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Biochemistry

CAROTENOIDS IN FISH. 49. *CYPRINIDAE* – BENTHOSOPHAGES: *CARASSIUS*  
*CARASSIUS*, *CARASSIUS AURATUS GIBELIO*, *TINCA TINCA*, *VIMBA VIMBA*,  
*BARBUS BARBUS* AND *BARBUS MERIDIONALIS PETENYI*

KAROTENOIDY U RYB. 49. *CYPRINIDAE* – BENTOSOFAGI: *CARASSIUS*  
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The column and thin-layer chromatography was used to test  
presence and total amount of carotenoids in some body parts of  
6 benthosophages fish species.

## INTRODUCTION

Among numerous representatives of the *Cyprinidae* family present in our waters, there are some species which feed on animals present within the sediments.

For two *Carassius carassius* species, feed, besides benthic organisms, includes also benthic plants; detritus in particular. Tench is another known benthosophage. Individuals of vimba, barbel, mediterranean barbel also feed on organisms present within the sediments. Individuals of previously mentioned species were subject of interest when carotenoids in fish were tested.

According to many surveys on particular carotenoids in fish, its presence and total amount is greatly influenced by the type of feed and the carotenoids level within the feed (Czczuga, 1981, Simpson et al. 1981, Storebakken et al. 1986).

Taking that into consideration I decided to analyse the carotenoids contents in various body parts of some *Cyprinidae* fish species for which the common feature was feeding on benthic organisms. If, then, the carotenoids content depends solely on feed taken, then the representatives of all tested benthophages species ought to have, pretty much the same, carotenoids. Besides, results give, among others, data on carotenoids level, as source of A vitamin, in individuals of economically usefull species. These

problems being more often sygnalized in the specialized literature (Torrissen et al. 1989).

## MATERIAL AND METHODS

Surveys were conducted on the following 6 benthophages fish species: *Carassius carassius* (L.) collected from various water basins at various periods) see Table 2–4), *Carassius auratus gibelio* (Bloch) from the Dojlida ponds near Białystok in November 1973, *Tinca tinca* (L.) collected from various water basins in various periods (see Table 6–9), *Vimba vimba* (L.) collected in July 1974 at the Lower Vistula, *Barbus barbus* (L.) collected in May 1976 from the Bug River near Drohiczyn and *Barbus meridionalis petenyi* (Heckel) collected from the Dunajec River near Niedzica in April 1981. In all collected individuals, fins, skin, muscles, liver, intestines and gonads were subjects of analysis.

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 (Czczuga 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 (Quickfit Co. – England) Particular fractions were eluated with various compositions of solvents (Czczuga 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 silicagel were used with different developing solvents (Czczuga 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) epiphese to hypophase relation determined in hexane and 95% methanol; d) comparison of the  $R_f$  values of thin-layer chromatogrammes; for identification of  $\beta$ -carotene,  $\beta$ -cryptoxanthin, canthaxanthin, lutein, zeaxanthin,  $\alpha$ -doradexanthin,  $\beta$ -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).

