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CAROTENOIDS IN FISH. XXXVI. CAROTENOID CONTENTS IN ADULT INDIVIDUALS OF SEA-TROUT *SALMO TRUTTA* L. DURING SPAWNING MIGRATION, SPAWNING AND POST-SPAWNING MIGRATION

KAROTENOIDY W RYBACH. XXXVI. ZAWARTOŚĆ KAROTENOIDÓW U WYROSŁYCH OSOBNIKÓW TROCI *SALMO TRUTTA* L. Z OKRESU WĘDRÓWKI TARŁOWEJ, TARŁA I WĘDRÓWKI POTARŁOWEJ

Carotenoids occurring in the adult sea-trout in the river Gowienica during spawning migration, spawning, and post-spawning migration are presented. Carotenoid contents in the fins, skin, muscules, liver, gonads, and intestines of 8 males and females were determined by means of column and thin layer chromatography.

INTRODUCTION

The trout (*Salmo trutta* L.) occurs in the river Gowienica catchment (Chełkowski, 1966; 1967 a, b). The Gowienica is a 53-km long Pomeranian river draining into the Szczecin Lagoon. The juvenile sea trout (smolts) descend to the Szczecin Lagoon and on to the Baltic Sea. After a feeding period in the sea, the adult sea trout come back to spawn in the Gowienica.

When studying carotenoid contents in various salmonids (Czeczuga, 1973, 1975, 1977, 1979a, b, 1982), we became interested in carotenoid levels in the adult trout caught in the Gowienica during their spawning migration, spawning, and post-spawning migration. So far, carotenoids in the sea trout have been dealt with by Andre (1926), Steven (1948, 1949). Thommen and Gloor (1965), Jarząbek (1970), Czeczuga (1975), and Matsumo et al. (1980 c).

MATERIALS AND METHODS

We studied carotenoid levels in fins, skin muscles, liver, gonads, and intestines of 8 male and 6 female *Salmo trutta* L. caught in the Gowienica during their spawning migration (14 December 1979; 3 October 1980), spawning (14 November 1980), and post-spawning migration (22 December 1980).

Carotenoid pigments were separated by means of column and thin layer chromatography. Prior to chromatography, the materials were hydrolysed for 24 h with 10% KOH in nitrogen at room temperature. The hydrolysed extract was run through an Al_2O_3-filled column. The columns (Quickfit, England) were 15–25-cm long. The fractions were eluted with various combinations of solvents (Czeczuga and Czerpak, 1976). The eluent was then evaporated and the evaporation residue dissolved in an appropriate solvent to obtain absorption curves, its maxima being used, i.a., to identify the carotenoids.

Apart from column chromatography, the acetone extract obtained was separated to individual bands by means of thin layer chromatography. Silicon gel (Merck)-covered glass plates (15 x 40 cm) were used for placement, by means of a micropipette, of acetone extracts, various solvents being used as well (Czeczuga and Czerpak, 1976). The R_t value was determined according to the generally accepted procedures.

The carotenoids were identified from absorption peaks in various solvents, the R_t value being compared to the reference, and also from the epi- vs. hypophase relationship. The references manufactured by F. Hoffman-LaRoche, Basle were used for β-carotene, β-cryptoxanthin, cantaxanthin, echinenon, lutein, zeaxanthin, tunaxanthin, and astaxanthin. The absorption peaks were determined on Spektromom-203 and Specol spectrophotometers.

Quantitative relationships between various carotenoids were determined as in Davies (1965).

RESULTS

a) Carotenoids occurring in sea trout during spawning migration (Tables 1, 2, 3)

Carotenoid levels were determined in 7 individuals (2 in 1979 and 5 in 1980). Results of chromatographic assays made on the 1979 individuals are presented in Table 1. Various organs of the females analysed were shown to contain 16 carotenoids, 12 being identified in males. No β-carotene epoxide, β-cryptoxanthin, lutein, and 3'-epilutein occurred in the male organs studied. Astaxanthin ester occurred in all the male and female organs tested. With regard to the total carotenoid content, the highest amounts in females were revealed in their skin, fins, and liver, the muscles showing the lowest contents. On the other hand, all the male organs contained, on the whole, less carotenoids, the highest contents being exhibited by the intestine and the lowest by the gonads.

