A C T A   I C H T H Y O L O G I C A   E T   P I S C A T O R I A
Vol. XXIII, Fasc. 2   Szczecin 1993

Bazyli CZECZUGA

Biochemisty

CAROTENOIDS IN FISH. 50. *CYPRINIDAE* – PLANKTONOPHAGES: *ALBURNUS ALBURNUS*, *ALBURNOIDES BIPUNCTATUS* AND *LEUCASPIUS DELINEATUS*

BADANIA KAROTENOIDÓW U RYB. 50. *CYPRINIDAE* – PLANKTONOFAGI: *ALBURNUS ALBURNUS*, *ALBURNOIDES BIPUNCTATUS* I *LEUCASPIUS DELINEATUS*

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The presence of carotenoids in several body parts of three planktonophagous species was investigated by means of columnar and thin–layer chromatography.

INTRODUCTION

Among various cyprinid fish species inhabiting inland waters of Poland, several feed on shellfish, a part of zooplankton. Numerous investigations have found astaxanthin to the main carotenoid in shellfish (Czeczuga and Czerpak 1968). Thus, the question arises whether fish species feeding on zooplankton also have this carotenoid as predominant. It is known that food abunding in carotenoids effects significantly their content in consumers, in this case in fish (Czeczuga and Czerpak 1976).

Cammon planktonophages of the *Cyprinidae* family in our inland waters are the break, spirling and sunbleak (Brylińska 1986). They mostly inhabit running waters, although bleak is also found in lakes and caught by fishermen.

MATERIAL AND METHODS

Fish species analysed in our investigations were: *Alburnus alburnus* L. caught in the Ełk Lake at the end of June 1975, *Alburnoides bipunctatus* Bloch in the Dunajec River (7th April 1981, Niedzica region), and *Leucaspius delineatus* Heckel in the Dojlidy ponds (collected in various periods). The fins, skin, muscles, liver, intestines and gonads were examined in all individuals.

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 uderwent hydrolysis with 10% KOH in N_2 atmosphere, at room temperature, within 24 hours. When hydrolised an extract was transferred into column filled up with Al_2O_3. The length of column ranged from 15 to 25 (Quickfit Co. – England) Particular fractions were eluted 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 plass covered with silicagel 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) appearence 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 allylohydroxy groups identified with acidic chloroform; f) epoxidic test. The quantity of particular carotenoids was estimated based upon a quantitive 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

Carotenoids identified in the fish species examined are given in Tab. 1–2. Data on the occurrence of carotenoids in various body parts of bleak are presented in Tab. 3.

