ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XV, Fasc. 1 Szczecin 1985

*Bazyli CZECZUGA**, *Andrzej WITKOWSKI***, *Mieczysław KOWALEWSKI****

Biochemistry

CAROTENOIDS IN FISH – XXXIX. PRESENCE OF SALMOXANTHIN IN *THYMALLUS THYMALLUS* (L.) SPECIMENS

KAROTENOIDY U RYB – XXXIX. OBECNOŚĆ SALMOKSANTYNY U OSOBNIKÓW *THYMALLUS THYMALLUS* (L.)

***Medical Academy, Białystok,**
****Museum of Natural History, Wrocław,**
*****Fish Culture Station, Łopuszna**

Column and thin-layer chromatography was taken advantage of to study the occurrence of particular carotenoids in different parts of grayling body. As a result, 20 carotenoids were identified, in this also salmoxanthin, i.e. the carotenoid typical of salmonid fishes.

INTRODUCTION

The only representative of *Thymallidae* family in Polish waters is characterized by intensive colouring of the body, this being especially true during the spawning season. Life cycle or *Thymallus thymallus* is very similar to that of brook trout (Witkowski et al. 1983). so that the representatives of *Thymallidae* family were classified in the past as belonging to *Salmonidae* family.

In the recent years, the so-called chemotaxonomic approach became more and more important in the systematics. It is based on the presence of certain chemical substances in particular species, in this also of carotenoids which are used both in plant (Liaaen-Jensen 1977) and animal world (Kayser 1982).

During our studies on the occurrence of carotenoids in fishes, in this also inin individuals belonging to the salmonid family (Czeczuga 1979) we became interested in the problem of possible similarities or differences as regards the presence of some carotenoids in *Thymallus thymallus* and other species of the salmonid family. Moreover, the results of such studies should be of importance for general knowledge on the occurrence of carotenoids in fishes, and on their abundance in biologically active substances, such as carotenoids which constitute a source of vitamin A.

MATERIAL AND METHODS

Individuals of *Thymallus thymallus* (L.)(two for each sex) were caught on 12 April 1980, in the Kamienica River, a tributary of Dunajec River, in Kamienica village. Total length (*longitudo totalis*) of the fishes ranged between 34.3 and 36.1 cm.

Equal weighed portions of the tissue collected from each individual of the given sex were mixed into one sample, homogenized and preserved in 95% acetone in dark-glass bottles. The samples were stored in a refrigerator untill the analysis. Separation of particular carotenoids was made with the use of column and thin-layer chromatography. The material was first hydrolysed in 10% KOH in nitrogen atmosphere, for 24 hours in room temperature.

Details of the procedure during chromatographic separation of the carotenoids have been described earlier (Czeczuga and Czerpak 1976). After the hydrolysis, the extract was transferred to a column filled with Al_2O_3. Length of the columns varied from 15 to 25 cm. produced by Quickfit, England). Particular fractions were eluted using different solvent systems (Czeczuga and Czerpak 1976).

Apart from the column chromatography, the acetone extracts were also separated into particular fractions taking advantage of thin-layer chromatography. Glass plates covered with silica gel were used. Also in this case different solvent systems were applied. R_f was determined according to standard methods.

Identification of particular carotenoids was carried out basing on the following methods: a) character of column chromatograms; b) maxima of pigment absorption in different solvents, determined using Beckman 2400-DU spectrophotometer; c) ratio between epiphase and hypophase, determined in hexane and 95% methanol; d) comparison of R_f values from thin-layer chromatograms, for the identification of α-carotene, β-carotene, α-cryptoxanthin, β-cryptoxanthin, canthaxanthin, lutein, zeaxanthin, adonixanthin, phoenicoxanthin, α-doradoxanthin, tunaxanthin and astaxanthin cochromatography was used with the standards of the firms Hoffman-La Roche and Co. Ltd., Basel, Switzerland, and Sigma Chemical Company; e) presence of allylohydroxyl groups determined with acid chloroform; f) epoxide test. Quantitatively concentrations of particular carotenoids were determined basing on quantitative spectra of absorption. These determinations took advantage of extinction coefficients E 1%/cm, at respective absorption maxima in petroleum benzin or hexane (Davies 1976).

