

Bazyli CZECZUGA

Fish biochemistry

**CAROTENOIDS IN FISH. 51. CYPRINIDAE - PHYTOPHAGUS: RUTILUS
RUTILUS, RUTILUS RUTILUS HECKELI, SCARDINUS
ERYTHROPTALMUS, CHONDROSTOMA NASUS AND RHODEUS
SERICEUS AMARUS**

**KAROTENOIDY U RYB. 51. CYPRINIDAE - FITOFAGI: RUTILUS
RUTILUS, RUTILUS RUTILUS HECKELI, SCARDINUS
ERYTHROPTALMUS, CHONDROSTOMA NASUS I RHODEUS
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The column and thin-layer chromatography was used to the presence and total amount of carotenoids in some body parts of 5 fish species

INTRODUCTION

Among cyprinid fish numerous inhabiting our waters there are species which feed mostly on tiny water plants and vegetable plankton, and in a less degree on small animals like shellfish or mollusca living on water plants. These species include the roach, rudd, beaked carp undermouth and bitterling. A relatively wide range of their occurrence (except bitterling) and very few environmental needs make them very useful economically in our inland fishing industry. Roach fishing, in particular, is still considerable in lakes and rivers, beaked carp undermouth being fished for in upper and middle parts of bigger rivers. Only bitterling has no economic significance.

MATERIAL AND METHODS

The fish species analysed in our investigations were collected: *Rutilus rutilus* L. individuals from the Augustów lakes in June and October 1988, *Rutilus rutilus heckeli* (Nordman) from the Bulgarian waters near Hisar in May 1989, *Scardinius erythrophthalmus* L. also from the Augustów lakes in June 1988, *Chondrostoma nasus* L. from the Bug River near Drohiczyn in May 1986, and *Rhodeus sericeus amarus* Bloch individuals from the Monety Lake near Wieliczki in Suwałki province in June 1990. The fins, skin, muscles, liver, intestines and gonads were analysed.

Each sample, when homogenized was flooded with 95% acetone in a dark glass bottle and kept until analysed in refrigerator. Separation of particular carotenoids was done by the column and thin-layer chromatography, described in details in our previous paper (Czeczuga and Czerpak 1976). Before, the material underwent hydrolysis with 10% KOH in N_2 atmosphere, at room temperature, within 24 hours. When hydrolysed an extract was transferred into column filled up with Al_2O_3 . The length of column ranged from 15 to 25 cm (Quickfit Co. - England). Particular fractions were eluated with various compositions of solvents (Czeczuga and Czerpak 1976).

Independantly from column chromatography, the acetone extract was splitting up into particular fractions by thin-layer chromatography. For that glass plates covered with sili-cagel were used with different developing solvents (Czeczuga and Czerpak 1968). Next R_f was counted according to the generally applied principles.

Identification of particular carotenoids was based on following methods: a) appearance of the column chromatogrammes; b) maximal carotenoids absorption in various solvents; c) epiphase to hypophase relation determined in hexane and 95% methanol; d) comparison of the R_f values of thin-layer chromatogrammes; for identification of β -carotene, β -cryptoxanthin, canthaxanthin, lutein, zeaxanthin, α -doradexanthin, β -doradexanthin and astaxanthin - the chromatography standards of Hoffman-La Roche Co., Co. Ltd. Basel, Switzerland and Sigma Chemical Co. USA were applied; e) presence of allyhydroxy groups identified with acidic chloroform; f) epoxide test.