Table 1

Per cent composition of carotenoids in some organs of pre-spawning Salmo trutta (14 December 1979)

Carotenoid	♀, l.c. = 49 cm w = 1.520 g						♂, l.c. = 69 cm, w = 3.275 g					
	fins	skin	mus-cles	liver	intes-tine	gonads	fins	skin	musc-les	liver	intes-tine	gonads
β – carotene			21.3	16.7		16.6				13.9		
β – carotene epoxide						37.4						
β – cryptoxanthin	23.9	14.7	18.9	40.0								
canthaxanthin		8.0		12.5	11.4				11.1	6.0		8.1
lutein		5.3				12.0						
3' – epilutein		3.2			3.8							
lutein epoxide	25.2	11.5	31.3				8.4			1.9		14.1
zeaxanthin	15.0	7.5		13.9	22.8		12.7	30.1			41.1	53.4
adonixanthin					49.1			7.3	45.0			
phoenicoxanthin		4.4					20.8		23.1	42.9	35.2	
diatoxanthin			18.4					30.1	20.8			
tunaxanthin				3.8						3.1		
neothxanthin	12.2						17.3					
α – doradexanthin	9.1	6.4	10.1	4.4	12.9						10.4	24.4
astaxanthin								5.8		27.7		
astaxanthin ester	14.6	39.0	trace	8.7	trace	34.0	40.8	26.7	trace	4.5	13.3	trace
Total carotenoid content in μg/g wet weight	1.699	1.739	0.375	1.475	1.283	0.412	0.442	0.464	0.340	0.582	0.663	0.121

Per cent composition of carotenoids in some organs of pre-spawning Salmo trutta (♀, (3 October 1980)

Table 2

Carotenoid	L. c. = 65 cm, w = 2.900 g						l. c. = 55 cm, w = 2.200 g				
	fins	skin	muscles	liver	intenstine	gonads	fins	skin	muscles	intenstine	gonads
β – carotene	7.9	6.8	13.6	6.1				3.4	11.1		21.0
α – cryptoxanthin		4.0		6.3		20.0	3.7				
β – cryptoxanthin	5.6	3.1	40.3	5.0	8.6	20.2		2.9	9.4	20.8	33.8
canthaxanthin		1.9	1.8		2.6			10.3		3.9	
3' – hydroxyechinenone									2.7		
lutein					4.7		5.2	17.9			
3' – epilutein	6.0	2.7	6.7	11.4	4.9	34.5	8.1				
lutein epoxide	25.5		5.3	4.0			46.3			4.1	
zeaxanthin	12.2	79.6	15.2	39.4	19.0	10.6			10.1	8.6	
adonixanthin	3.0							25.6	6.7	54.2	
diatoxanthin	22.1	1.9		13.1					37.0		
salmoxanthin			2.6				13.3				
tunaxanthin						14.7			3.8		11.1
α – doradexanthin	17.7		8.6	7.0	6.8		5.5	39.9	5.9		
astaxanthin					7.4						
astaxanthin ester			5.9	7.7	46.0		17.9		13.3	8.4	34.1
Total carotenoid content in μg/g wet weight	1.100	2.317	0.931	0.749	1.335	0.144	1.365	4.833	0.960	3.357	0.177

Table 3

Per cent composition of carotenoids in some organs of pre-spawning Salmo trutta (♂) (3 October 1980)

Carotenoid	l.c. = 52 cm, w = 1.700 g						l.c. = 56 cm, w = 1.900 g						l.c. = 42 cm, w = 0.880 g					
	fins	skin	mus-cles	liver	inten-stine	gonads	fins	skin	mus-cles	liver	inten-stine	gonads	fins	skin	mus-cles	liver	inten-stine	gonads
β – carotene	20.5		49.9	10.9	4.5	29.6	18.0		4.3						6.1	4.9	7.2	21.4
α – cryptoxanthin								13.6						30.2				
β – cryptoxanthin	14.7			27.8	8.6		10.7	5.8			25.3	19.7	12.9	30.6	2.4	18.2	11.1	
canthaxanthin		27.6				14.7	3.9		24.2	16.8	17.2	55.9	6.6		1.6	6.9		5.9
echinenone	16.9																	
3' – hydroxyechinenone										2.4		5.5						
lutein	20.4			5.1			15.6	7.4	3.0							8.9		11.6
3' – epilutein	16.3			5.2			7.9								3.8	13.2	3.8	5.0
Lutein epoxide	3.8	12.6	15.3						18.0		10.6		45.0	17.9	44.1	19.9	10.7	9.3
zeaxanthin				10.8	19.1		1.7	11.4	31.1	27.0	4.8		12.5		17.2	6.5	25.8	11.8
phoenicoxanthin				14.6				4.4					3.1					
adonixanthin		15.7					14.0		7.2	6.0	4.0		5.1					
diatoxanthin										8.2								
salmoxanthin							7.7											11.1
tunaxanthin													14.8	21.3				
α – doradexanthin	7.4	33.4					8.6				24.4				20.6			8.2
astaxanthin						6.0	3.0	21.8		26.3						5.4	6.2	
astaxanthin ester		10.7	34.8	25.6	67.8	49.7	9.6	35.4	12.2	13.3	13.7	18.9			4.2	16.1	35.2	15.7
Total carotenoid content in μg/g wet weight	7.575	0.846	1.408	1.049	2.279	0.150	3.369	1.699	3.389	1.603	8.131	0.132	4.973	1.014	4.233	2.461	6.973	0.714

