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CAROTENOIDS IN FISH. XXXVIII. CAROTENOID CONTENTS IN
HUCHO HUCHO (L.) INDIVIDUALS

KAROTENOIDY U RYB. XXXVIII. ZAWARTOŚĆ KAROTENOIDÓW
U OSOBNIKÓW *HUCHO HUCHO* (L.)

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Using column and thin layer chromatography the authors studied the occurrence and total contents of various carotenoids in some selected body parts of females and males of *Hucho hucho* in the river Dunajec.

INTRODUCTION

A salmonid species called the Danube salmon (*Hucho hucho*) inhabits the rivers Czarna Orawa and Dunajec (West Beskidy Mountains, southern Poland) (Witkowski and Kowalewski, 1980, 1982). The species is found in waters of the Prut and Danube catchment areas (Kulmatycki, 1934, 1935; Kirka, 1963; Svetina, 1970; Jungwirth, 1977, 1978, 1979; Holcik, 1982). The body of the fish is basically brown, often brownred with a greenish hue. The species is predacious and feeds mainly on other fish (Pravochensky and Kolder, 1969; Nagy, 1976; Witkowski and Kowalewski, 1982, 1983).

When studying the occurrence of carotenoids in fish in general, attention was paid to the amount of these substances in commercially important species (Czczuga, 1975, 1979 a-c), including the salmonids (Czczuga, 1973, 1975, 1977, 1979 a, b, 1982; Czczuga and Chełkowski, 1983). With respect to the carotenoid content in *Hucho hucho*, their

occurrence in eggs only has been studied so far (Czczuga, 1975). Therefore, in our opinion, data on the occurrence of some carotenoids and their amounts in various organs of the adult Danube salmon should be valuable in broadening our knowledge, particularly with regard to salmonids.

MATERIALS AND METHODS

Assays were made on 2 individuals of each sex of *Hucho hucho* (L.) caught in the first decade of April 1982 in the River Dunajec where another river, the Białka Tatrzańska, merges with it near the village of Dębno. The total length of the individuals caught ranged within 54–100 cm. Assays were made on fins, skin, muscles, liver, and intestine. Additionally, the carotenoid content was determined in non-fertilised and fertilised eggs, larvae, and in 1-mo-old fry.

Tissue samples of the same weight from both individuals of each sex were pooled, homogenised, covered with 95% acetone in a dark bottle, and kept in a refrigerator until analysed. Prior to the analyses, the materials were hydrolysed for 24 h in 10% KOH in nitrogen at room temperature. To separate various carotenoids, column and thin layer chromatography were used; the details of the techniques applied were described elsewhere (Czczuga and Czerpak, 1976). The hydrolysed extract was transferred into an Al_2O_3 -filled 15–25 cm long column (Quickfit, England). Individual fractions were eluted with various solvents (Czczuga and Czerpak, 1976).

Regardless of column chromatography, the acetone extract obtained was separated into various thin layer chromatography fractions. Silicone gel-covered glass plates and various combinations of solvents were used. R_f values were determined according to the generally accepted procedures.

Individual carotenoids were identified based on the following: a) column chromatograms; b) absorption peaks of various pigments in different solvents as determined in a Beckman 2400-DU spectrophotometer; c) epi- to hypophase ratio as determined in hexane and 95% methanol; d) comparison of thin layer chromatogram R_f 's with standards; Hoffman-La Roche (Switzerland) and Sigma Chemical Company (USA) standards were used to identify α -carotene, β -carotene, ϵ -carotene, β -cryptoxanthin, canthaxanthin, lutein, zeaxanthin, adonixanthin, phaeonicoxanthin, α -doradexanthin, diatoxanthin, and astaxanthin; e) the presence of allohydroxy groups determined with acid chloroform; f) epoxide test. Quantitative determinations of individual carotenoid contents were made from quantitative absorption spectra. The determinations were based on extinction coefficients $E_{1\%/\text{cm}}$ at appropriate absorption peaks in paraffin ether or hexane (Davies, 1976).

