ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XXVIII, Fasc. 1 Szczecin 1998

Bazyli *CZECZUGA* [1], Ryszard *BARTEL* [2]

THE OCCURRENCE OF CAROTENOIDS IN DIFFERENT-AGE INDIVIDUALS OF *PELECUS CULTRATUS* (L.) FROM THE VISTULA LAGOON

WYSTĘPOWANIE KAROTENOIDÓW U RÓŻNYCH WIEKOWO OSOBNIKÓW CIOSY (*PELECUS CULTRATUS* L.) Z ZALEWU WIŚLANEGO

[1] **Department of General Biology, Medical University, Białystok, Poland**
[2] **Institute of Inland Fisheries, Gdańsk**

The occurrence and contents of various carotenoids in body parts of different-age individuals of *Pelecus cultratus* from the Vistula Lagoon were studied with column and thin layer chromatography.

INTRODUCTION

Biologically active carotenoids are synthesised by plants. When taken up with food they accumulate in animal organs. Some of them are used as a source of vitamin A. In fishes, carotenoids acquired with food accumulate mainly in the liver and intestines. In some species they give reddish colour to muscles (salmonids) or to fins. Recently fish-farmers have taken much interest in carotenoids (Torrissen et al. 1989). It has been found that feeding fishes, salmonids in particular, with artificial food causes their muscles to get pale, thus making them less attractive to consume (Simpson et al. 1981). Therefore, in many countries, feeds with supplementary carotenoids (mainly astaxanthin or canthaxanthin) are used, which makes the fish more attractive for the market.

In our studies on the content of carotenoids in respective fish species of the Polish waters, this time we concentrate on *Pelecus cultratus* individuals found in the Vistula Lagoon near the Baltic Sea, a species never examined for carotenoids. We are particularly interested in carotenoids specific to different-age individuals of this species in natural conditions.

MATERIAL AND METHODS

The investigations included *Pelecus cultratus* (L.) individuals collected for analysis in the second half of May 1994 from the Vistula Lagoon near the Baltic Sea. They were divided into three age groups according to the measurements of the body length compared with the data of Terlecki (Brylińska 1986) for individuals of this species from the Vistula Lagoon. In all groups the following organs were examined: fins (separately—dorsal (D), caudal (C), and the remaining ones), skin, muscles, liver, intestine, and in females from the last age group—also eggs.

Tissue samples from each individual were homogenised, placed in a dark bottle, soaked in 95-% acetone, and 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, 1.8 cm in diameter. The fractions were eluted with different solvent combinations (Czeczuga and Czerpak 1976).

In addition the column chromatography, the acetone extracts were separated with layer chromatography as well. Silica gel covered glass plates and different solvents 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-carotene, β-cryptoxanthin, canthaxanthin, lutein, zeaxanthin, 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 based on the absorption coefficient E 1%/cm in corresponding peaks of absorbance in kerosene ether or hexane (Davies 1976).

RESULTS

Thirteen carotenoids were found in the individuals of *Pelecus cultratus* from the Vistula Lagoon (Tab. 1). All individuals from the age group II shared β-carotene, tunaxanthin, lutein epoxide, and astaxanthin (Tab. 2). Group IV individuals had β-carotene,

lutein epoxide, β-doradexanthin, and astaxanthin in common (Tab. 3). Individuals of group V shared only β-carotene and astaxanthin (Tab. 4). The highest carotenoid content in groups II and IV was found in fins D and C, and liver, while in group V—in fins D and C and eggs. Astaxanthin was most frequently a dominant in the respective parts of the body (in 12 cases), followed by tunaxanthin and β-doradexanthin (in 8), and lutein epoxide (in 6 cases). The data obtained, indicate that in all of the body parts examined ketocarotenoids were predominant in every age group and included hydroxyechinenone, canthaxanthin, α- and β-doradexanthin, and astaxanthin. Their total content in the material examined ranged from 21.6% to 72.8% of all carotenoids (Tab. 2–4).