Table 1

List of the carotenoids from *Thymallus thymallus*

No	Carotenoid	Structure (see Fig. 1)	Semisystematic name
I	α – carotene	A – X – B	β, ε – carotene
II	β – carotene	A – X – A	β, β – carotene
III	α – cryptoxanthin	B – X – C	β, ε – caroten – 3′ – ol
IV	β – cryptoxanthin	A – X – C	β, β – caroten – 3 – ol
V	canthaxanthin	H – X – H	β, β – carotene – 4,4′ – dione
VI	4′ – hydroxyechinenone	E – X – H	4′ – hydroxy – β, β – caroten – 4 – one
VII	lutein	C – X – D	β, ε – carotene – 3,3′ – diol
VIII	3′ – epilutein	C – X – D	β, ε – carotene – 3,3′ – diol (stereoisomeric)
IX	lutein epoxide	D – X – K	5,6 – epoxy – 5,6 – dihydro – β, ε – carotene – 3,3′ – diol
X	salmoxanthin	G – X – K	5,6 – epoxy - 5,6 – dihydro – β, ε – carotene – 3,3′,6′ – triol
XI	zeaxanthin	C – X – C	β, β – carotene – 3,3′ – diol
XII	neothxanthin	B – X – D	ε, ε – caroten – 3 – ol
XIII	tunaxanthin	D – X – D	ε, ε – carotene – 3,3′ – diol
XIV	idoxanthin	F – X – I	3,3′,4′ – trihydroxy – β, β – caroten – 4 – one
XV	phoenicoxanthin	H – X – I	3 – hydroxy – β, β – carotene – 4,4′ – dione
XVI	α – doradexanthin	D – X – I	3,3′ – dihydroxy – β, ε – caroten – 4 – one
XVII	adonixanthin	C – X – I	3,3′ – dihydroxy – β, β – caroten – 4 – one
XVIII	astaxanthin	I – X – I	3,3′ – dihydroxy – β, β – carotene – 4,4′ – dione
XIX	mutatochrome	A – Y – L	5,8 – epoxy - 5,8 – dihydro – β, β – carotene
XX	β – apo – 2′ – carotenal	A – Z	3′,4′ – didehydro – 2′ – apo – β – caroten – 2′ – al

Table 2

Carotenoid content found in the investigated parts of the body female, eggs and fry of *Thymallus thymallus* (in %)

Carotenoid	Fins	Skin	Muscles	Intestine	Liver	Eggs	Unfer-tilized eggs	ferti-lized eggs	month old fry	fry
α – carotene						13.7				
β – carotene	8.4				11.7	10.0		15.4		
α – cryptoxanthin		6.0				11.3		4.4		
β cryptoxanthin	12.5	15.4					3.8	10.9		
canthaxanthin		20.0	17.4	19.1	4.3		4.4		1.9	
4 – hydroxyechinenone			7.3					2.2		
lutein	3.5	28.3			9.8				12.6	
3′ – epilutein	5.4						8.6			
lutein epoxide	13.3			49.4	21.1	7.6	66.2	47.1	5.6	28.0
zeaxanthin	7.7	6.1			4.9		3.0		13.9	56.1
adonixanthin	20.5							20.0		
idoxanthin			5.1							
phoenicoxanthin				14.9						
tunaxanthin			55.0			8.2			25.0	15.9
neothxanthin						13.7				
salmoxanthin						10.4				
α – doradexanthin	16.4	18.4			10.7				41.0	
astaxanthin	12.3	5.8	15.2	16.6	37.5	25.1	14.1	trace	trace	trace
Total content in μg/g wet weight	0.665	0.374	0.198	0.891	0.508	0.855	1.784	0.929	0.735	0.427

RESULTS

20 carotenoids (Tab. 1, Fig. 1) were found in *Thymallus thymallus* specimens under study, in this 18 carotenoids in the females (Tab. 2). The group of most frequent carotenoids contained canthaxanthin, lutein, lutein epoxide, zeaxanthin and astaxanthin. Attention should be given to the presence of 4'-hydroxyechinenone, 3'-epilutein, idoxanthin, and most of all salmoxanthin in the females under study. Total content of carotenoids in particular parts of female bodies varied from 0.198 (muscles) to 1.784 μg/g fresh weight (unfertilized eggs). As regards grayling males (Tab. 3), their bodies also contained 18 carotenoids. The most frequent were: β-carotene, β-cryptoxanthin, lutein, lutein epoxide, zeaxanthin, α-doradoxanthin and astaxanthin. It should be noted that some body parts of grayling males contained 3'-epilutein, idoxanthin, and salmoxanthin. The highest content of carotenoids was found in fish muscles (1.323 μg/g fresh weight).

