A C T A I C H T H Y O L O G I C A E T P I S C A T O R I A

Abbrev.: Acta Ichthyol. Piscat. 32 (1): 3–21, Ann. 2002

Bazyli *CZECZUGA* [1], Ryszard *BARTEL* [2], Ewa *CZECZUGA-SEMENIUK* [1]

Carotenoids in fish

CAROTENOID CONTENT IN EGGS OF ATLANTIC SALMON (*SALMO SALAR* L.) AND BROWN TROUT (*SALMO TRUTTA* L.) ENTERING POLISH RIVERS FOR SPAWNING OR REARED IN FRESH WATER

ZAWARTOŚĆ KAROTENOIDÓW W IKRZE ŁOSOSI (*SALMO SALAR* L.) I TROCI (*SALMO TRUTTA* L.) WCHODZĄCYCH NA TARŁO DO RZEK POLSKI I HODOWANYCH W WODZIE SŁODKIEJ

[1] Department of General Biology, Medical University in Białystok, Poland
[2] River Fishery Department, Institute of Inland Fisheries, Gdańsk, Poland

The authors investigated the carotenoids content in yellow, yellow-orange, and orange eggs of salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) entering Polish rivers for spawning or reared in fresh water. The study shows the presence of females of both salmon and brown trout with the M74 syndrome.

INTRODUCTION

Rapid advances in industry and agricultural chemicalisation in the second half of the 20th century brought about negative effects in a number of trophic links. The first symptoms in humans, being the terminal trophic link, appeared in the form of itai-itai disease caused by excessive cadmium concentration and as minamata disease induced by high lead concentration. Chemical pollution of water had also an effect on lower links of trophic chains, including fish. In 1974 in Sweden a disease called M74 syndrome was officially registered in the hatch of "Baltic" salmon caught in the river Mörrum. The name of the disease comes from a Swedish word Miljö meaning the environment and the year the disease was observed for the first time (Bengtsson et al. 1994). According to the authors, its characteristic feature in the hatch of salmon is:

- Lack of normal motoric activity, apathy and only weak avoidance reactions. There is little swimming movement, but erratic rushes may lead to exhaustion.
- Greyish colour due to discoloration of the skin.

- Ruptures of blood vessels in the form of hemorrhages close to the heart and white precipitates in the yolk sac next to the fat droplet.
- Swollen yolk sac and exophthalmia ("pop-eye").
- Yolk sac fry may show an elevation in the activity of 7-ethoxyresorufin-0-deethylase (EROD), which is dependent on the liver enzyme cytochrome P450.
- Livers of fry suffering from M74 have more vacuoles and a lower glycogen content.

High mortality was noted when the hatch started to feed actively. In the subsequent years, this phenomenon became intensified and was observed in many rivers of Sweden and Finland (Soivio 1996). Hatch mortality in some rivers reached as much as 96% (Karlström 1999). Later observations have revealed that the intensely coloured dark-orange eggs were only slightly affected and the loss of colour was in direct proportion to the mortality of hatch. It is assumed that the disease is a result of pollution of the aquatic environment of the Baltic Sea (Wulff et al. 2001).

The colour of the salmon eggs depends on ketocarotenoid content, mainly astaxanthin and canthaxanthin (Czeczuga 1975, 1979; Craik 1985). Based on the content of these carotenoids in the eggs, attempts have been made to diagnose the M74 syndrome in salmon (Lingell 1994; Pettersson and Lingell 1999).

Therefore, we decided to investigate whether carotenoid content in the eggs of salmon and brown trout females, entering Polish rivers for spawning or reared in fresh water, triggers symptoms of the M74 syndrome in the hatching and if so, to what extent it affects the populations of salmon and brown trout.

MATERIAL AND METHODS

The eggs for analyses were collected at the end of October and in the first half of November 1998 from 74 females Atlantic salmon, *Salmo salar* L. and 14 females of brown trout, *Salmo trutta* L. caught during their spawning migration in Darłowo on the Wieprza River and in Świbno on the Vistula River, and from those bred in fresh water in hatcheries at Miastko and Rutki. The eggs collected were divided according to their colour into three groups: yellow, yellow-orange, and orange using a four-stage scale developed in the Swedish Salmon Research Institute SR-814 94 Alvkarleby, Sweden (Börjeson and Norrgren 1997). No dark-orange eggs were found in the material, which was the case in the years 1996 and 1997 (Bartel 1996 and unpublished data).

Following a one week storage, the refrigerated material (–4°C) was sent to the laboratory where it was analysed a week later.

The carotenoid pigments were isolated using column and thin-layer chromatography. Prior to chromatography, the material was homogenized and then subjected to hydrolysis in a 10% KOH solution in nitrogen atmosphere and at room temperature for 24 hours. Then,

the extract was placed on the column of Quickfit Co. filled with Al_2O_3. The individual fractions were eluted using various solvent systems (Czeczuga 1993). The eluent was evaporated, and the remainder was dissolved in a suitable solvent to draw the maximum of absorption, necessary, among other things, to identify a particular carotenoid (Fig. 1).

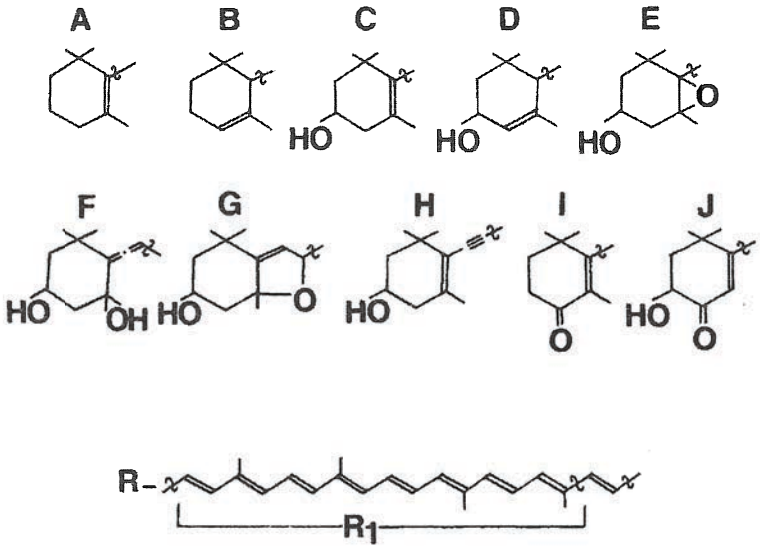

Fig. 1. Structural features of carotenoids from investigated materials

In addition to column chromatography, the acetone extract was divided into fractions by means of thin-layer chromatography. Silicon gel-covered glass plates (Merck Co.) and various solvent systems (Czeczuga 1988) were used. The R_f values were established according to commonly accepted criteria.