Table 1

List of the carotenoids from the investigated material

	Carotenoid	Structure (see Fig. 1)	Semisystematic name
1	β-carotene	A - r - A	β,β-carotene
2	ε-carotene	B - r - B	ε,ε-carotene
3	β-cryptoxanthin	A - r - C	β,β-caroten-3-ol
4	neothxanthin	B - r - D	ε,ε-caroten-3-ol
5	lutein	C - r - D	β,ε-carotene-3,3'-diol
6	zeaxanthin	C - r - C	β,β-carotene-3,3'-diol
7	tunaxanthin	D - r - D	ε,ε-carotene-3,3'-diol
8	lutein epoxide	D - r - E	5,6-epoxy-5,6-dihydro-β,ε-carotene-3,3'-diol
9	hydroxyechinenone	A - r - G	3-hydroxy-β,β-carotene-4-one
10	canthaxanthin	F - r - F	β,β-carotene-4,4'-dione
11	α-doradexanthin	D - r - G	3,3'-dihydroxy-β,ε-caroten-4-one
12	β-doradexanthin	C - r - G	3,3'-dihydroxy-β,β-caroten-4-one
13	astaxanthin	G - r - G	3,3'-dihydroxy-β,β-carotene-4,4'dione

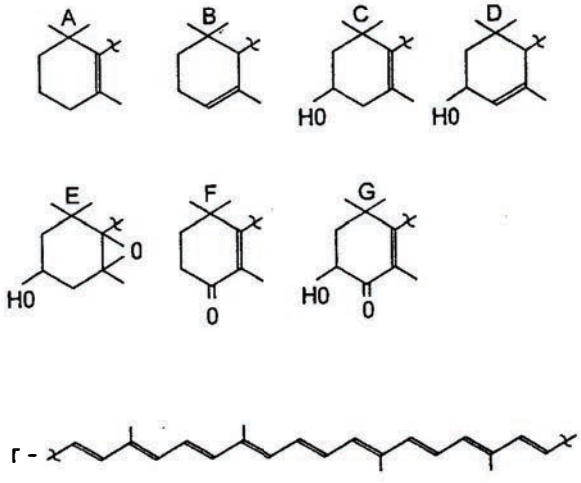

Fig. 1. Structural features of carotenoids from the material investigated

Table 2

Carotenoid content in the investigated parts of the body of *Pelecus cultratus*—II age group (total length 19.3–22.5 cm)

Part of body	Carotenoids (see Table 1)	Major carotenoids (%)	Keto-carotenoids (%)	Total content ($\mu g \cdot g^{-1}$ dry weight)
First part (n – 9)				
Fins D and C	1, 3, 7, 8, 10, 12, 13	12 (33.7)	44.5	14.54
Other fins	1, 3, 7, 8, 11, 12, 13	12 (37.9)	37.9	5.32
Skin	1, 3, 7, 8, 11, 12, 13	8 (40.2)	31.8	1.38
Muscles	1, 3, 4, 7, 8, 12, 13	4 (18.4)	31.0	0.57
Liver	1, 3, 6, 7, 8, 10, 11, 12, 13	7 (23.7)	35.1	10.41
Intenstine	1, 5, 7, 8, 10, 11, 12, 13	8 (45.9)	43.5	4.91
Second part (n – 9)				
Fins D and C	1, 3, 4, 5, 6, 7, 8, 10, 13	8 (27.1)	27.0	17.10
Other fins	1, 3, 6, 7, 8, 10, 13	13 (26.7)	40.8	5.16
Skin	1, 3, 7, 8, 10, 11, 12, 13	13 (23.9)	56.5	1.00
Muscles	1, 3, 4, 7, 8, 13	13 (47.1)	24.6	0.25
Liver	1, 3, 4, 7, 8, 11, 12, 13	8 (19.5)	27.9	7.15
Intenstine	1, 4, 5, 6, 7, 8, 10, 11, 12, 13	13 (34.9)	60.0	5.43

Table 3

Carotenoid content in the investigated parts of the body of *Pelecus cultratus*—IV age group (total length 30.2–32.8 cm)