The results concerning those individuals migrating to spawn in October 1980 are presented in Table 2. The females and males examined showed the presence of 16 and 18 carotenoid pigments, respectively. Most female and male organs analysed conteined β-carotene, β-cryptoxanthin, various forms of lutein, zeaxanthin, α-doradexanthin (females), and estaxanthin ester.

The highest total content of carotenoids were revealed in female skin, fins, and intestine, and the lowest in gonads. In males, the carotenoid-richest organs were fins, muscles, and the intestine.

b) Carotenoids occurring in sea trout during spawning (Table 4)

The three individuals analysed yielded a total of 19 carotenoids. Most female and male organs tested were found to contain β-cryptoxanthin, cantaxanthin, lutein epoxide, zeaxanthin, α-doradexanthin, and astaxanthin ester. The presence of salmoxanthin should be emphasized. The total carotenoid content in both sexes was at its highest in fins, skin, and the intestine. It was only in one male that the muscles conteined a relatively high amount of carotenoids.

c) Carotenoids occurring in sea trout during post-spawning migration (kelts)

A total of 17 carotenoids were identified in both females and males, 2 individuals of each sex being tested. Most organs were found to contain β-cryptoxanthin, cantaxanthin, 3'-epilutein, lutein epoxide, zeaxanthin, α-doradexanthin, and astaxanthin ester. The presence of salmoxanthin as well as of parasiloxanthin should be particularly stressed here, too. The gonads of one female yielded β-apo-2'carotenal.

With respect to the total carotenoid content, the carotenoidrichest female organs were fins, skin, and the intestine, and in males additionally muscles and the liver (in one individual).

DISCUSSION

Most carotenoids found in the *Salmo trutta* individuals tested had been already shown several times in numerous other fish species (Tanaka, 1978). On the other hand, some of the carotenoids found belong to the so-called rare carotenoids, e.g., echinenone and its derivatives 3'-hydroxyechinenone, salmoxynthin, idoxynthin, and parasiloxynthin.

Echinenone was shown to occur in some individuals of carp, *Cyprinus carpio* (Czeczuga, 1979 c). In salmonids, echinenone was found in *Salmo gairdneri* from culture ponds (Czeczuga, 1979 b), in some other freshwater salmonids (Matsuno et al., 1980 c), and in certain marine species (Matsuno et al., 1980 a; Czeczuga, 1982). The echinenone derivative, i.e., 3'-hydroxyechinenone, was found in some marine salmonids only (Matsuno et al., 1980 a).

Salmoxanthin has so far been regarded as a carotenoid specific of salmonids only; it was identified for the first time in *Oncorhynchus keta* (Matsuno et al., 1980).

