A C T A I CH T H Y O L O G I C A E T P I S C A T O R I A
Vol. XXI, Fasc. 2 Szczecin 1991

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Fish biochemistry

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CAROTENOIDS IN *COREGONUS LAVARETUS* L. INDI VIDUALSF VARIOUS POPU LATI ONS
BADANIA KAROTENOIDÓW U OSOBNIKÓW *COREGONUS LAVARETUS* L. RÓŻNYCH POPU LACJI

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Presence and total amount of carotenoids in muscles of *Coregonus lavaretus* males and females as well as in fish roe from the three Alpins lakes were tested based on column and thin-layer chromatography.

INTRODUCTION

So far conduced surveys on the carotenoids presence and their total amount in the *Salmonidae* family representatives have concerned mostly salmon and trout. For salmon, carotenoids were estimated mostly, in muscles prior to, during and after spaawning (Crozier 1970, Kitachara 1983, 1984, 1985; Czeczuga and Chełkowski 1984). There were also some works done on carotenoids contents in roe of some species (Czeczuga 1979a, 1982; Craik 1985). Surveys on trouts were directed, primarily on presence of some carotenoids, in fish, next on effect of various feed types on carotenoids accumulation in various fish organs and finally were concentrated, mainly, on effects of carotenoids, canthaxanthin, in particular, applied as a feed additive in aquaculture, on trout's muscles colour intensity (Steven 1948, Hata and Hata 1975, Jutariu et al. 1978, Czeczuga 1979b, Quantz 1980, Matsuno et al. 1980a,b Storebakken et al. 1986).

As for the species belonging to *Coregonidae* genus, only few works have been published so far. Czeczuga (1975) presence of particular carotenoids in the *Coregonus albula* roe as well as in roe and some organs of *Coregonus lavaretus* (fragmentaric data) and also within muscles of *Coregonus autumnalis migratorus* (G.) from the Bajkał Lake (Czeczuga 1976). Besides, presence and amount of carotenoids in each body part of the *Coregonus albula* males and females and *Coregonus peled* individuals, acclimatized in the Mazurskie Lake District waters, were analysed (Czeczuga 1977a). The data published lately on carotenoids in roe and muscles of *Coregonus albula* were directed on effect of carotenoids on eggs survival of that species, in between (Dąbrowski et al. 1987). Among numerous publications on carotenoids presence in various Salmonidae species (see Torrissen et al. 1989). Czeczuga (1975) is the only one to mention the carotenoids presence in *Coregonus lavaretus* from the Suwalskie Lake District. In that connection we decided to test individuals of the very species, from various populations, for presence and amount of carotenoids. For it was well known for the *Coregonus lavaretus* species to differ in muscle colour intensity, from whitish to reddish-resembling the one for the salmon muscle, depending on population, in between (Hartmann 1980, Rösch, 1980, Rösch and Dąbrowski 1986).

MATERIAL AND METHODS

Surveys were conducted on the *Coregonus lavaretus* L. individuals collected during November-December 1983, at the three Alpin's Lakes: Konstance-Untersee Lake, Bodensee Lake and Lac de Genève. Besides for samples collected from the Bodensee, carotenoids content was measured separately for population spawning within the pelagic part of the Lake (Local name-Blaufelchen) - the one with whitish muscles, and the near-shore spawning population (local name-Gangfish) whose muscles are reddish in colour (Eckmann et al. 1988,Eckmann 1989). Surveys were conducted on muscles of males and females and roe.

The muscles and or roe (5 g) samples collected from each fish flooded with 95% acethone after homogenisation, were kept in dark glass botles in refrigerator until tested. Separation of particular carotenoids was done by column and thin layer chromatography, details of which were presented in our previous paper (Czeczuga and Czerpak 1976). Prior to that materials were hydrolysed with 10% KOH, under nitrogen atmosphere, at room temperature, within 24 hours. When hydrolysed, an extract was tranferred to column packed up with Al_2O_3. The column length ranged from 15 to 25 cm (Quickfit-Co.- England). Separate fractions were eluated by various solvent patterns (Czeczuga and Czerpak 1976).

Independently of column chromatography, the acethone extract was devided into separate fractions by thin-layer chromatography. For that the glass plates covered with silic-gel, with different solvent patterns were used (Czeczuga and Czerpak 1968).

Next the R_f value was calculated according to obligatory standards.

