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CAROTENOIDS IN FISH XXXIV. CAROTENOIDS IN UNEVEN AGED INDIVIDUALS OF CERTAIN ANTARCTIC SPECIES OF FISH

KAROTENOIDY U RYB. XXXIV. KAROTENOIDY U RÓŻNYCH WIEKOWO OSOBNIKÓW NIEKTÓRYCH GATUNKÓW RYB Z ANTARKTYDY

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By means of columnar and thin-layer chromatography, the author investigated the presence of various carotenoids in six fish species from the Antarctic (*Dissostichus eleginoides, Notothenia gibberifrons, Pseudochaenichtys georgianus, Trematomus hansoni, Trematomus bernacchii* and *Bathydraco macrolepis*).

The presence of the 20 carotenoids has been found.

The total content of carotenoids ranged from 0.011 (*Notothenia gibberifrons*, 35.0 cm body length to 1.291 μg/g wet weight (fins of *Bathydraco macrolepis*).

INTRODUCTION

Our previous studies on the occurrence of carotenoids in fish dealt mainly with the presence of various carotenoids in different parts of the body of several fish species, both freshwater and marine (Czeczuga 1973, 1975). Attention was also paid to the effect of different types of food on the occurrence and the total content of carotenoids in the various parts of the fish body; the significance of natural and artificial food was in studied on *Salmo trutta* (Czeczuga 1979 a) whereas effect of parasites or pollution of the habitat were investigated in the case and the carp (Czeczuga 1979 b). Whilst carrying out investigations on carotenoids in Antartic fish species (Czeczuga and Kłyszejko 1978), we felt that studies on the presence and amounts of carotenoids in individuals of various age, belonging to economically significant species in that region would be of interest since these species providing, as they do a source of animal protein are now being caught in increasing numbers by fishing fleets from various countries of the world.

MATERIAL AND METHODS

The material were brought to the laboratory in deep-freeze containers (–20°C) after being caught during the 8sh cruise of RV "Profesor Siedlecki" in 1978. The material, thus obtained comprised the following fish species: *Dissostichus eleginoides, Notothenia gibberifrons, Pseudochaenichthys georgianus, Trematomus hansoni, Trematomus bernacchii* and *Bathydraco macrolepis.* For analysis of the carotenoid content assays 30–32 g samples of the flesh from the middle part of the body were removed from each individual of the species under study. A sample consisting of muscular tissue together with the skin from the same part of the body was also studied. These samples were taken from all the fishes, from the youngest to the oldest individual of a given species. Such a sampling method was dictated by the assumption of expressed by Shust and Pinskaya (1978) that the linear growth of Antarctic fishes was a manifestation of their age. The brain and fins, skin and muscles of each species were analysed.

The material were prepared immediately on collection by placing them into dark glass containers and covering them with 96% acetone. They were kept in a refrigerator until the spectrophotometric determinations were made.

The carotenoid pigments were extracted by means of 96% acetone in a dark room. Saponification was carried out by means of 10% KOH in ethanol at about 20°C for 24 for hours in the dark in a nitrogen atmosphere.

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

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

Quantitative determinations of carotenoid concentrations were made the quantitative absorption spectra. These determinations were based on the extinction coefficient E 1%/cm at maximum absorbance wavellength in petroleum ether or hexane.

RESULTS

The carotenoids found to be present in the *Dissostichus eleginoides* individuals investigated are shown in Table 1, from which it will be seen that 11 carotenoids were identified in this material. The commonest carotenoids were zeaxanthin and asthaxanthin. The presence of α-doradexanthin and phoenicoxanthin in worth mentioning. The analysis of carotenoid contents in individuals of different body lengths (Table 2) revealed that up to 49.0 cm body length the total carotenoid content increased with fish growth, after which a slight fall was observed. Different fish growth groups showed the domination of various carotenoids. It is of interest that the total carotenoid content was higher in the brain of this species than in the muscles (Table 7).

