svensk kem. Tidskr, № 43, 1931.
39. G l o v e r M., M o r t o n R., R o s e n G. Astaxanthin, Cholesterol and Lipids in Developing Salmon Eggs. Bioch. J. V. 50, № 3, 1951.
40. G o o d w i n T. W. Carotenoids in Fish. Biochem. Soc. Symp, № 6, 1951.
41. H a r t m a n n H., M e d e n F., K u h n R., B i e l i g H. Untersuchungen über die Befruchtungsstoffe der Regenbogenforelle. Z. Naturforsch. Bd. 2, H 9/10, 1947..
42. H i r a o Sh., Y a m a d a I., K i k u c h i R. Carotenoids in Fish. Bull. Tokai Reg. Fish. Res. Lab. № 16, 1957.
43. H u b b s C., S t a v e n h a g e n J. Effect of Maternal Carotenoid Deficiency on Viability of Darter Offspring. Phys. Zool. 31, 1958.
44. K a n e m i t s u T., A o e H. The Studies of Carotenoids of the Salmon. Bull. Jap. Soc. Sci. Fish. V. 24, N 6, 1958.
45. L e d e r e r E. Recherches sur les carotenoides des invertebres. Bull. Soc. Chim. Biol. V. 20, 1938.
46. M y r a y a m a S., Y a n a s e M. The Amounts of Chemical Constituents of Eyed Eggs of Rainbow Trout from Various Sources. Bull. Tokai. Reg. Fish. Res. Lab. № 31, 1961.
47. S t e v e n D. M. Carotenoid Pigmentation of Trout. Nature, 160, 1947.
48. S t e v e n D. M. Carotenoids in the Reproductive Cycle of the Brown Trout. J. Exp. Biol. V. 26, N 3, 1949.
49. W i s l y W. I. Survival Behaviour. Sea front. V. 10, № 1, 1964.