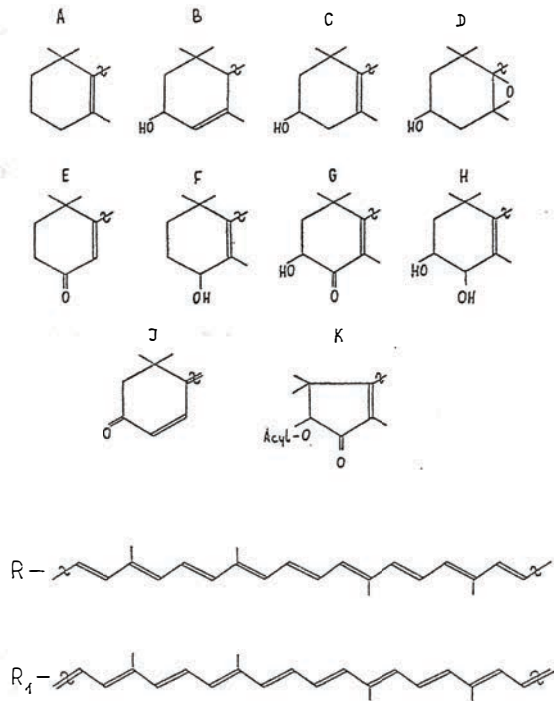


Fig 1. Structural features of carotenoids from materials investigated

zeaxanthin, α -doradexanthin, β -doradexanthin and astaxanthin - the chromatography standards of Hoffman-La Roche Co., Co. Ltd. Basel, Switzerland and Sigma Chemical Co. USA were applied; e) presence of allyhydroxy groups identified with acidic chloroform; f) epoxide test.

The quantity of particular carotenoids was estimated based upon a quantitative aspects of absorption. The calculation was based upon the extinction coefficients $E 1\%/cm$ for adequate absorption maxima with ether or hexane (Davies 1976). The chemical structure of particular carotenoids was presented according to Straub (1987).

RESULTS

Within the tested materials 18 carotenoids were identified (Tab. 1-2).

Table 1

List of carotenoids from the investigated materials

Carotenoid	Structure (see Fig. 1)	Semisystematic name
1 β -carotene	A - R - A	β,β -carotene
2 α -cryptoxanthin	A - R - B	β,ϵ -caroten-3-ol
3 β -cryptoxanthin	A - R - C	β,β -caroten-3-ol
4 lutein	B - R - C	β,ϵ -carotene-3,3'-diol
5 zeaxanthin	C - R - C	β,β -carotene-3,3'-diol
6 tunaxanthin	B - R - B	ϵ,ϵ -carotene-3,3'-diol
7 lutein epoxide	B - R - D	5,6-epoxy-5,6-dihydro- β,ϵ -carotene-3,3'-diol
8 echinenone	A - R - E	β,β -caroten-4-on
9 3'-hydroxyechinenone	C - R - F	3'-hydroxy- β,β -caroten-4-one
10 4'-hydroxyechinenone	E - R - F	4'-hydroxy- β,β -caroten-4-one
11 canthaxanthin	E - R - E	β,β -carotene-4,4'-dione
12 α -doradexanthin	B - R - G	3,3'-dihydroxy- β,ϵ -caroten-4-one
13 β -doradexanthin	C - R - G	3,3'-dihydroxy- β,β -caroten-4-one
14 phoenicoxanthin	E - R - G	3-hydroxy- β,β -carotene-4,4'-dione
15 idoxanthin	G - R - H	3,3',4'-trihydroxy- β,β -caroten-4-one
16 astaxanthin	G - R - G	3,3'-dihydroxy- β,β -carotene-4,4'-dione
17 rhodoxanthin	I - R ₁ - I	4',5'-didehydro-4,5'-retro- β,β -carotene-3,3'-dione
18 2'-norastaxanthin ester	G - R - K	3,3'-dihydroxy-2-nor- β,β -carotene-4,4'-dione-3-acylate