Table 1

## List of the carotenoids from the investigated materials

| Carotenoid                    | Structure<br>(see Fig. 1) | Semisystematic name  |
|-------------------------------|---------------------------|--|
| $\alpha$ -carotene            | A - R - B                 | $\beta$ , $\epsilon$ -carotene                                     |
| $\beta$ -carotene             | B - R - B                 | $\beta$ , $\beta$ -carotene  |
| $\epsilon$ -carotene          | A - R - A                 | $\epsilon$ , $\epsilon$ -carotene                                  |
| $\alpha$ -cryptoxanthin       | B - R - D                 | $\beta$ , $\epsilon$ -caroten-3-ol                                 |
| $\beta$ -cryptoxanthin        | B - R - C                 | $\beta$ , $\beta$ -caroten-3-ol                                    |
| $\beta$ -carotene monoepoxide | B - R - L                 | 5,6-epoxy-5,6-dihydro- $\beta$ , $\beta$ -carotene                 |
| lutein                        | C - R - D                 | $\beta$ , $\epsilon$ -carotene-3,3'-diol                           |
| 3'-epilutein                  | C - R - D                 | $\beta$ , $\epsilon$ -carotene-3,3'-diol (stereoisomeric)          |
| zeaxanthin                    | C - R - C                 | $\beta$ , $\beta$ -carotene-3,3'-diol                              |
| neothxanthin                  | A - R - D                 | $\beta$ , $\epsilon$ -caroten-3-ol                                 |
| lutein epoxide                | D - R - F                 | 5,6-epoxy-5,6-dihydro- $\beta$ , $\epsilon$ -carotene-3,3'-diol    |
| echinenone                    | B - R - G                 | $\beta$ , $\beta$ -caroten-4-one                                   |
| 3'-hydroxyechinenone          | C - R - G                 | 3'-hydroxy- $\beta$ , $\beta$ -caroten-4-one                       |
| canthaxanthin                 | G - R - G                 | $\beta$ , $\beta$ -carotene-4,4'-dione                             |
| phoenicoxanthin               | G - R - H                 | 3-hydroxy- $\beta$ , $\beta$ -carotene-4,4'-dione                  |
| $\alpha$ -doradexanthin       | D - R - H                 | 3,3'-dihydroxy- $\beta$ , $\epsilon$ -carotene-4-one               |
| $\beta$ -doradexanthin        | C - R - H                 | 3,3'-dihydroxy- $\beta$ , $\beta$ -carotene-4-one                  |
| idoxanthin                    | H - R - I                 | 3,3',4'-trihydroxy- $\beta$ , $\beta$ -caroten-4-one               |
| astaxanthin                   | H - R - H                 | 3,3'-dihydroxy- $\beta$ , $\beta$ -carotene-4,4'-dione             |
| diatoxanthin                  | C - R <sub>1</sub> - K    | 7,8-didehydro- $\beta$ , $\beta$ -carotene-3,3'-diol               |
| mutatochrome                  | B - R <sub>1</sub> - L    | 5,8-epoxy-5,8-dihydro- $\beta$ , $\beta$ -carotene                 |
| mutatoxanthin                 | C - R <sub>1</sub> - M    | 5,8-epoxy-5,8-dihydro- $\beta$ , $\beta$ -carotene-3,3'-diol       |
| rhodoxanthin                  | N - R <sub>2</sub> - N    | 4',5'-didehydro-4,5'-retro- $\beta$ , $\beta$ -carotene-3,3'-dione |

## RESULTS

Within the tested material 23 carotenoids were identified (Table 1).

From organs of tested individuals of *Carassius carassius* 15 carotenoids were isolated and identified (Table 2-4). Such carotenoids as lutein epoxide, zeaxanthin and astaxanthin were present within all tested body parts of all individuals from various water basins. Total amount of carotenoids ranged from 0.131 (muscles) up to 11.643  $\mu\text{g/g}$  wet weight (liver) (Table 4).

Table 2

Carotenoid content in the *Carassius carassius* (l.t. 17 cm) (in %)  
(June 10, 1982; ponds in Poryta Jabłoni)

| Carotenoid                                | Fins  | Skin and Muscles | Liver | Intenstine |
|---|-------|------------------|-------|------------|
| $\beta$ -carotene                         | 13.5  | 8.2              | 3.6   | 9.2        |
| $\beta$ -cryptoxanthin                    | 20.9  | 12.6             | 8.3   | 5.6        |
| canthaxanthin                             |       | 1.0              | 20.3  |            |
| lutein                                    |       |                  | 12.3  | 3.4        |
| lutein epoxide                            | 26.7  | 18.4             | 9.2   | 9.2        |
| zeaxanthin                                | 10.8  | 6.2              | 18.4  | 4.4        |
| $\beta$ -doradoxanthin                    |       |                  | 20.6  |            |
| phoenicoxanthin                           |       | 14.8             | 3.4   | 11.2       |
| astaxanthin                               | 28.1  | 38.8             | 3.9   | 37.1       |
| Total content in $\mu\text{g/g}$ fresh wt | 0.349 | 0.162            | 2.890 | 0.734      |

Table 3

Carotenoid content in some parts of (*Carassius carassius*) (l.t. 18 cm) (in %)  
(October 24, 1983; pond Czechowizna)

| Carotenoid                                | Fins  | Skin  | Muscles | Liver | Intestine |
|---|-------|-------|---------|-------|-----------|
| $\beta$ -cryptoxanthin                    | 37.1  | 42.7  | 7.6     |       |           |
| zeaxanthin                                | trace | 3.5   | 23.8    | 5.1   | 62.5      |
| lutein                                    |       |       |         | 10.1  |           |
| 3'-epilutein                              |       |       |         | 10.1  |           |
| lutein epoxide                            | 15.9  | trace | 28.3    | 5.1   | 27.8      |
| $\alpha$ -doradoxanthin                   |       |       |         | 24.6  |           |
| phoenicoxanthin                           |       |       |         | 19.8  |           |
| canthaxanthin                             |       | 2.7   |         | 4.7   |           |
| astaxanthin                               | trace | 47.5  | 48.8    | 15.3  | 9.7       |
| rhodoxanthin                              | 31.2  |       |         | 5.2   |           |
| mutatoxanthin                             | 15.7  | 3.5   |         |       |           |
| Total content in $\mu\text{g/g}$ fresh wt | 0.195 | 0.750 | 0.165   | 0.568 | 0.370     |