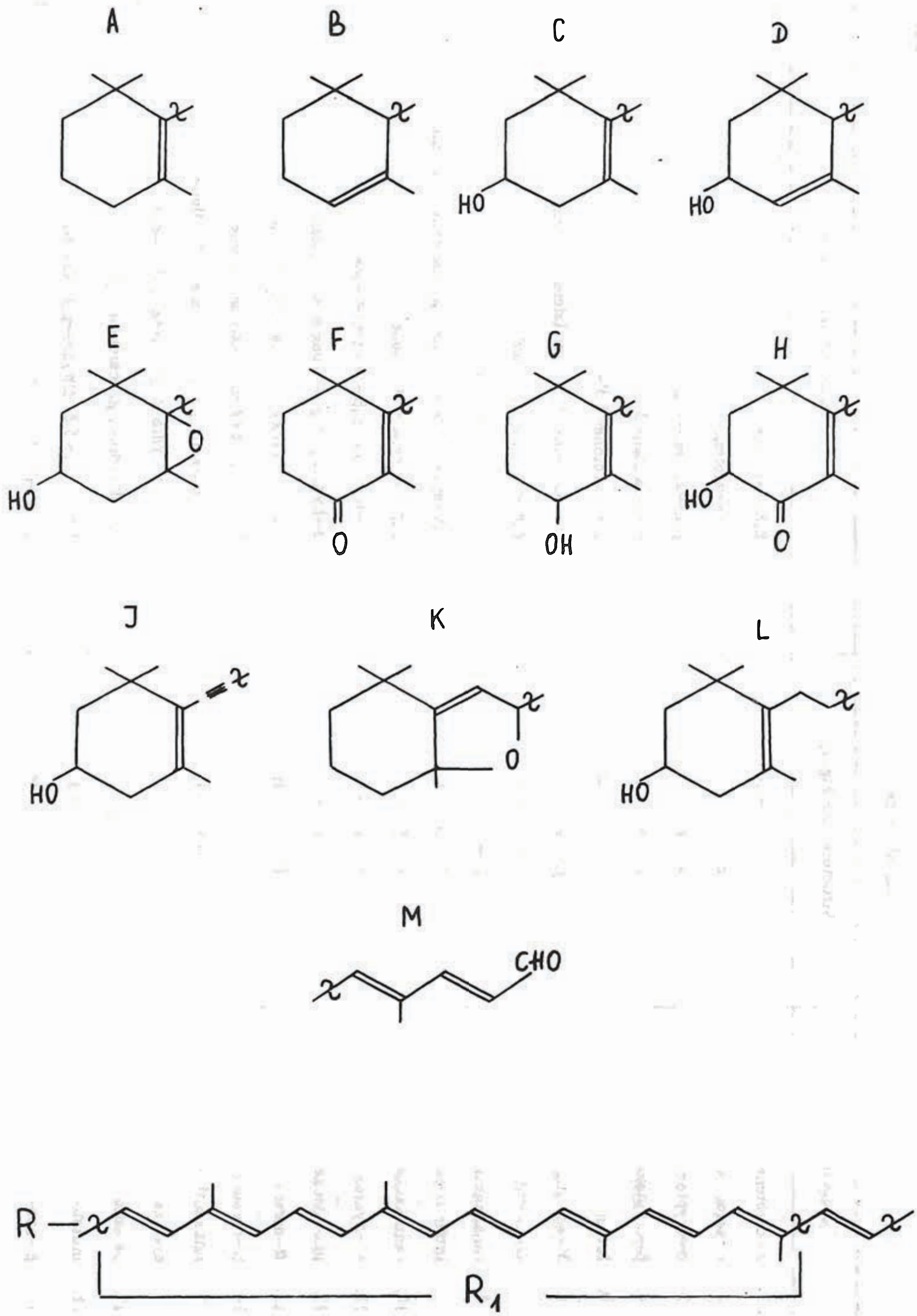

Fig. 1. Structural features of carotenoids from materials investigated

Table 1

List of the carotenoids from materials investigated

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. α–cryptoxanthin	A – R – D	β,ε–caroten–3–ol
4. β–cryptoxanthin	A – R – C	β,β–caroten–3–ol
5. lutein	C – R – D	β,ε–carotene–3,3'–diol
6. 3'–epilutein	C – R – D	β,ε–carotene–3,3'–diol (stereoisomeric)
7. zeaxanthin	C – R – C	β,β–carotene–3,3'–diol
8. tunaxanthin	D – R – D	ε,ε–carotene–3,3'–diol
9. lutein epoxide	D – R – E	5,6–epoxy–5,6–dihydro–β,ε–carotene–3,3'–diol
10. canthaxanthin	F – R – F	β,β–carotene–4,4'–dione
11. 4'–hydroxyechinenone	F – R – G	4'–hydroxy–β,β–caroten–4–one
12. phoenicoxanthin	F – R – H	3–hydroxy–β,β–carotene–4,4'–dione
13. α–doradexanthin	D – R – H	3,3'–dihydroxy–β,ε–carotene–4–one
14. β–doradexanthin	C – R – H	3,3'–dihydroxy–β,β–caroten–4–one
15. astaxanthin	H – R – H	3,3'–dihydroxy–β,β–carotene–4,4'–dione
16. diatoxanthin	C – R_1 – I	7,8–didehydro–β,β–carotene–3,3'–diol
17. parasiloxanthin	C – R_1 – L	7,8–dihydro–β,β–carotene–3,3'–diol
18. mutatochrome	A – R_1 – K	5,8–epoxy–5,8–dihydro–β,β–carotene
19. β–apo–2'–carotenal	A – R – M	3'–4'–didehydro–2'–apo–β–caroten–2'–al

Table 2

Presence of carotenoids in particular investigated species

Carotenoid	*Alburnus alburnus*	*Leucaspius delineatus*	*Alburnoides bipunctatus*
1. β–carotene	x	x	x
2. ε–carotene		x	
3. α–cryptoxanthin	x	x	
4. β–cryptoxanthin		x	x
5. lutein		x	
6. 3'–epilutein			x
7. zeaxanthin	x	x	x
8. tunaxanthin	x	x	
9. lutein epoxide	x	x	x
10. canthaxanthin	x	x	x
11. 4'–hydroxyechinenone	x		
12. phoenicoxanthin	x		x
13. α–doradexanthin	x	x	x
14. β–doradexanthin	x		
15. astaxanthin	x	x	x
16. diatoxanthin		x	
17. parasiloxanthin			x
18. mutatochrome		x	
19. β–apo–2'–carotenal			x
Number of carotenoids	11	13	11