The carotenoids and range of their percentages in the *Notothenia gibberifrons* individuals are presented in Table 3. Thirteen carotenoids were identified, of which the most noteworthy of which were α-cryptoxanthin and tunaxanthin. In the specimens of

Table 1

Carotenoid content in the body of the *Dissostichus eleginoides*

Carotenoid	fluctuations in %
β– carotene epoxide	18.8
β–cryptoxanthin	29.0
lutein (free and epoxide)	10.0–16.5
zeaxanthin	21.3–39.2
phoenicoxanthin	28.6
α–doradexanthin	17.5–28.1
astaxanthin	13.7–40.5
astaxanthin ester	10.7–15.0
aurochrome	7.2–18.5
mutatochrome	7.0–34.8
mutatoxanthin	6.4– 7.8

Table 2

Carotenoid content in the *Dissostichus eleginoides* and different body lengths

length of body in cm	total content of carotenoids in μg/g f.w.	dominating carotenoids
28.5	0.043	astaxanthin (21.8%), zeaxanthin (23.3%)
35.0	0.077	zeaxanthin (39.2%), β–cryptoxanthin (30.6%)
49.0	0.083	β–cryptoxanthin (29.0%), astaxanthin (18.0%)
60.0	0.080	zeaxanthin (35.1%), α–doradexanthin (28.1%)
68.0	0.077	mutatochrome (34.8%), zeaxanthin (24.6%)

Table 3

Carotenoid content in the body of the *Notothenia gibberifrons*

Carotenoid	fluctuations in %
β–carotene	9.6–15.7
β–carotene epoxide	9.9–36.6
α–cryptoxanthin	5.3– 5.6
β–cryptoxanthin	8.2–19.3
canthaxanthin	3.1–18.2
lutein (free and epoxide)	5.8–25.7
zeaxanthin	6.1–39.3
phoenicoxanthin	22.9
tunaxanthin	19.0
astaxanthin	5.2–33.8
astaxanthin ester	7.6–17.4
aurochrome	60.7
mutatochrome	3.2–43.4

different lengths the total carotenoid content was found to rise in the fishes of 35 cm to 50 cm (Table 4). While the smaller specimens contained 0.011 μg/g on the average, the specimens 50 cm length contained 0.283 μg/g body weight. In the brain of the individuals studied, zeaxanthin and astaxanthin predominated and the total carotenoid content was 0.262 μg/g.

The results of the analyses for *Pseudochaenichthys georgianus* are shown in Table 5, which gives the carotenoids determined and percentage ranges. Fourteen carotenoids were

Table 4

Carotenoid content in the *Notothenia gibberifrons* and different body lengths

length of body in cm	total content of carotenoids in $\mu g/g$ f.w.	dominating carotenoids
35.0	0.011	aurochrome (60.7%)
36.0	0.022	β–carotene epoxide (36.3%), mutatochrome (32.0%)
37.5	0.037	lutein (36.2%)
43.0	0.054	mutatochrome (43.7%)
45.5	0.194	lutein (23.9%), β-cryptoxanthin (19.3%)
48.0	0.220	astaxanthin (22.3%), zeaxanthin (27.5%)
50.0	0.283	zeaxanthin (22.5%), canthaxanthin (18.2%)

Table 5

Carotenoid content in the body of the *Pseudochaenichthys georgianus*

Carotenoid	fluctuations in %
β–carotene	8.2–12.6
β–carotene epoxide	12.3
β–cryptoxanthin	16.5–46.5
canthaxanthin	4.2–21.7
lutein (free and epoxide)	4.0–37.2
zeaxanthin	22.8–47.8
phoenicoxanthin	10.3–23.8
neothxanthin	9.6
astaxanthin	8.3–18.8
astaxanthin ester	2.6–29.2
aurochrome	11.5–12.7
auroxanthin	13.2
mutatochrome	8.6–24.3
mutatoxanthin	7.7–11.6

Table 6

Carotenoid content in the *Pseudochaenichthys georgianus* and different body lengths

length of body in cm	total content of carotenoids in $\mu g/g$ f.w.	dominating carotenoids
38.0	0.041	zeaxanthin (26.2%), astaxanthin ester (24.3%)
43.0	0.040	zeaxanthin (48.8%), astaxanthin ester (21.9%)
44.0	0.049	zeaxanthin (22.7%), astaxanthin ester (18.6%)
48.0	0.082	astaxanthin ester (29.2%), mutatochrome (24.3%)
51.0	0.155	zeaxanthin (31.6%), lutein (21.9%)
54.0	0.148	β–cryptoxanthin (49.8%), zeaxanthin (37.9%)
56.0	0.125	isozeaxanthin (22.9%), β–cryptoxanthin (19.4%)

Table 7

Carotenoid content in the brain of some fish (in %)

