ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XIX Fasc. 1 Szczecin 1989

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Biochemistry

STUDIES ON CAROTENOIDS IN SPAWNING *SALMO TRUTTA MORPHA LACUSTRIS* L.*

BADANIA KAROTENOIDOW U OSOBNIKÓW *SALMO TRUTTA MORPHA LACUSTRIS* L. W OKRESIE TARŁA*

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The occurence and contents of various carotenoids in different body parts of spawning Salmo trutta morpha lacustris from the Lake Wdzydze were studied with column and thin layer chromatography.

INTRODUCTION

Due to their body pigmentation, salmonids have for a long time attracted of researchers interested in carotenoids in fish (see Simpson et al., 1981). The studies dealt mainly with the species of the genus *Salmo*, particularly the salmon, *Salmo salar* L. (Glover et al., 1962; Jarząbek, 1970; Czeczuga, 1975; Schiedt et al., 1981; Craik and Harvey, 1986; Skrede and Storebakken, 1986). nother representative of the genus is the trout, *Salmo trutta* L., occuring in some Polish waters.

Three biological varieties of trout are known from the Polish inlad waters. Under natural conditions, the varieties differ somewhat in their habitat preferences , body shape and pigmentation as well as in biological characteris-

* Part 47 in te series "Carotenoids in fish"

tics (Brylińska, 1986). The varieties are: *Salmo trutta m. trutta* L., *Salmo trutta m. fario* L., and *Salmo trutta m. lacustris* L., the latter still present in some lakes within the drainage of upper reaches of rivers Wda and Brda (Kaj, 1961) Sakowicz, 1961a, c). Recently the variety has been introduced into other Polish waters (Backiel and Bartel, 1967; Epler and Bieniarz, 1974; Jakubowski and Penczak, 1976; Wajdowicz, 1976; Bartel and Zieliński, 1987). However, the largest population of *S. trutta m. lacustris* occurs in the Lake Wdzydze.

Studies on carotenoids in the three trout varieties have been so far limited mainly to the sea trout, *S. trutta m. trutta* (Steven, 1949; Jitariu et al., 1968; Czeczuga, 1975; 1979; Czeczuga and Chełkowski, 1984; Storebakken et al., 1986) and, to some extent, concerned *S. trutta m. fario* (Czeczuga, 1975, 1979 b). On the other hand, individuals of *S. trutta m. lacustris* have not been examined from this point of view. Therefore data on carotenoids in *S. trutta m. lacustris* will supplement the present knowledge about carotenoids in fish in general, and in salmonids in particular.

MATERIALS AND METHODS

S. trutta m. lacustris individuals (2 males and 2 females) were caught with an electric apparatus on November 12, 1987 from Lake Wdzydze tributaries Wda and Trzebiocha during the fish spawning migration. Certain biometric data of the individuals examined (body length and weight) are presented in Tables 2-3. Assays were made on fins, skin, muscles, intestines, liver, and gonads.

Tissue samples from each individual were homogenized, placed in a dark bottle and covered with 95% acetone, after which they were kept in a refrigerator until analysed. Carotenoid pigments were separated by means of column and thin layer chromatography. Prior to separation, each sample was hydrolysed for 24 h with 10% KOH in nitrogen at room temperature. The details of the chromatographic techniques used are given in one of the previous papers of the series (Czeczuga and Czerpak, 1976). The hydrolysed extract was transferred to an AL_2O_3–filled column, 15-25 cm long (Quickfit, England). The fractions were eluted with different solvent combinations (Czeczuga and Czerpak, 1976).

Irrespectively of the column chromatography, the acetone extracts were separated with layer chromatography as well. Silica gel covered glass plates and different solvent combinations were used (Czeczuga and Czerpak, 1976). The R_f values were determined according to the commonly used procedures.

Carotenoids were identified on the basis of:

a) the nature of column chromatograms,

b) absorption peaks in various solvents,

c) epi– to hypophase ratio determined in hexane and 95—methanol,
d) comparisons of thin layer chromatogram R_f values with standards (Hoffman-La Roche and Co. LTD, Switzerland and Sigma Chemical Co. USA) to identify β-csrotene, β-cryptoxanthin, cantaxanthin, lutein, zeaxanthin, adnixanthin, α-doradexanthin, and astaxanthin,
e) presence of allohydroxy groups determined with acidic chloroform,
f) epoxy test.

Carotenoid contents were determined from quantitative absorption spectra. The assays were basedon thr extinction coefficient E 1%/cm in corresponding peaks of absorption in kerosene ether or hexane (Davies, 1976).