Table 3

Carotenoid content in the *Alburnus alburnus* L. (in %)

Carotenoid	Fins	Skin	Muscles	Liver	Intenstine	Eggs
β—carotene				4.4		
α—cryptoxanthin		4.3		6.4		10.9
4'—hydroxyechinenone			59.5	13.8		25.0
canthaxanthin	27.8	38.1			44.6	3.7
lutein epoxide		5.8		7.0	19.3	26.1
zeaxanthin		11.4		9.3		9.5
phoenicoxanthin	33.4					
tunaxanthin			10.4			
α—doradexanthin	5.8	8.7	8.5	7.0		
β—doradexanthin	7.2				13.4	
astaxanthin	25.8	31.7	21.6	52.1	22.7	24.8
Total content in μg/g fresh wt	77.073	11.792	0.477	386.017	174.528	57.329

Table 4

Carotenoid content in the *Alburnoides bipunctatus* Bloch (in %)

Carotenoid	Fins	Skin	Muscles	Liver	Intestine	Gonads ♀	Gonads ♂
β–carotene	12.9		39.3	20.8			
β–cryptoxanthin			10.4	23.4			
canthaxanthin		22.8				36.1	
3'–epilutein		14.2	11.9	13.4	7.6		22.6
lutein epoxide	22.8	9.6		31.5	17.4	16.8	9.6
zeaxanthin	64.3				15.9	33.5	19.8
phoenicoxanthin					6.4		
parasiloxanthin					11.2		22.1
α–doradexanthin						13.6	
astaxanthin	trace	53.4	38.4	10.9	9.6		25.9
β–apo–2'–carotenal					31.9		
Total content in µg/g fresh wt	0.162	0.196	0.260	0.337	0.889	0.167	0.409

Table 5

Carotenoid content in the *Leucaspius delineatus* Heckel (in %)

Carotenoid	Spawning*			Fry**
	Eggs	Fins, Skin, Muscles	Liver, Intenstine	
β—carotene				27.4
α—cryptoxanthin	19.6	18.4	5.3	1.6
β—cryptoxanthin		37.5	12.4	37.3
canthaxanthin	17.5	2.0	18.5	
lutein epoxide		5.0	8.7	
zeaxanthin	32.4	15.2	14.6	36.1
α—doradexanthin	trace	2.7	4.6	3.1
astaxanthin	23.1	19.2	32.4	17.0
mutatochrome	7.4		3.5	
Total conten in μg/g fresh wt	7.617	2.292	1.435	1.932

* – May 4, 1976

** – July 3, 1976

Table 6

Carotenoid content in the *Leucaspius delineatus* Heckel (in %), (November 3, 1973)

Carotenoid	Fins	Skin	Muscles	Liver	Intestine
β–carotene	10.4			26.6	
ε–carotene					5.6
α–cryptoxanthin		5.9			8.6
β–cryptoxanthin			5.4	46.1	6.9
canthaxanthin		3.3			
lutein		24.0	26.4		37.3
lutein epoxide		32.9	14.3		
zeaxanthin	5.5				17.8
tunaxanthin	47.9				
diatoxanthin	12.0				
astaxanthin	16.0	33.9	53.9	20.7	16.8
α–doradexanthin	5.0				
unknown				6.6	7.1
Total content in μg/g fresh wt	12.496	10.377	1.478	8.716	10.743

Twelve carotenoids were identified, of which the most plentiful were 4'–hydroxyechinenone (muscles), canthaxanthin (intestines and skin), phoenicoxanthin (fins) and astaxanthin (liver). Total carotenoid content in the *Alburnus alburnus* was found to be very high, ranging from 0.477 (muscles) to 386.017 μg/g fresh weight (liver).

In the *Alburnoides bipunctatus* specimens, the presence of twelve carotenoids was noted (Tab. 4), among which the most noteworthy was parasiloxanthin found in the male intestine and gonads. Predominant carotenoids were: zeaxanthin (fins, and gonads in female), astaxanthin (muscles, skin and gonads in male) and β–apo–2'–carotenal (intestines). Total carotenoid content ranged from 0.162 (fins) to 0.889 μg/g fresh weight (intestines).

Data on the *Leucaspius delineatus* specimens examined are given in Tab. 5–6. In those collected in 1973, the presence of fourteen carotenoids was noted. Noteworthy is ε–carotene found in the intestines. The most abundant were β–cryptoxanthin (liver), lutein (intestines), tunaxanthin (fins) and astaxanthin (skin and muscles). Total carotenoid content ranged from 1.478 (muscles) to 12.496 μg/g fresh weight (fins).

