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CAROTENOID CONTENT IN VARIOUS BODY PARTS OF ATLANTIC SALMON (*SALMO SALAR* L.) AND ARCTIC CHARR (*SALVELINUS ALPINUS* L.) SPECIMENS FROM AN OCEAN RANCHING FARM

BAZYLI CZECZUGA[1*], EWA CZECZUGA-SEMENIUK[1],
SOFFIA VALA TRYGGVADOTTIR[2]

[1]*Department of General Biology, Medical University*
Kilińskiego 1, 15-089 Białystok, Poland
e-mail: [*] *bazzylio@poczta.onet.pl*

[2]*Icelandic Fisheries Laboratories*
P.O. Box 1405, Skulagotu 4
121 Reykjavik, Iceland

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Abstract

Using column (CC), thin-layer (TLC), and high-performance liquid chromatography (HPLC), carotenoid content was examined in various body parts (fins, skin, muscles, liver, intestines, and gonads) of Atlantic salmon (*Salmo salar* L.) and Arctic charr (*Salvelinus alpinus* L.) specimens from an ocean ranching farm. Sixteen carotenoids were identified in the specimens examined - 11 in Atlantic salmon individuals and 15 in Arctic charr. In both the Atlantic salmon and Arctic charr individuals, ketocarotenoids were the predominant group, of which β-doradexanthin, canthaxanthin, and astaxanthin were the most numerous in Atlantic salmon and

phoenicoxanthin, canthaxanthin, and astaxanthin in Arctic charr. Total carotenoid content in all the body parts examined, apart from the gonads, was higher in the males of both species compared to the females. A comparison of the total carotenoid content in the muscles (with the skin) of both fish species with data obtained by other authors for these and other salmonid species indicates that, conversely to other breeding methods, breeding Atlantic salmon and Arctic charr in an ocean ranching farm does not significantly reduce carotenoid content in the muscles.

INTRODUCTION

Conditions for the reproduction and growth of many fish species, especially salmonids, have deteriorated considerably due to water pollution in aquatic reservoirs (Elliott 1994, Bagliniere and Maisse 1999). To meet the growing demand for fish meat (Halver 1989, Wilson 1991), numerous fish species are bred on a large-scale in so-called aquaculture, using pools, ponds and even ocean ranching farms (Stirling 1985, Penell and Barton 1996). This type of industrial breeding of many fish species is stressogenic and results in slower growth (Iwama *et al.* 1997), poorer meat quality (Wedemeyer 1997), and affects reproduction (Pankhurst and Van Der Kraak 1997). Moreover, in industrial breeding, artificial instead of natural feeds are applied, which significantly affects the quality and appearance of the meat (Czeczuga 1979b, Woźniak 2004). Since artificial feed contains small amounts of carotenoids, especially the red type (astaxanthin, canthaxanthin), the salmonid meat assumes a whitish color instead of its natural reddish tint, which reduces demand for it. Therefore, carotenoids are added to artificial feed thus increasing production costs considerably (Torrissen *et al.* 1989).

In this context, the authors decided to assess the effects of breeding in an ocean ranching farm, which most resembles natural conditions (Laird and Needham 1988), on the carotenoid content in various body parts of two salmonid fish species, namely the Atlantic salmon and the Arctic charr.

MATERIALS AND METHODS

The assays were performed on the fins, skin, muscles, liver, intestines, and gonads dissected from five males and five females of *Salmo salar* L. and *Salvelinus alpinus* L. (aged 2+) caught in 1998. These salmon were from an ocean ranching farm located in the Atlantic Ocean near the southwestern coastline of Iceland. The fish were taken just after they had migrated into fresh water. These fish had been released as smolts at 60-70 g and returned to the fish farm two years later.

The presence of the respective carotenoids in the particular body parts of the specimens assayed was identified by column and thin–layer chromatography

(CC and TLC, respectively) with different solvent systems (Czeczuga 1981) as well as high–performance liquid chromatography (HPLC).

Prior to chromatography, the material was homogenized and hydrolyzed for 24 hours in a 10% KOH solution in nitrogen, at room temperature. The extract was subsequently placed on an Al_2O_3-filled Quickfit Co. column. The individual fractions were eluted using various solvent systems. The eluent was evaporated, and the residue was dissolved in an appropriate solvent to draw the maximum of absorption. This was necessary, among other reasons, to identify a particular carotenoid. In addition to column chromatography, an acetone extract was divided into fractions with thin-layer chromatography. Silicon gel-covered glass plates (Merck Co.) and various solvent systems were used. The R_f values were established according to commonly accepted criteria (Kraus and Koch 1996). Column and thin-layer chromatography are described in detail in Czeczuga (1986).