Carotenoids were identified based on the absorption maximum in different solvents, R_f values according to the standards of F. Hoffman-La Roche Co., Basel and Sigma Chemical Co. USA, and the obtained ratios of epiphase to hypophase. In order to distinguish tunaxanthin from lutein and canthaxanthin from astaxanthin, spectral analysis was used to determine the presence of hydroxyl and epoxide groups at the iononic rings (end group) of these carotenoids (Wetter et al. 1971). The absorption maxima were determined with a spectrophotometer Spektromom-203 and Specol.

Quantitative ratios of the respective carotenoids were estimated according to the Davies method (Czeczuga 1988), while the structure of carotenoids was estimated according to Straub (1987).

RESULTS

The eggs of salmon and brown trout examined contained 15 and 14 carotenoids, respectively (Table 1). Mutatoxanthin was found only in the eggs of salmon females. The total carotenoid content in the eggs of salmon ranged from 3.978 to 15.583 $\mu g \cdot g^{-1}$ of fresh eggs (Darłowo) (Table 2). In brown trout, the total carotenoid content varied between 3.762 and 10.473 $\mu g \cdot g^{-1}$ (Świbno) (Table 3). The total carotenoid content in the eggs of salmon females reared in fresh water was lower than in females in the Baltic water. No such differences were observed in brown trout. The mean content of astaxanthin and canthaxanthin (red carotenoids) in the yellow eggs ranged from 12.23 (Miastko) to 14.89% (Świbno) of all carotenoids. In the yellow-orange eggs, the mean content of these carotenoids ranged from 24.65 (Świbno) to 32.15% (Miastko), while in the orange eggs—from 37.89 (Świbno) to 56.64% (Miastko). The mean values of astaxanthin and canthaxanthin in the yellow eggs of brown trout were 13.90–4.52%, in the yellow-orange eggs they amounted to 25.40–34.44%, while in the orange eggs the values were 41.63–54.51% of all carotenoids.

The content of lutein and zeaxanthin (yellow carotenoids) in the eggs of salmon females ranged from 14.22 (orange eggs, Miastko) to 51.99% of all carotenoids (yellow eggs, Darłowo). The highest percentage of these carotenoids were found in the yellow eggs, the lowest in the orange eggs. A similar tendency was observed in the sea trout eggs examined. The content of these two carotenoids ranged from 20.24 (orange eggs, Świbno) to 46.31% of all carotenoids (yellow eggs, Świbno).

DISCUSSION

As demonstrated in the present study, the colour of eggs in salmon and brown trout did not depend on the total quantity of carotenoids but on their reciprocal proportions which particularly refers to the presence of astaxanthin and canthaxanthin on the one hand and lutein and zeaxanthin on the other. When astaxanthin and canthaxanthin are predominant, the eggs are dark orange, while if lutein and zeaxanthin prevail, the eggs are yellow. According to Craik (1985), the critical level of carotenoids (astaxanthin) for eggs development is 1–3 $\mu g \cdot g^{-1}$ per egg, depending on the salmonid species; higher values guarantee healthy hatch in hatcheries. Pettersson and Lignell (1999), however, suggest that if the level of astaxanthin in the eggs of *Salmo salar* females is below 2.22 $\mu g \cdot g^{-1}$, symptoms of M74 will develop. In our study, the yellow eggs of both salmon and brown trout had astaxanthin and canthaxanthin below 2.22 μg; in the yellow-orange eggs this carotenoid content was higher, compared to the threshold level, only in 3 females. In the orange eggs, this carotenoid content exceeded the border value for the M74 syndrome in 15 salmon and 3 brown trout females (Table 4).

Table 1

Carotenoid list from the investigated material

No.	Carotenoid		Structure (see Fig. 1)	Semisystematic 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
6	Tunaxanthin	$C_{40}H_{56}O_2$	D - R - D	ε,ε-carotene-3,3'-diol
7	Zeaxanthin	$C_{40}H_{56}O_2$	C - R - C	β,β-carotene-3,3'-diol
8	Antheraxanthin	$C_{40}H_{56}O_3$	C - R - E	5,6-epoxy-5,6-dihydro-β,β-carotene-3,3'-diol
9	Deepoxyneoxanthin	$C_{40}H_{56}O_3$	C - R - F	6,7-didehydro-5,6-dihydro-β,β-carotene-3,3'-diol
10	Mutatoxanthin	$C_{40}H_{56}O_3$	C - R_1 - G	5,8-epoxy-5,8-dihydro-β,β-carotene-3,3'-diol
11	Diatoxanthin	$C_{40}H_{54}O_2$	C - R_1 - H	7,8-didehydro -β,β-carotene-3,3'-diol
12	3'-Hydroxyechinenone	$C_{40}H_{54}O_2$	C- R - I	3-hydroxy-β,β-carotene-4-one
13	Adonixanthin	$C_{40}H_{54}O_3$	C - R - J	3,3'-dihydroxy-β,β-caroten-4-one
14	Canthaxanthin	$C_{40}H_{52}O_2$	I - R - I	β,β-carotene-4,4'-dione
15	Astaxanthin	$C_{40}H_{52}O_4$	I - R - J	3,3'-dihydroxy-β,β-carotene-4,4'-dione

Table 2

Carotenoid content in eggs of diffrent colour of *Salmo salar* L.