Table 1

List of the carotenoids from *Hucho hucho*

Carotenoid	Structure (see Fig. 1)	Semisystematic name
α -carotene	A - X - B	β, ϵ - carotene
β - carotene	A - X - A	β, β - carotene
ϵ - carotene	B - X - B	ϵ, ϵ - carotene
α - cryptoxanthin	B - X - C	β, ϵ - caroten - 3' - ol
β - cryptoxanthin	A - X - C	β, β - caroten - 3 - ol
canthaxanthin	H - X - H	β, β - carotene - 4,4' - dione
4' - hydroxyechinenone	E - X - H	4' - hydroxy - β, β - caroten - 4 - one
lutein	C - X - D	β, ϵ - carotene-3,3'-diol (iso...)
3' - epilutein	C - X - D	β, ϵ - 3,3' - diol (stereoisomeric)
lutein epoxide	D - X - K	5,6 - epoxy - 5,6 - dihydro - β, ϵ - carotene - 3,3' - diol
salmoxanthin	G - X - K	5,6 - epoxy - 5,6 - dihydro - β, ϵ - carotene - 3,3', 6' - triol
zeaxanthin	C - X - C	β, β - carotene - 3,3' - diol
diatoxanthin	C - Y - N	7,8 - didehydro - β, β - carotene - 3,3' - diol
neothxanthin	B - X - D	ϵ, ϵ - caroten - 3' - ol
tunaxanthin	D - X - D	ϵ, ϵ - carotene - 3,3' - diol
idoxanthin	F - X - I	3,3', 4' - trihydroxy - β, β - caroten - 4 - one
phoenicoxanthin	H - X - I	3 - hydroxy - β, β - carotene - 4,4' - dione
α - doradexanthin	D - X - I	3,3' - dihydroxy - β, ϵ - caroten - 4 - one
adonixanthin	C - X - I	3,3' - dihydroxy - β, β - caroten - 4 - one
astaxanthin	I - X - I	3,3' - dihydroxy - β, β - carotene - 4,4' - dione
mutatochrome	A - Y - L	5,8 - epoxy - 5,8 - dihydro - β, β - carotene
mutatoxanthin	C - Y - M	5,8 - epoxy - 5,8 - dihydro - β, β - carotene - 3,3' - diol
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
of male *Hucho hucho* (L.) in %

Name of carotenoid	Skin	Muscles	Liver	Milt	Gonad	Fins D	Fins V	Fins C	Fins ad	Fins A	Fins P
α - carotene								1.4			
β - carotene	25.5	9.7	39.0		11.8	11.2	15.1	21.0		28.9	39.6
ϵ - carotene											18.0
α - cryptoxanthin	9.8					8.1		11.9			
β - cryptoxanthin		37.3		13.8		9.7	14.4	19.1	19.9	12.8	12.0
canthaxanthin		8.3		2.4					2.5		
lutein					16.1	9.2	5.9				
3' - epilutein		4.7				11.1	3.5		6.5		19.6
lutein epoxide	18.3	10.7	17.5	7.7	4.9	33.8	18.9	23.1	4.9		
zeaxanthin	27.0	10.4	17.7		17.3	16.9	21.3	14.6	23.6	36.6	
4' - hydroxyechinenone					22.4				2.2		
adonixanthin			7.4						16.9		
phoenicoxanthin					18.0						
salmoxanthin		3.9									
tunaxanthin				36.7							10.8
neothxanthin	12.1									21.7	
idoxanthin			18.4								
α - doradexanthin		10.4		24.4				8.9			
astaxanthin	7.3	4.6		15.0			11.5		23.5		
mutatoxanthin							9.4				
unknown					9.5						
Total content in $\mu\text{g/g}$ fresh weight	0.236	0.788	0.301	0.332	0.398	0.264	0.225	0.389	1.288	0.135	0.373