Pigments were also determined by ion-pairing in reverse–phase HPLC. A 300 µl volume of ion-pairing reagent was added to 1000 µl of clear extract, according to Mantoura and Llewellyn (1983). The HPLC equipment consisted of a Shimadzu SCL-6B gradient programmer and a Rheodyne 7125 injector equipped with a 20 µl loop. Detection was achieved in a Shimadzu SPD – 6AV UV-VIS spectrophotometric detector set at 440 nm and a Shimadzu RF-535 fluorescence detector.

Table 1

List of the carotenoids from the investigated materials.

Carotenoid		Summary formula	Structure (see Fig. 1)	Semi-systematic name
1.	β-carotene	$C_{40}H_{56}$	a - r – a	β,β-Carotene
2.	β-cryptoxanthin	$C_{40}H_{56}O$	a - r – c	β,β-Caroten-3-ol
3.	neothxanthin	$C_{40}H_{56}O$	b -r - d	ε,ε-Caroten-3-ol
4.	lutein	$C_{40}H_{56}O_2$	c - r - d	β,ε-Carotene-3,3'-diol
5.	3'-epilutein	$C_{40}H_{56}O_2$	c - r - d	β,ε-Carotene-3,3'-diol (stereoizomeric)
6.	zeaxanthin	$C_{40}H_{56}O_2$	c - r - c	β,β-Carotene-3,3'-diol
7.	tunaxanthin	$C_{40}H_{56}O_2$	d - r - d	ε,ε-Carotene-3,3'-diol
8.	lutein epoxide	$C_{40}H_{56}O_3$	b - r - e	5,6-Epoxy-5,6-dihydro-β,ε-carotene-3,3'-diol
9.	antheraxanthin	$C_{40}H_{56}O_3$	c - r - e	5,6-Epoxy-5,6-dihydro-β,β-carotene-3,3'-diol
10.	deepoxyneoxanthin	$C_{40}H_{56}O_3$	c - r_1 - f	6,7'-Didehydro-5,6-dihydro-β,β-carotene-3,5,3'-triol
11.	idoxanthin	$C_{40}H_{54}O_4$	g - r - i	3,3',4'-Trihydroxy-β,β-caroten-4-one
12.	3'-hydroxyechinenone	$C_{40}H_{54}O_2$	c - r - h	3'-Hydroxy-β,β-caroten- 4-one
13.	β-doradexanthin	$C_{40}H_{54}O_3$	c - r - i	3,3'-Dihydroxy-β,β-caroten-4-one
14.	canthaxanthin	$C_{40}H_{52}O_2$	h - r - h	β,β-Carotene-4,4'-dione
15.	phoenicoxanthin	$C_{40}H_{52}O_3$	g - r - h	3-Hydroxy-β,β-caroten- 4,4'-dione
16	astaxanthin	$C_{40}H_{52}O_4$	i - r - i	3,3'-Dihydroxy-β,β-carotene-4,4'-dione

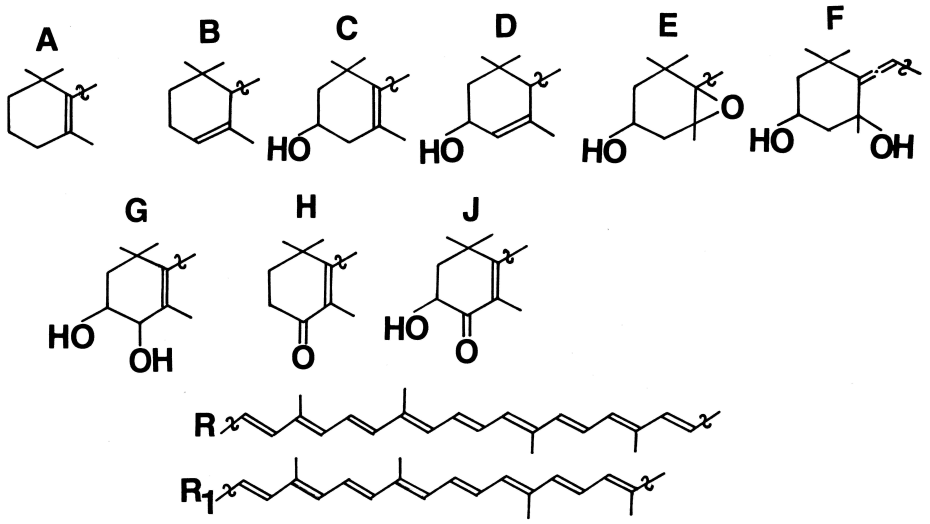

Fig. 1. Structural features of carotenoids from the investigated materials (see Table 1).

