

Bazyli Czeczuga

## Carotenoids in fish. 25. *Cobitidae* from Polish waters

### Karotenoidy u ryb. 25. *Cobitidae* z polskich wód

Wpłynęło 23 maja 1979 r.

**Abstract:** Using column and thin-layer chromatography the content of carotenoids in the separate parts of the body of the thunder-fish, loach, and spined loach was investigated.

The analyses showed qualitative and quantitative differences between the investigated fish species.

In the investigation on the carotenoid content in various parts of the body of different fish species the problem of the occurrence of these biologically active substances in the representatives of the *Cobitidae* family, living in Polish waters, has aroused special interest. It is known that out of *Cobitidae* thunder-fish (*Misgurnus fossilis*), loach (*Nemachilus barbatulus*) and spined loach (*Cobitis taenia*) are encountered in Polish waters. These fish species show characteristic yellow brown body colouring, their environments being also specific. The thunder-fish is most numerous in stagnant river overflow-arms, overgrown bays and marshy grounds and swamps with a soft bottom where the fishes frequently dig themselves in. The other two species live in similar habitats. They can be most frequently encountered both in stagnant and in flowing waters, more rarely in mountainous streams. The specimens of the spined loach like those of the thunder-fish dig themselves in sandy bottoms. The specimens of the discussed 3 species chiefly feed on benthos fauna, above all they consume *Mollusca*, worms and insect larvae.

## Material and method

The investigation included the following specimens of the genus *Cobitidae*: *Misgurnus fossilis* (L.) collected from different environments during the year 1978, the two sexes of *Nemachilus barbatulus* (L.), and *Cobitis taenia* L. females. The acetone extracts of fins, skin, muscles, liver, intestines and reproductive organs were chromatographically analysed. Moreover, the carotenoid content was determined also in the brain of *Misgurnus fossilis* specimens.

The collected material was homogenized, treated with 95% acetone in dark bottles under nitrogen atmosphere, and kept in a refrigerator. The separation of particular carotenoid pigments was carried out by means of column and thin-layer chromatography. Before analysis the material was hydrolysed with 10% KOH in nitrogen atmosphere at room temperature during 24 hrs. After hydrolysis the extract was passed through a 15—25 cm long column filled with  $Al_2O_3$ . The separate fractions were eluted with various system of solvents, the eluates were evaporated and after evaporation the residue was dissolved in the best suited solvent (see Czeczuga, Czepak 1976) in order to draw the absorption curve whose maxima among other uses served for the identification of particular carotenoids. For the identification of  $\beta$ -,  $\gamma$ -carotene, canthaxanthin, lutein, zeaxanthin, and astaxantin the standards produced by F. Hoffmann-La Roche, Basle, were used. The absorption maxima were determined by means of a Unicam spectrophotometer.

Apart from column chromatography the obtained acetone extract was separated into individual strains with thin-layer chromatography. The glass plates 15 × 40 cm in size were covered with silica gel (Merck produce) and the acetone extract was then put with a micropipette on the starting line, various solvent systems being again used (Czeczuga, Czepak 1976). The  $R_f$  value was determined according to the generally accepted rules.

The identification of the individual carotenoids was carried out on the basis of the absorption maxima of separate fractions, the  $R_f$  values, the epoxide test, and also of the obtained epiphase and hypophase relations (Czeczuga, 1972). The quantitative relations of the separate carotenoids were determined according to Davies's method (Czeczuga, Czepak 1976).

## Results

Table I contains the results of the chromatographic analysis of carotenoids found in the acetone extract of the different parts of the body of three thunder-fish specimens. 10 carotenoids were found with females

Table I. Percentage of carotenoids found in the investigated parts of the body of *Misgurnus fossilis* (L.) sampled from three environments in 1978.

a - Wilanow farm ♀ (Oct., 18); b - ponds at Szprawy fish farm ♀ (Nov., 3); c - Dojlidy farm ♂ ♀ (Nov., 8)

Tablica I. Procentom zawartość sterydowych karotenoidów w badanych częściach ciała *Misgurnus fossilis* (L.) pobranych z trzech różnorodnych środowisk w 1978 r.

a - z gospodarstwa Wilanowo ♀ (18.X.); b - ze stawów w Szprawy ♀ (3.XI.); c - z gospodarstwa Dojlidy ♂ ♀ (8.XI.)