Table 4

Per cent composition of carotenoids in some organs of spawning Salmo trutta (14 November 1980)

Carotenoid	♀, l. c. – 69 cm, w – 3.640 g						♂, l. c. – 50 cm, w – 1.310 g						♂, l. c. – 47 cm, w – 1.270 g					
	fins	skin	mus-cles	liver	inten-stine	gonads	fins	skin	mus-cles	liver	inten-stine	gonads	fins	skin	mus-cles	liver	inten-stine	gonads
β – carotene		8.4	6.8	12.2		19.7						10.5		5.6		13.2		
β – carotene epoxide					10.8													
α – cryptoxanthin									7.7			9.5			7.5		11.0	4.0
β – cryptoxanthin	24.6	8.5	19.1	21.1	10.1	10.0		7.6		8.6	9.0	11.9	31.3	22.9	11.8	17.8	10.9	
canthaxanthin	6.3	2.4		21.4			4.0	12.0	10.0	17.9	15.6		24.8	23.3	3.0	16.6	2.0	26.8
lutein	9.1		7.9		11.5	9.2										10.5		11.0
3' – epilutein	5.7					11.0					4.0					12.0		27.8
lutein epoxide	6.0		13.4	11.6	24.4		8.0			4.3	6.0	12.1	1.4	11.3	34.9			2.0
zeaxanthin	3.5	31.6	12.3	8.7	13.0	5.2			31.2	8.0	43.0	22.1	19.3	27.0	23.6	7.1	9.0	10.0
diatoxanthin	9.1																	
adonixanthin	13.8	9.5	11.7	4.6			18.0		26.9			14.4					29.0	
phoenicoxanthin					3.7								8.3					
idoxanthin								36.5		49.8								1.0
tunaxanthin		4.8																
neothxanthin				7.4				4.9										
salmoxanthin	9.5						18.0		2.8		4.0							4.9
α – doradoxanthin		13.8	6.8	8.4		2.3	20.3	14.0	10.7	7.9	17.0	6.7	3.2	2.9	6.6		26.3	6.7
astaxanthin			7.8									12.8						
astaxanthin ester	12.4	21.0	14.2	4.6	26.5	42.6	31.7	25.0	10.7	3.5	1.4		11.7	7.0	12.6	22.8	11.8	5.0
Total carotenoid content in μg/g wet weight	1.178	4.761	0.687	0.565	1.002	0.403	2.786	6.574	0.322	0.815	1.872	0.505	5.742	11.490	3.211	2.399	2.562	1.064