Carotenoids were identified by comparisons with standards from: a) the behavior in column chromatography; b) their UV-VIS spectra; c) their partition between n-hexane and 95% ethanol; d) their R_f-values in thin-layer chromatography; e) the presence of the allylic OH group determined by the acid $CHCl_3$ test; f) the epoxide test; g) the mass spectrum (see Vetter *et al.* 1971).

Carotenoid pigment standards were purchased from the Hoffman-La Roche Company, Switzerland; the International Agency for ^{14}C Determinations, Denmark, and Sigma Chemical Company, USA.

Quantitative analyses were performed with UV-VIS spectroscopy according to the Davies method (Czeczuga 1981). The structure of carotenoids was described by Straub (1987) and Czeczuga (1988).

RESULTS

Sixteen carotenoids were identified in the specimens examined (Table 1, Fig.1) - 11 in the Atlantic salmon individuals and 15 in the Arctic charr. Although 3'-epilutein, antheraxanthin, idoxanthin, 3'-hydroxyechinenone and phoenicoxanthin were not detected in Atlantic salmon, they were present in Arctic charr. β-doradexanthin was found only in Atlantic salmon specimens. In both the Atlantic salmon and Arctic charr individuals, ketocarotenoids were the predominant group (idoxanthin, 3'-hydroxyechinenone, β-doradexanthin, phoenicoxanthin, canthaxanthin, and astaxanthin), of which β-doradexanthin,

canthaxanthin, and astaxanthin were the most numerous in Atlantic salmon and phoenicoxanthin, canthaxanthin, and astaxanthin in Arctic charr (Tables 2, 3).

Table 2

Carotenoid content in *Salmo salar* L. (in %) (F-female, M-male)

Carotenoid	Fins		Skin		Muscles		Liver		Intestine		Gonads	
	F	M	F	M	F	M	F	M	F	M	F	M
β-carotene	1.4				17.3	13.0	13.9				15.5	5.0
β-cryptoxanthin	16.8			20.6		11.3		10.5				8.5
neothxanthin			4.5	3.2		3.7						
tunaxanthin	18.8			9.5	1.1	5.5			17.6	4.7	3.9	10.7
zeaxanthin							10.0	10.5			6.2	
lutein epoxide	5.3		4.7		2.0				3.6		4.4	
canthaxanthin	12.0	26.6	25.4	6.7	30.1	27.8	22.6	46.3	59.2	55.8	2.0	17.9
β-doradexanthin	16.1	26.5	24.7	23.0		13.5	15.3	9.4		21.5		28.1
astaxanthin	29.4	45.5	37.0	37.0	49.5	24.2	37.2	23.3	18.5	11.6	66.9	12.6
deepoxyneoxanthin	0.2	1.4	1.7			1.0	1.0		1.1	6.4	1.1	2.2
Total ketocarotenoids content	57.5	98.6	87.1	66.7	79.6	65.5	75.1	79.0	77.7	88.9	68.9	58.6
Total content in μg g^{-1} fresh wt	2.944	3.826	2.780	4.581	3.507	3.610	4.584	4.682	5.598	7.464	5.372	0.135

Table 3

Carotenoid content in *Salvelinus alpinus* L. (in %) (F - female, M - male)