Table 4

Carotenoid content in the *Carassius carassius* (l.t. 19 cm) (in %)  
(October 19, 1973; pond Dojlidy)

| Carotenoid                                   | Fins  | Skin  | Muscles | Liver  | Intenstine |
|--|-------|-------|---------|--------|------------|
| $\beta$ -carotene                            | 3.4   | 5.8   |         |        | 11.8       |
| $\alpha$ -cryptoxanthin                      | 6.0   | 5.9   | 20.9    | 7.5    | 4.9        |
| $\beta$ -cryptoxanthin                       |       | 32.0  |         | 12.9   | 11.2       |
| neothxanthin                                 |       | 3.8   |         |        |            |
| canthaxanthin                                | 3.8   | trace | 6.0     | 9.0    | 10.9       |
| lutein                                       | 13.0  | trace | 32.8    | 18.2   | 28.4       |
| lutein epoxide                               | 39.6  | 24.3  | 24.5    | trace  | 5.8        |
| zeaxanthin                                   | 18.9  | 18.5  | trace   | 18.7   | 10.0       |
| $\alpha$ -doradexanthin                      | 6.3   | 3.6   | trace   | 12.4   | 8.2        |
| diatoxanthin                                 |       | 1.5   | 2.5     |        |            |
| astaxanthin                                  | trace | 2.0   | 13.3    | 31.3   | 8.0        |
| unknown                                      | 7.7   | 2.6   |         |        |            |
| Total content in $\mu\text{g/g}$<br>fresh wt | 2.876 | 1.052 | 0.131   | 11.643 | 4.174      |

Table 5

Carotenoid content in the *Carassius auratus gibelio* (l.t. 15 cm) (in %)

| Carotenoid                                   | Fins  | Skin  | Muscles | Liver | Intenstine | Eggs  |
|--|-------|-------|---------|-------|------------|-------|
| $\beta$ -carotene                            | 6.3   |       | 5.0     |       | 4.1        | 3.7   |
| $\alpha$ -carotene                           | 13.3  |       |         |       |            |       |
| $\varepsilon$ -carotene                      |       | 3.4   |         |       |            |       |
| $\alpha$ -cryptoxanthin                      | 6.2   |       |         |       |            |       |
| $\beta$ -cryptoxanthin                       | 4.1   |       | 10.9    | 6.8   | 12.6       |       |
| echinenone                                   |       | 2.9   |         |       |            |       |
| neothxanthin                                 |       | 19.9  |         |       |            |       |
| canthaxanthin                                |       |       |         |       |            | 19.5  |
| lutein                                       | 14.7  |       |         |       | 11.7       | 35.5  |
| lutein epoxide                               | 24.7  | 45.6  |         |       | 12.3       | 7.4   |
| zeaxanthin                                   |       |       | 26.5    | 24.1  | 38.2       |       |
| diatoxanthin                                 | 16.2  | 9.4   |         |       |            | 22.5  |
| $\alpha$ -doradexanthin                      | 2.3   |       | 4.0     |       | 12.9       | 5.6   |
| astaxanthin                                  | 12.2  | 18.8  | 53.6    | 69.2  | 8.2        | 9.5   |
| Total content in $\mu\text{g/g}$<br>fresh wt | 5.741 | 2.887 | 0.301   | 5.027 | 4.555      | 2.011 |

Table 6

Carotenoid content in the *Tinca tinca* (l.t. 20 cm) (in %) June 10, 1982; ponds in Poryta Jabłoń

| Carotenoid                                | Fins  | Liver | Intenstine |
|---|-------|-------|------------|
| $\beta$ -carotene                         | 2.3   |       |            |
| $\beta$ -cryptoxanthin                    | 18.7  | 15.8  | 2.9        |
| canthaxanthin                             | 3.2   | 11.1  | 27.6       |
| lutein                                    | 7.1   |       | 7.5        |
| 3'-epilutein                              |       |       | 4.5        |
| lutein epoxide                            | 34.4  | 3.9   | 9.0        |
| zeaxanthin                                | 21.4  | 11.9  | 8.9        |
| rhodoxanthin                              | 6.6   | 2.8   |            |
| diatoxanthin                              | 3.1   | 17.4  | 12.2       |
| $\alpha$ -doradexanthin                   |       | 3.2   | 20.1       |
| astaxanthin                               | 3.2   | 13.7  | 7.3        |
| mutatoxanthin                             |       | 20.2  |            |
| Total content in $\mu\text{g/g}$ fresh wt | 1.701 | 5.600 | 1.249      |