Name of Carotenoid Nazwa Karotenoidu	Skin - Skóra		Plac - Płetwy			Muscles - Mięśnie			Liver - Wątroba			Intestine - Jelito			Eggs - Jaja			Brain Mózg	Milt Mięso	
	a	b	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c			
β-carotene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β-carotene epoxide	56.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β-caroten epoksy	-	-	46.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β- cryptoxanthin	-	-	9.1	-	6.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β- kryptoksantyna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bixinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bixinonon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Canthaxanthin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kantaksantyna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lutein	-	-	70.7	-	43.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lutein	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lutein epoxide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α - doradexanthin	4.8	-	21.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α - doradeksantyna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Peraxanthin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tunakantyna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zeaxanthin	9.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zeksanantyna	-	-	15.0	-	12.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isosaxanthin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoeksantyna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diatoxanthin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diatoksantyna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Astaxanthin	28.7	72.6	3.2	10.7	6.3	13.9	67.2	45.6	8.5	-	-	-	-	-	-	-	-	-	-	-
Astaxantyna	-	1.5	17.0	-	66.3	-	7.8	-	12.7	100.0	11.1	-	-	-	-	-	-	-	-	-
Astaxanthin ester	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Astaksantyna estrowa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthocyanin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antocyjanin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unknown xanthophylls	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xantofiny nieznanego typu	-	25.7	-	-	6.0	-	-	-	1.8	-	-	-	-	-	-	-	-	-	-	-
Total content in $\mu\text{g/g}$ fresh weight Ogólna zawartość w $\mu\text{g/g}$ mrozonej wagi	12.454	0.782	3.467	4.194	2.781	7.875	0.174	0.224	0.320	4.183	2.475	1.614	3.063	0.477	0.832	1.658	0.440	0.407	8.419	0.052

caught in October in the fish farm at Wilamów (where municipal wastes are fed to the pond). Zeaxanthin and astaxanthin were found in all parts of the thunder-fish body. The presence of  $\alpha$ -doradoxanthin was also noted in every parts of the body except for the intestine. However,  $\beta$ -carotene epoxide,  $\beta$ -cryptoxanthin, lutein, lutein epoxide, tunaxanthin, isozeaxanthin, astaxanthin ester and mutatochrome were only found in some parts of the fish body. The greatest total carotenoid content was found in the skin and the least one in the muscles (Table Ia). The specimens sampled in the first decade of November from the ponds at Supraśl fish farm (natural food base) contained only 7 carotenoids (Table Ib). Astaxanthin (in the free or ester form) was found in every part of the body, astaxanthin ester amounting to 100% of all carotenoids in the liver and intestines. The highest carotenoid content was in this case noted in the fins and liver, the lowest one in the muscles. In the first decade of November thunder-fish specimens were sampled from the ponds at Dojlidy (intense carp culture), 13 carotenoids having been noted in the fishes (Table Ic). Among the carotenoids frequently noted in the different parts of the body were  $\beta$ -cryptoxanthin, lutein, zeaxanthin, and astaxanthin ester. The presence of echinone in the muscles and of diatoxanthin in the milt of specimens from Dojlidy should be stressed. The highest total content of carotenoids was found in the brain, fins, and in the skin.

Table II contains the results of the chromatographic analysis of carotenoids found in the acetone extract of the different parts of the body of three *Nemachilus barbatulus* specimens. The presence of 12 carotenoids was found in the females of this species (Table II a).  $\beta$ -carotene, lutein and astaxanthin ester most frequently occurred in the investigated specimens of *N. barbatulus*. Moreover, carotenoids such as  $\gamma$ -carotene,  $\epsilon$ -carotene, diatoxanthin and asterin acid which are rather infrequently found in fish, were also observed. The highest content of carotenoids was found in such parts of *N. barbatulus* body as the roe, intestines, liver and fins. In the investigated specimens of *N. barbatulus* (Table II b) 8 carotenoids were only observed, while the most frequently occurring carotenoids were  $\beta$ -carotene epoxide and astaxanthin (the pure and ester forms). Similarly as with females  $\gamma$ -carotene,  $\epsilon$ -carotene and asterin acid were found in males, the highest carotenoid content being observed in the intestines and the liver.