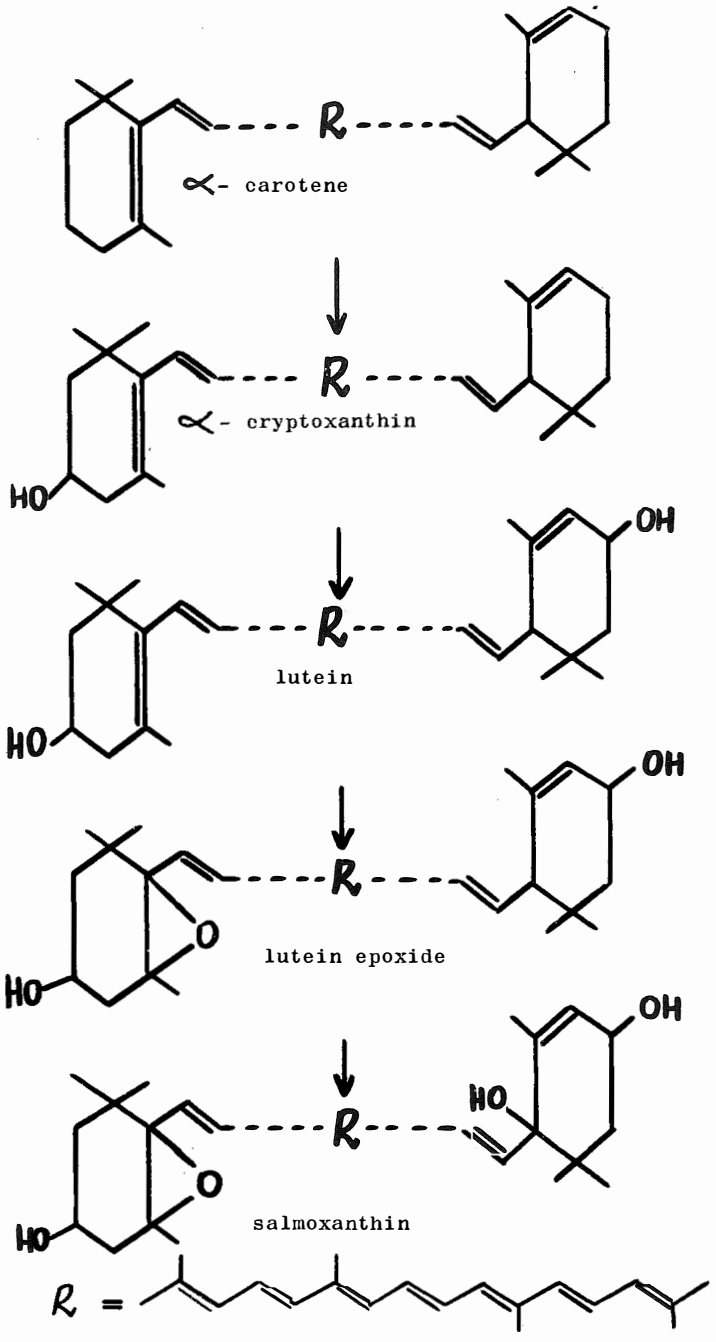

Fig. 1. Biosynthesis of salmoxanthin in fish

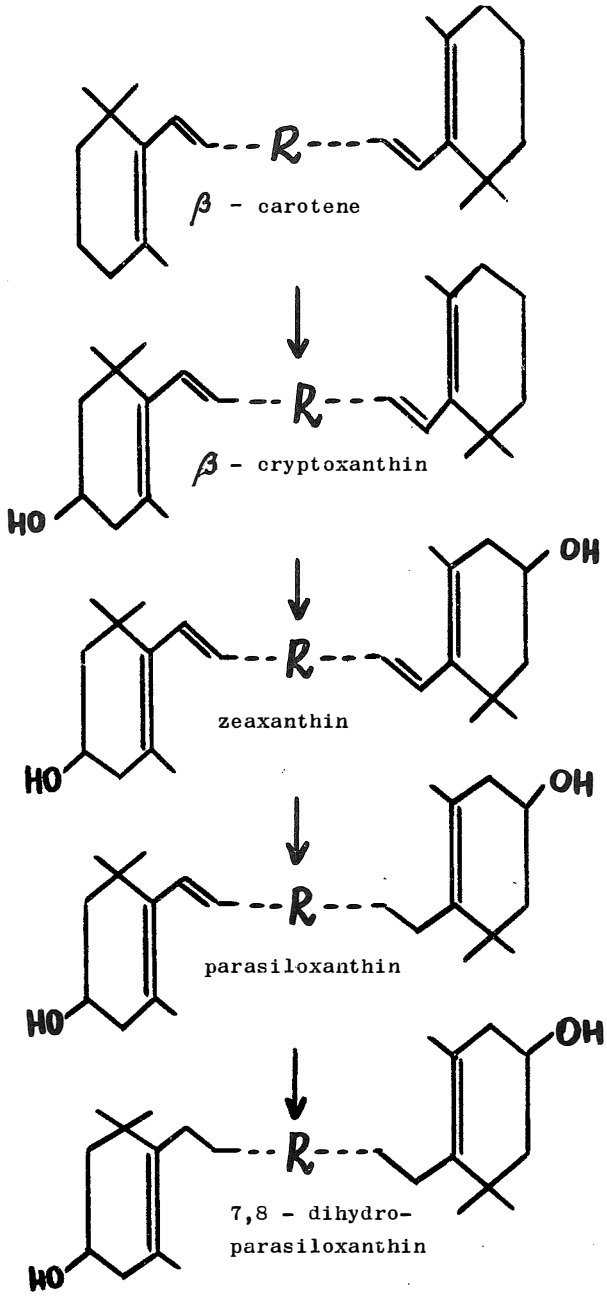

Fig. 2. Biosynthesis of parasiloxanthin in fish

Table 5

Per cent composition of carotenoids in some organs of post-spawning Salmo trutta (22 December 1980)