Specification	Colour of eggs		
	yellow	yellow-orange	orange
Darłowo, 30 Oct–13 Nov 1998			
Number of females	20	17	12
Carotenoid (see Table 1)	1–9, 11–15	1–15	1, 2, 4, 5, 7–9, 11–15
Total content in $\mu g \cdot g^{-1}$	8.179 (4.069–14.563)	7.284 (3.978–15.583)	8.327 (4.581–13.479)
Astaxanthin and canthaxanthin (A) in $\mu g \cdot g^{-1}$	0.993 (0.382–1.685)	1.971 (1.223–4.295)	3.629 (1.923–5.974)
in %	12.71 (3.44–18.05)	27.10 (20.17–33.63)	43.91 (37.71–58.18)
Lutein and zeaxanthin (B) in $\mu g \cdot g^{-1}$	4.276 (1.591–10.092)	3.469 (1.161–8.033)	2.650 (1.444–4.939)
in %	51.99 (14.83–77.61)	46.34 (26.83–61.84)	32.04 (18.54–38.99)
Ratio A/B	0.23	0.57	1.37
Miastko, 30 Oct 1998			
Number of females	2	4	2
Carotenoid (see Table 1)	1–9, 15	1–9, 12, 14, 15	1–6, 9, 12, 14, 15
Total content in $\mu g \cdot g^{-1}$	4.689 (4.392–4.985)	5.284 (4.631–6.054)	5.146 (4.733–5.559)
Astaxanthin and canthaxanthin (A) in $\mu g \cdot g^{-1}$	0.573 (0.545–0.600)	1.462 (1.236–1.810)	2.930 (2.503–3.357)
in %	12.23 (12.03–12.42)	32.15 (20.41–34.10)	56.64 (52.89–60.39)
Lutein and zeaxanthin (B) in $\mu g \cdot g^{-1}$	2.334 (2.281–2.387)	2.042 (1.587–2.703)	0.745 (0.518–0.972)
in %	49.92 (47.89–51.94)	38.71 (30.79–44.64)	14.22 (10.95–17.49)
Ratio A/B	0.24	0.72	3.93
Świbno, 27 Oct 1998			
Number of females	8	7	2
Carotenoid (see Table 1)	1–11, 13–15	1–9, 11, 12, 14, 15	1–5, 7, 8, 10–12, 14, 15
Total content in $\mu g \cdot g^{-1}$	5.060 (4.384–5.820)	5.641 (4.476–6.202)	5.822 (5.805–5.839)
Astaxanthin and canthaxanthin (A) in $\mu g \cdot g^{-1}$	0.760 (0.546–1.019)	1.379 (1.082–1.652)	2.206 (2.154–2.257)
in %	14.89 (12.55–17.50)	24.65 (19.98–32.09)	37.89 (37.11–38.66)
Lutein and zeaxanthin (B) in $\mu g \cdot g^{-1}$	2.230 (1.701–2.662)	2.411 (1.579–3.193)	1.476 (1.012–1.940)
in %	44.26 (36.29–53.48)	42.14 (32.38–50.32)	25.35 (17.43–33.22)
Ratio A/B	0.34	0.57	1.49

Table 3

Carotenoid content in eggs of diffrent colour of *Salmo trutta* L.

Specification	Colour of eggs		
	yellow	yellow-orange	orange
Rutki, 13 Nov 1998			
Number of females	2	5	
Carotenoid (see Table 1)	1, 2, 4–9, 11, 12, 14, 15	1–9, 11, 12, 14, 15	
Total content in $\mu g \cdot g^{-1}$	5.594 (5.292–5.895)	5.809 (4.097–7.042)	
Astaxanthin and canthaxanthin (A) in $\mu g \cdot g^{-1}$	0.772 (0.707–0.837)	1.458 (1.123–1.800)	
in %	13.90 (12.00–15.80)	25.40 (19.62–28.38)	
Lutein and zeaxanthin (B) in $\mu g \cdot g^{-1}$	2.383 (2.010–3.156)	2.770 (0.949–4.546)	
in %	45.76 (37.99–53.53)	45.53 (23.17–64.56)	
Ratio A/B	0.32	0.52	
Świbno, 26 Nov 1998			
Number of females	2	4	2
Carotenoid (see Table 1)	1–4, 6–9, 13, 15	1–4, 6–9, 12, 14, 15	1, 2, 4, 6–9, 11, 13–15
Total content in $\mu g \cdot g^{-1}$	5.228 (3.392–6.693)	4.829 (4.566–4.987)	7.369 (4.265–10.473)
Astaxanthin and canthaxanthin (A) in $\mu g \cdot g^{-1}$	0.754 (0.558–0.950)	1.658 (1.295–1.964)	3.342 (2.325–4.359)
in %	14.52 (14.20–14.83)	34.44 (25.97–39.79)	48.07 (41.63–54.51)
Lutein and zeaxanthin (B) in $\mu g \cdot g^{-1}$	2.229 (2.223–2.235)	2.106 (1.821–2.657)	1.405 (0.988–1.821)
in %	46.31 (33.21–59.41)	43.47 (32.27–53.27	20.24 (17.39–23.17)
Ratio A/B	0.34	0.78	2.38

Table 4

Number of females from love and above of difference limen (2.22 $\mu g \cdot g^{-1}$ eggs) astaxanthin content in eggs

Hatchery	Colour of eggs					
	yellow		yellow-orange		orange	
	love	above	love	above	love	above
Salmo salar L.						
Darłowo	20	—	14	3	1	11
Miastko	2	—	4	—	—	2
Świbno	8	—	7	—	—	2
Salmo trutta L.						
Rutki	2	—	4	1		
Świbno	2	—	3	—	—	2

As reported by Börjeson and Norrgren (1997), among salmon females coming to the Swedish rivers in 1992/93 for spawning, only those with dark orange eggs were all healthy. Of 348 *Salmo salar* females examined approximately 65% of those with orange eggs, 42% with yellow-orange and only 15% with yellow eggs were healthy. The authors underline the fact that compared to 1976/1977 the incidence of M74 syndrome among salmon coming for spawning to the rivers of Sweden has increased rapidly.

In 1997 in Polish hatcheries during incubation of salmon eggs mean losses reached 30%; considerable losses of hatched larvae were also observed, especially during yolk-sac resorption. Carotenoids assimilated from food consumed by fish are accumulated in the respective organs, but mainly in the liver (Czeczuga et al. 2000). This refers to lampreys (Czeczuga and Bartel 2000) and to fish, both freshwater and marine despite of taxonomic status. Although carotenoids are stored in the liver, the level of oxygenated carotenoids is low there (Hata and Hata 1973, 1975; Sivtseva 1982). It is thus believed that the main metabolism of carotenoids in salmonids occurs in the skin and in sexually maturing salmon also in flesh (Hata and Hata 1975, Kitahara 1983, Schiedt et al. 1985, Ando 1986). In the digestive tract of salmonids astaxanthin and canthaxanthin are partly degraded (Foss et al. 1987, Storebakken et al. 1987), while at the time of anadromous migrating of *Oncorhynchus keta* reductive metabolism takes place of astaxanthin through adonixanthin to zeaxanthin, and of canthaxanthin through echinenone to β-carotene (Kitahara 1983, Schiedt et al. 1985, Ando 1986). Systematic studies of the carotenoid content in the respective body parts of a number of fish species have revealed a carotenoid shift in the organism throughout the year, the changes differing between males and females. This usually occurs before and after the spawning time. Data have been reported for Pacific salmon of the genus *Oncorhynchus*. Crozier (1970) was the first to focus on this problem while studying carotenoids in *Oncorhynchus nerka* specimens. The shift was confirmed in *Oncorhynchus keta* (cf. Kitahara 1983, Ando 1986) and *Oncorhynchus masou* specimens (Kitahara 1985). In *Salmo trutta* specimens changes in carotenoid in the respective body parts of both sexes have been examined by Czeczuga and Chelkowski (1984) and Czeczuga and Bartel (1989). In the species of the genus *Salmo* carotenoids accumulate in the muscles, while in the genera *Oncorhynchus* and *Salvelinus* also in the skin. Carotenoid shift refers not only to the salmonid representatives as it was previously assumed, but has a wider range—from lampreys (Czeczuga and Bartel 2000) to eels (Czeczuga and Czeczuga-Semeniuk 2000a). Carotenoid shift prior to the spawning time has been observed in *Pelecus cultratus*, a cyprinid fish (Czeczuga and Bartel 1998) and in two representatives of freshwater species—burbot, *Lota lota* and pike, *Esox lucius* (Czeczuga and Czeczuga-Semeniuk 1999a, 2000b), and other species of fish (Czeczuga and Czeczuga-Semeniuk 1999b). In females, carotenoids shift

mainly to the gonads, in males to the fins and skin, taking part in the so called nuptial plumage change.

