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Carotenoids in fish
35. *Cyprinidae*: *Abramis brama*, *Abramis ballerus*, and *Blicca björkna*

Bazyli Czeczuga

Medical Academy, Department of General Biology, ul. Kilińskiego 1, 15-230 Białystok, Poland

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Abstract — The author investigated the presence of various carotenoids in certain organs of *Abramis brama*, *A. ballerus* and *Blicca björkna*.

The presence of the following carotenoids has been stated: β-carotene, γ-carotene, α-cryptoxanthin, β-cryptoxanthin, canthaxanthin, lutein, lutein epoxide, zeaxanthin, tunaxanthin, mutatochrome, idoxanthin, α-doradexanthin, astaxanthin and astaxanthin ester.

The total content of carotenoid ranged from 0.093 (muscles of *Abramis brama*) to 6.688 μg/g fresh weight (skin of *Abramis ballerus*).

Key words: carotenoids in *Abramis brama*, *Abramis ballerus* and *Blicca björkna*.

1. Introduction

Among the economically valuable fish species, the *Cyprinidae* occupy an important position. The carp, for example, plays a significant role in fish farming while a number of other cyprinoids are of importance in inland fisheries. Of these species, the bream and two others, very similar in their morphology to young bream, are important fish foods. The bream, in particular, constitutes a considerable proportion of the fish catches because of its abundance and the fact that it grows to quite a large size.

In our studies on the presence of various carotenoids in different fish species living in Polish waters, we have become interested in the

problem of the occurrence of carotenoids in certain organs of individuals of species belonging to this group.

Investigating the occurrence of α-doradexanthin in fishes from Polish waters (Czeczuga 1981b), this carotenoid in *Abramis brama* and *Blicca björkna* has been found to occur.

2. Materials and methods

Individuals of the *Abramis brama* (Linné) coming from two different environments were studied. Some were caught in a lake, typical of the kind inhabited by bream, belonging to the Ełk group of lakes (April) and the others were taken from a pond of the old river-bed type (March). In addition, specimens of *Abramis ballerus* (Linné) from Stettin Bay (June), and of the *Blicca björkna* (Linné) species from the Ełk lakes (April) were investigated.

The material was prepared immediately on collection by placing it into dark glass containers and covering it with 96% acetone. It was kept in a refrigerator until the spectrophotometric determinations were made. The carotenoid pigments were extracted with 96% acetone in a dark room. Saponification was carried out with 10% KOH in ethanol at a temperature of about 20°C for 24 hours in the dark in a nitrogen atmosphere.

Columnar and thin-layer chromatography, described in detail in our previous papers (Czeczuga, Czerpak 1976) were used for the separation of the various carotenoids. A glass column (Quickfit-England), approximately 1 cm dia. and 15—20 cm in length, filled with Al_2O_3 was used in column chromatography. The extract was passed through the column after which the different fractions were eluted with the solvent. Silica gel used for the thin-layer chromatography, with appropriate solvent systems, the R_f values being determined for each spot.

The pigments were identified by the following methods: a) behaviour on column chromatography; b) absorption spectra of the pigments in various solvents, recorded with a Beckman 2400 DU spectrophotometer model; c) the partition characteristic of the carotenoid between hexane and 95% methanol; d) comparison of R_f on thin-layer chromatography; for identification of β-carotene, β-cryptoxanthin, canthaxanthin, lutein, zeaxanthin, and astaxanthin co-chromatography were applied using identical carotenoids (made by Hoffman-La Roche and Co. Ltd., Basel, Switzerland, and Sigma Chemical Company, USA); e) the presence of allylic hydroxyl groups, determined with acid chloroforms; and f) the epoxide test.

Quantitative determinations of the concentrations of carotenoid solution were made from the quantitative adsorption spectra. These de-

terminations were based on the extinction coefficient E $1^{0}/_{0}$/cm at the wave-lengths of maximal absorbance in petroleum ether or hexane.