Identification of particular carotenoids was based on: a) character of the column chromatogramms; b) an absorption maximum of carotenoids in different solvents; c) epiphase to hipophase relation estimated in hexane and 50% methanol; d) comparison of the thin-layer chromatography R_f values; for identification of values; for identification of β-carotene, β-cryptoxanthin, canthaxanthin, lutein, zeaxanthin, adonixanthin, α-doradexanthin an astaxanthin standards produced by Hoffman-La Roche and Co.Ltd Basel, Switzerland and by Sigma Chemical Co.USA were used; e) presence of the allylohydroxylic groups identified with an acidic chloroform; f) the epoxidic test.

A quantitative estimation of each carotenoid concentration was based on quantitative absorption aspects. The estimations were based on extinction coefficient E 1%/cm for required absorption maximums in oil ether or hexane (Davies 1976). Chemical structure of each carotenoid was presented accodring to Straub (1987).

RESULTS

Within the tested material 16 carotenoids were identified (Tab. 1, Fig. 1). According to data in Table 2 in all 10 females from the Konstance-Untersee Lake, tested, β-cryptoxanthin and astaxanthin were present, with an astaxanthin being a dominant carotenoid in 7 cases and lutein (together with 3'-epilutein), lutein epoxide and adonixanthin dominating, each, in one fish. Total amount of carotenoids in muscles ranged from 0.338 to 0.889 μg/g, average being 0.604 μg per g of wet matter. As for the roe, an astaxanthin happened to be the carotenoid typical for all tested female gonads, being the dominant one in 5 cases. The carotenoids concentration in in the roe ranged from 0.454 to 0.740; average 0.552 μg/g.

Results of analysis done on muscles and roe of *Coregonus lavaretus* from the Bodensee are presented in Table 3 and 4. Muscles of all the males from pelagic spawning population have astaxanthin, which proved, also, to be a dominat carotenoidn in 7 males on 9 tested. Concentration of carotenoids within the males muscles of that population ranged from 0.632 to 2.040, with average being 1.051 μg/g of wet matter. As for the females' muscles, all have β-cryptoxanthin, astaxanthin and lutein epoxide, however dominating carotenoids were β-cryptoxanthin, zeaxanthin, canthaxanthin, astaxanthin and lutein epoxide. Carotenoids, in total ranged from 0.419 to 1.394 μg/g (average 0.937 μg/g.

All roe samples from this very population had astaxanthin, while dominant carotenoids were canthaxanthin, astaxanthin and lutein epoxide. Total amount of carotenoids ranged from 0.362 to 1.364 with average being 0.748 μ/g.