It takes a few or several hours, depending on the salmonid species, for astaxanthin or canthaxanthin absorbed from food to get to the blood (Choubert et al. 1987). In the plasma it is bound to a high-density lipoprotein and very high density lipoprotein produced in the liver and in this form is transported to the skin and muscles (Nakamura et al. 1985). Part of astaxanthin binds with vitellogenin, egg yolk protein precursor which takes part in astaxanthin transport from the muscles to the ovaries of maturing females (Ando et al. 1986). In the ovary carotenoids combine with lipovitellin (Schiedt et al. 1985; Ando 1986).

It is now believed that pollution of the Baltic waters by certain organochlorines (mainly polychlorinated debenzofurans and coplanar PCBs) and accumulation of these substances in the salmon organism cause a decrease in thiamine and in red-colour carotenoids such as astaxanthin and canthaxanthin (Lignell 1994; Pettersson and Lignell 1996, 1998, 1999; Vuorinen et al. 1997; Pickova et al. 1998). The eggs obtained from a female suffering from M74 syndrome are yellow and not orange or dark orange like in healthy females, because it contains a small amount of astaxanthin and canthaxanthin and a greater amount of yellow carotenoids such as lutein and zeaxanthin. Most of the fish fry hatched from such eggs die (Norrgren et al. 1994). Fish fry with M74 syndrome show neurological symptoms, discoloration of the skin, and exopthalmia (pop-eye). Morphological and physiological changes also include blood vessel lesions, precipitates in the yolk sac and disturbed liver function (Börjeson et al. 1994; Lundström et al. 1996, 1999a). Mortality can be maximally reduced by bathing such eggs and newly hatched fry in thiamine (Bengtsson and Hill 1966; Amcoff et al. 1998). While at the sea, salmons accumulate carotenoids from food in the respective body parts, mainly in the muscles and liver, which during spawning migration to the river shift to the gonads.

Carotenoid content in general and carotenoid composition, including the amount of astaxanthin and canthaxanthin in the organism of females which begin their spawning migration depends on the kind of food consumed before the migration (Satio 1969; Schiedt et al. 1981; Craik and Harvey 1986; Skrede and Storebakken 1986; Storebakken et al. 1986; Torrissen and Naevdal 1988). This also refers to the carotenoid content in general and the level of astaxanthin and canthaxanthin in particular in the eggs of salmonids (Czeczuga 1975, 1979; Czeczuga et al. 1991; Torrissen 1984; Torrissen and Torrissen 1985). Examinations of stomach contents of specimens caught north of Faroe Islands have revealed that over 80% of food consumed by salmon consists of crustaceans mainly of the genus *Themisto* as well as euphausids and shrimps. Fish, including herring and mackerel constitute less than 20% (Jacobsen and Hansen 1996; Karlsson et al. 1999). The herring, *Clupea harengus* and the sprat, *Sprattus sprattus* are the main food species of salmon in the Baltic Sea

(nearly 90%) (Ikonen 1996). In the southern part of the Baltic Sea sprat predominate in food (Soivio and Hartikainen 1999). The eggs and muscles of females from the region of Faroe Island are dark orange. Females from the Baltic Sea have pale colour of the flesh and less pigmented eggs (Chełkowski 1965; Chełkowska 1982), compared to the North Atlantic salmon (Pettersson and Lignell 1996, 1998). In crustaceans, astaxanthin and canthaxanthin are the main carotenoid component (Négre-Sadargues 1978; Czeczuga and Czeczuga-Semeniuk 1999c); in herring and sprat general carotenoid content is low, and astaxanthin together with canthaxanthin are not predominant (Czeczuga 1976, 1980). It has been established that low levels of astaxanthin in eggs and in yolk-sac of the Baltic salmon fry correlate with elevated mortality due to M74 (Lignell 1994). The effect of carotenoids, particularly astaxanthin, on reproduction and embryonic growth of fish, including salmon, is known (Mikulin and Soin 1975; Christensen and Torrissen 1997). Using isotopes with 14C-labeled astaxanthin (Torrissen et al. 1999), a comparably high concentration of astaxanthin was found in the skin in salmon fry that had started to feed. Yellow eggs contain small amounts of astaxanthin, thus discolouration, mainly of the skin, can be observed in larval forms.

The question arises concerning the role of astaxanthin in the growth of salmon larvae. Astaxanthin is assumed to be the most potent antioxidative agent (Kurashige et al. 1990; Nishigaki et al. 1994; Christensen et al. 1995) due to a high number of conjugated double bonds in the astaxanthin molecule and the ability to quench singlet oxygen and other radicals without being chemically consumed, compared to other carotenoids (Edge et al. 1997; Mortensen and Skibste 1998).

According to many investigators, the etiology of M74 syndrome is associated with high concentration of organochlorines in the Baltic, causing thiamine and astaxanthin deficiency in salmon (Cowey et al. 1985; Niimi et al. 1997; Amcoff et al. 1998; 1999b; Lundström et al. 1999: Pettersson and Lignell 1999). Thiamine is known to be a cofactor in the whole series of enzymatic reactions in metabolic processes, including energy release. Thiamine bath of the eggs obtained from females with M74 syndrome causes an increase in the concentration of α-tocopherol and ubiquinon, antioxidant agents, in the liver (Börjeson et al. 1996). Thiamine is found in the food of the Baltic salmon in different amounts, depending on the clupeid species being their main food. Sprat contain twice as much of this vitamin (0.88), compared to herring (0.34 $\mu g \cdot g^{-1}$). Moreover, the activity of thiaminase, the enzyme that degrades thiamine in herring is considerably higher compared to that of sprat (Soivio and Hartikainen 1999).

Apart from food, also disturbed liver plays a role in specimens with M74 syndrome. In all fish, the liver is the reservoir of carotenoids which prior to the spawning time shift to the gonads. If the liver of the fish with M74 syndrome does not function properly, carote-

noid accumulation in this organ is limited and thus conversion of less oxygenated carotenoids such as β-cryptoxanthin, lutein, and zeaxanthin may not take place. Thus, these carotenoids, particularly dihydroxy ones (lutein, zeaxanthin) shift to the eggs before the spawning time and cause yellow coloration.