RESULTS

Table 1 presents carotenoids identified in varoiurs body parts of the *S. truatta m. lacustries* individuals examined. Fig. 1 shows the structure of the carotenoids found.

The presence of 21 carotenoid pigments was revealed. Their partitioning among various organs of males and females is shown in Tables 2 – 3.

Table 1

List of carotenoids from materials investigated

Carotenoid	Structure (see Fig. 1)	Semisystematic name
1. β-carotene	A–R–A	β, β-carotene
2. β-cryptoxanthin	A–R–C	β, β-caroten-3-ol
3. α-cryptoxanthin	A–R–C	β, β-caroten-3-ol
4. zeaxantin	C–R–C	β, β-carotene-,3'-diol
5. lutein	C–R–D	β, ε-carotene-3,3'-diol
6. 3'-epilutein	C–R–D	β,ε-carotene-3,3'-diol (stereoisometric)
7. neothxanthin	B–R–D	ε,ε-caroten-3-ol
8. tunaxanthin	D–R–D	ε,ε-carotene-3,3'-diol
9. diatoxanthin	C–R_1–H	7,8-didehydro-β,β-carotene-3,3'-diol
10. echinenone	A–R–E	β,β-carotene-4-one
11. hydroxyechinenone	A–R–F	3-hydroxy-β,β-carotene-4-one
12. adonixanthin	C–R–F	3,3'-dihydroxy-β,β-caroten-4-one
13. α-doradexanthin	D–R–F	3,3'-dihydroxy-β,ε-caroten-4-one
14. idoxanthin	F–R–G	3,3',4'-trihydroxy-β,β-caroten-4-one
15. canthaxanthin	E–R–E	β,β-carotene-4,4'-dione
16. astaxanthin	F–R–F	3,3'-dihydroxy-β,β-carotene-4,4'-dione
17. rhodoxanthin	L–R_2–K	4',5'-dihydro-4,5-'-retro-β,β-carotene-3,3'-dione
18. antheraxanthin	C–R–L	5,6-epoxy-5,6-dihydro-β,β-carotene-3,3'-diol
19. lutein epoxide	D–R–L	5,6-epoxy-5,6-dihydro-β,ε-carotene-3,3'-diol
20. salmoxanthin	N–R–I	5,6-epoxy-5,6-dihydro-β,ε-carotene-3,3',6'-triol
21. mutatoxanthin	C–R_1–M	5,8-epoxy-5,8-dihydro0β,β-carotene-3,3'-diol

Astaxanthin was observed in all the organs examined in both sexes. The major carotenoids in females were: β-carotene (skin of female A), zeaxanthin (intestine of female A), β-doradexanthin (skin of female B), and astaxanthin (fins, liver and muscles of both females; intestine and eggs of female B). In males, the following carotenoids were dominating: β-cryptoxanthin (muscles and gonads