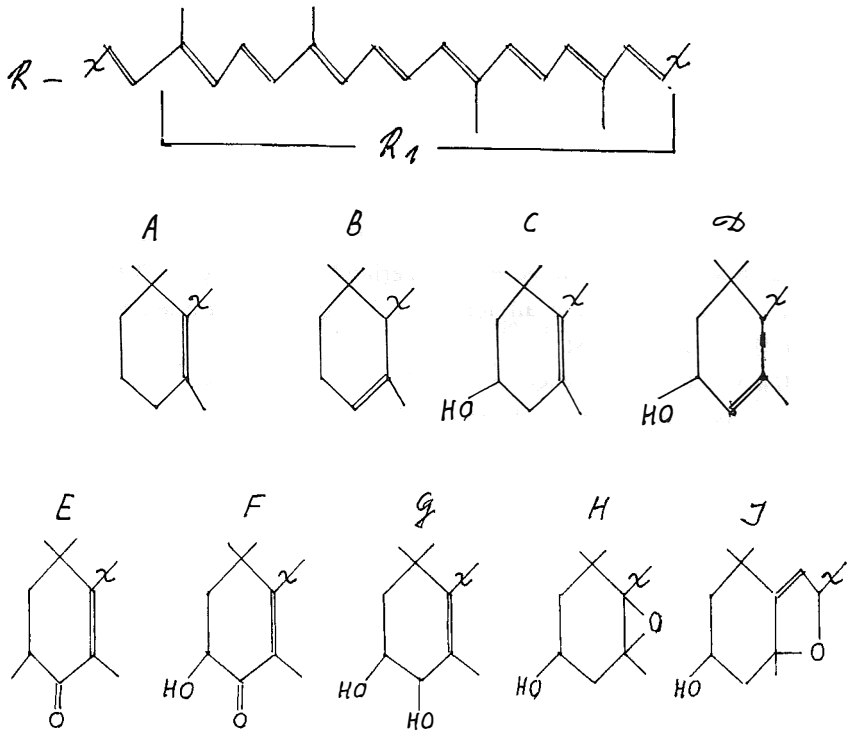

Fig. 1. Structural features of carotenoids from investigated materials

Table 4 presents results of surveys done on muscles and roe of fish from the near-shore spawning population. Muscles of all males had β-cryptoxanthin and astaxanthin, however the dominant carotenoids were β-cryptoxanthin, zeaxanthin and in 8 fish on 11 tested, astaxanthin. Carotenoids, in total, ranged from 0.797 to 1.825 μg/g (average – 1.187 μg/g). Muscles of all the tested females, alike males included β-cryptoxanthin and astaxanthin. In the muscles of 2 female on 6 fish tested, the dominant carotenoid happened to be zeaxanthin, while in 4 cases-astaxanthin. Total carotenoids amount in muscles ranged from 0.699 to 1.374 with average being 1.089 μg/g of wet matter.

In case of the roe samples, in all 12 gonad's samples astaxanthin was present, while dominating carotenoids were zeaxanthin – in 1 case, canthaxanthin in 4 and astaxanthin in 7 cases. Carotenoids level changed from 0.603 to 1.547 μg/g (average 0.932 μg/g).

Results of surveys on carotenoids level in males' muscles and muscles and roe of females from the Genewesee Lake are presented in Table 5. Muscles of all males tested induced zeaxanthin and astaxanthin, while muscles of females accumulated

Table 1

List of the carotenoids from investigated materials

Carotenoid	Structure (see Fig. 1)	Semisytematic name
1. β-carotene	A – R – A	β,β-carotene
2. β-cryptoxanthin	A – R – C	β,β-caroten-3-ol
3. zeaxanthin	C – R – C	β,β-carotene-3-3'-diol
4. lutein (3'-epilutein)	C – R – D	β,β-carotene-3-3'-diol (isomeric)
5. neothxanthin	B – R – D	ε,ε-caroten-3-ol
6. tunaxanthin	D – R – D	ε,ε-carotene-3,3'-diol
7. echinenone	A – R – E	β,β-carotene-4-one
8. hydroxyechinone	A – R – F	3-hydroxy-β,β-carotene-4-one
9. adonixanthin	C – R – F	3,3'-dihydroxy-β,β-carotem-4-one
10. α-doradexanthin	D – R– F	3,3'-dihydroxy-β,ε-caroten-4-one
11. idoxanthin	F – R – G	3,3',4'-trihydroxy-β,β-caroten-4-one
12. canthaxanthin	E – R – E	β,β-carotene-4'-dione
13.astaxanthin	F – R – F	3,3'-dihydroxy-β,β-carotene-4,4'-dione
14.lutein epoxide	D – R – H	5,6-epoxy-5,6-dihydro-β,ε-carotene-3,3'-diol
15. mutatoxanthin	C – R_1 – I	5,8-epoxy-5,8-dihydro-β,β-carotene-3,3-diol

Table 2

Carotenoid composition and content in lavaret muscle and eggs from Konstance-Untersee (9.11.1983)

A – female

No	Total content (µg/g fresh wt)	Carotenoid (see Table 1)	Major carotenoid (%)
1.	0.375	2,3,9,10,13,14	13 (36,9)
2.	0.889	1,2,9,10,13,14	13 (34.1)
3.	0.626	2,9,I3,14	13 (46,5)
4.	0.734	2,3,9,10,12,13	13 (43.0)
5.	0.658	1,2,3,4,9,11,13,14	4 (30.5)
6.	0.556	2,3,I2,13,14	13 (24.2)
7.	0.495	2,4,9,10,12,13	13 (42.1)
8.	0.731	1,2,4,9,10,13,14	9 (29.8)
9.	0.338	2,3,13,14	14 (35.5)
10.	0.642	1,2,3,9,10,12,13,15	13 (53.3)
	0.604 ± 0.167		

B – eggs

1.	0.525	3,9,10,12,13,14	I3 (40.5)
2.	0.559	1,3,6,13	13 (51.8)
3.	0.521	1,2,13,15	13 (40.1)
4.	0.454	1,2,4,13,14	2 (47.5)
5.	0.465	2,3,9,10,13	13 (32.5)
6.	0.740	1,2,3,12,13	13 (37.6)
7.	0.598	2,3,4,9,10,12,13,14	14 (36.7)
8.	0.552 ± 0.095		