As it has been already mentioned, reductive metabolism of astaxanthin into yellow zeaxanthin and of canthaxanthin into β-carotene takes place in *Oncorhynchus keta* during anadromous migration (Kitahara 1983; Ando 1986). This process may become intensified in the specimens with M74 syndrome, causing diverse shades of yellow colour of the eggs. This seems likely, as the general carotenoid content in the yellow, yellow-orange and orange eggs is practically the same (Tables 2, 3), the ratio of astaxanthin and canthaxanthin to lutein and zeaxanthin being changed. In the yellow eggs the ratio ranges between 0.23 and 0.34, in the yellow-orange eggs 0.52–0.72,while in the orange ones is greater than one and in the material examined changes from 1.37 to 3.93.

The effect chemical pollution of water on larval forms of other salmonid species is known in other latitudes. High mortality of early life-stage salmonids from some of the Great Lakes of North America has been reported since 1968 under the name of Early Mortality Syndrome (EMS) and is also associated with the lack of thiamine (Marcquenski 1996; Brown et al. 1998) but not with the lack of astaxanthin in the eggs of feral broodfish (McDonald 1995). The occurrence of EMS in the Pacific salmon specimens, including *Oncorhynchus kisutch* is negatively correlated with alewife (*Alosa pseudoharengus*) abundance in Lake Michigan (Fitzsimons et al. 1999). This may be due to a change in food quality connected with a shortage of alewife, being the main food item this species. 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 can be observed in Atlantic salmon specimens (*Salmo salar*) with Cayuga syndrome in the New York Finger Lakes (Fisher et al. 1996a, b). All these three disorders responsible for mortality in early life stages of salmonids are characterised by a low level of thiamine in eggs (Fitzsimons et al. 1999).

Until now, the M74 syndrome has been investigated in the specimens of the Atlantic salmo, *Salmo salar*. However, another species of this genus—brown trout, *Salmo trutta* living in the Baltic Sea can be affected. Results of analysis of carotenoids in the eggs of *Salmo trutta* females at the beginning of spawning in Polish rivers are similar to those obtained for salmon females. According to Bengtsson et al. (1999), in the 1990s the reproduction rare of a number of other fish species in the Baltic, including the brown trout, decreased. Preliminary studies of sea-run Baltic brown trout showed that yolk-sac fry with symptoms that resembled those of M74, such as lethargy, skin darkening, and mortality, had low mean thiamine concentration compared to healthy specimens (Landergren et al.

1999). Considerable oscillations in the thiamine content in the liver and gonads are also observed in some specimens of Baltic cod, *Gadus morhua* (cf. Amcoff et al. 1999a). All these disturbances associated with the reproduction of many fish species are due to pollution of the Baltic, particularly by organochlorines (Wulff et al. 2001).

CONCLUSIONS

1. The authors investigated carotenoid content in the yellow, yellow-orange, and orange eggs of salmon (*Salmo salar*) and brown trout (*Salmo trutta*) females entering Polish rivers to spawn or reproducing in fresh water.
2. The eggs of salmon examined contained 15 carotenoids an the eggs of brown trout—14. Mutatoxanthin was found only in the eggs of salmon females.
3. The total carotenoid content in the eggs of salmon ranged from 3.978 to 15.583 $\mu g \cdot g^{-1}$ of fresh eggs and in brown trout varied between 3.762 and 10.473 $\mu g \cdot g^{-1}$.
4. The mean content of astaxanthin and canthaxanthin (red carotenoids) in the yellow eggs was from 12.23 to 14.89%, in the yellow-orange eggs ranged from 24.65 to 32.15%, while in the orange eggs from 37.89 to 56.64% of all carotenoids. The mean values of astaxanthin and canthaxanthin in the yellow eggs of brown trout were 13.90–14.52%, in yellow-orange eggs: 25.40–34.44%, while in the orange eggs: 41.63–54.51% of all carotenoids.
5. The highest percentage of lutein and zeaxanthin (yellow carotenoids) were found in the yellow eggs, the lowest in the orange ones. A similar tendency was observed in the brown trout eggs examined.
6. The colour of eggs in salmon and brown trout does not depend on the total quantity of carotenoids but on their reciprocal proportions, which particularly refers to the presence of astaxanthin and canthaxanthin on the one hand and lutein and zeaxanthin on the other. When astaxanthin and canthaxanthin are predominant, the eggs are dark-orange, while lutein and zeaxanthin prevail, the eggs are yellow.
7. Pettersson and Lignell (1999) suggest that if the level of astaxanthin and canthaxanthin in the eggs of Salmo salar females is below 2.22 $\mu g \cdot g^{-1}$, M74 syndrom will develop. In our study, the yellow eggs of both salmon and brown trout had astaxanthin and canthaxanthin below 2.22 μg; in the yellow orange eggs the carotenoid content was higher compared to the threshhold level, only in 3 females. In the orange eggs, the carotenoid content exceeded the border value for the M74 syndrome in 15 salmon and 3 trout females.

REFERENCES

Amcoff P., H. Börjeson, R. Eriksson, L. Norrgren, 1998: Effects of thiamine treatments on survival of M74-affected feral Baltic salmon. In: Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and Baltic Sea [McDonald G., J.D Fitzsimons, D.C. Honeyfield (eds)]. American Fisheries Society. Symposium 21, Bethesda, Maryland: 26–30.

Amcoff P., H. Börjeson, P. Landergren, L. Vallin, L. Norrgren, 1999a: Thiamine (vitamin B1) concentrations in salmon (*Salmo salar*), brown trout (*Salmo trutta*) and cod (*Gadus morhua*) from the Baltic Sea. Ambio, **28**: 48–54.

Amcoff P., J. Lundström, L. Teimert, H. Börjeson, L. Norrgren, 1999b: Physiological and morphological effects of microinjection of oxythiamine and PCBs in embryos of Baltic salmo (*Salmo salar*): A comparison with the M74 syndrome. Ambio, **28**: 55–66.

Ando S., 1986: Studies on the food biochemical aspect of changes in chum salmo *Oncorhynchus keta* spawning migration: mechanisms of muscle deterioration and nuptial coloration. Men. Facult. Fish. Hokkaido Univ., **33**: 1-95.

Ando S., T. Takeyama, M. Hatano, 1986: Transport associated with serum vitellogenin of carotenoid salmon (*Oncorhynchus keta*). Agric. Biol. Chem., **50**: 557–564.

Bartel R., 1996: Does M.-74 syndrome occur in Polish wild sea trout and reared salmon? In: Report from the Second Workshop on Reproduction Disturbances in Fish [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Swedish Environmental Protection Agency, Stockholm, Report No. 4534: 90–100.