Part of body	Carotenoids (see Table 1)	Major carote-noids (%)	Ketocarote-noids (%)	Total content ($\mu g \cdot g^{-1}$ dry weight)
First part (n – 7)				
Fins D and C	1, 3, 4, 5, 7, 8, 10, 12, 13	12 (24.4)	53.4	9.86
Other fins	1, 4, 5, 8,10, 11, 12, 13	13 (17.6)	42.7	2.29
Skin	1, 3, 4, 7, 8, 10, 11, 12, 13	12 (17.8)	38.8	1.57
Muscles	1, 3, 7, 8, 10, 11, 12, 13	7 (24.6)	39.9	0.59
Liver	1, 3, 6, 8,10, 11, 12, 13	13 (28.7)	61.6	9.86
Intenstine	1, 2, 3, 8, 10, 11, 12, 13	13 (30.4)	72.8	3.61
Second part (n – 7)				
Fins D and C	1, 2, 3, 5, 6, 7, 8, 11, 12, 13	13 (24.4)	38.5	6.21
Other fins	1, 4, 6, 7, 8, 10, 11, 12, 13	13 (29.9)	46.1	3.53
Skin	1, 3, 4, 6, 7, 8, 9, 11, 12, 13	6 (23.8)	36.0	1.27
Muscles	1, 3, 4, 5, 6, 8, 11, 12, 13	4 (21.7)	21.6	0.65
Liver	1, 3, 6, 7, 8, 10, 11, 12, 13	13 (22.8)	49.4	11.56
Intenstine	1, 2, 4, 6, 7, 8, 10, 11, 12, 13	12 (41.6)	56.6	2.47

Table 4

Carotenoid content in the investigated parts of the body of *Pelecus cultratus*—V age group (total length 33.4–36.5 cm)

Part of body	Carotenoids (see Table 1)	Major carotenoids (%)	Ketocarotenoids (%)	Total content ($\mu g \cdot g^{-1}$ dry weight)
First part (n – 5)				
Fins D and C	1, 2, 5, 6, 8, 10, 11, 12, 13	8 (31.1)	39.7	13.29
Other fins	1, 3, 5, 6, 7, 8, 10, 11, 12, 13	12 (20.2)	51.2	3.22
Skin	1, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13	8 (26.1)	36.0	3.78
Muscles	1, 3, 7, 8, 10, 11, 12, 13	10 (30.8)	66.3	0.61
Liver	1, 3, 5, 6, 10, 11, 12, 13	10 (24.9)	59.5	4.65
Intenstine	1, 3, 4, 6, 7, 11, 12, 13	7 (33.8)	40.2	2.94
Eggs	1, 2, 3, 5, 7, 9, 10, 12, 13	9 (27.1)	69.3	6.36
Second part (n – 5)				
Fins D and C	1, 4, 5, 6, 7, 8, 11, 12, 13	12 (32.4)	43.9	10.58
Other fins	1, 4, 6, 7, 8, 10, 13	7 (36.5)	33.5	3.14
Skin	1, 2, 4, 6, 7, 8, 12, 13	7 (38.5)	23.7	3.42
Muscles	1, 3, 4, 7, 8, 11, 12, 13	7 (40.4)	37.6	1.04
Liver	1, 3, 4, 7, 8, 9, 10, 11, 12, 13	12 (21.5)	55.1	2.40
Intenstine	1, 4, 6, 8, 10, 13	13 (37.8)	66.5	2.76
Eggs	1, 3, 4, 6, 7, 8, 10, 13	7 (36.7)	38.6	6.26

DISCUSSION

The knife (*Pelecus cultratus*) spends most of its life in desalted areas of the Baltic Sea, the Black Sea, the Sea of Azov, the Caspian Sea, and the Aral Sea (Berg 1948; Gąsowska 1962). Individuals of this fish are encountered in such lakes in Russia as Ladoga, Onega, or Ilmien (Koncevaja 1972) or Balaton in Hungary (Jokiel 1956) and in some dammed reservoirs (Tanasijčuk 1973). However, for the spawning time it always enters a river. In Poland, it is mainly found in the Vistula Lagoon, the Baltic Sea (Jokiel 1956; Terlecki 1979), and in the Polish rivers distant from the Baltic Sea. It has been caught in the Vistula River in its mouth (Pliszka et al. 1951), near Tczew (Gąsowka 1962), and even near Warsaw (Backiel and Kossakowski 1949; Pliszka et al. 1951). It has been also observed at various sites of the river Warta (Jaśkowski 1962; Penczak 1969) and in the Wirynka, a left tributary of the Warta (Kaj 1958). Moreover, it has been found in the river San near Przemyśl (Rolik 1971). In all reservoirs, the knife starts to feed on plankton, mainly on crustaceans, while in older age—on small fishes (Koncevaja 1972; Tanasijčuk 1973), mainly the gobid fish supplemented with invertebrates. In the Vistula Lagoon, the gammarids and chironomids are the supplementary food (Brylińska 1986).