Female individuals in their nuptial period revealed the greatest quantities of β–cryptoxanthin, and their spawn – zeaxanthin. Total carotenoid content was the highest in the spawn (7.617 μg/g fresh weight).

DISCUSSION

On comparing the results obtained it should be noted that the species examined reveal varied composition and total content of carotenoids, despite the same food range. In the bleak individuals analysed, carotenoids of the ketocarotenoid group dominate (canthaxanthin, 4–hydroxyechinenone, phoenicoxanthin, α–doradexanthin and astaxanthin), but not in the other two species examined. In the sunbleak specimens only muscles contained astaxanthin which as ketokarotenoid constituted over half of all the carotenoids. In other body parts carotenoids of other groups prevailed. In the fins almost half of all the carotenoids was occupied by tunaxanthin, a derivative of ε–carotene, in the liver – β–cryptoxanthin and in the intestines – lutein. In the spirling specimens examined, carotenoids of the ketocarotenoid group constituted over half of all carotenoids in the skin (canthaxanthin and astaxanthin) and in the female gonads (canthaxanthin, α–doradoxanthin). In the remaining body parts of this species, carotenoids of other groups were found to dominate. In the fins, zeaxanthin of the hydroxycarotenoid group constituted 64.3% of all carotenoids.

Despite these differences, all planktonophages examined had some typical carotenoids in common. These were β–carotene, zeaxanthin, tunaxanthin, lutein epoxide, α–doradexanthin, canthaxanthin and astaxanthin. Excepting tunaxanthin and α–doradexanthin they are all commonly met in shellfish, plankton shellfish included. Therefore, they may be suggested to be of food origin.

Total carotenoid content was found to be relatively high in all body parts examined, except muscles. In the bleak individuals it was over 300 μg/g fresh weight found in the liver, being a very high content in fish. In the case of muscles, the most abundant in carotenoids were the sunbleak's muscles, with 1.478 μg/g fresh weight.

An interesting finding noted is the identification, in the investigated planktonophages, of the carotenoids not frequently revealed in fish. To this group belong: 4'–hydroxyechinenone in the bleak individuals, ε–carotene and diatoxanthin in the sunbleak individuals, parasiloxanthin and β–apo–2'–carotenal in the spirlings. 4'–hydroxyechinenone has been hitherto reported, among others, in *Carassius carassius* (Czeczuga and Czerpak 1976). ε–carotene, however, has been noted in several representatives of *Pleuronectidae,* in *Limanda limanda* and *Platichthys flesus* from the Baltic Sea (Czeczuga 1980).

Diatoxanthin was found in pond smelt individuals in Japan (Matsuno et al. 1974), and in some European freshwater species – *Salmo gairdneri* and *Salmo trutta morpha fario* (Czeczuga 1976). Parasiloxanthin was first reported by Matsuno et al. (1976) in *Parasilus asotus* individuals, and then by Czeczuga and Chełkowski (1984) in *Salmo trutta.* β–apo–2'–carotenal is suggested to be of food origin, that means it did not come from any precursor in the fish body, but was introduced with food, just as it was in the case of the carp fry fed on food wit rape–cake addition (Czeczuga and Dąbrowski 1983). Carotenoids of the apocarotenal group quite frequently occur in seeds of various plants (Goodwin 1980).

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STRESZCZENIE

Autor stosując chromatografię kolumnową i cienkowarstwową badał występowanie poszczególnych karotenoidów w płetwach, skórze, mięśniach, wątrobie, jelitach i gonadach trzech gatunków – planktonofagów wód słodkowodnych.

Ustalono obecność następujących karotenoidów: β–carotene, ε–carotene, α–cryptoxanthin, β–cryptoxanthin, lutein, 3'–epilutein, zeaxanthin, tunaxanthin, lutein epoxide, canthaxanthin, 4'–hydroxyechinenone, phoenicoxanthin, α–doradexanthin, β–doradexanthin, astaxanthin, diatoxanthin, parasiloxanthin, mutatochrome oraz β–apo–2'–carotenal.

Wspólnymi karotenoidami dla wszystkich trzech planktonofagów okazały się: β–carotene, zeaxanthin, tunaxanthin, lutein epoxide, α–doradexanthin, canthaxanthin oraz astaxanthin.

Podano również ogólną zawartość karotenoidów w poszczególnych częściach ciała oraz stosunki procentowe poszczególnych karotenoidów.

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Received: 1993.03.09

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