Table III contains the results of the chromatographic analysis of carotenoids found in the acetone extract of different parts of the body of three *Cobitis taenia* specimens. The occurrence of 12 carotenoids with  $\epsilon$ -carotene, phoenicoxanthin and asterin acid was found. With the investigated *C. taenia* specimens the most frequent carotenoids were canthaxanthin, lutein and astaxanthin ester. In every part of the fish body except for the muscles, the total carotenoid content was relatively high, attaining 249.93  $\mu\text{g/g}$  of fresh weight in the intestines.

Table II. Percentage of carotenoids found in the investigated parts of the body of *Nemachilus barbatus* (L.) sampled from the ponds at Supraśl fish farm on October 18, 1978

Tabela II. Procentowa zawartość stwierdzonych karotenoidów w badanych częściach ciała *Nemachilus barbatus* (L.) pobranych ze stawów w Supraślu 18.X.1978 r.

a - ♀; b - ♂

Name of carotenoid Nazwa karotenoidu	Skin Skóra		Fins Płetwy		Muscles Mięśnie		Liver Wątroba		Intestine Jelitko		Eggs Ikra		Milt Mlecz	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
β-carotene β-karoten	58.7		69.6		11.7		33.2					2.0		
δ-carotene δ-karoten		12.4	5.2	5.1		5.6	12.0	14.7						2.9
ε-carotene ε-karoten							8.5	11.3						
β-carotene epoxide β-karoten epoksy	10.1	74.9	10.6	21.3		57.1			69.4		20.8			
β-oryptoxanthin β-kryptoksantyna					48.2				18.7					
Isocryptoxanthin Isokryptoksantyna					8.4		27.7	37.6			34.5			
Lutein Luteina	21.7		14.4		18.6		12.2	11.2						
Diatoxanthin Diatoxantyna												9.4		
Astaxanthin Astaksantyna				35.1		12.8		23.5	7.3			2.2		46.9
Astaxanthin ester Astaksantyna estrowa	6.0		8.0	28.2		9.4	24.5	50.5		11.9		29.9		50.2
Asterin acid Asterynowy kwas	3.5	3.8										1.2		
Matateochrome Matateochrom				9.7			5.1		35.8					
Unknown xanthophylls Nieokreślone ksantofille		8.9	2.8		3.7		1.3		8.1					
Total content in µg/g fresh weight Ogólna zawartość w µg/g surowej wagi	0.397	0.664	1.069	2.289	0.191	0.354	1.826	5.758	2.378	6.168	8.608	2.825		

Table III. Percentage of carotenoids found in the investigated parts of the body of *Cobitis taenia* L. ♀ sampled from the River Supraśl on April 22, 1978

Tabela III. Procentowa zawartość stwierdzonych karotenoidów w badanych częściach ciała *Cobitis taenia* L. ♀ pobranych z rzeki Supraśl 22.IV.1978 r.

Name of carotenoid Nazwa karotenoidu	Skin Skóra	Fins Płetwy	Muscles Mięśnie	Liver Wątroba	Intestine Jelitko	Eggs Ikra
$\beta$ -carotene $\beta$ -karoten	23.4					
$\xi$ -carotene $\xi$ -karoten	3.4					
Canthaxanthin Kantaksantyna	18.8	49.1	20.9	21.5	12.5	12.9
$\beta$ -carotene epoxide $\beta$ -karoten epoksy			14.6			14.3
Phoenicoxanthin Foenikoksantyna			37.2	18.0		
Lutein Luteina	8.8	19.3	20.0	7.3	8.7	17.0
$\alpha$ -doradoxanthin $\alpha$ -doradeksantyna	5.3	6.4				5.6
Isozeaxanthin Isozeaksantyna	5.3					
Tunaxanthin Tunaksantyna		8.0		5.8		
Astaxanthin Astaksantyna				30.2	1.9	
Astaxanthin ester Astaksantyna estrowa	30.3	17.2	7.3		70.1	13.2
Asterin acid Asterynowy kwas						27.1
Unknown xanthophylls Nieokreślone ksantofile	4.7			3.9	6.8	9.9
Total content in $\mu\text{g/g}$ fresh weight Ogólna zawartość w $\mu\text{g/g}$ surowej wagi	13.106	36.277	0.160	64.427	249.930	34.636