Table 3

Carotenoid compositions and content in lavaret (pelagic spawning) muscle and eggs from Boden lake (30.11.1983)

A – male

No	Total content (µg/g fresh wt)	Carotenoid (see Table 1)	Major carotenoid (%)
1.	0.632	2,3,4,13,14	13 (60.9)
2.	0.867	1,2,4,9,10,12,13,14	13 (29.2)
3.	1.764	I,2,9,10,12,13,14	13 (45.0)
4.	2.040	3,9,10,13,14	14 (39.8)
5.	0.967	2,3,9,10,13	13 (61.4)
6.	0.968	3,12,13	13 (69.9)
7.	0.736	9,10,12,13,14,15	13 (52.6)
8.	0.829	1,2,3,9,10,13,14	2 (34.1)
9.	0.656	2,6,13,14	13 (29.1)
	1.051 ± 0.509		

B – female

1.	0.419	1,2,3,4,13,14,15	13 (28.1)
2.	1.249	2,3,4,12,13,14	3 (30.7)
3.	1.147	2,3,4,12,13,14	12 (45.8)
4.	1.394	2,6,9,10,13,14)	13 (55.0)
5.	0.891	1,2,3,13,14,15	2 (32.9)
6.	0.521	2,I3,14	14 (60.5)
	0.937 ± 0.399		

C – eggs

1.	0.409	2,9,10,12,13,15	12 (44.2)
2.	0.713	1,9,10,12,13,14,15	13 (53.4)
3.	1.364	3,9,10,13,14	13 (29.7)
4.	0.877	2,3,12,13,15	12 (29.6)
5.	0.762	2,3,9,10,12,13	12 (32.1)
6.	0.469	2,3,9,10,13,14	13 (35.4)
7.	0.651	1,2,3,9,10,13,14	14 (35.5)
8.	0.362	2,3,4,13,14	13 (48.1)
9.	0.753	2,3,4,9,10,13	13 (32.2)
10.	1.116	3,9,10,13,14	13 (33.8)
	0.748 ± 0.313		

Table 4

Carotenoid composition and content in lavaret (near shore spawning) muscle and eggs from Boden lake (7.12.1983)

A – male

No	Total content (μg/g fresh wt)	Carotenoid (see Table 1)	Major carotenoid (%)
1.	0.927	1,2,6,9,13,14	13 (38.3)
2.	0.987	2,4,9,10,12,13	2 (28.8)
3.	0.917	1,2,3,9,12,13,14	13 (29.1)
4.	1.740	2,9,10,12,13,14,15	13 (28.4)
5.	0.805	2,3,4,12,13,14	2 (31.7)
6.	1.698	2,3,7,9,10,13,14	13 (33.5)
7.	0.798	1,2,3,13,14	3 (39.2)
8.	1.189	2,3,9,10,13	13 (37.9)
9.	0.999	2,3,9,10,13	13 (38.4)
10.	1.825	1,2,3,9,13,14	13 (39.8)
11.	1.169	1,2,3,12,13,14	13 (27.4)
	1.187 ± 0.382		

B – female

1.	0.981	2,3,9,10,13,14	3 (30.9)
2.	1.150	2,3,4,9,10,13	13 (28.2)
3.	0.699	2,4,12,13,14	13 (58.2)
4.	1.483	2,4,9,10,13,14,15	13 (59.1)
5.	1.374	2,3,9,10,12,13	13 (46.0)
6.	0.836	1,2,3,4,12,13,14,15	3 (22.6)
	1.089 ± 0.305		

C – eggs

1.	0.674	2,4,13,14	13 (55.4)
2.	1.547	4,13,14,15	13 (40.2)
3.	1.264	1,2,3,4,13,14	13 (41.1)
4.	0.883	2,3,12,13,14,15	13 (33.6)
5.	0.882	2,3,4,13,14,15	13 (54.5)
6.	0.707	2,3,9,10,13,14	3 (48.5)
7.	0.671)	2,4,12,13	12 (38.5)
8.	0.603	1,2,3,12,13,14	12 (33.7)
9.	0.740	2,4,9,10,12,13	12 (25.8)
10.	1.321	1,2,3,12,13,14	13 (36.6)
11.	0.605	2,3,12,13	13 (51.3)
12.	1.282	2,4,12,13,14	12 (38.8)
	0.932 ± 0.330		