Table 7

Carotenoid content in some parts of *Tinca tinca* (l.t. 17 cm) (in %)  
(October 24, 1983; pond Czechowizna)

| Carotenoid                                | Fins  | Skin  | Muscles | Liver | Intenstine |
|---|-------|-------|---------|-------|------------|
| $\beta$ -cryptoxanthin                    | trace | 48.9  | 5.6     | 16.0  | 10.4       |
| zeaxanthin                                | trace | 7.3   | 21.3    | 19.5  | 55.2       |
| lutein                                    | 5.1   |       |         |       |            |
| lutein epoxide                            | 71.3  |       | 13.6    |       | 3.9        |
| 3'-hydroxyechinenone                      |       | 7.7   |         |       |            |
| phoenicoxanthin                           |       | 13.3  | 5.3     |       |            |
| canthaxanthin                             | 4.7   | 7.6   | 36.8    | trace | 26.0       |
| astaxanthin                               | 18.9  |       | 13.5    | 61.4  |            |
| neothxanthin                              |       | 15.2  |         |       |            |
| rhodoxanthin                              |       |       | 4.0     |       |            |
| mutatoxanthin                             |       |       |         | 3.1   | 4.5        |
| Total content in $\mu\text{g/g}$ fresh wt | 0.730 | 0.092 | 0.320   | 0.855 | 0.954      |

Table 8

Carotenoid content in the *Tinca tinca* (l.t. 21 cm) (in %)  
(October 19, 1973; pond Dojlidy)

| Carotenoid                                | Fins  | Skin  | Brain | Muscles | Liver | Inten-<br>stine | Eggs  |
|---|-------|-------|-------|---------|-------|-----------------|-------|
| $\beta$ -carotene                         |       |       |       | 10.3    |       |                 |       |
| $\beta$ -carotene monoepoxide             |       |       |       |         |       |                 | 12.6  |
| $\alpha$ -cryptoxanthin                   | 2.8   | trace | 9.3   | 9.9     | 9.5   | 10.7            | 24.7  |
| $\beta$ -cryptoxanthin                    |       |       | 9.6   | 6.4     |       |                 | 8.4   |
| neothxanthin                              |       |       | 8.9   |         |       |                 |       |
| canthaxanthin                             | 13.9  | 2.2   | 7.7   |         | 8.3   |                 | 5.7   |
| lutein                                    |       |       | 21.9  | 4.9     |       |                 |       |
| lutein epoxide                            | 24.6  | 57.9  | 12.2  | 21.7    |       |                 | 16.3  |
| zeaxanthin                                | 37.5  | 34.8  |       | 26.6    |       | 14.8            |       |
| $\alpha$ -doradexanthin                   | 5.8   | 3.2   | 5.3   | 5.0     | 2.4   | trace           | 3.7   |
| phoenicoxanthin                           |       |       |       |         | 19.0  |                 |       |
| astaxanthin                               | 15.4  | 1.9   | 25.1  | 15.2    | 60.6  | 66.1            | 28.7  |
| mutatoxanthin                             |       |       |       |         |       | 8.4             |       |
| Total content in $\mu\text{g/g}$ fresh wt | 2.436 | 2.003 | 3.681 | 0.189   | 1.547 | 3.281           | 5.428 |

Table 9

Carotenoid content in some parts *Tinca tinca* (l.t. 38 cm) (in %)  
(December 20, lake Necko)

| Carotenoid                                | Fins  | Skin  | Muscles | Liver | Intenstine | Gonad ♀ |
|---|-------|-------|---------|-------|------------|---------|
| $\beta$ -carotene                         |       | 4.9   | 2.5     | 27.9  |            | 25.2    |
| $\beta$ -cryptoxanthin                    | 16.5  |       |         |       | 23.5       |         |
| lutein                                    | 35.1  | 24.6  | 10.0    | trace | 13.7       | 8.3     |
| 3'-epilutein                              |       |       |         |       | 11.5       |         |
| lutein epoxide                            | 37.4  | 52.1  | 62.5    | 33.4  | 17.3       | 14.2    |
| neothxanthin                              |       |       |         | 1.8   |            |         |
| zeaxanthin                                | 0.6   |       |         |       |            |         |
| echinenone                                |       |       |         |       |            | 28.7    |
| 3'-hydroxyechinonone                      |       |       |         |       |            | 4.1     |
| canthaxanthin                             |       |       |         | 6.4   | 29.6       | 4.1     |
| idoxanthin                                |       |       |         |       |            | 6.3     |
| $\alpha$ -doradexanthin                   |       | 1.5   |         | 2.1   | 4.5        | 9.1     |
| astaxanthin                               |       | 9.1   |         | 20.7  |            |         |
| mutatoxanthin                             | 10.4  | 7.8   | 25.0    | 7.7   |            |         |
| Total content in $\mu\text{g/g}$ fresh wt | 2.790 | 2.750 | 0.145   | 1.780 | 0.945      | 0.404   |