Carotenoid	*D. eleginoides*	*N. gibberifrons*	*P. georgianus*
β–cryptoxanthin			21.7
canthaxanthin			9.2
lutein		5.8	37.6
phoenicoxanthin	28.6		23.8
zeaxanthin	11.1	33.9	
astaxanthin	37.5	33.8	
astaxanthin ester	15.0	17.4	
mutatoxanthin	7.8	9.1	7.7
Total content of carotenoids in μg/g fresh weight	0.138	0.262	0.214

Table 8

Carotenoid content in the body of the *Trematomus hansoni*

Carotenoid	fluctuations in %
α–carotene	5.9–29.1
β–carotene	7.9–13.4
α–cryptoxanthin	18.2
β–cryptoxanthin	8.1– 9.4
β–carotene epoxide	11.2
lutein	11.7–18.8
zeaxanthin	11.2–55.2
canthaxanthin	15.7
astaxanthin	6.7–19.9
astaxanthin ester	23.1
aurochrome	14.2
mutatochrome	7.4–21.1
mutatoxanthin	8.9

Table 9

Carotenoid content in the *Trematomus hansoni* and different body lengths

length of body in cm	total content of carotenoids in μg/g f.w.	dominating carotenoids
31–33	0.043	α–carotene (19.1%), β–carotene (19.0%), zeaxanthin (55.2%)
41–42	0.069	zeaxanthin (32.9%), astaxanthin ester (23.1%)

identified, the most noteworthy being the ε-carotene derivative, neothxanthin. The commonest carotenoids in this species were β-cryptoxanthin, zeaxanthin and astaxanthin. The studies on the total carotenoids content in individuals of various lengths (Table 6) revealed the content to rise, beginning with the smallest specimens up to those 51 cm long, past which a tendency to fall was observed. The dominant carotenoids were, in most cases zeaxanthin and astaxanthin. The brain carotenoid was higher than that of the muscles even in those specimens found to contain the largest amount of carotenoids.

Two growth classes of *Trematomus hansoni* were also studied for their carotenoid content. The carotenoids are listed in Table 8 and their total amount found in individuals from different growth groups are shown in Table 9. It should be noted that α-carotene was present in this species. The commonest carotenoid was zeaxanthin.

Table 10

Carotenoid content in the body of the *Trematomus bernacchii* (in %)

Carotenoid	%
α–carotene	9.3
β–cryptoxanthin	9.4
lutein	12.3
tunaxanthin	7.1
zeaxanthin	14.8
astaxanthin	28.9
aurochrome	5.0
Total content of carotenoids in μg/g fresh weight	0.142

Table 11

Carotenoid content in some parts of the body of the *Bathydraco macrolepis* (in %)

Carotenoid	fins	skin	muscles
β–carotene epoxide		10.9	
β–cryptoxanthin		15.6	34.7
α–doradexanthin	10.1		
canthaxanthin			3.8
lutein (free and epoxide)	12.4		25.4
isolutein	6.5		
zeaxanthin	21.3	46.3	8.0
astaxanthin	28.5	27.2	5.3
astaxanthin ester	21.2		16.5
mutatoxanthin			6.3
Total content of carotenoids in μg/g fresh weight	1.291	1.272	0.138

The presence of these pigments was also investigated in one specimen of the *Trematomus bernacchii* (Table 10) and *Bathydraco macrolepis* (Table 11), the species yielding a single individual each. The most interesting finding was the presence of α-carotene in the *Trematomus bernacchii* individual and isolutein in the *Bathydraco macrolepis* one.