Carotenoid	♂, l.c. = 59 cm, w = 1.800 g						♂, l.c. = 43 cm, w = 0.940 g						♀, l.c. = 57 cm, w = 1.700 g						♀, l.c. = 67 cm, w = 2.550					
	fins	skin	muscles	liver	intestine	gonads	fins	skin	muscles	liver	intestine	gonads	fins	skin	muscles	liver	intestine	gonads	fins	skin	muscles	liver	intestine	gonads
β – carotene			4.9	12.1																8.4	20.0	13.5		11.3
α – cryptoxanthin							26.3			13.7									7.4		5.1	5.7		
β – cryptoxanthin	5.0	10.4	22.4	12.8	8.3	21.1	3.8			11.5	20.4	15.4	21.5	20.9	10.8	17.2	39.0	15.7	17.7	12.2	16.4	48.0	33.5	23.7
canthaxanthin	7.0	6.2	48.0	3.3					1.6	47.5		25.9	12.7	11.0	19.9	5.0	13.2		2.5				17.6	2.7
lutein	19.0	8.3							29.8															
3' – epilutein	9.0						8.6				13.9			8.7	17.0	8.0	16.5	6.5		11.1	11.2			
lutein epoxide				5.8	18.7	36.6	3.6	7.0	4.6		16.5			12.0	5.6				14.1	13.3	12.0	21.3	5.0	
zeaxanthin	13.0	16.5	4.0	31.3	12.6		9.4	9.8	9.5	23.7	12.8	32.4	30.0	6.0	5.9	25.0	21.5	38.9	23.8	18.0			9.2	12.8
salmoxanthin	23.8													12.3	6.0			3.8		10.6				7.2
tunaxanthin		6.6																						
adonixanthin	2.2	15.7			7.1		8.3	10.0						11.7							10.3		3.2	
diatoxanthin												5.9	2.0											
phoenicoxanthin						23.5								7.2							13.7			
parasiloxanthin																							6.6	7.0
α – doratexanthin	13.4			5.0	2.9			5.6	4.0	3.6	36.4	20.4	7.8	7.7	25.8	14.8	7.1	7.0				11.5		6.0
astaxanthin							14.7	9.0										5.6	9.9					
astaxanthin ester	7.6	36.3	20.7	29.7	50.4	18.8	25.2	58.6	50.5				26.0	2.5	9.0	30.0	2.7	22.5	24.6	26.0	11.4		24.9	17.9
β –apo–2'–carotenal																								11.3
Total carotenoid content in μg/g wet weight	1.445	3.342	3.877	0.824	7.350	0.242	4.003	2.518	2.192	3.393	0.998	0.500	1.651	1.115	0.484	0.321	2.236	0.451	1.035	1.257	0.568	0.647	0.979	0.317

Table 6

Mean carotenoid in some organs of Salmo trutta males and females in different periods
(in μg/g wet weight)

Period	Fins	Skin	Muscles	Liver	Intenstine	Gonads
			Males			
Before spawning	4.089	1.005	2.342	1.423	4.511	0.297
Spawning	4.264	9.032	1.766	1.607	2.217	0.784
After spawning	2.724	2.930	3.034	2.108	4.174	0.371
			Females			
Before spawning	1.388	2.963	0.755	1.058	1.564	0.244
Spawning	1.178	4.761	0.687	0.565	1.002	0.403
After spawning	1.343	1.186	0.526	0.484	1.607	0.384

Subsequently, it was found to occur in a number of other marine salmonids (Matsuno et al., 1980 a; Czeczuga, 1982). According to the present knowledge, this carotenoid pigment is a derivative of lutein epoxide (Fig. 1).

Idoxanthin was identified in a marine crustacean *Idothea metallica* (Herring, 1969) and was subsequently found in carp and sea lamprey (Nagata and Matsuno, 1979; Matsuno and Nagata, 1979), and also in *Micropterus salmoides* (Czeczuga, 1981).

Parasiloxanthin was found for the first time by Matsuno et al. (1976) in *Parasilus asotus*; later, the presence of this carotenoid was revealed in *Lota lota* (Czeczuga, 1983). This carotenoid is a derivative of zeaxanthin (Fig. 2).

The highest total contents of such biologically active compounds as carotenoids varied in various organs of *Salmo trutta*, depending on the life history stage and sex. In the spawning males, carotenoids seem to aggregate particularly heavily in skin, fins, and less so in gonads, the contents decreasing at this time mostly in muscles and in the intestine. The spawning females show the highest contents of the pigments studied in skin and gonads, the contents in fins, muscles, the liver and intestine decreasing. General comments about carotenoid dynamics in the spawning *Salmo trutta* are given by Steven (1949). Additionally, the spawning *Oncorhynchus nerka* showed carotenoids to be transferred from muscles to skin, particularly in males (Crozier, 1970; Jarząbek, 1970).