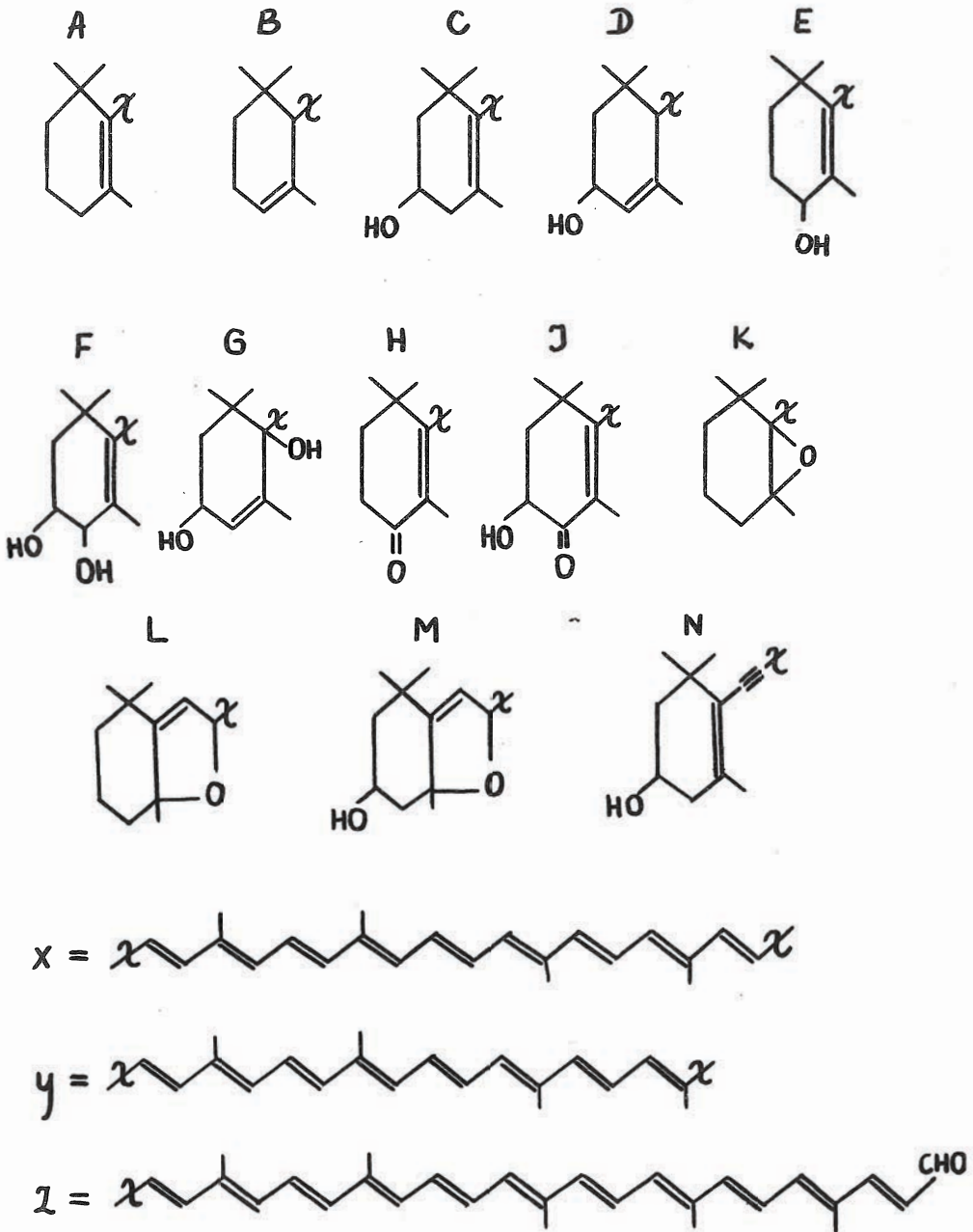


Fig. 1. Structural features of carotenoids from materials studied

Table 3

Carotenoid content found in the investigated parts of the body of female *Hucho hucho* (L.) in %

Name of carotenoid	Skin	Intestine	Liver	Muscles	Fins A	Fins D	Fins ad	Fins C	Fins P
β - carotene	34.0	19.3					31.2	34.3	
ϵ - carotene								16.5	
α - cryptoxanthin		6.6			32.5		20.8		14.6
β - cryptoxanthin	14.3	19.7	27.0	27.2		11.4	1.6		
canthaxanthin			12.2	11.3					
4' - hydroxyechinenone						19.1			
3' - epilutein	5.0			19.2		22.6		14.0	
lutein epoxide	16.7	6.3			53.2	36.5			8.5
zeaxanthin	21.2	10.3		42.3			13.8		33.2
adonixanthin		16.9							
phoenicoxanthin			10.4						
diatoxanthin			12.0				10.1		
tunaxanthin								14.1	
neothxanthin								15.7	
astaxanthin		11.0	38.4		14.3		12.5	5.4	
α - doradexanthin	8.9	9.8							32.2
mutatochrome						10.4			
β - apo - 2' - carotenal									11.5
Total content in $\mu\text{g/g}$ fresh weight	0.956	1.237	0.391	0.505	0.511	1.908	10.538	1.131	1.041

RESULTS

The carotenoids found in the *Hucho hucho* individuals examined were listed in Table 1 and Fig. 1.

The *H. hucho* males were found to contain 20 carotenoids (Table 2). β -carotene, β -cryptoxanthin, lutein epoxide, and zeaxanthin occurred in most of the body parts examined. Particularly noteworthy is the presence of α - and ϵ -carotene, 3'-epilutein, 4'-hydroxyechinenone, salmoxanthin, neothxanthin, and idoxanthin in some organs. The total carotenoid content in *H. hucho* males ranged from 0.135 (anal fins) to 1.288 $\mu\text{g/g}$ wet weight (adipose fins). Muscles were relatively rich in carotenoids (0.788 $\mu\text{g/g}$ wet weight).

The presence of 18 carotenoid was found in the Danube salmon females examined (Table 3). The most frequent carotenoid were β -cryptoxanthin, lutein epoxide, and zeaxanthin. The females, too, contained ϵ -carotene, 4'-hydroxyechinenone, 3'-epilutein, and neothxanthin. The liver showed the lowest and the adipose fin the highest contents of carotenoids (0.391 and 10.538 $\mu\text{g/g}$ wet weight, respectively).