According to the data obtained, carotenoids found in different body parts of *Pelecus cultratus* individuals from the Vistula Lagoon are derivatives of β-carotene (β-crypto-

xanthin, zeaxanthin, hydroxyechinenone, canthaxanthin, β-doradexanthin, and astaxanthin), ε-carotene (neothxanthin, tunaxanthin), and α-carotene (lutein, lutein epoxide, and α-doradexanthin). All these three carotenoids and their derivative have been found in the living organisms of the Baltic Sea. Those organisms were found to be food items of *Pelecus cultratus* (Czeczuga 1976, 1978). Only α-carotene was not found in the examined individuals of the knife, though its derivatives were present in all parts examined. α-Carotene is transformed into lutein or its epoxy form both in plants and animals (Goodwin 1980). ε-Carotene and its derivatives—neothxanthin and tunaxanthin are common in freshwater fishes (Czeczuga 1979, 1981) and in sea fishes (Bingham et al. 1979). However, in the individuals examined, β-carotene and its derivatives prevail, mainly ketocarotenoids with astaxanthin constituting the majority. These carotenoids are known to depend on the transformation pathway of β-carotene into astaxanthin. However, both in marine fishes (Miki et al. 1984; Matsuno et al. 1985), anadromic fishes (Kitahara 1983), and freshwater fishes (Katsuyama et al. 1987) such ketocarotenoids as astaxanthin and canthaxanthin can be reduced; astaxanthin—to zeaxanthin and canthaxanthin—to β-carotene. In the group of carotenoids found in the *Pelecus cultratus* individuals, β-carotene and β-cryptoxanthin are provitamins of vitamin A. One molecule of β-cryptoxanthin gives one molecule of vitamin A, while one molecule of β-carotene produces two vitamin A molecules (Bauernfeind 1972).

A comparison made of the total carotenoids content in the respective body parts of the *Pelecus cultratus* individuals examined, indicates that in all age groups the highest concentration of carotenoids occurred in fins D and C, while the lowest in the muscles. In the remaining body parts in the first two age groups, the concentrations were similar. However, in group V (females with mature eggs) the distribution of carotenoids content in the respective parts changed compared to the other age groups. A significant decrease in carotenoids was observed in the liver, while their amount increased in the skin. The highest concentration in sexually mature females was still maintained in dorsal (D) and caudal (C) fins, and very high in the eggs. It seems that the carotenoids accumulated in the liver in sexually immature *Pelecus cultratus* individuals in the prespawning season shift to other organs, in the case of females—to the eggs and skin. Carotenoid shift in the prespawning time has been already observed only in salmonid fish species. It was found by Crozier (1970) in *Oncorhynchus nerka* individuals, by Czeczuga and Chełkowski (1984) in *Salmo trutta* morpha *trutta* before and after the spawning time, and by Czeczuga and Bartel (1989) in *Salmo trutta* morpha *lacustris* in the spawning time. Hence, the literature data have attributed this phenomenon to the salmonids (Torrissen et al. 1989). The present studies support the assumption that carotenoid shift in the spawning time occurs also in the cyprinid fishes, a representative of which is the knife. It should be assumed that carote-

noids in the knife, like in salmonids (Ando 1986), move in the organism together with blood, combined with particular serum proteins.

CONCLUSIONS

A total of 13 carotenoids was found in the individuals of knife (*Pelecus cultratus* L.) from the Vistula Lagoon. The highest carotenoid content was found in fins D and C, liver, and intestines, while the lowest—in muscles. In sexually mature females in the prespawning season carotenoids shift from the liver to the eggs and skin.

REFERENCES

Ando S., 1986: Studies on the food biochemical aspect of changes in chum salmon, *Oncorhynchus keta* spawning migration: mechanisms of muscle deterioration and nupital coloration. Mem. Facult. Fish. Hokkaido Univ., **33**: 1–95.