Carotenoid	Fins		Skin		Muscles		Liver		Intestine		Gonads	
	F	M	F	M	F	M	F	M	F	M	F	M
β-carotene	0.2	5.1	8.1	3.7	2.7	4.5	0.5	2.9	4.6	5.1	5.4	2.8
β-cryptoxanthin	13.4	11.9	5.7	8.1	10.1	21.0	7.1	7.2	0.2	6.7	12.4	7.6
neothxanthin			20.3		6.7	7.2				14.6		6.3
lutein					5.2						3.1	
3'-epilutein					4.6						1.8	
zeaxanthin							13.1	14.7		11.3		
tunaxanthin	6.9			14.2	14.2	7.4	23.8		20.5			7.2
lutein epoxide	11.8				5.0	11.5			4.6		4.0	12.1
antheraxanthin											15.9	
deepoxyneoxanthin			14.2		17.9				3.4	11.0	6.1	
idoxanthin									5.3		6.2	
3'-hydroxyechinenone							6.1		10.3			
canthaxanthin	52.3	17.4	27.6	27.4	8.3	19.7	9.3	20.8	20.7	23.1	31.5	17.0
phoenicoxanthin		21.9	10.4	4.5		10.2	9.0	7.0	20.9	12.4		13.9
astaxanthin	15.4	43.7	13.7	42.1	25.3	18.5	31.1	47.4	9.5	15.8	13.6	33.1
Total ketocarotenoids content	67.7	83.0	51.7	74.0	33.6	48.4	55.5	75.2	66.7	51.3	51.3	64.0
Total content in μg g^{-1} fresh wt	5.612	6.732	6.724	7.874	3.225	4.642	7.836	9.436	6.218	8.125	7.326	0.114

The total carotenoid content in all the body parts examined, apart from the gonads, was higher in the males of both species compared to the females. Both male and female specimens of Atlantic salmon had the most carotenoids in the intestines, while Arctic charr had the most in the liver. The gonads of the males of both species were the least abundant in carotenoids as were the skin of Atlantic salmon females and the muscle of Arctic charr females.

DISCUSSION

The carotenoids detected in the present study material have been noted previously in these and other salmonid fish species (Jarząbek 1970, Czeczuga 1979a, Czeczuga and Chełkowski 1984, Choubert 1986, Czeczuga and Bartel 1989, Czeczuga *et al.* 2002, Torrissen *et al.* 1989). In the literature on this subject, alloxanthin or cyanthiaxanthin, a zeaxanthin derivative, is also reported to occur in salmonid species. This carotenoid was noted in the Pacific salmon species of the genus *Oncorhynchus* (Kitahara 1983). In the present study, all the examined body parts of both species showed a predominance of ketocarotenoids, including canthaxanthin and particularly astaxanthin. A similar pattern was observed by other researchers in both Atlantic salmon specimens (Jarząbek 1970, Torrissen *et al.* 1989, Czeczuga *et al.* 2002) and Arctic charr individuals (Christiansen and Wallace 1988, Scalia *et al.* 1989). The astaxanthin that occurs naturally in salmon of different origin consists of a mixture of three configurational isomers: 78 to 85% (3S,3'S)-astaxanthin, 12 to 17% (3R, 3'R)-astaxanthin, and 2 to 6% of the optically inactive meso-astaxanthin (Schiedt *et al.* 1981). The co-occurrence of the mixture of all three configurational isomers has also been reported in crustaceans (Rønneberg *et al.* 1980, Renstrøm *et al.* 1981).

The predominance of ketocarotenoids in the specimens of the salmonid species examined is associated with the intake of food rich in ketocarotenoids, especially astaxanthin. After reaching the ocean, *Salmo salar* specimens feed on crustaceans and insects from the water surface and on small fish. They also consume sprat and herring, Euphausiidae, and pelagic Amphipoda (Jacobsen and Hansen 1996). Crustaceans constitute over 80% of the food they consume. *Salvelinus alpinus* is also a predacious fish, and in natural conditions adolescent specimens eat insect larvae, insects, and crustaceans, while adults also feed on fish. Like the Atlantic salmon individuals, there was a predominance of crustaceans in the food consumed by Arctic charr (Grainger 1953, Johnson 1980, Behnke 1984). Ketocarotenoids, especially astaxanthin, are predominant in both the crustaceans belonging to Euphausiidae, Amphipoda, and other taxons (Czeczuga 1975a, 1984).

The carotenoid content varies in the respective fish body parts; this is especially evident prior to reproduction. In females, carotenoids shift from the liver mainly to the gonads (eggs), while in the males the shift is to the skin and fins and is known as the nuptial cover. This spawning behavior is particularly significant in Arctic charr males (Fabricius 1953, Fabricius and Gustafson 1954). In this species, carotenoids play a role in sexual signals (Skarstein and Folstad 1996, Olson and Owens 1998).