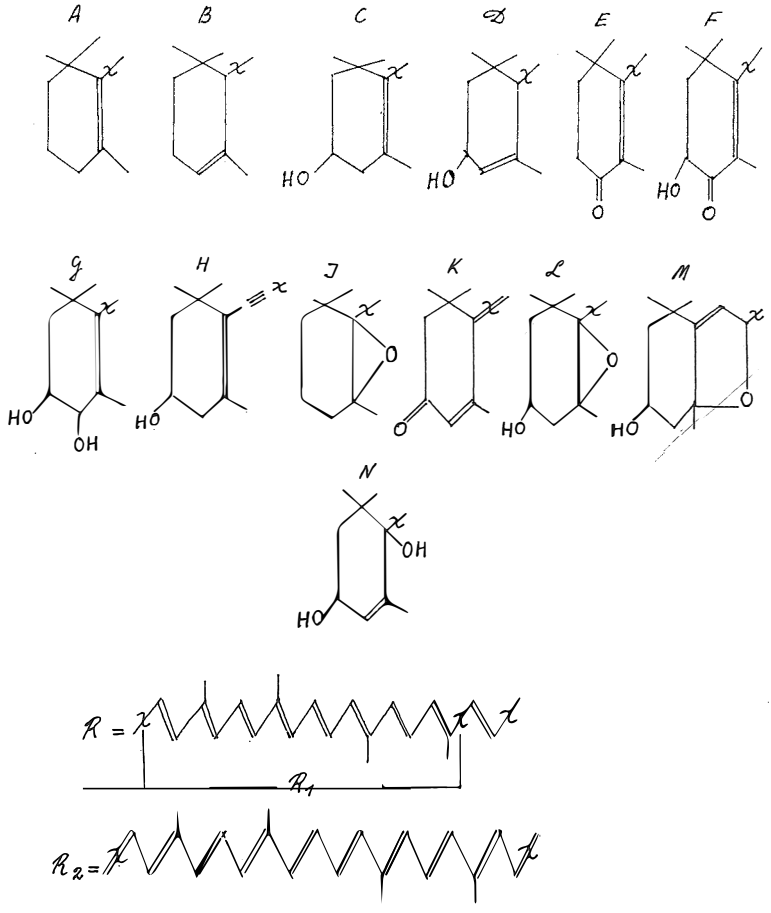

Fig.1. Structural features of carotenoids from materials investigated

of male C), neothxanthin (fins of male C), tunaxanthin (fins of male D), canthaxanthin (skin of male C), α-doradexanthin (skin and gonads of male D), and astaxanthin (liver and intestine of both males; muscles of male D). The total carotenoid content in females was found to range from 0.918 (intestine of female B) to 1.437 μg/g wet weight (liver of female B). The range in males covered 0.125 (gonads of male D) to 1.463 μg/g wet weight (intestine of male D).

Table 2

Carotenoid content (%) found in the investigated parts of the body of female *Salmo trutta* m. *lacustris* L.

Part of body	Carotenoid (see Table 1)	Major caro-tenoid (%)	Total content (μg/g wet wt)
Female A – longitude caudalis – 47.0 cm; weight – 2.070 g			
Fins	1,2,4,8,13,15,16,20	16(36.6)	0.531
Skin	1,2,4,6,8,13,16,19,21	1(19.0)	0.562
Muscles	2,4,6,13,16,17,19	16(29.0)	0.772
Intenstine	2,4,7,13,16,17,19	4(32.1)	1.289
Liver	2,4,12,13,16,19	19(83.0)	1.036
Eggs	2,4,5,8,13,15,16,19	19(27.8)	1.058
Female B – longitude caudalis – 53.0 cm; weight – 1.725 g			
Fins	4,5,9,16,17,19,20	16(55.3)	0.833
Skin	2,4,6,9,13,15,16,17,19	13(25.4)	0.737
Muscles	4,6,7,8,13,16,19	16(25.2)	0.777
Intenstine	4,14,15,16,17	16(52.8)	0.918
Liver	2,4,8,12,15,16,17	16(42.4)	1.437
Eggs	4,5,6,12,16,17,19	16(40.7)	1.072

Table 3

Carotenoid content (%) found in the investigated parts of the body of male *Salmo trutta* m. *lacustris* L.

Part of body	Carotenoid (see Table 1)	Major caro-tenoid (%)	Total content (μg/g wet wt)
Male C– longitude caudalis – 43 cm; weight – 1.200 g			
Fins	1,2,3,6,7,8,13,16,17,19,21	7(27.7)	0.934
Skin	2,8,13,15,16,19,	15(31.5)	1.298
Muscles	2,3,4,5,6,713,16	2(28.1)	0.317
Intenstine	2,8,12,14,16,17,19,21	16(40.9)	0.939
Liver	1,2,4,7,13,15,16,18,19,20	16(41.8)	1.091
Gonad	2,4,7,11,15,16,18,19,21	2(17.5)	0.234
Male D – longitude caudalis – 40 cm; weight – 1.000 g			
Fins	2,4,7,8,11,13,16,17	8(26.4)	0.533
Skin	2,4,7,8,13,15,16,17	13(21.7)	1.300
Muscles	2,4,7,15,16,17,21	16(25.1)	0.256
Intenstine	5,7,8,13,15,16,17,18,19	16(59.7)	1.463
Liver	4,7,8,10,13,15,16,17,18	16(33.4)	0.672
Gonad	2,4,7,13,16,21	13(40.6)	0.125

DISCUSSION

The carotenoids recorded in *S. trutta m. lacustris* were often reported from other salmonid species (Czeczuga 1975, 1976, 1977, 1979a, 1982; Matsuno et al., 1980; Kitahara, 1984; Craik, 1985; Czeczuga et al., 1986). Comparisons with data *S. trutta m. fario* (Czeczuga, 1979b) and sea trout, *S. trutta m. trutta* (Czeczuga and Chełkowski 1984) reveal differences with respect to the occurence of certain carotenoids (Table 4). Such carotenoids as astaxanthin,rhodoxanthin, and mutatoxanthin are known only from *S. trutta m.*

Table 4

Carotenoid content in *Salmo trutta* L.