Backiel T., J. Kossakowski, 1949: Materiały do charakterystyki stosunków rybackich na Wiśle [Characterization of fishing conditions on Vistula]. Przegl. Ryb., **16**: 146–152. (In Polish).

Bauernfeind J.C., 1972: Carotenoid vitamin A precursors and analogs in food and feeds. J. Agr. Food. Chem., **20**: 456–473.

Berg L.S., 1948: Ryby presnych vod SSSR i sopredelnych stran [Freshwater fishes of SSSR and neighbonring states]. Izd. AN SSSR, Moskva-Leningrad: 810–814. (In Russian).

Bingham A., D.W. Wilkie, H.S. Mosher, 1979: Tunaxanthin: occurrence and absolute sterochemistry. Comp. Biochem. Physiol., **62B**: 489–495.

Brylińska M., (ed.) 1986: Ryby słodkowodne Polski [Freshwater fish of Poland]. PWN, Warszawa: 282–285. (In Polish).

Crozier G.F., 1970: Tissue carotenoids in prespawning and spawning sockeye salmon (*Oncorhynchus nerka*). J. Fish Res. Bd. Canada, **27**: 937–975.

Czeczuga B., 1976: Carotenoids content in some crustaceans from the Baltic Sea. Bull. Acad. Pol. Sci. Cl. II, **24**(6): 349–353.

Czeczuga B., 1978: Carotenoid content in some animals of the Baltic Sea benthos. Bull. Acad. Pol. Sci. Cl. II, **26**(6): 383–387.

Czeczuga B., 1979: Changes in carotenoids in *Cyprinus carpio* L. Hydrobiologia, **65**: 233–240.

Czeczuga B., 1981: Rhodoxanthin in *Ctenopharyngodon idella* Val. (Cyprinidae). Comp. Biochem. Physiol., **69B**: 885 887.

Czeczuga B., R. Bartel, 1989: Studies on carotenoids in spawning *Salmo trutta* morpha *lacustris* L. Acta Ichth. Piscat., **19**, 1: 49–58.

Czeczuga B., Z. Chełkowski, 1984: Carotenoid contents in adult individuals of sea-trout *Salmo trutta* L. dnring spawning migration, spawning and post-spawning migration. Acta Ichth. Piscat., **14**, 1–2: 187–201.

Czeczuga B., R. Czerpak, 1976: The kind of food and the content of carotenoids and vitamin A in *Carassius carassius* (L.) and *Leucaspius delineatus* (Heck.). Acta Hydrobiol., **18**: 1–21.

Czeczuga B., R. Czerpak, 1968: Carotenoids in the carapaces of the *Orconectes limosus* (Raf.), Crustacea, Decapoda. Eur. J. Biochem., **5**: 429–432.

Davies B.H., 1976: Carotenoids. In: Chemistry and biochemistry of plant pigments [T.W. Goodwin (ed.)], Academic Press, London-New York-San Francisco: 38–165.

Gąsowska M., 1962: Krągłouste i ryby – Cyclostomi et Pisces. Klucze do oznaczania kręgowców Polski [Cyclostomes and Fishes. Keys for identification of the vertebrates of Poland], PWN, Warszawa: 62–64. (In Polish).

Goodwin T.W., 1980: The biochemistry of the carotenoids. 1 Plants. Chapman and Hall, London-New York: 223–248.

Jaśkowski J., 1962: Materiały do znajomości ichtiofauny Warty i jej dopływów [Contribution materials to knowledge of ichthyofauna of the Warta and its tributaries]. Fragm. Faun., **9**: 449–499. (In Polish).

Jokiel J., 1956: Dziesięcioletnia ciosa z Zalewu Wiślanego [Ten-year-old knife from the Vistula Lagoon]. Gosp. Ryb., nr **7**: 20. (In Polish).

Kaj J., 1958: Rzadsze i fizjograficznie interesujące gatunki ryb północno-zachodniej Polski [Rare and physiographically interesting fish species of north-western Poland]. - Przyr. Pol. Zach., **2**: 270–277. (In Polish).

Katsuyama M., T. Komori, T. Matsuno, 1987: Metabolism of three stereoisomers of astaxanthin in the fish rainbow trout and tilapia. Comp. Biochem. Physiol., **86B**: 1–5.