Bengtsson B.-E., Å. Bergman, I. Brandt, C. Hill, N. Johansson, A. Sodergren, J. Thulin, 1994: Reproductive disturbances in Baltic fish. Swedish Environmental Protection Agency, Stockholm.

Bengtsson B.-E., C. Hill, 1996: Review of recent research efforts on reproductive disturbances in Baltic fish. In: Report from the Second Workshop on Reproduction Disturbances in Fish [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Swedish Environmental Protection Agency, Stockholm, Report No. 4534: 20-23.

Bengtsson B.-E., C. Hill, A. Bergman, I. Brandt, N. Johansson, C. Magnhagen, A. Sodergren, J. Thulin, 1999: Reproductive disturbances in Baltic fish: A synopsis of the RiRe project. Ambio, 28: 2–8.

Börjeson H., L. Förlin, L. Norrgren, 1996: Investigation of oxidants and prooxidants in salmon affected by the M74 syndrome. In: Report from the Second Workshop on Reproduction Disturbances in Fish [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Swedish Environmental Protection Agency, Stockholm, Report No. 4534: 95-96.

Börjeson H., L. Norrgren, 1997: The M74 syndrome, a review of potential etiological factors. In: Chemically Induced Alterations in Functional Development and Reproduction of Fishes [Rolland M.R., M. Gilbertson, Peterson F.E., (eds)]. SETAC (Society for Environmental Toxicology and Chemistry) Press, Pensacola, Florida: 153–166.

Börjeson H., L. Norrgren, T. Andersson, P.-A. Bergqvist, 1994: The Baltic salmon—situation in the past and today. In: Report from the Uppsala Workshop on Reproduction Disturbances in Fish [Norrgren L. (ed.)]. Swedish Environmental Protection Agency. Stockholm, Report No. 4346: 14–25.

Brown S.B., D.C. Honeyfield, D. Vandenbyllaard, 1998: Thiamine analysis in fish tissues. In: Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and Baltic Sea [McDonald G., J.D Fitzsimons, D.C. Honeyfield (eds)]. American Fisheries Society. Symposium 21, Bethesda, Maryland: 18–25.

Chełkowska B., 1982: Studies on morphology and biology of salmon, *Salmo salar* L. in the river Drawa. Acta Ichthyol. Piscat., **12** (Suppl.): 3–69.

Chełkowski Z., 1965: Salmon from the West Pomerania rivers, debouching into the Baltic Sea. Prz. Zool., **9**: 275–280. (In Polish).

Choubert G., A. Guillou, B. Fauconneau, 1987: Absorption and fate of labeled canthaxanthin 15.15'-3H_2 in rainbow trout (*Salmo gairdneri* Rich.). Comp. Biochem. Physiol., **87**A: 717–720.

Christensen R., J. Glette, Ø. Lic, O.J. Torrissen, R. Waagbo, 1995: Antioxidant status and immunity in Atlantic salmon *Salmo salar* L., fed semi-purified diets with and without astaxanthin supplementation. J. Fish Diseases, **18**: 317–328.

Christensen R., O.J. Torrissen, 1997: Effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (*Salmo salar* L.). Aquaculture, **153**: 51–62.

Cowey C.B., J.G. Bell, D. Knox, A. Fraser, A. Youngson, 1985: Lipids and lipid antioxidant systems in developing eggs of salmon (*Salmo salar*). Lipids, **20**: 567–572.

Craik J.C.A., 1985: Egg quality and egg pigment content in salmonid fishes. Aquaculture, **47**: 61–88.

Craik J.C.A., S.M. Harvey, 1986: The carotenoids of eggs of wild and farmed Atlantic salmon, and their changes during development of the start of feeding. J. Fish Biol., **29**: 549–565.

Crozier G.F., 1970: Tissue carotenoids in prespawning and spawning sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada, **27**: 973–975.

Czeczuga B., 1975: Carotenoids in fish. Salmonidae and Thymallidae from Polish water. Hydrobiologia, **46**: 223–239.

Czeczuga B., 1976: Carotenoids in fish. *Clupea harengus* L. and *Spratus sprattus* L. (Clupeidae) of the Baltic Sea. Pol. Arch. Hydrobiol., **23**: 123–129.

Czeczuga B., 1979: Carotenoids in the eggs of *Oncorhynchus keta* (Walb.). Hydrobiologia, **63**: 45–47.

Czeczuga B., 1980: Carotenoids in fish. *Sardina pilchardus* Walb. (Clupeidae). Hydrobiologia **69**: 277–279.

Czeczuga B., 1988: Carotenoids. In: CRC Handbook of Lichenology [Galun M. (ed.)]. CRC Press, Boca Raton, Florida: 25–34.

Czeczuga B., 1993: Carotenoids in lichens. In: Phytochemistry and Chemotaxonomy of Lichenized Ascomycetes [Feige G.B. and Lumbsch H.T. (eds)]. J. Cramer Press, Berlin–Stuttgart: 53–66.

Czeczuga B., R. Bartel, 1989: Studies on carotenoids in spawning *Salmo trutta* morpha *lacustris* L. Acta Ichthyol. Piscat., **19**: 49–58.

Czeczuga B., R. Bartel, 1998: The occurrence of carotenoids in different-age individuals of *Pelecus cultratus* (L.) from the Vistula Lagoon. Acta Ichthyol. Piscat, **28**, 1: 15–23.

Czeczuga B., R. Bartel, 2000: Carotenoid resources in lampreys (Petromyzontidae). Bull. Lampetra, **4**: 105–117.

Czeczuga B., Z. Chełkowski, 1984: Carotenoid contents in adult individuals of sea-trout *Salmo trutta* L. during spawning migration, spawning and post-spawning migration. Acta Jchthyol. Piscat., **14**, 1–2: 187–201.

Czeczuga B., E. Czeczuga-Semeniuk, 1999a: Carotenoid content in *Lota lota* (L.) individuals in various biological activity periods. Folia Biol., **47**: 67–72.

Czeczuga B., E. Czeczuga-Semeniuk, 1999b: Carotenoids in fillets of spiny dogfish shark *Squalus acanthias* L. (Chondrichthyes, Squalidae). Bull. Sea Fish. Inst. Gdynia, 2 (147): 3–10.

Czeczuga B., E. Czeczuga-Semeniuk, 1999c: Comparative studies of carotenoids in four species of crayfish. Crustaceana, **72**: 693–700.

Czeczuga B., E. Czeczuga-Semeniuk, 2000a: Carotenoid content in *Anguilla anguilla* (L.) individuals undertaking spawning migration. Folia Biol., **48**: 1–6.

Czeczuga B., E. Czeczuga-Semeniuk, 2000b: Carotenoid content in some body parts of pike (*Esox lucius* L.) before, during, and post-spawning. Acta Ichthyol. Piscat., **30**, 1: 101–115.