Table 5

Carotenoid composition and content in lavaret muscle and eggs from Geneva lake (20.12.1983)

A – male

No	Total content (µg/g fresh wt)	Carotenoid (see Table 1)	Major carotenoid (%)
1.	0.851	1,2,3,13,14,15	3 (42.8)
2.	0.738	3,9,12,13,14	12 (25.1)
3.	0.813	1,2,3,9,12,13,15	12 (32.9)
4.	1.166	1,2,3,9,12,13	2 (29.2)
5.	1.166	2,3,9,12,13,14	14 (26.5)
6.	0.538	1,2,3,12,13,14	2 (40.5)
	0.879 ± 0.247		

B – female

1.	0.545	2,3,4,9,12,13	2 (33.0)
2.	0.619	2,3,12,13	13 (69.3)
3.	0.507	2,3,9,10,12,13,14	3 (37.1)
4.	0.520	2,3,9,10,12,13	3 (25.2)
5.	0.521	2,3,9,12,13	13 (47.5)
6.	0.948	2,3,4,9,10,12,13,14	13 (40.9)
7.	1.633	1,2,3,13	13 (75.4)
8.	0.790	2,9,10,12,13,14	13 (59.1)
9.	0.563	2,3,9,10,12,13,15	12 (27.1)
10.	0.822	1,2,3,9,10,13	13 (49.1)
	0.747 ± 0.346		

C – eggs

1.	1.191	1,2,3,4,12,13,14	12 (28.9)
2.	1.607	2,3,12,13,14	13 (58.3)
3.	1.859	3,12,13	12 (48.8)
4.	1.134	2,3,9,10,12,13	3 (37.4)
5.	1.489	2,3,12,13	13 (60.8)
6.	0.891	3,12,13,14,15	12 (51.8)
7.	3.048	2,3,12,13	12 (64.6)
8.	1.339	3,8,9,10,12,13,14	12 (39.8)
	1.570 ± 0.209		

β-cryptoxanthin and astaxanthun, with zeaxanthin, canthaxanthin and astaxanthin present in roe samples. Carotenoids dominant in the males muscles were β-cryptoxanthin, zeaxanthin, cathaxanthin and astaxanthin were dominant carotenoids in females 'muscles. As for the roe, zeaxanthin dominated in 1 case, canthaxanthin and astaxanthin in 5 and 2 cases, respectively. Total carotenoids level in muscles of males from the Lac de Genève ranged from 0.538 to 1.666; an average being 0.879 μg/g. Total carotenoids in muscles of females ranged from 0.520 to 1.633 μg/g, with while in raw oscilated between 0.891 and 3.048, with an average amount equal to 1.570 μg/g of wet weight.

DISCUSSION

Fishes like all other animals do not form carotenoids "de novo", but intake them with feed and transform some of them into more oxygenated xanthophils (Isler 1971). Besides, some fish species, mainly from the salmonidae family, accumulate carotenoids are accumulated mostly in liver and intestines, while for the salmonidae fish species a great amount of carotenoids in accumulated also in muscles, which effects its colour (Kanemitsu and Aoe 1958). The carotenoids level in each part of fish body depends on feed and how rich in carotenoids it is (Choubert 1979, Simpson et al. 1981, Czeczuga and Dąbrowski 1983, Czeczuga and Kiziewicz 1985). A natural nutrition effects accumulation course of carotenoids in each body part of fish (Craik and Harvey 1986, Storebakken et al. 1986). That is why in the aquaculture of salmonidae fish fed artifical feed, mostly, carotenoids are added-canthaxanthin in particular- which restores accumulation abbility in particular body parts, muscles in particular. Colour of muscles resemble than muscles of the natural population (Deufel 1965, Schmidt and Baker 1969, Choubert and Luquet 1982, Choubert 1983).