## Discussion

Of the carotenoids found in the investigated representatives of *Cobitidae* the presence of those which were relatively rare in fish should be stressed. This group included:  $\beta$ -carotene epoxide, echinenone, diatoxanthin,  $\gamma$ -carotene,  $\varepsilon$ -carotene, phoenicoxanthin, asterin acid,  $\alpha$ -doradoxanthin, mutatoxanthin, and mutatochrome.

Carotenes such as  $\gamma$ - and  $\varepsilon$ -carotene were found in the specimens of *Salmo gairdneri* (Czeczuga 1979 b) while the  $\varepsilon$ -carotene was also noted in the specimens of *Coregonus albula* (Czeczuga 1977). Weber et al. (1973) claimed the occurrence of  $\varepsilon$ -carotene in the specimens of *Cichlasoma citrinellum*. The occurrence of xanthophylls such as mutatochrome, mutatoxanthin, echinenone, diatoxanthin, and asterin acid was also observed in the specimens of *Salmo gairdneri*. Echinenone and diatoxanthin were also observed in other fish species. Tanaka et al. (1976) found echinenone in the sea bream, while Rodriguez et al. (1973) reported it from the goldfish. Matsuno and Katsuyama (1976) reported the occurrence of diatoxanthines in several fish species of the *Percichthes* group. Phoenicoxanthin was noted in the

specimens of *Coregonus peled* (Czeczuga 1977) and  $\beta$ -carotene epoxide in the specimens of *Cyprinus carpio* (Czeczuga 1979 c). K a t a y a m a et al. (1970) were first to find the occurrence of  $\alpha$ -doradexanthin in fish.

As far as the investigated specimens of *Misgurnus fossilis* are concerned, zeaxanthin and astaxanthin are among the carotenoids found in greater amounts in the population from Wilamowo: in the specimens from the Supraśl fish farm, a greater content of the two forms of astaxanthin and in the specimens from the Dojlidy ponds, of lutein, zeaxanthin, and astaxanthin (also the two forms) was observed.

In the investigated specimens of *Nemachilus barbatulus* significant differences in the occurrence of carotenoids as depending on sex were observed. Moreover, the highest content of  $\beta$ -carotene,  $\beta$ -carotene epoxide and the two forms of astaxanthin (males) was noted here. In the specimens of *Cobitis taenia* the most frequent carotenoids were canthaxanthin, lutein, and the two forms of astaxanthin. Moreover, in the skin of these specimens  $\beta$ -carotene constituted 23.4% of all carotenoids while asterin acid amounted to 27.1% in the roe.

In considering the total content of carotenoids in the specimens of the investigated *Cobitidae* species, it was found that in the specimens of *Misgurnus fossilis* the fins, skin and liver contained the greatest amounts of these substances. The total carotenoid content in the muscles was within the range of 0.174—0.320  $\mu\text{g/g}$  of fresh weight. The greatest content of carotenoids was noted in the muscles of specimens from the Dojlidy ponds, where fish were constantly given additional fodder. In the specimens from these ponds the variation of carotenoids was greater than in the fish from other farms. In the specimens of *Nemachilus barbatulus* the highest carotenoid content was found in the intestine, liver and reproductive cells, the lowest content being noted in the muscles: 0.191 in females and 0.354  $\mu\text{g/g}$  in males. The muscles of *Cobitis taenia* specimens contained only 0.160  $\mu\text{g/g}$  while in other parts of the body of these fishes the carotenoid content ranged from 13.105 (skin) to 249.930  $\mu\text{g/g}$  of fresh weight (intestines).

In general, the specimens of the investigated species of *Cobitidae* contained much more carotenoids than the species of such economical importance as *Coregonus albula* (Czeczuga 1977). It is a not infrequent example that the „weed” fish contain much more carotenoids than economically important species. Among other species it was found with *Leucaspis delineatus* (Czeczuga, Czerpak 1976) and also with *Acerina cernua* L. (Czeczuga 1979 a).