Table 3

Carotenoid content found in the investigated parts of the body male *Thymallus thymallus* (in %)

Carotenoid	Fins	Skin	Muscles	Liver	Intestine	Gonad	Milt
β – carotene		8.7	78.8	13.9			7.7
α – cryptoxanthin						16.8	
β – cryptoxanthin	14.9	28.0			12.3	16.3	
canthaxanthin					7.4	2.8	7.5
lutein	4.5	5.5	6.2			5.6	11.2
3' – epilutein	4.5			17.8	24.8		
lutein epoxide	19.7		10.3		24.6		4.8
zeaxanthin	12.1	17.7	4.8			41.1	
adonixanthin		8.6					10.7
phoenicoxanthin				10.6	11.6		
idoxanthin				11.9	12.8	1.7	
salmoxanthin	10.3						24.0
α – doradexanthin	14.4	5.2				7.2	12.1
tunaxanthin						6.6	
neothxanthin		18.6		22.1			
astaxanthin	12.2	7.7	trace	17.2	6.5	1.9	22.0
mutatochrome	7.4						
β – apo – 2' – carotenal				6.5			
Total content in μg/g wet weight	0.718	1.323	0.200	0.505	0.978	0.990	0.371

DISCUSSION

Most carotenoids formed in *Thymallus thymallus* specimens under study belonged to a group most frequent in fishes, and only some were those occuring less frequently. The latter were α-carotene, 4'-hydroxyechinenone, 3'-epilutein, idoxanthin salmoxanthin and neoxanthin. The last carotenoid is a precursor of tunaxanthin, which has been previously found in *Thymallus thymallus* eggs (Czeczuga 1975). As regards α-carotene, it was present only in *Thymallus thymallus* eggs. This carotene has been found only in a few fish species (Czeczuga 1980). With respect to echinenone derivative, viz. to 4'-hydroxyechinenone, this carotenoid occurs in salmonid species, similarly as echinenone. Among others Matsuno et al. (1980 b) found 4'-hydroxyechinenone in a few trout species in Japanese waters, and Czeczuga and Chełkowski (1984) – in *Salmo trutta.* Similarly, 3'-epilutein, an isomer of lutein, and salmoxanthin, a lutein derivative, were found in some salmonid species. Salmoxanthin was even recognized as the carotenoid characteristic of salmonid species only, due to the fact that so far it was found in some freshwater as well as marine salmonids (Matsuno et al. 1980a, 1980b, Czeczuga 1982).

As regards the two last carotenoids in this group, viz. idoxanthin and neothxanthin, they were also found in *Salmo trutta* (Czeczuga and Chełkowski 1984). Moreover, idoxanthin was also identified in some cyprinids. Nagata and Matsuno (1979) found it for