3. Results

The results of the chromatographic analysis of the *Abramis brama* individuals are presented in Tables I and II. The carotenoids β, γ-carotene, α- and β-cryptoxanthin, canthaxanthin, lutein epoxide, zeaxanthin,

Table I. Carotenoid content in some parts of the body of Abramis brama from river (March) in % of the total carotenoid content (3 specimens, ♀, age 2+)

Carotenoid	Fins	Skin and muscles	Liver	Fat	Eggs	Heart
β - carotene				11.5	33.7	
γ - carotene					3.2	
α - cryptoxanthin	28.8	15.8	42.7	14.3	16.8	
β - cryptoxanthin			8.3			
Lutein epoxide	54.0	60.5	35.5	24.1	14.6	14.9
Zeaxanthin	17.8		10.1		5.8	34.6
α - doradexanthin	4.4	15.3	3.4	21.9	13.6	28.1
Mutatochrome		6.4		28.2		
Astaxanthin ester					12.3	22.4
Total content of carotenoids in μg/g fresh weight	1.041	0.013	1.152	0.367	1.624	0.738

Table II. Carotenoid content in some parts of the body of Abramis brama from lake (April) in % of the total carotenoid content (3 specimens, ♀, age 2+)

Carotenoid	Fins	Skin	Muscles	Liver	Intestine	Eggs
β - carotene	76.6		67.6			
Canthaxanthin		48.6				3.3
Lutein epoxide			22.3		62.0	15.3
Zeaxanthin				18.9		
Idoxanthin				13.1		6.4
α - doradexanthin			trace			trace
Astaxanthin			10.1		20.1	
Astaxanthin ester	23.4	51.4		68.0	17.9	75.0
Total content of carotenoids in μg/g fresh weight	0.392	0.267	0.128	1.244	0.427	1.749

α-doradexanthin, idoxanthin, astaxanthin, astaxanthin ester and mutatochrome were identified. Differences were observed in the various carotenoids in the *A. brama* specimens caught in March from the old river-bed and those from the lake collected during the third decade of April. In the March specimens, lutein epoxide, α-doradexanthin and α-crypto-

xanthin were found in all the parts of the body studied with the exception of the heath, whereas those caught at the end of April from the lake were found to have only astaxanthin (free and ester forms taking things altogether) in all parts of the body. In both the former and latter specimens it was found that the spawn contained the largest amount of carotenoids.

Table III. Carotenoid content in some parts of the body of Abramis ballerus in % of the total carotenoid content (5 specimens, ♀, age 1+) [x] - maximum absorption in nm 400, 425

Carotenoid	Skin	Fins	Muscles	Liver	Intestine	Eggs
β - carotene					22.9	
α - cryptoxanthin		14.4				8.3
β - cryptoxanthin	62.9	44.2	15.4	40.2	39.0	16.3
Canthaxanthin			2.4			
Lutein		6.7	19.8			26.0
Lutein epoxide	7.1	9.0	13.0		1.9	
Zeaxanthin			6.9			
Tunaxanthin		13.6				
Mutatochrome		12.1		11.1		
Astaxanthin	9.7	trace	34.7	26.0	36.2	35.8
Astaxanthin ester	20.3		7.8	22.7		10.2
Unknown						3.4[x]
Total content of carotenoids in μg/g fresh weight	6.688	2.158	1.601	2.308	3.938	1.598

Table IV. Carotenoid content in some parts of the body of Blicca björkna in % of the total carotenoid content (5 specimens, ♂♂, age 1+)

Carotenoid	Fins	Skin	Gills	Muscles	Liver	Intestine
β - carotene	2.0	24.6	12.5	14.8	6.5	8.5
α - cryptoxanthin	9.5		5.7		7.6	
β - cryptoxanthin	8.1			17.8	28.3	12.7
Canthaxanthin	9.1					
Lutein						33.8
Lutein epoxide	12.4					
Zeaxanthin	13.7	25.2		67.5		9.7
α - doradexanthin	19.6		13.0		10.6	11.8
Astaxanthin	22.2	42.6	51.7	trace	38.9	23.5
Astaxanthin ester	3.4	7.6	17.1		8.1	
Total content of carotenoids in μg/g fresh weight	5.031	0.943	4.442	0.601	5.901	1.803

In our investigation of the *Abramis ballerus* individuals it was found that these fishes contained β-carotene, α- and β-cryptoxanthin, canthaxanthin, lutein, lutein epoxide, zeaxanthin, tunaxanthin, astaxanthin (free and ester forms) and mutatochrome (Table III). β-cryptoxanthin and astaxanthin were found to be present in all the parts of the body. The

total carotenoid content ranged from 1.598 μg/g in the gonads, to 6.688 μg/g in the skin.