Table 10

Carotenoid content in the *Vimba vimba* (l.t. 37 cm) (in %)

| Carotenoid                                | Fins  | Skin and muscles | Liver | Intenstine |
|---|-------|------------------|-------|------------|
| $\beta$ -carotene                         | 3.0   | 3.5              | 6.8   | 4.0        |
| $\beta$ -cryptoxanthin                    | 16.9  | 17.2             | 25.7  | 17.0       |
| lutein                                    | trace | 9.2              | 7.2   | 5.6        |
| zeaxanthin                                |       |                  |       | 16.2       |
| lutein epoxide                            | 2.8   | 20.9             | 3.3   | 8.9        |
| astaxanthin                               | 47.3  | 45.0             | 57.0  | 48.3       |
| canthaxanthin                             |       | 4.2              |       |            |
| Total content in $\mu\text{g/g}$ fresh wt | 1.210 | 0.221            | 1.255 | 0.531      |

Table 11

Carotenoid content in the *Barbus barbus* (l.t. 32 cm) (in %)

| Carotenoid                                | Fins  | Skin  | Muscles | Liver | Inten-<br>stine | Gonads $\sigma$ |
|---|-------|-------|---------|-------|-----------------|-----------------|
| $\beta$ -carotene                         |       | 16.7  |         |       |                 |                 |
| $\alpha$ -cryptoxanthin                   | 26.7  | 34.1  | 13.0    | 15.4  | 32.7            |                 |
| $\beta$ -cryptoxanthin                    |       | 49.2  |         | 15.6  | 16.9            |                 |
| canthaxanthin                             |       |       | 2.0     | 6.2   |                 |                 |
| lutein                                    |       |       |         | 24.2  |                 |                 |
| zeaxanthin                                | 10.0  |       |         |       |                 |                 |
| mutatochrome                              |       |       |         |       | 13.2            | 10.0            |
| astaxanthin                               | 63.3  | trace | 85.0    | 38.6  | 37.2            | 90.0            |
| Total content in $\mu\text{g/g}$ fresh wt | 1.661 | 0.161 | 0.341   | 5.250 | 5.859           | 0.584           |

In table 5 results of the *Carassius auratus gibelio* body parts analysis are listed. Presence of 14 carotenoids was noted. In all body parts tested, the astaxanthin occurred. Worth mentioning is presence of  $\epsilon$ -carotene and echinenone in the skin samples. Lutein (fish roe), lutein epoxide (fins and skin), zeaxanthin (muscles, liver and intestines) and astaxanthin (muscles and liver) were highest in quantities. Total carotenoids content for tested individuals of *Carassius auratus gibelio* ranged from 0.301 (muscles) to 5.741  $\mu\text{g/g}$  of wet weight (fins).

For the *Tinca tinca* individuals presence of 19 carotenoids was stated (Table 6–9). Similarly, as for the *Carassius carassius* individuals, differences in the carotenoids contents within the samples from various body parts of tench, from various water basins were noted. The total carotenoids content ranged from 0.092 (skin) (Table 7) to 5.428  $\mu\text{g/g}$  of wet weight (fish roe) (table 8). A relatively high amount of carotenoids (3.681  $\mu\text{g/g}$ ) in brain samples of tench individuals from the Dojlida pond is worth to be