Table 2

Carotenoid identification in particular species of the fishes

	Carotenoid	<i>Rutilus rutilus</i>	<i>Rutilus rutilus heckeli</i>	<i>Scardinius erythro- phthalmus</i>	<i>Chondro- stoma nasus</i>	<i>Rhodeus sericeus amarus</i>
1	β -carotene			×	×	×
2	α -cryptoxanthin	×			×	
3	β -cryptoxanthin	×	×	×	×	×
4	lutein	×		×		×
5	zeaxanthin	×	×	×	×	
6	tunaxanthin	×		×		
7	lutein epoxide	×		×	×	×
8	echinenone	×				
9	3'-hydroxyechinenone	×				×
10	4'-hydroxyechinenone	×		×		
11	canthaxanthin	×	×	×		×
12	α -doradexanthin	×	×	×	×	
13	β -doradexanthin					×
14	phoenicoxanthin	×				×
15	idoxanthin					×
16	astaxanthin	×	×	×	×	×
17	rhodoxanthin	×		×		
18	2'-norastaxanthin ester	×		×		
	Number of carotenoids	15	5	12	7	10

The results of carotenoid analysis in the *Rutilus rutilus* individuals examined are given in Tab. 3-5. Fifteen carotenoids were identified, of which the most numerous in the young were astaxanthin, phoenicoxanthin and lutein epoxide. Adult body parts examined contained only astaxanthin in greatest quantities, ranging from 68.0% in the fins to 94.5% of total carotenoid content in the intestines. Total carotenoid content in young individuals was 0.198, in adults ranging from 0.180 (muscles) to 36.890 $\mu\text{g g}^{-1}$ fresh weight (intestines). In the roach fins 2'-norastaxanthin ester was found in small amount.

Table 3

Carotenoid content (in %) in the *Rutilus rutilus* L.* (October)

Carotenoid	Small specimens**	Fins	Skin and muscles	Gill	Liver	Intestine
α -cryptoxanthin	9.5					
β -cryptoxanthin				9.9	3.1	
phoenicoxanthin	27.3	4.4	2.7			
canthaxanthin		1.5	6.0		3.6	
lutein epoxide	26.1	1.1				
zeaxanthin		2.4		6.0		1.4
tunaxanthin		22.1		5.1	7.3	
4'-hydroxyechinenone			0.9	3.5		4.1
rhodoxanthin		2.0	7.4	5.3	4.5	6.2
astaxanthin	37.2	66.0	83.0	65.2	81.5	88.3
α -doradexanthin				5.0		
2'-norastaxanthin ester		0.5				
Total content ($\mu\text{g g}^{-1}$ fresh weight)	0.196	2.925	0.699	2.270	6.420	36.890

* 25.5 cm length of body; ** 7.6 cm length of body

Table 4

Carotenoid content (in %) in some parts of *Rutilus rutilus* L. (October) (length 17.2 cm)

Carotenoid	Fins	Skin	Muscles	Liver	Intestine
β -cryptoxanthin	39.3	19.1	7.9		
zeaxanthin		16.3	34.0	13.1	32.2
lutein		20.8		4.3	
lutein epoxide	19.2		27.1	12.6	59.8
α -doradexanthin	3.8		7.8	21.8	
phoenicoxanthin				19.4	
echinenone				14.4	
canthaxanthin		2.4	10.3	4.0	
astaxanthin	29.1	41.3	4.9	10.4	4.1
2'-norastaxanthin ester	0.5				
rhodoxanthin	8.1		8.0		3.9
Total content ($\mu\text{g g}^{-1}$ fresh weight)	0.930	0.320	0.180	1.245	1.230

Table 5
Carotenoid content (in %) in the fins
of the old *Rutilus rutilus* L.

Carotenoid	A	B
β -cryptoxanthin	8.1	
3'-hydroxyechinenone	5.6	
zeaxanthin	4.9	16.1
lutein	1.7	7.2
lutein epoxide	38.7	21.3
tunaxanthin		18.0
astaxanthin	39.2	20.1
rhodoxanthin		14.3
2'-norastaxanthin ester	1.8	3.0
Total content ($\mu\text{g g}^{-1}$ fresh weight)	0.734	0.807

A - Fins C (pinna caudalis),
B - Fins D (pinna dorsalis), P (pinnae pectorales), V (pinnae ventrales).