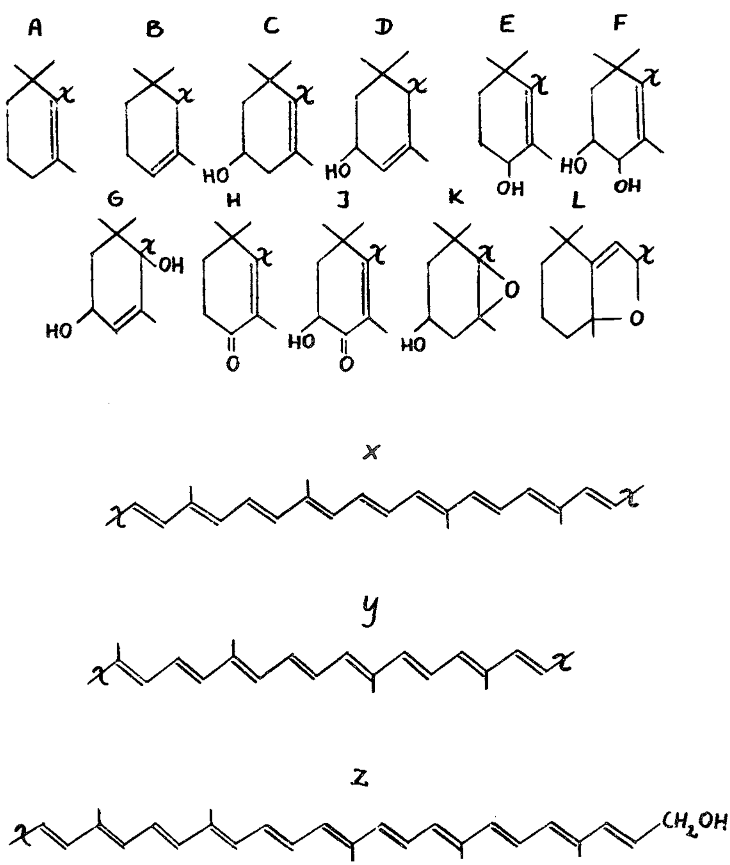

Fig. 1. Structural features of carotenoids from *Thymallus thymallus* specimens.

the first time in fancy red carp, and Czeczuga (1981) in specimens of *Micropterus salmoides*, as also in *Cyprinus carpio* from heated waters (Czeczuga and Dąbrowski 1983). With respect to neothxanthin, it is a precursor of tunaxanthin, and is formed from ε-carotene, the latter being rather rare in fishes. On the other hand, tunaxanthin is fairly frequent in fishes belonging to various systematic units (Bingham et al. 1979).

Many carotenoids identified in grayling are also fairly frequent in salmonid fishes, this being especially true of salmoxanthin, i.e. the carotenoid characteristic of the salmonids. This fact would justify former taxonomic classification of *Thymallus thymallus* into the family *Salmonidae*.

As regards total content of carotenoids in the specimens under study, there were some differences between males and females. In males maximal carotenoid contents were found in skin, gonads and food tract, while in case of females the highest levels were recorded in unfertilized eggs and food tract. Moreover, male fins contained much less carotenoids than the skin, whereas the opposite was found in case of females. Differences in carotenoid content between males and females during the spawning season have been observed previously also in case of *Salmo trutta* (Czeczuga and Chełkowski 1984);

Translated: Dr. Maria Bnińska

REFERENCES

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

Czeczuga B., 1975: Carotenoids in fish. IV. Salmonidae and Thymallidae from polish waters.-Hydrobiologia 46: 223–239.

Czeczuga B., 1979: Carotenoids in fish. XX. Carotenoids in Salmo gairdneri Rich. and Salmo trutta morpha fario L.-Hydrobiologia 64: 251–259.

Czeczuga G., 1980: Carotenoids in fish. XXVI. Pungitius pungitius (L.) and Gasterosteus aculeatus L. (Gasterosteidae).-Hydrobiologia 74: 7–10.

Czeczuga B., 1981: Carotenoids in fish. XXVIII. Carotenoids in Micropterus salmonides (Lacépède) Centrarchidae.-Hydrobiologia 78: 45–48.

Czeczuga B., 1982: Carotenoids in fish. XXXII. Content of carotenoids in eggs utilized in the form of caviar.-Folia Histochem. et Cytochem. 20: 63–68.

Czeczuga B., Chełkowski Z., 1984: Carotenoids in fish. XXVI. Carotenoids content in prespawning and spawning Salmo trutta.-Acta Ichthyol. Piscat. 14, 1–2.

Czeczuga B., Czerpak R., 1976: Carotenoids in fish. 7. 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., and Dąbrowski K., 1983: Rapeseed meal in the diet of common carp reared in heated waters V. Carotenoids in diets and fish tissues.-Tierphysiol. Tierernährg. Futtermittelkde 50: 52–61.

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

Kayser H., 1982: Carotenoids in insects. (In: Carotenoid Chemistry and Biochemistry, Ed: G. Britton and T.W. Goodwin. Pergamon Press, Oxford and New Jork): 195–210.