The eggs, larvae, and fry were found to contain 14 carotenoids (Table 4). Lutein epoxide and astaxanthin occurred in all three groups. The larvae contained the highest amounts of carotenoids (1.738 $\mu\text{g/g}$ wet weight) while non-fertilised eggs were the poorest in carotenoids (0.364 $\mu\text{g/g}$ wet weight).

Table 4

Carotenoid content found in the eggs and the fry of the *Hucho hucho* (L.) in %

Name of carotenoid	unfertilized eggs	fertilized eggs	week - old fry	monthold fry
β - carotene			7.7	
α - cryptoxanthin		8.1		
β - cryptoxanthin	16.7	10.0		
canthaxanthin		6.3		2.0
4' - hydroxyechinenone				2.2
lutein		15.4		
3' - epilutein	5.7			33.0
lutein epoxide	6.3	9.1	59.0	13.1
zeaxanthin	39.5	28.7		12.8
adonixanthin			6.3	3.4
salmoxanthin	5.4			
tunaxanthin			20.9	
α - doradexanthin			6.1	14.5
astaxanthin	25.4	22.4	trace	19.0
Total content in $\mu\text{g/g}$ fresh weight	0.364	0.432	1.738	0.538

common, having been recorded so far i.a. in *Limanda limanda* (Czeczuga, 1980). Idoxanthin, apart from crustaceans in which it was first described (Herring, 1969), was recorded in sea lamprey (Matsuno and Nagala, 1979) and in some fish species, *Micropterus salmoides* among the others (Czeczuga, 1981).

Relatively high carotenoid contents in muscles of *H. hucho* males (0.788 µg/g wet weight) and in adipose fins of both sexes (1.288–10,538 µg/g wet weight) are worth emphasising. Muscles of other freshwater salmonids, except for *Coregonus lavaretus*, were found to contain much less carotenoids than the *H. hucho* individuals studied. The following contents, respectively, were recorded in muscles of *Coregonus albula*, *C. peled*, *Salmo gairdneri*, and *S. trutta morpha fario*: 0.162–0.460; 0.002–0.170; 0.018–0.400; 0.037–0.230 µg/g wet weight (Czeczuga, 1975, 1977, 1979 b). These data point out that *H. hucho* belongs to the carotenoid-richest salmonids.

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KAROTENOIDY U RYB. XXXVIII. ZAWARTOŚĆ KAROTENOIDÓW U OSOBNIKÓW

Hucho hucho (L.)

STRESZCZENIE

Autorzy stosując chromatografię kolumnową i cienkowarstwową badali występowanie poszczególnych karotenoidów w płetwach, skórze, mięśniach, wątrobie, jelitach, ikrze i narybku głowacicy – *Hucho hucho* (L.).

W wyniku badań ustalono obecność takich karotenoidów, jak: α -, β -, ϵ -karotenu, α -, β -kryptoksantyny, kantaksantyny, 4'-hydroksyechinenonu, luteiny, 3' - epiluteiny, luteiny epoksy, zeaksantyny, adoniksantyny, foenikoksantyny, salmoksantyny, tunaksantyny, neotksantyny, idoksantyny, α -doradoksantyny, astaksantyny, mutatochromu, mutatoksaantyny i β - apo - 2'-karotenu.

Podano również ogólną zawartość karotenoidów dla poszczególnych części ciała głowacicy oraz stosunki procentowe poszczególnych karotenoidów. Najzasobniejszymi w karotenoidy u osobników obu płci okazały się płetwy tłuszczowe.

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KAROTINOIDY U RYB. XXXVIII. СОДЕРЖАНИЕ

KAROTINOIDOV U OSOBEИ HUSHO HUSHO (L.)

Р е з ю м е

Авторы исследовали присутствие отдельных каротиноидов в плавниках, коже, мышцах, печени, кишках, икре и мальков дунайского лосося - *Hucho hucho* (L.) с применением колоночной и тонкослойной хроматографии.

В результате исследований установлено наличие таких каротиноидов, как: α -, β -, ϵ -каротин, α -, β -криптоксантин, кантаксантин, 4' - гидроксиэхиненон, лютеин, 3' - эпилютеин, лютеин эпо-

кси, зеаксантин, адониксантин, феоеникоксантин, салмоксантин, тунаксантин, неоксантин, идоксантин, α - дорадоксантин, астаксантин, мутатохром, мутатоксантин и β -апо-2'-каротинал.

Дано также общее содержание каротиноидов для отдельных частей тела дунайского лосося, а также процентное отношение отдельных каротиноидов. У особей обоих полов наиболее богатыми каротиноидами оказались жировые плавники.

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