The results of the chromatographic analysis of the *Blicca björkna* individuals are given in Table IV. In these specimens β-carotene, α- and β-cryptoxanthin, canthaxanthin, lutein, lutein epoxide, zeaxanthin, α-doradexanthin and the free and ester forms of astaxanthin were determined. As Table IV shows, β-carotene and astaxanthin were found in all the parts of the body studied. The total carotenoid content varied between 0.601 μg/g in the muscles and 5.901 μg/g in the liver.

4. Discussion

On comparing the results obtained from the analysis of the bream specimens, differences are observed in the occurrence of certain carotenoids. While in the specimens caught in the river environment the most frequently observed carotenoids were lutein, α-doradexanthin and α-cryptoxanthin in the specimens from the lakes the commonest carotenoid was astaxanthin. In addition canthaxanthin and idoxanthin were found in the individuals from the lakes. γ-carotene and α-, β-cryptoxanthin were noted in those from river. The author observed similar differences in his previous studies on the carotenoid content in *Misgurnus fossilis* specimens from various environments (Czeczuga 1980). A different carotenoid composition and differences in the total carotenoid content were also noted in studies carried out on the trout from rivers and ponds (Czeczuga 1979a) and on carp specimens breed on food of varying quality (Czeczuga 1979b). It was interesting to note that idoxanthin was found to be present in the liver and spawn of the bream from the lakes since this carotenoid is, as we known, a β-carotene derivate (3.3', 4'-trihidoxy-4-keto-β-carotene). This carotenoid was first described by Herring (1969) who found it in the marine crustacean, *Idothea metallica*. In fish, on the other hand, it was found by Nagata and Matsuno (1979) in the fancy red carp and in individuals of the arctic lamprey (Matsuno, Nagata 1979). Recently it has been found to be present in individuals of the *Micropterus salmonides* (Czeczuga 1981a).

On comparing the carotenoids present in the individuals of the *Abramis ballerus* and *Blicca björkna* under study, it can be seen that the carotenoids present in all the parts of the body were in the former species, β-cryptoxanthin and astaxanthin, whereas in the latter species they were β-carotene and astaxanthin.

As we know, all the three species studied in our investigations feed on more or less the same type of food but, as our studies have revealed, quite significant differences occur in their carotenoid content. There are

also differences in the amount of carotenoids in the muscles of these species.

The bream from the rivers proved to be the poorest as regards carotenoids, they contained only 0.093 μg, whereas the bream from the lake had somewhat more, namely 0.128 μg as compared with the *Blicca björkna* containing 0.601 μg carotenoids in 1 g of muscle and the *Abramis ballerus* which contained as much as 1.601 μg/g muscle. In addition to the above, while in both populations of bream, the spawn has the highest carotenoid content, the *A. ballerus* specimens had the highest content in the skin, whereas in the *Blicca björkna*, in the liver and fins.

It has frequently been noted that numerous factors affect the presence and the amount of carotenoids occurring in various fish species. The most important of these factors are the biological nature of the individuals of a given species and their physiological state. As is known, just before spawning, the carotenoids are frequently translocated to the gonads and often to the skin and fins. An important role in the accumulation of certain carotenoids is also played by the type of food on which the fish live, as previously reported (Czeczuga, Czerpak 1976). Since, without doubt, the nutrient cources differ in various types of water, this affects the carotenoid content in different individuals of the same species.

5. Polish summary

Karotenoidy u ryb. 35. *Cyprinidae*: *Abramis brama*, *Abramis ballerus* i *Blicca björkna*

Stosując adsorpcyjną chromatografię kolumnową i cienkowarstwową, badano obecność poszczególnych karotenoidów w różnych narządach *Abramis brama*, *A. ballerus* oraz *Blicca björkna*.

Stwierdzono obecność następujących karotenoidów: β-, γ-karoten, α-, β-kryptoksantynę, kantaksantynę, luteinę oraz jej formę epoksydową, zeaksantynę, α-doradeksantynę, tunoksantynę, mutatochrom, idoksantynę oraz wolną i estrową formę astaksantyny.

Stwierdzono jakościowe i ilościowe różnice w występowaniu poszczególnych karotenoidów nie tylko u przedstawicieli badanych gatunków, ale również u osobników *Abramis brama* pobranych z rzeki i jeziora (tabele I—IV).

Ogólna zawartość karotenoidów wahała się od 0,083 (mięśnie — *Abramis brama*) do 6,688 μg/g surowej wagi (skóra — *A. ballerus*).

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