Liaaen-Jensen S., 1977: Algal carotenoids and chemosystematics.-Mar. Natur. Products Chem. **1**: 239–259.

Matsuno T., Nagata S., Katsuyama M., 1980a: The structure of a new carotenoid, salmoxanthin from the salmon, Oncorhynchus keta Walbaum.-Bull. Jap. Soc. Sci. Fish. **46**: 911.

Matsuno T., Nagata S., Katsuyama M., Matsutaka H., Maoka T., Akita T., 1980b: Comparative biochemical studies of carotenoids in fishes. – XVIII. Carotenoids of cultured fishes, japanese char, brook trout, lake trout, masu trout, red-spotted masu trout, rainbow trout and brown trout.-Bull. Jap. Soc. Sci. Fish. **46**: 473–478.

Nagata S., and Matsuno T., 1979: The occurrence of idoxanthin in fancy red carp Cyprinus carpio.-Bull. Jap. Soc. Sci. Fish. **45**: 537.

Witkowski A., Kowalewski M., Kokurewicz B., 1983: Lipień. [The grayling]. PWRiL. Warszawa.

Translated: Dr. Teresa Radziejewska

B. Czeczuga, A. Witkowski, M. Kowalewski

KAROTENOIDY U RYB. XXXIX. OBECNOŚĆ SALMOKSANTYNY U OSOBNIKÓW *THYMALLUS THYMALLUS* (L.)

STRESZCZENIE

Autorzy stosując chromatografię kolumnową i cienkowarstwową badali występowanie karotenoidów w poszczególnych częściach ciała samic i samców lipienia.

W wyniku badań ustalono obecność takich karotenoidów jak: α- i β-karotenu,, α- i β-kryptoksantyny, kantaksantyny, 4'-hydroksyechinenonu, luteiny, 3'-epiluteiny, epoksydową formę luteiny, salmoksantyny, zeaksantyny, neotksantyny, tunaksantyny, idoksantyny, foenikoksantyny, α – doradeksantyny, adoniksantyny, astaksantyny, mutatochromu oraz β–apo–2'–karotenalu. Podano również stosunki procentowe poszczególnych karotenoidów oraz ogólną ich zawartość w badanych częściach ciała lipieni. U samic najwięcej karotenoidów zawierała ikra, zaś u samców – mięśnie. Ponadto autorzy zwracają uwagę na obecność u badanych osobników lipieni salmoksantyny – typowego karotenoidu ryb łososiowatych.

Чечуга Б., Витковски А,
Ковалевски М.

КАРОТИНОИДЫ У РЫБ XXXIX. ПРИСУТСТВИЕ САЛЬМОКСАНТИНА У ОСОБЕЙ THYMALLUS THYMALLUS

Р е з ю м е

Авторы, применяя колонную и тонкослойную хроматографию, исследовали присутствие ряда каротиноидов в некоторых органах самок и самцов хариуса.

Установлено присутствие следующих каротиноидов: α, β - каротин , α , β - криптоксантин, кантаксантин, 4' - гидроксиэхиненон, лютеин, 3' -эпилютеин, эпоксидная форма лютеина, сальмоксантин, зеаксантин, неотксантин, тунаксантин, идоксантин, фоэникоксантин, α -дорадоксантин, адониксантин, астаксантин, мутатохром, а также β -апо-2'-каротеналь.

Авторы приводят также процентное и общее содержание каротиноидов в различных органах хариуса. Оказалось, что у самок наиболее богатой каротиноидами является икра, у самцов - мышцы.

Кроме этого, авторы обращают внимание на присутствие у особей хариуса сальмоксантина - типичного каротиноида лососевых рыб.

Author's addresses:
Prof. Dr Bazyli CZECZUGA
Deprtment of General Biology,
Medical Academy
Białystok 15–230

Dr. Andrzej WITKOWSKI
Museum of Natural History
Wrocław University
Wrocław 50–335

M.Sc. Mieczysław KOWALEWSKI
Fish Culture Station,
Polish Anglers Association
Łopuszna near Nowy Targ 34–432

Received: 1 VI 1983