Czeczuga B., K. Dąbrowski, R. Rösch, A. Champigneulle, 1991: Carotenoids in *Coregonus lavaretus* L. ndividuals of various populations. Acta Ichthyol. Piscat., **21**, 2: 3–16.

Czeczuga B., B. Klyszejko, E. Czeczuga-Semeniuk, 2000: The carotenoid content in certain fish species from the fisheries of New Zealand. Bull. Sea Fish. Inst. Gdynia, 1 (149): 35–42.

Edge R., D.J. McGarvey, T.G. Truscott, 1997: The carotenoids as anti-oxidants—a review. Photochem. Photobiol. B: Biology, **41**: 189–200.

Fisher J.P., S. Brown, P.R. Browser, G.A. Wooster, T. Chiotti, 1996a: Continued investigations into the role of thiamine and thiaminase-rich forage in the Cayuga syndrome of New York's landlocked Atlantic salmon. In: Report from the Second Workshop on Reproduction Disturbances in Fish [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Swedish Environmental Protection Agency, Stockholm, Report No. 4534: 79–81.

Fisher J.P., B. Bush, J.M. Spitsbergen, 1996b: Contrasting pathologies associated with Cayuga syndrome and PCB-induced mortality in early life stages of Atlantic salmon. In: Report from the Second Workshop on Reproduction Disturbances in Fish [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Swedish Environmental Protection Agency, Stockholm, Report No. 4534: 87–89.

Fitzsimons J.D., S.B. Brown, D.C. Honeyfield, J.G. Hnath, 1999: A review of early mortality syndrome (EMS) in Great Lakes salmonids: Relationship with thiamine deficiency. Ambio, **28**: 9–15.

Foss P., T. Storebakken, E. Austreng, S. Liaaen-Jensen, 1987: Carotenoids in diets for salmonids. V. Pigmentation of rainbow trout and sea trout with astaxanthin and astaxanthin dipalmitate in comparison with canthaxanthin. Aquaculture, **65**: 293–302.

Hata M., M. Hata, 1973: Studies on astaxanthin formation in some fresh-water fishes. Tohoku J. Agric. Res., **24**: 192–196.

Hata M., M. Hata, 1975: Carotenoid pigments in rainbow trout *Salmo gairdneri irideus*. Tohoku J. Agric. Res., **26**: 35–40.

Ikonen E., 1995: Feeding of salmon during the spawning run, preliminary information. In: Report from the Second Workshop on Reproduction Disturbances in Fish [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Swedish Environmental Protection Agency, Stockholm, Report No. 4534: 47–48.

Jacobsen J.A., L.P. Hansen, 1996: The food of Atlantic salmon, *Salmo salar* L., north of the Faroe Islands. ICES. Anadromous and Catadromous Fish Committee CM 1996/M, 10, 21 pp.

Karlsson L., E. Ikonen, A. Mitans, S. Hansson, 1999: The diet of salmon (*Salmo salar*) in the Baltic Sea and connections with the M74 syndrome. Ambio, **28**: 37–42.

Karlström Ö., 1999: Development of the M74 syndrome in wild populations of Baltic salmon (*Salmo salar*) in Swedish rivers. Ambio, **28**: 82–86.

Kitahara T., 1983: Behaviour of carotenoids in the chum salmon (*Oncorhynchus keta*) during anadromus migration. Comp. Biochem. Physiol., **76B**: 97–101.

Kitahara T., 1985: Behaviour of carotenoids in the masu salmon *Oncorhynchus masou* during anadromus migration. Bull. Jap. Soc. Sci. Fish., **51**: 253–255.

Kurashige M., E. Okimasu, M. Inoue, K. Utsumi, 1990: Inhibition of oxidative injury of biological membranes by astaxanthin. Physiol. Chem., Phys. Med. NMIR, **22**: 27–38.

Landergren P., L. Vallin, L. Westin, P. Amcoff, H. Börjeson, B. Ragnarsson, 1999: Reproductive failure in Baltic Sea trout (*Salmo trutta*) compared with the M74 syndrome in Baltic salmon (*Salmo salar*). Ambio, **28**: 87–91.

Lignell A., 1994: Astaxanthin in yolk-sac fry from feral Baltic salmon. In: Report from the Uppsala Workshop on Reproduction Disturbances in Fish [Norrgren L. (ed.)]. Swedish Environmental Protection Agency. Stockholm, Report No. 4346: 94–96.

Lundström J., H. Börjeson, L. Norrgren, 1999: Histopathological studies of yolk-sac fry of Baltic salmon (*Salmo salar*) with the M74 syndrome. Ambio, **28**: 16–23.

Lundström J., B. Camey, P. Amcoff, A. Pettersson, H. Börjeson, L. Forlin, L. Norrgren, 1999: Antioxidative systems, detoxifying enzymes and thiamine leaves in Baltic salmon (*Salmo salar*) that develop M74. Ambio, **28**: 24–29.

Lundström J., L. Norrgren, H. Börjeson, 1996: Clinical and morphological studies of Baltic salmon yolk-sac fry suffering from the M74 syndrome. In: Report from the Second Workshop on Reproduction Disturbances in Fish [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Swedish Environmental Protection Agency, Stockholm, Report No. 4534: 26–27.

Marcquenski S.V., 1996: Characterization of Early Mortality Syndrome (EMS) in salmonids from the Great Lakes. In: Report from the Second Workshop on Reproduction Disturbances in Fish [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Swedish Environmental Protection Agency, Stockholm, Report No. 4534: 73–75.

McDonald G., 1995: Early mortality syndrome (EMS) in Baltic salmon, Background notes. In: Early mortality syndrome (EMS) in Great Lakes Fishes, Summary of workshop and task area proposal. Research workshop, Detriot, Michigan 2–3 February 1995: 10–12.

Mikulin A.Y., S.G. Soin, 1975. The functional significance of carotenoids in the embryonic development of teleosts. J. Ichthyol., **15**: 749–759.

Mortensen S., L.H. Skibste, 1998: Relative stability of carotenoid radical cations and homologue tocopheroxyl radicales. A real time kinetic study of antioxidant hierarchy. FEBS Letters, **417**: 261–266.

Nakamura K., M. Hata, M. Hata, 1985: A study on astaxanthin in salmon Oncorhynchus keta serum. Bull. Jap. Soc. Sci. Fish., **51**: 979–983.

Négre-Sadargues G., 1978: Sur les transformation métaboliques des pigments caroténoides chez les Crustacés: synthese bibliographique. Ann. Biol., **17**: 415–454.

Niimi A.J., C.-J. Jackson, J. Fitzsimons, 1997: Thiamine dynamics in aquatic ecosystems and its biological implications. Int. Rev. ges. Hydrobiol., **82**: 47–56.