Table 6
Carotenoid content (in %) in the
Rutilus rutilus heckeli (Nordman)

Carotenoid	Fins, skin and muscles	Liver and intestine
β -cryptoxanthin	14.5	10.7
canthaxanthin	1.5	4.7
zeaxanthin	46.9	34.3
α -doradexanthin	4.2	6.8
astaxanthin	32.9	43.5
Total content ($\mu\text{g g}^{-1}$ fresh weight)	0.493	0.825

the skin, zeaxanthin in the female gonads and astaxanthin in the skin, liver and intestine. Total carotenoid content in various body parts ranged from 0.064 (muscles) to 1.191 $\mu\text{g g}^{-1}$ fresh weight (intestines).

In the *Rhodeus sericeus amarus* individuals (Tab. 9) carotenoids were analysed separately in males (nuptial garment) and females. The carotenoids were identified. Lutein epoxide, idoxanthin (fins, skin, muscles together) and zeaxanthin (inner organs) dominated in males. Male inner organs contained 12.398 $\mu\text{g g}^{-1}$ of carotenoids, while the remaining body parts - 0.381 $\mu\text{g g}^{-1}$ fresh weight. In the female inner organs β -cryptoxanthin and zeaxanthin, and in the remaining body parts - astaxanthin and zeaxanthin were found in the largest quantities. The inner organs carotenoid 1.478 $\mu\text{g g}^{-1}$ of carotenoids, other body parts - 0.298 $\mu\text{g g}^{-1}$ fresh weight. In one female bitterling, *Ligula intestinalis* was found, it con-

In the *Rutilus rutilus heckeli* individuals examined five carotenoids were identified, of which the most abundant was zeaxanthin, constituting 46,9% of all carotenoids. Total carotenoid content reached 5.493 $\mu\text{g g}^{-1}$ fresh weight (Tab. 6).

Table 7 presents the results of chromatographic analysis of the *Scardinius erythrophthalmus* specimens. Twelve carotenoids were identified in their bodies. Those, constituting the largest quantities were canthaxanthin (skin with muscles) and astaxanthin. Total carotenoid content in the individuals studied ranged from 0.087 (skin with muscles) to 4.553 $\mu\text{g g}^{-1}$ fresh weight (liver). The rudd fins, just like the roach fins also revealed the presence of 2'-norastaxanthin ester.

The results of chromatographic analysis of *Chondrostoma nasus* are presented in Table 8. Seven carotenoids were identified α -, β -cryptoxanthin, lutein epoxide and astaxanthin were found in nearly all body parts examined. β -cryptoxanthin was found in the greatest amount in the liver, lutein epoxide in

tained 15.059 $\mu\text{g g}^{-1}$ of carotenoids, among which β -carotene (30.6%) and β -cryptoxanthin (28.3%) dominated.

Table 7

Carotenoid content (in %) in the *Scardinus erythrophthalmus* L.

Carotenoid	Fins	Skin and muscles	Gill	Liver	Intestine
β -carotene				0.8	
canthaxanthin	22.4	43.2		18.6	
lutein	31.1				
lutein epoxide					24.3
zeaxanthin		39.8			
4'-hydroxyechinenone					12.3
tunaxanthin	1.7	6.0	9.6		
rhodoxanthin	2.4	1.5	15.7		8.2
astaxanthin	41.2	9.5	74.7	80.6	54.2
α -doradexanthin					1.0
2'-norastaxanthin ester	1.2				
Total content ($\mu\text{g g}^{-1}$ fresh weight)	1.230	0.087	0.595	4.553	1.678

Table 8

Carotenoid content (in %) in the *Chondrostoma nasus* L.