DISCUSSION

The results of these studies show that the list of carotenoids found to date in fish of the Antarctic (Czeczuga and Kłyszejko 1978) should be extended to include α-carotene, β-cryptoxanthin, phoenicoxanthin, α-doradexanthin, neothxanthin and isolutein. Neothxanthin is a derivative of ε-carotene (3-hydroxy-ε-carotene) which lies in the conversion pathway of ε-carotene to tunaxanthin. This carotenoid was first reported to be present in fish by Tanaka et al. (1977), who found it in marine species, *Neothunnus albacora*. The present author (1980) noted its presence in some species, representatives of the family *Pleuronectidae*, from the Baltic Sea. As noted in a previous paper (Czeczuga 1979 b), grows the total carotenoid content of *Cyprinus carpio* increases with fish growth since a number of carotenoids are accumulated in the various parts of the body. On the whole this has been confirmed in the present study on Antarctic fishes particularly as regards *Notothenia gibberifrons.* On he other hand, it was found that in two other species, the increase in carotenoid content occured at a definite age past which a tendency to decline was observed. This may be related to changes in the dietary spectrum of these fish as they grow older. Previous studies on the *Notothenia gibberifrons* (Shust and Pinskaya 1978) have shown that these fishes permanently inhabit the sea bottom and feed mainly on benthos. The alimentary canal of the individuals of this species were filled in 75% with *Polychaeta* fragmented crustaceans, representatives of the *Echinodermata* and molluscs. Krill, however, is found far less frequently in the alimentary canal of this species than in other representatives of this family. In two other species in which growth – related changes in the carotenoid content were studied the dietary spectrum underwent changes. Particularly in the adult *Pseudochaenichthys georgianus*, a large proportion of the alimentary canal content consitend of krill; schools of these fish searching for food are often observed (Permitin, 1969; Permitin and Tarverdieva, 1972; Permitin and Sazanov, 1974; Tarverdieva, 1972).

It was of interest to note that the brain tissue of some of the fish species under study contained comparatively large amounts of carotenoids. This would confirm our previous observations concerning the quite high carotenoid concentrations in the brain of certain freshwater fish (Czeczuga 1977), especially those inhabiting waters not very rich in oxygen (thunder fish).

Considering the carotenoids found in the fish species studied here, one is tempted to suggest the astaxanthin synthesis pathway in these fishes. The biosynthesis of astaxanthin is likely to occurs by way of β-carotene conversion into echinenone which is then converted into canthaxanthin; this is then converted into phoenicoxanthin which is, in turn, converted into astaxanthin. Though echinenone has not been found in fishes of the Anctarctic, yet it has nevertheless been found quite often in other fish species both freshwater (Czeczuga 1979 a, b) and marine (Tanaka, 1978). The conversion described form probably one of the fundamental pathway of astaxanthin biosyntesis in fish.

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KAROTENOIDY U RYB. XXXIV. KAROTENOIDY U RÓŻNYCH WIEKOWO OSOBNIKÓW NIEKTÓRYCH GATUNKÓW RYB Z ANTARKTYDY

Streszczenie

Autor stosując chromatografię kolumnową i cienkowarstwową badał zawartość poszczególnych karotenoidów w mięśniach różnych wiekowo sześciu gatunków ryb z wód Antarktydy.

W wyniku badań ustalono obecność takich karotenoidów jak: α-, β-carotene, β-carotene epoxide, α-, β-cryptoxanthin, cantaxthin lutein, lutein epoxide, isolutein, zeaxanthin, neothxanthin, astaxanthin, astaxanthin ester, mutatochrome, mutatoxanthin, aurochrome oraz auroxanthin.

Podano również wahania procentowe poszczególnych karotenoidów oraz ogólną ich zawartość dla badanych gatunków ryb. Okazało się, że mięśnie osobników niektórych gatunków ryb najzasobniejsze są w karotenoidy w wieku najstarszym (*Notothenia gibberifron, Trematomus hansoni*) osobniki zaś pozostałych gatunków, najwięcej zawierają karotenoidów w wieku dojrzałym (średnim).

Б. Чечуга

КАРОТИНОИДЫ У РЫБ. XXXIУ. КАРОТИНОИДЫ У РАЗЛИЧНЫХ ПО ВОЗРАСТУ ОСОБЕИ НЕКОТОРЫХ ВИДОВ РЫБ АНТАРКТИКИ

Р е з ю м е

Применяя колонную и тонкослойную хроматографию автор исследовал содержание отдельных каротиноидов в мясе различающихся возрастом 6-ти видов рыб Антарктики.

доказали наличие таких каротиноидов как: α-, β- carotene, β - carotene epoxide, α-, β- oryptoxanthin, cantaxthin, lutein, lutein epoxide, isolutein, zeaxanthin, neothxanthin, astaxanthin, astaxanthin ester, mutatochrome, mutatoxanthin, aurochrome, auroxantin.

Приводятся также процентные колебания отдельных каротиноидов, а также их общее содержание для исследованных видов рыб. Обнаружили, что мышцы некоторых видов рыб являются богатыми содержанием каротиноидов в самом старшем возрасте (Notothenia gibberifrons, Trematomus hansoni), особи других видов содержат наибольшее количество каротиноидов в зрелом(среднем) возрасте.

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