Table 12

Carotenoid content in the *Barbus meridionalis petenyi* (l.t. 18 cm) (in %)

| Carotenoid                     | Fins  | Skin  | Muscles | Inten-<br>stine | Liver | Gonads ♀ |
|--------------------------------|-------|-------|---------|-----------------|-------|----------|
| β-carotene                     |       | 12.2  |         |                 |       |          |
| α-cryptoxanthin                |       |       |         |                 |       | 11.0     |
| β-cryptoxanthin                |       | 10.0  | 44.6    | 18.2            |       |          |
| canthaxanthin                  | 12.9  |       | 5.4     | 6.4             | 19.5  |          |
| 3'-hydroxyechinenone           |       |       |         |                 |       | 4.1      |
| lutein                         |       | 10.8  |         | 16.1            |       | 26.4     |
| 3'-epilutein                   |       |       |         |                 | 16.0  |          |
| lutein epoxide                 | 20.8  | 18.9  |         |                 |       | 16.0     |
| zeaxanthin                     | 30.0  | 18.7  |         | 18.5            |       | 42.5     |
| neothxanthin                   | 22.6  |       |         |                 |       |          |
| α-doradexanthin                | 9.7   |       |         |                 | 12.2  |          |
| β-doradexanthin                |       | 11.4  | 43.1    | 3.9             | 5.6   |          |
| phoenicoxanthin                |       |       |         |                 | 2.7   |          |
| diatoxanthin                   |       | 18.0  |         | 33.5            | 28.0  |          |
| astaxanthin                    | 4.0   | trace | 6.9     | 3.4             | 16.0  |          |
| Total content in µg/g fresh wt | 0.472 | 0.456 | 0.158   | 1.291           | 0.759 | 0.589    |

underlined (Table 8).

Table 10 presents results of analysis on carotenoids presence in the tested *Vimba vimba* individuals. Seven carotenoids were identified, with such carotenoids as β-carotene, β-cryptoxanthin, lutein, lutein epoxide and astaxanthin being present within all tested body parts of vimba. The astaxanthin was present in all body parts, in highest quantities and ranged from 45% (skin + muscles) to 57.0% (liver), while total carotenoids amount ranged from 0.221 (skin + muscles) to 1.255 µg/g of wet weight (liver).

In the tested *Barbus barbus* individuals 8 carotenoids were identified, with α-cryptoxanthin and astaxanthin being present in all body parts (Table 11) tested. Carotenoids such as β-cryptoxanthin (skin) and astaxanthin (fins, muscles, liver, intestines and milt) dominated quantitatively.

Total carotenoids content ranged from 0.161 (muscles) to 5.859 µg/g of wet weight (intestines).

Table 12 gives list of carotenoids identified in various body parts of *Barbus meridionalis petenyi* individuals, tested. Among 15 identified carotenoids, the dominating ones were: β-cryptoxanthin (muscles), lutein (gonads), zeaxanthin (fins and gonads), neothxanthin (fins), β-doradexanthin (muscles) and diatoxanthin (intestines and liver). The total carotenoids content ranged from 0.158 (muscles) to 1.291 µg/g of wet weight (intestines).

Table 13

Carotenoid content in the investigated fishes

| Carotenoid                    | <i>Carassius carassius</i> | <i>Carassius auratus gibelio</i> | <i>Tinca tinca</i> | <i>Vimba vimba</i> | <i>Barbus barbus</i> | <i>Barbus meridionalis petenyi</i> |
|-------------------------------|----------------------------|----------------------------------|--------------------|--------------------|----------------------|------------------------------------|
| $\alpha$ -carotene            |                            | x                                |                    |                    |                      |                                    |
| $\beta$ -carotene             | x                          | x                                | x                  | x                  | x                    | x                                  |
| $\epsilon$ -carotene          |                            | x                                |                    |                    |                      |                                    |
| $\alpha$ -cryptoxanthin       | x                          | x                                | x                  |                    | x                    | x                                  |
| $\beta$ -cryptoxanthin        | x                          | x                                | x                  | x                  | x                    | x                                  |
| $\beta$ -carotene monoepoxide |                            |                                  | x                  |                    |                      |                                    |
| lutein                        | x                          | x                                | x                  | x                  | x                    | x                                  |
| 3'-epilutein                  | x                          |                                  | x                  |                    |                      | x                                  |
| zeaxanthin                    | x                          | x                                | x                  | x                  | x                    | x                                  |
| neothxanthin                  | x                          | x                                | x                  |                    |                      | x                                  |
| lutein epoxide                | x                          | x                                | x                  | x                  |                      | x                                  |
| echinenone                    |                            | x                                | x                  |                    |                      |                                    |
| 3'-hydxoxyechinenone          |                            |                                  | x                  |                    |                      | x                                  |
| canthaxanthin                 | x                          | x                                | x                  | x                  | x                    | x                                  |
| phoenicoxanthin               | x                          |                                  | x                  |                    |                      | x                                  |
| $\alpha$ -doradexanthin       | x                          | x                                | x                  |                    |                      | x                                  |
| $\beta$ -doradexanthin        | x                          |                                  |                    |                    |                      | x                                  |
| idoxanthin                    |                            |                                  | x                  |                    |                      |                                    |
| astaxanthin                   | x                          | x                                | x                  | x                  | x                    | x                                  |
| diatoxanthin                  |                            | x                                | x                  |                    |                      | x                                  |
| mutatochrome                  |                            |                                  |                    |                    | x                    |                                    |
| mutatoxanthin                 | x                          |                                  | x                  |                    |                      |                                    |
| rhodoxanthin                  | x                          |                                  | x                  |                    |                      |                                    |