## STRESZCZENIE

Autor stosując chromatografię kolumnową i cienkowarstwową badał występowanie poszczególnych karotenoidów w płetwach, skórze, mięśniach, wątrobie, jelitach i w ikrze piskorza, śliza i kozy.

W wyniku badań ustalono obecność takich karotenoidów jak:  $\beta$ -carotene,  $\gamma$ -carotene,  $\epsilon$ -carotene, echinenone,  $\beta$ -carotene epoxide, canthaxanthin,  $\beta$ -cryptoxanthin, isocryptoxanthin, phoenicoxanthin, lutein, lutein epoxide,  $\alpha$ -doradexanthin, tunaxanthin, zeaxanthin, isozeaxanthin, diatoxanthin, astaxanthin, astaxanthin ester, asterin acid, mutatochrome i mutatoxanthin. Podano również stosunki procentowe poszczególnych karotenoidów dla badanych części ciała w/w gatunków *Cobitidae*. Między innymi osobniki kozy okazały się najbardziej zasobne w karotenoidy w porównaniu do dwóch pozostałych badanych gatunków *Cobitidae*.

## REFERENCES

- Czeczuga B., 1972. Astaxanthin — the carotenoid predominant in *Eylais hamata* (Koenike, 1897) (*Hydracarina, Arachnoidea*). Comp. Biochem. Physiol., 32 B, 137—141.
- Czeczuga B., 1977. Karotenoidy u ryb. 13. *Coregonus peled* (Gmel.) z polskich wód — Carotenoids in fish. 13. *Coregonus poled* (Gmel.) from Polish waters. Acta Hydrobiol., 19, 183—190.
- Czeczuga B., 1979 a. Karotenoidy u ryb. 21. *Percidae* z polskich wód — Carotenoids in fish. 21. *Percidae* from Polish waters. Acta Hydrobiol., 21, 1—7.
- Czeczuga B., 1979 b. Carotenoids in fish. 20. Carotenoids in *Salmo gairdneri* Rich. and *Salmo trutta morpha fario* L. Hydrobiologia, 63, 15—23.
- Czeczuga B., 1979 r. Carotenoids in fish. 22. Changes in carotenoids in *Cyprinus carpio* L. Hydrobiologia, 64, 233—240.
- Czeczuga B., R. Czerpak, 1976. Karotenoidy u ryb. 7. Rodzaj pokarmu i zawartość karotenoidów i witaminy A u *Carassius carassius* (L.) i *Leucaspis delineatus* (Heck.) — Carotenoids in fish. 7. The kind of food and the content of carotenoids and vitamin A in *Carassius carassius* (L.) and *Leucaspis delineatus* (Heck.) Acta Hydrobiol., 18, 1—21.
- Katayama T., Yokoyama, C. O. Chichester, 1970. The biosynthesis of astaxanthin. 1. The structure of  $\alpha$ -doradexanthin and  $\beta$ -doradexanthin. Int. J. Biochem., 1, 438—444.
- Matsumo T., M. Katsuyama, 1976. Comparative biochemical studies of carotenoids in fishes. 9. On the nineteen species of fishes in the division *Percichthyees*. Bull. Jap. Soc. Sci. Fish., 42, 645—649.
- Rodríguez D. B., K. L. Simpson, C. O. Chichester, 1973. The biosynthesis of astaxanthin. 17. Intermediates in the conversion of  $\beta$ -carotene. Int. J. Biochem., 4, 213—222.
- Tanaka Y., T. Katayama, K. L. Simpson, C. O. Chichester, 1976. The biosynthesis of astaxanthin. 20. The carotenoids in marine red fish and the meta-



bolism of the carotenoids in sea bream, *Chrysophrys major* Temminck and Schlegel. Bull. Jap. Soc. Sci. Fish., 42, 1177—1182.  
Weber R., G. W. Barlow, A. H. Brush, 1973. Pigments of a color polymorphism in a cichlid fish. Comp. Biochem. Physiol., B, 44, 1127—1135.

Author's address — Adres autora

Prof. dr hab. Bazyli Czeczuga

Zakład Biologii Ogólnej, Akademia Medyczna, ul. Kilińskiego 1, 15-230 Białystok