In summary, when examining the total carotenoid contents in various organs of the 8 males and 6 females studied, carotenoid transfer among various organs is observed (Table 6). The carotenoidrichest organs of *Salmo trutta* during their stay in the Gowienica differ from one individual to another, depending on their sex and physiologic condition. During the spawning migration, carotenoids tend to aggregate in the male intestine and fins, and in the female skin and intestine. During spawning itself, the highest levels of carotenoid pigments are found in skin and fins of both males and females. At the kelt stage, males show the highest amounts of carotenoids in the intestine and muscles, while females – in the intestine and fins. At this time, large amounts of carotenoids remain in the skin of both males and females. A similar type of changes in female and male carotenoid contents during the spawning migration, spawning, and post-spawning migration was found in the intestine and gonads only, the remaining tissues tested showing sex-dependent differences. They may be accounted for by sex specificity involving, perhaps, a differential inter-organ transfer velocity of various carotenoids, or differing carotenoid-aggregation patterns in various organs.

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ZAWARTOŚĆ KAROTENOIDÓW U WYROSŁYCH OSOBNIKÓW *SALMO TRUTTA* Z OKRESU WĘDRÓWKI TARŁOWEJ, TARŁA I WĘDRÓWKI POTARŁOWEJ

STRESZCZENIE

Stosując chromatografię kolumnową i cienkowarstwową autorzy badali występowanie poszczególnych karotenoidów u wyrosłych osobników troci (*Salmo trutta* L.) obu płci, będących w okresie wędrówki tarłowej, tarła i wędrówki potarłowej (u keltów). Badaniami objęto płetwy, skórę, mięśnie, wątrobę, jelito i gonady 8 samców i 6 samic pozyskanych w pomorskiej rzece Gowienicy.

W wyniku badań ustalono obecność takich karotenoidów jak: β-carotene, β-carotene epoxide, α-cryptoxanthin, β-cryptoxanthin, echinenone, 3'-hydroxyechinenone, canthaxanthin, lutein, 3'-epilutein, lutein epoxide, zeaxanthin, diatoxanthin, adonixanthin, α-doradexanthin, phoenicoxanthin, salmoxanthin, tunaxanthin, neothaxanthin, idoxanthin, parasiloxanthin, astaxanthin, astaxanthin ester i β-apo-2'-carotenal.

Podano również ogólną zawartość karotenoidów oraz stosunki procentowe poszczególnych z nich. Okazało się, że w okresie życia rzecznego troci nasttępuje przemieszczenie się karotenoidów między poszczególnymi częściami ciała. Ogólna zawartość karotenoidów w badanych częściach ciała troci jest różna przed, po i podczas tarła i zależy to również od płci. Jeśli chodzi o tarło, to u osobników obu płci najzasobniejsze w karotenoidy są płetwy i skóra.

Чечуга Б. и Хелковски З.

СОДЕРЖАНИЕ КАРОТИНОИДОВ У ВЗРОСЛЫХ ОСОБЕЙ КУМЖИ SALMO TRUTTA В ПЕРИОДЫ; НЕРЕСТОВОЙ МИГРАЦИИ, НЕРЕСТА И ПОСЛЕНЕРЕСТОВОЙ МИГРАЦИИ

Р е з ю м е

Применения колонную и тонкослойную хроматографию авторы исследовали присутстве отдельных каротиноидов у взрослых особей кумжи (Salmo trutta L. обеих полов, которые находились в периоде нерестовой миграции, нереста и послеперестовой миграции (у кельтов). Исследованиями охватывали плавники, кожу, мышцы, печень кишку и половые железы 8 самцов и 6 самок отловленных в реке Гоеницы на взморье.

В результате исследований установили наличие таких каротиноидов β-каротин, β-каротин эпоксиде, α- криптоксантин, β-криптоксантин, эхиненоне, 3'-гидроксиэхиненоне, кантексантин, лютеин, эпилютеин, лютеин эпоксид, зеаксантин, диаксантин, адониксантин, α-дорадексантин, ксантин, сальмоксантин, тюнаксантин, неоэксантин, идоксантин, парасилоксантин, астаксантин, астаксантин эстер и β-апо-2' альдегид кароноида.

В статье приведены также; общее содержание каротиноидов и процентные отношени отдельных каротиноидов. Нашли, что в речном периоде жизни кумжи имеет место переиещение каротиноидов между отдельными частями тела. Общее содержание каротиноидов в исследуемых частях тела кумжи различное перед, после и во время нереста. Зависит это также от пола. Что касается нереста, то наиболее богатыми каротиноидами у особей обеих полов являются плавники и кожа.

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