In comparison to the other parts of the body, the smallest amounts of astaxanthin were noted in the intestines of both the Atlantic salmon and Arctic charr specimens of each sex. According to March *et al.* (1990) and Choubert *et al.* (1994), that the smallest amounts of astaxanthin were noted in the salmonid intestines suggests it is absorbed rapidly in the intestines and shifted to other body parts. The absorption of carotenoids occurs along the length of the intestine and this is why it is so rapid (Hardy *et al.* 1990). The astaxanthin that reaches the muscles shows a higher affinity to actiomyosin than canthaxanthin and forms a complex in the salmon muscle (Henmi *et al.* 1989). Part of the astaxanthin reaches the cell plasma (March *et al.* 1990), giving these cells a reddish color. The remaining astaxanthin is reduced (Ando and Hatano 1987) and transformed into vitamin A (Schiedt *et al.* 1985, Guillon *et al.* 1989).

Table 4

Carotenoids levels reported in wild salmonids

	Species	Carotenoids $\mu g\ g^{-1}$	Ref.
1.	Atlantic salmon (*Salmo salar*)	3.1 – 4.1*	this paper
2.	Atlantic salmon (*S. salar*)	3.0 – 11.0	Satio 1969, Schiedt *et al.* 1981, Skrede & Storebakken 1986
3.	Brown trout (*S. trutta* m. *fario*)	1.4 – 8.6	Czeczuga 1979b
4.	Lake trout (*S. trutta* m. *lacustris*)	0.7 – 1.3	Czeczuga & Bartel 1989
5.	Sea trout (*S. trutta* m. *trutta*)	1.5 – 9.7	Czeczuga & Chełkowski 1984
6.	Danube salmon (*Hucho hucho*)	0.8 – 1.7	Czeczuga *et al*. 1986
7.	Arctic charr (*Salvelinus alpinus*)	5.0 – 6.3	this paper
8.	Arctic charr (*S. alpinus*)	1.1 – 2.2	Christiansen & Wallace 1988
9.	Arctic charr (*S. alpinus*)	8.6	Scalia *et al.* 1989
10.	Brook trout (*S. fontinalis*)	0.9 – 1.9	Czeczuga 1975b
11.	Chinook salmon (*Oncorhynchus tschawytscha*)	8.0 – 9.0	Kanemitsu & Aoe 1958
12.	Chum salmon (*O. keta*)	3.0 – 8.0	Kanemitsu & Aoe 1958, Schiedt *et al.* 1981
13.	Coho salmon (*O. kisutch*)	9.0 – 21.0	Kanemitsu & Aoe 1958, Schiedt *et al*. 1981
14.	Pink salmon (*O. gorbuscha*)	4.0 – 6.0	Kanemitsu & Aoe 1958
15.	Rainbow trout (*O. mykiss*)	1.0 – 3.0	Czeczuga 1975b, Schiedt *et al*. 1986
16.	Sockeye salmon (*O. nerka*)	26.0 – 37.0	Kanemitsu & Aoe 1958, Schiedt *et al.* 1981

* mean for muscles from skin

The comparison of total carotenoid content in the muscles (including skin) of both fish species with data obtained by other authors (Table 4) for these and other salmonid species indicates that breeding Atlantic salmon and Arctic charr in an ocean ranching farm does not significantly reduce the carotenoid content in the muscles, conversely to other breeding methods (Torrissen *et al.* 1989, 1990). However, it should be remembered that the total carotenoid content in the muscles and the amount of red carotenoids (astaxanthin, canthaxanthin) in the respective body parts of fish, including salmonids, depends on the type of food it consumes. The total carotenoid content is also decreased by parasites (Czeczuga 1980). Water polluted with organochlorine substances causes the so-called M74 syndrome in salmon (Vuorinen *et al*. 1997); the eggs of afflicted females are pale yellow as they have a low red carotenoid content, especially of astaxanthin (Pettersson and Lignell 1999, Czeczuga *et al.* 2002). Most of the larvae that develop from such eggs die when they begin active feeding. M74 syndrome occurs not only in Baltic salmon but has also recently been noted in the sea trout *Salmo trutta* m. *trutta* (Czeczuga *et al.* 2002).

The effect of chemical water pollution on the larval forms of other salmonid species is known in other latitudes. The high mortality of early life-stage salmonids including Pacific salmon from some of the Great Lakes of North America has been reported under the name of Early Mortality Syndrome (EMS) (McDonald 1995). Clinical symptoms similar to those noted in M74 in salmon from the Baltic Sea or in EMS in other salmonid species from the North American Great Lakes were observed in Atlantic salmon specimens with Cayuga syndrome in the New York Finger Lakes (Fisher *et al.* 1996). All three of these disorders responsible for mortality in early life-stage salmonids are characterized by a low level of astaxanthin and thiamine in eggs (Fitzsimons *et al.* 1999).

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