Carotenoid	*morpha*		
	lacustris	*fario*	*trutta*
β-carotene	×	×	×
ε-carotene		×	
zeaxanthin	×		×
lutein	×		×
lutein epoxide	×	×	×
astaxanthin	×	×	×
canthaxanthin	×	×	×
4-keto-4-hydroxy-β-carotene		×	
isozeaxanthin		×	
tunaxanthin	×	×	×
α-cryptoxanthin	×	×	×
isocrytoxanthin		×	
mutatochrome		×	
β-cryptoxanthin	×	×	×
aurochrome		×	
diatoxanthin	×	×	×
α-doradexanthin	×	×	×
β-carotene epoxide			×
echinenone	×		×
3'-hydroxyechinenone	×		×
3'-epilutein	×		×
adonixanthin	×		×
phoenicoxanthin			×
salmoxanthin	×		×
neothxanthin	×		×
idoxanthin	×		×
parasiloxanthin			×
β-apo-2'-carotenal			×
rhodoxanthin	×		
mutatoxanthin	×		
antheraxanthin	×		
Number of carotenoids	21	15	22

lacustris. On the other hand, the species studied does not contain several carotenoids found in the remaining two *S. trutta* biological varieties. The individuals of *S. trutta m. fario* examined earlier showed the least diversity of their set of carotenoids (15), while the other two varieties contained much richer sets (21 in *S. trutta m. lacustris* and 22 in *S. trutta m. trutta)*. The differences may be perhaps for by different habitat preferences of the three varieties and hence different diets. There is a greater variation in lacustrine and marine food resources available to fish than is the case in rivers. Both the sea trout and *S. trutta m. lacustris* migrate to spawn in upper reaches of rives to streams (Sakowicz, 1961b; Szczerbowski, 1973; Brylińska, 1966), the migrations making it possible for the fish to consume diversified food. A number of publications have already demonstrated the carotenoid composition in may fish, including salmonids, to be significantly affected by the type of food consumed (Deufel, 1975; Choubert, 1979; Quantz, 1980; Czeczuga and Dąbrowski, 1983; Czeczuga and Kiziewicz, 1985; Skrede and Storebakken, 1986).

The food of *S. trutta m. lacustris* varies, too, depending on the habitat. The juveniles living in streams feed on benthic fauna dominated by amphipods and insect larvae (Różański, 1961). When in rivers, the growing fish feed on snails, oligochaetes, and other small fish, e. g. the stickleback (Bartel, 1965).During its lacustrine phase, the species feeds mainly on other fish; roach, perch, bleak, and stickleback were the principal diet items depending on the season and te nature of a water body in question (Wojno, 1961; Bartel, 1965; Bryliński and Brylińska, 1964).

The *S. trutta m. lacustris* males and females examined were shown to differ in their total carotenoid contents. The highest carotenoid content in females was found in the liver, eggs, and intestine; the carotenoid-richest organs in males were: skin, liver, and intenstine, the male gonads showing the lowest contents. Differences in carotenoid concentrations in various organs were also observed between males and females in the spawning sea trout (Czeczuga and Chełkowski, 1984). Immediately before spawning, carotenoids in the are transported from the carotenoid-richest organs (intenstine and liver) to gonads and skin in females and to skin and fins in males. As shown by Ando (1986), carotenoids in spawning salmonids are transferred as complexes, mostly those of astaxanthin with lipoproteins. The complexes are formed in the carotenoid-richest organ, the intenstine; the blood system carries them to the liver and, depending on the species biology, to various other parts of the body.

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BADANIA KAROTENOIDÓW U OSOBNIKÓW *SALMO TRUTTA MORPHA LACUSTRIS* L. W OKRESIE TARŁA

STRESZCZENIE

Autorzy stosując chromatografię kolumnową i cienkowarstwową badali występowanie poszczególnych karotenoidów w płetwach, skórze, mięśniach, wątrobie, jeelitach i gonadach osobników obu płci *Salmo trutta morpha lacustris* L. w okresie tarła.

Ustalono obecność następujących karotenoidów: β-karoten, α-, β-kryptoksantyna, kantaksantyna, echinenon, hydroechinenon, luteina, 3'-epiluteina lutein epoksy, zeaksantyna, anteraksantyna, adoniksantyna, diatoksantyna, salmoksantyna, tunaksantyna, neoksantyna, idoksantyna, α-doradeksantyna, astaksantyna, rodoksantyna i mutatoksantyna.

Podano również ogólną zawartość karotenoidów w poszczególnych częściach ciała troci jeziorowej oraz stosunki procentowe poszczególnych karotenoidów. Najzasobniejszymi w karotenoidy u samic okazały się wątroba, jelita i gonady, u samców zaś skóra, jelita i wątroba.

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Received: 1988.05.25