The *Coregonus lavaretus,* feedling on benthos, feeds also on crustacian plancton. Surveys conducted on carotenoids in molluscs and crustacians proved carotenoits of the ketocarotenoids group (astaxanthin, canthaxanthin) and β carotene and its derivative β-craptoxanthin to dominate, with lutein epoxide present in smaller amounts (Green 1957, Herring 1968 a,b, Czeczuga 1977b, 1984). Because astaxanthin and canthaxanthin dominated in muscles and roe samples of many *Coregonus lavaretus* individuals tested it is possible for the carotenoids taken within the feed to accumulate in the fish body. Besides it can not be excluded for β-carotene and its derivative β-cryptoxanthin and also lutein to be transform, within the fish body, into astaxanthin, which was proved in the case of *Coregonus lavaretus.*

Muscles of males both from the Lac de Genève and the Bodeńskie Lake, accumulated more carotenoids than did the muscles of females from the same population. Simillar phenomenon was observed earlier for *Salmo trutta* L. (Czeczuga and Chełkowski 1984) and *Hucho hucho* L. individuals (Czeczuga et al. 1986). Prior to spawning, for

many fish species, shifting of carotenoids to gonads, fins and skin takes place (species which are characterised by nuptial coloring) (Steven 1949, Crozier 1970, Kitahara 1983, 1984, 1985, Czeczuga and Chełkowski 1984). In *Salmo trutta* individuals ketacarotenoids are main carotenoids to migrate from intestines, liver and, to a lesser extend, from muscles. It concerns *Salmo trutta* males, in particular, for which some carotenoids from muscles, during spawning period, move into skin. In females this phenomenon is less visible (Czeczuga and Chełkowski 1984). According to surveys of Ando (1988) carotenoids migration in *Oncorhynchus keta* individuals, during spawning migration, concerns complex compound-result of linkage between carotenoids and lipoproteins. This complex is being formed within an intestine wall where accumulated carotenoids make complexes with lipoproteins transfered there by plasma. Complexes formed are than transferred with blood to muscles, next from muscles into liver and finally to gonads and body covers. The most important role in creation of carotenoid-lipoprotein complexes play, among carotenoids, ketocarotenoids-mostly astaxanthin. It is well known for ketocarotenoids to dominate quite often among carotenoids, in salmonidae fish, (Glover et al. 1952, Thommen and Gloor 1965, Jarząbek 1970, Czeczuga 1976, 1979a, 1982, Schiedt et al. 1981) which is, also, true in the case of *Coregonus lavaretus.*

Comparison of total carotenoids amount in *Coregonus lavaretus* roe from the Alpin's Lakes proved the smallest concentration of carotenoids to be in roe samples collected at the beginning of November (Konstance-Untersee Lake) with the highest concentration noted for roe sampoles collected by the end of December (Lac de Genève). Above differences resulted from carotenoids migration phenomenon, due to which, the closer the spawning time the carotenoids increase in roe more visible.

When comparing carotenoids contents in muscles and roe of the *Coregonus lavaretus* two populations from the Bodensee, it becomes clear for the near-shore spawning population to accumulate more carotenoids than do the pelagic spawning population to accumulate more carotenoids than do the pelagic spawning population individuals. Nevertheless, reddish colour of muscles and moree intensive roe colour, in a near-shore spawning population individuals, is caused by higher concentration of carotenoids. It could be explained by the carotenoids role, ksanthophils in particular, in oxygen deposit when it is at a low level in water (Sharevich and Prokhodskaya 1977). Oxygen conditions within a near-shore area, in almost each lake, are ussually worse than in its pelagic, open part.

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BADANIA KAROTENOIDÓW U OSOBNIKÓW *COREGONUS LAVARETUS* RÓŻNYCH POPULACJI

STRESZCZENIE

Autorzy stosując chromotografię kolumnową i cienkowarstwową badali występowanie i zawartość poszczególnych karotenoidów w mięśniach osobników obu płci i w ikrze *Coregonus lavaretus* z trzech jezior alpejskich. Zwrócono także uwagę na karotenoidy u osobników dwóch populacji tego gatunku z jeziora Bodeńskiego różniących się miejscem rozmnażania się, kolorem mięśni i ikry.

W wyniku badań ustalono obecność następujących karotenoidów: β-karoten, β-kryptoxanthin, zeaxanthin, lutein (wraz z 3'epilutein), neothxanthin, tunaxanthin, echinenone, hydroxyechinenone, adonixanthin, α-doradexanthin, idoxanthin, canthaxanthin, astaxanthin, lutein epoxide oraz mutatoxanthin.

Podano również ogólną zawartość karotenoidów dla mięśni i ikry oraz stosunki procentowe poszczególnych karotenoidów. Osobniki populacji *Coregonus lavaretus* rozmnażające się w strefie przybrzeżnej jeziora Bodeńskiego okazały się zasobniejsze w karotenoidy w porównaniu do osobników populacji rozmnażającej się w otwartej części Jeziora Bodeńskiego.

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Received: 1990.06.08