Carotenoid	Fins	Skin	Muscles	Liver	Intestine	Ovaries
β -carotene			15.5		22.4	
α -cryptoxanthin	11.5	18.2	28.4	19.1		
β -cryptoxanthin	19.5	15.4	13.1	33.8		29.7
lutein epoxide	39.0		16.9	9.6	17.8	16.6
zeaxanthin						30.2
α -doradexanthin		19.4				
2'-norastaxanthin ester	30.0	31.0	26.1	37.5	59.8	23.5
Total content ($\mu\text{g g}^{-1}$ fresh weight)	0.075	0.160	0.064	0.705	1.191	0.177

Table 9

Carotenoid content (in %) in the *Rhodeus sericeus amarus* (Bloch)

Carotenoid	Males		Females		Plerocercoids <i>Ligula intestinalis</i>
	Fins, skin and muscles	Liver and intestine	Fins, skin and muscles	Liver and intestine	
β -carotene	21.6	12.2			30.6
β -cryptoxanthin				25.3	28.3
3'-hydroxyechinenone				11.8	
zeaxanthin		55.7	28.3	44.3	18.6
lutein epoxide	34.5			8.3	
canthaxanthin	7.8	7.5		7.7	6.6
β -doradexanthin			19.5		
idoxanthin	26.7				
phoenicoxanthin					10.5
astaxanthin	9.4	24.6	40.9		5.4
unknown			11.3	2.6	
Total content ($\mu\text{g g}^{-1}$ fresh weight)	0.381	12.398	0.298	1.478	15.059

DISCUSSION

In the phytophagous species studied, a few rare carotenoids were found - idoxanthin in the skin with muscles of the male bitterling and 2'-norastaxanthin ester in the roach and rudd. The presence of idoxanthin has been already reported in the *Cyprinus carpio* (Nagata and Matsuno 1979), and in the arctic lamprey (Matsuno and Nagata 1979). Czczuga (1981) noted its presence in the *Micropterus salmoides*, in the trout (Czczuga and Chelkowski 1984) and grayling (Czczuga et al. 1985). Idoxanthin is known to be a link in a specific sequence of changes of β -carotene into astaxanthin (Czczuga 1988).

A particularly interesting finding was that of 2'-norastaxanthin ester, being a derivative of astaxanthin, in the fins of roach and rudd. A member of the norcarotenoid group, it is most probably produced as the result of astaxanthin oxidation. Previously it has been reported in bright red marine representatives of *Coelenterates* (Hertzberg and Liaaen-Jensen 1968; LeBoeuf et al. 1981a, b) and also in red colour representatives of several marine species of *Copepoda* (Bandaranayake and Gentien 1982). In fish, 2'-norastaxanthin ester has been found in several marine fish species of the Falkland Islands (Czczuga and Kłyszajko 1986). Thus, this would be the first report on the presence of this carotenoid in freshwater fish representatives. It should be noted that in the bright red marine fish species *Mullidae* - *Mullus barbatus*, Czygan and Krtüger (1982) revealed the presence of actinioerythrin, which also belongs to the norcarotenoid group and is known (Hertzberg and Liaaen-Jensen 1968) to be the main carotenoid of the bright red *Actinia equina* individuals, in which also 2'-norastaxanthin ester has been found. On comparing the fish species examined and the carotenoids of defined groups found we can see the relation between roach and rudd individuals, in which ketocarotenoids constituted over half of all the carotenoids noted. In the roach, these were: phoenicoxanthin, canthaxanthin, 4'-hydroxyechinenone, and astaxanthin, the last one being the main carotenoid found in the roach individuals examined (31.1% - 94.5%). In the rudd, this carotenoid group contained canthaxanthin, 4'-hydroxyechinenone, astaxanthin and α -doradexanthin, which in all body parts examined constituted over half of all the carotenoids found.