Kitahara T., 1983: Behaviour of carotenoids in the chum salmon (*Oncorhynchus keta*) during andromous migration. Comp. Biochem. Physiol., **76B**: 97–101.

Koncevaja N.J., 1972: Sozrevanie i nerest cechoni (*Pelecus cultratus* L.) v ozere Ilmen [Maturation and spawning-time of knife in Ilmen lake]. Rybnochoz. izuč. vnutr. vodoemov, **10**: 24–27. (In Russian).

Matsuno T., Katsuyama, T. Maoka, T. Hirono, T. Komori, 1985: Reductive metabolic pathways of carotenoids in fish (3S, 3'S) – astaxanthin to tunaxanthin A, B and C. Comp. Biochem. Physiol., **80B**: 779–789.

Miki W., K. Yamaguch, S Konosu, T. Watanabe, 1984: Metabolism of dietary carotenoids in eggs of red sea bream. Comp. Biochem. Physiol., **77B**: 665–668.

Penczak T., 1969: Ichtiofauna rzek Wyżyny Łódzkiej i terenów przyległych. Część III. Przegląd i charakterystyka gatunków [Ichthyofauna of rivers of the Łódź Upland and adjacent areas. Part. III. Review and characterization of species]. Acta Hydrobiol., **11**: 339–360. (In Polish).

Pliszka F., T. Backiel., J. Dziekońska., J. Kossakowski., St. Włodek, 1951: Badania nad odżywianiem się ryb na Wiśle [Studies on feeding of fishes from the Vistula River]. Rocz. Nauk. Roln., **57**: 205–236. (In Polish).

Rolik H., 1971: Ichtiofauna dorzecza górnego i środkowego Sanu [Ichthyofauna of upper and middle part of river San basin]. Fragm. Faun., **17**: 559–584. (In Polish).

Simpson K.L., T. Katayama, C.O. Chichester, 1981: Carotenoids in fish feeds. In: Carotenoids as Colorants and Vitamin A Precursors [J.C. Baurenfeind (ed.)], Academic Press, New York, Chapt. 4: 463–538.

Straub O. (ed.), 1987: Key to carotenoids. Birkhauser Verlag, Basel-Boston: 11–162.

Tanasijčuk L.N., 1973: Cechon Kachovskogo i Kremenčugskogo Vodochranilišč [Knife of Kachovski and Kremencugski reservoirs]. Ryb. Choz., **17**: 67–71. (In Russian).

Terlecki J., 1979: Charakterystyka cech merystycznych i biomerystycznych ciosy – *Pelecus cultratus* z Zalewu Wiślanego [Characterization of meristematic and biomeristematic features of knife from the Vistula Lagoon]. Zesz. Nauk. AR-T w Olsztynie, **10**: 194–203. (In Polsh).

Torrissen O.J., R.W. Hardy, K.D. Schearer, 1989: Pigmentation of Salmonids—carotenoid deposition and metabolism. Critical Rev. Aquatic Sci., **1**: 209–225.

Bazyli CZECZUGA, Ryszard BARTEL

WYSTĘPOWANIE KAROTENOIDÓW U RÓŻNYCH WIEKOWO OSOBNIKÓW CIOSY (*PELECUS CULTRATUS* L.) Z ZALEWU WIŚLANEGO

STRESZCZENIE

Autorzy stosując chromatografię kolumnową i cienkowarstwową badali występowanie poszczególnych karotenoidów w płetwach, skórze, mięśniach, wątrobie, jelitach i gonadach osobników trzech grup wiekowych *Pelecus cultratus* (L.).

Ustalono obecność następujących karotenoidów: β-karoten, ε-karoten, β-kryptoksantyna, neoksantyna, luteina, zeaksantyna, tunaksantyna, lutein epoksyde, hydroksyechinenon, kantaksantyna, α-doradeksantyna, β-doradeksantyna i astaksantyna.

Podano również ogólną zawartość karotenoidów w poszczególnych częściach ciała ciosy oraz udział procentowy poszczególnych karotenoidów. Wraz z wiekiem ryb wzrasta ilość karotenoidów oraz ogólna ich zawartość.

Received: 18 December 1997

Authors' address:

Bazyli Czeczuga DSc Prof Tit
Department of General Biology
Medical University in Białystok
Kilińskiego 1, 15-230 Białystok, Poland