## DISCUSSION

It has become evident from the obtained results, that in spite of great variety in carotenoids present in the tested benthophages fish individuals some carotenoids were common for all of them (Table 13). These are:  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, zeaxanthin, canthaxanthin, and astaxanthin. These carotenoids being quite commonly present in benthic invertebrate organisms (Czczuga 1977a) state a nutritive basis for the tested fish species (Brylińska 1986).

Besides, after comparing carotenoids of the crucian carp (Table 2-4) and tench (Table 6-9), collected for analysis at various seasons from various water basins, it is to be said, that independently from fish age and origin, all have groups of, so called steady carotenoids. For *Carassius carassius* individuals this carotenoids group in-

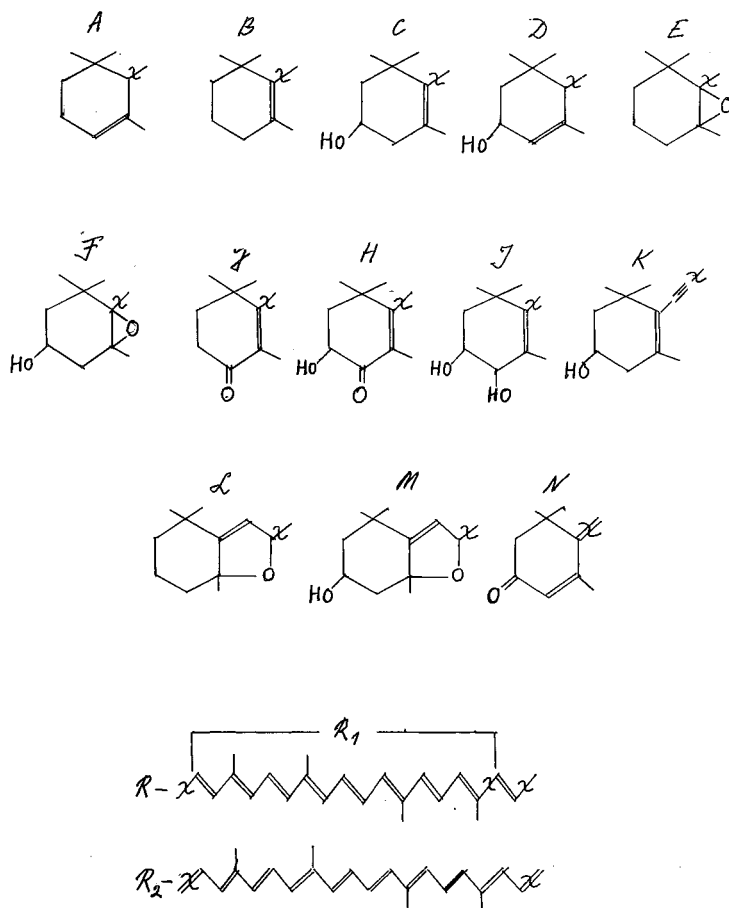


Fig. 1. Structural features of carotenoids from materials investigated

cludes:  $\beta$ -cryptoxanthin, lutein, lutein epoxide, canthaxanthin and astaxanthin while  $\beta$ -cryptoxanthin, lutein, lutein epoxide, zeaxanthin, canthaxanthin, astaxanthin and mutatoxanthin make up group of carotenoids characteristic for *Tinca tinca* individuals. The other carotenoids isolated from the crucian carp and the tench, not included into so called steady carotenoids, are carotenoids, which presence in fish depends, probably, on environmental conditions.