In the beaked carp undermouth, however, only α -doradexanthin and astaxanthin of the ketocarotenoid group were found. And although ketocarotenoids occurred in all body parts studies, only in the fins and intestines they constituted over half of the carotenoids found. In the bitterling the ketocarotenoid group consisted of canthaxanthin, astaxanthin, 3'-hydroxyechinenone, β -doradexanthin, idoxanthin and phoenicoxanthin, constituting a considerable part of all the carotenoids found in males and females, and over half in the female skin and muscles.

Interesting differences were noted between carotenoids present in the *Rutilus rutilus* and its variety *Rutilus rutilus heckeli*. As it has been already mentioned main carotenoid of

the ketocarotenoid group found in the *Rutilus rutilus* was astaxanthin, while in the *Rutilus rutilus heckeli* - zeaxanthin (over 46% of all the carotenoids). All carotenoids constituted about 38%.

Total carotenoid content in the fish species of economic value was the highest in roach and the lowest in beaked carp undermouth individuals. In bitterlings, it was considerably higher in males in the nuptial garment than in females.

Similar phenomenon was observed when studying carotenoids in the stickleback of both sexes (Czczuga 1980a). Also very high was total carotenoid content in the body of plerocercoid *Ligula intestinalis* found in one of the bitterling specimens examined. In the larval form of this tapeworm β -carotene dominated. It should be stressed that the data obtained in this field confirm our earlier observations referring to carotenoids in *Ligulidae* representatives (Czczuga 1972, 1974). Higher carotenoid content in the parasite body than in the host has been observed previously both in tapeworms and parasitic shellfish. This was reported by Lenel (1961) when studying carotenoids in the relationship of *Sacculina carcini* as a parasite and *Carcinum meenas* (crab) as a host.

The same observations were made with reference to *Argulus foliaceus* shellfish being an ectoparasite of sticklebacks (Czczuga 1971) or *Ergasilus sieboldi* shellfish spanging on the tench branchia (Czczuga 1980b).

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Bazyli CZECHUGA

KAROTENOIDY U RYB. 51. CYPRINIDAE - FITOFAGI: *RUTILUS RUTILUS*, *RUTILUS RUTILUS HECKELI*, *SCARDINUS ERYTHROPTALMUS*, *CHONDROSTOMA NASUS* I *RHODEUS SERICEUS AMARUS*

STRESZCZENIE

Stosując chromatografię kolumnową i cienkowarstwową badano występowanie poszczególnych karotenoidów w niektórych częściach ciała 5 gatunków ryb fitofagów. Badaniami objęto takie gatunki jak: *Rutilus rutilus*, *Rutilus rutilus heckeli*, *Scardinus erythrophthalmus*, *Chondrostoma nasus* oraz *Rhodeus sericeus amarus*.

W wyniku badań ustalono obecność takich karotenoidów jak: β -karoten, α -kryptoksantyna, β -kryptoksantyna, luteina, zeaksantyna, tunaksantyna, epoksyd luteiny, echinenon, 3'-hydroksyechinenon, 4'-hydroksyechinenon, kantaksantyna, α -doradeksantyna, β -doradeksantyna, fenikoksantyna, idoksantyna, astaksantyna, rodoksantyna, oraz ester 2'-norastaksantyny.

Wspólnymi karotenoidami dla wszystkich badanych gatunków ryb okazały się β -kryptoksantyna, zeaksantyna oraz astaksantyna.

Na podkreślenie zasługuje wykazanie w płetwach płoci i wzdęgi obecności estru 2'-norastaksantyny. Byłoby to pierwsze wykazanie tego karotenoidu u przedstawicieli ryb słodkowodnych.

Podano również ogólną zawartość karotenoidów oraz stosunki procentowe poszczególnych z nich. Ogólna zawartość karotenoidów wahała się od 0,075 (płetwy *Chondrostoma nasus*) do 36,890 $\mu\text{g g}^{-1}$ świeżej masy (jelita *Rutilus rutilus*). Jeśli chodzi o badane części ciała to najzasobniejszymi w karotenoidy okazały się wątroby i jelita badanych gatunków ryb.

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