It is to be stated, that most of the identified carotenoids belong to the group of, so called, common carotenoids. So far, only such carotenoids as  $\epsilon$ -carotene, echinenone, rhodoxanthin and diatoxanthin were the one rarely noted in fish.  $\epsilon$ -carotene, for example, was identified, for the first time, in the *Cichlasoma citrinellum* individuals, and in some varieties of this species was even the main carotenoid present (Webber et al. 1973). Later on  $\epsilon$ -carotene was isolated from some fish species of marine (Czeczuga

1980b) and freshwater (Czczuga 1980a,c) origin. As for echinenone in the *Cyprinidae* fish species this carotenoid was noted in *Cyprinus carpio* individuals (Czczuga 1979b, Czczuga and Dąbrowski 1983). Presence of echinenone was also noted in both marine and freshwater fish species belonging to the *Salmonidae* family (Czczuga 1979a, 1982a; Matsuno et al. 1980). Particular attention is to be paid to presence of rhodoxanthin, noted in the crucian carp and tench collected in the spring time. So far, rhodoxanthin was noted only in few fish species. Katsuyama and Matsuno (1979) first isolated the rhodoxanthin from some phytophagous fish species belonging to the *Tilapia* genus, while Czczuga (1981) proved, that carotenoid to be present in *Ctenopharyngodon idylus* – a phytophagous fish species present in our waters, as well as in *Leuciscus* genus (Czczuga 1989) representatives. Presumably, the rhodoxanthin presence in the crucian carp and tench individuals is due to a nourishment taken. We proved it once with aquarium fish, on *Carassius auratus*, in between, feeding on various feed. Rhodoxanthin was noted only in the individuals fed on feed including this particular carotenoid (Czczuga and Kiziewicz 1985).

Another carotenoid worth mentioning is diatoxanthin noted in some tested fish species. As a derivative of zeaxanthin, diatoxanthin is quite often present in some algae species (Czczuga 1979c). As for the fish, it was isolated in several cases, in the *Cyprinidae* fish, in between. *Cyprinus carpio* individuals were the one, where diatoxanthin was isolated from (Czczuga 1979b).

When comparing the total carotenoids content in the tested fish – benthophages, in the main, liver was the body part richest in carotenoids, and, to a lesser degree, – intestines. This phenomenon was noted, also, for the other fish species feeding on other animal groups – for *Leucaspis delineatus* individuals, in between (Czczuga and Czerpak 1976). Accumulated carotenoids are then, during reproduction time transferred either to skin or fins or to reproductive cells, as it is in case of the *Salmonidae* fish species females (Czczuga and Chełkowski 1984, Czczuga and Bartel 1989, Czczuga et al. 1991).

Attention is to be paid also to differences in presence of particular carotenoids and their total amount in the crucian carp and tench individuals collected in spring and autumn. That phenomenon was observed, already, for the individuals of *Misgurnus fossilis* (Czczuga 1980b) and *Abramis brama* (Czczuga 1982b).

Worth mentioning is, also, a relatively high carotenoids content in the tench's brain. It might confirm our earlier findings for fish species living in waters with relatively low oxygen level to have more carotenoids in brain, than the fish from well oxygenated waters (Czczuga 1977b). It, again, might give evidence for a numerous biological role of carotenoids; in this particular case for their share in oxygen accumulation (Karnaukov 1973).

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#### KAROTENOIDY U RYB KARPIOWATYCH. 49. CYPRINIDAE – BENTOSOFAGI

##### STRESZCZENIE

Stosując chromatografię kolumnową i cienkowarstwową autor badał występowanie poszczególnych karotenoidów w niektórych częściach ciała 6 gatunków ryb bentosofagów. Badaniami objęto takie gatunki jak *Carassius carassius*, *Carassius auratus gibelio*, *Tinca tinca*, *Vimba vimba*, *Barbus barbus* oraz *Barbus meridionalis petenyi*.

W wyniku badań ustalono obecność takich karotenoidów jak  $\alpha$ -carotene,  $\beta$ -carotene,  $\epsilon$ -carotene,  $\alpha$ -cryptoxanthin,  $\beta$ -cryptoxanthin,  $\beta$ -carotene monoepoxide, lutein, 3'-epilutein, zeaxanthin, neothxanthin, lutein epoxide, echinenone, 3'-hydroxyechinenone, canthaxanthin, phoenicoxanthin,  $\alpha$ -doradexanthin,  $\beta$ -doradexanthin, idoxanthin, astaxanthin, diatoxanthin, mutatochrome, mutatoxanthin oraz rhodoxanthin.

Wspólnymi karotenoidami u wszystkich badanych ryb bentosofagów okazały się  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, zeaxanthin, canthaxanthin oraz astaxanthin.

Podano również ogólną zawartość karotenoidów oraz stosunki procentowe poszczególnych z nich. Ogólna zawartość karotenoidów wahała się od 0,092 (skóra u *Tinca tinca* ze stawu Czechowizna) do 11.643  $\mu\text{g/g}$  świeżej masy (wątroba *Carassius carassius* ze stawu Dojlidy). Jeśli chodzi o badane części ciała to najzasobniejszymi w karotenoidy okazały się wątroby i jelita badanych gatunków ryb.

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