Nishigaki I., A.A. Dmitrovskii, W. Miki, K. Yagi, 1994: Suppressive effect of astaxanthin on lipid peroxidation induced in rats. J. Clin. Biochem. Nutr., **16**: 161–166.

Norrgren L., B.-E. Bengtsson, H. Börjeson, 1994: Reproduction Disturbances in Fish. In: Report from the Uppsala Workshop on Reproduction Disturbances in Fish [Norrgren L. (ed.)]. Swedish Environmental Protection Agency. Stockholm, Report No. 4346: 7–11.

Pettersson A., Å. Lignell, 1996: Decreased astaxanthin leaves in the Baltic salmon and the M74 syndrome. In: Report from the Second Workshop on Reproduction Disturbances in Fish [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Swedish Environmental Protection Agency, Stockholm, Report No. 4534: 28–29.

Pettersson A., Å. Lignell, 1998: Low astaxanthin leaves in Baltic salmon exhibiting the M74 syndrome. In: Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and Baltic Sea [McDonald G., J.D Fitzsimons, D.C. Honeyfield (eds)]. American Fisheries Society. Symposium 21, Bethesda, Maryland: 26–30.

Pettersson A., Å. Lignell, 1999: Astaxanthin deficiency in eggs and fry of Baltic salmon (*Salmo salar*) with the M74 syndrome. Ambio, **28**: 43–47.

Pickova J., A. Kiessling, A. Pettersson, P.C. Dutta, 1998: Comparison of fatty acid composition and astaxanthin content in healthy and by M74 affected salmon eggs from three Swedish river stocks. Comp. Biochem. Physiol., **120**: 256–261.

Satio A., 1969: Color in raw and cooked Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd Canada, **26**: 2234–2242.

Schiedt K., F.J. Leuenberger, M. Vecchi, 1981: Natural occurrence of enantiomeric and mesoastaxanthin 5. Ex-wild salmon (*Salmo salar* and *Oncorhynchus*). Helv. Chim. Acta, **64**: 449–452.

Schiedt K., F.J. Leuenberger, M. Vecchi, E. Glinz, 1985: Absorption, retention and metabolic transformations of carotenoids in rainbow trout, salmon and chicken. Pure Appl. Chem., **57**: 685–692.

Skrede G., T. Storebakken, 1986: Characteristics of color in raw, baked and smoked wild and pen-reared Atlantic salmon. J. Food Sci., **51**: 804–816.

Scott W.B., E.J. Crossman, 1973: Freshwater fishes of Canada. Fish. Res. Bd Canada, 184: 1–966.

Sivtseva L.V., 1982: Qualitative composition and distribution of carotenoids and vitamin A in the organs and tissues of rainbow trout (*Salmo gairdneri*). J. Ichthyol., **22**: 96–103.

Soivio A., 1996: M74 in Finland. In: Report from the Second Workshop on Reproduction Disturbances in Fish [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Swedish Environmental Protection Agency, Stockholm, Report No. 4534: 42–43.

Soivio A., K. Hartikainen, 1999: Thiaminase activity in the forage fish of Baltic salmon (*Salmo salar*). In: Nordic Research Cooperation on Reproductive Disturbances in Fish. Report from the Redfish project [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Tema Nord, 530: 63–66.

Storebakken T., P. Foss, I. Huse, A. Wandsvik, T.B. Lea, 1986: Carotenoids in diets for salmonids. III. Utilization of canthaxanthin from dry and wet diets by Atlantic salmon, rainbow trout and sea trout. Aquaculture, **51**: 245–253.

Storebakken T., P. Foss, K. Schiedt, E. Austreng, S. Liaaen-Jensen, U. Mainz, 1987: Carotenoids in diets for salmonids. IV. Pigmentation of Atlantic salmon with astaxanthin, astaxanthin dipalmitae and canthaxanthin. Aquaculture, **65**: 279–292.

Straub O. (ed.), 1987: Key to Carotenoids. Birkhäuser Verlag, Basel–Boston.

Torrissen O.J., 1984: Pigmentation of salmonids—effect of carotenoids in eggs and start-feeding diets on survival and growth rate. Aquaculture, **43**: 185–203.

Torrissen O.J., L. Balk, K. Ingebrigsten, 1999: Astaxanthin metabolism in eggs and larvae of Atlantic salmon (*Salmo salar*). In: Nordic Research Cooperation on Reproductive Disturbances in Fish. Report from the Redfish project [Bengtsson B.-E., C. Hill, S. Nellbring (eds)]. Tema Nord, 530: 89–92.

Torrissen O.J., G. Naevdal, 1988: Pigmentation of salmonids-variation in flesh carotenoids of Atlantic salmon. Aquaculture, **68**: 305–310.

Torrissen K.K., O.J. Torrissen, 1985: Protease activities and carotenoid leaves during the sexual maturation of Atlantic salmon (*Salmo salar*). Aquaculture, **50**: 113–120.

Vuorinen P.J., J. Paasivirta, M. Keinänen, J. Kostinen, T. Rantio, T. Hyötyläinen, L. Welling, 1997: The M74 syndrome of Baltic salmon (*Salmo salar*) and organochlorine concentrations in the muscle of female salmon. Chemosphere, **34**: 1151–1166.

Wetter W., G. Englert, N. Rigassi, U. Schwieter, 1971: Spectroscopic Methods. In: Carotenoids [Isler O. (ed.)]. Birkhäuser Verlag, Basel–Boston: 189–229.

Wulff F.V., L.A. Rahm, P. Larsson, 2001: A Systems Analysis of the Baltic Sea. Springer-Verlag, Berlin–Heidelberg–New York.

Bazyli *CZECZUGA*, Ryszard *BARTEL*, Ewa *CZECZUGA-SEMENIUK*

ZAWARTOŚĆ KAROTENOIDÓW W IKRZE ŁOSOSI (*SALMO SALAR* L.) I TROCI (*SALMO TRUTTA* L.) WCHODZĄCYCH NA TARŁO DO RZEK POLSKI I HODOWANYCH W WODZIE SŁODKIEJ

STRESZCZENIE

Autorzy stosując chromatografię kolumnową i cienkowarstwową badali zawartość karotenoidów w żółtej, żółto-pomarańczowej i pomarańczowej ikrze 74 samic łososia bałtyckiego (*Salmo salar* L.) i 14 samic troci (*Salmo trutta* L.) wchodzących na tarło do rzek Polski i hodowanych w wodzie słodkiej.

W ikrze badanych łososi stwierdzono występowanie 15 karotenoiów, zaś w ikrze troci 14. Mutatoksantyna występowała jedynie w ikrze samic łososi.

Ogólna zawartość karotenoidów w ikrze samic łososi wahała się od 3,978 do 15,583 $\mu g \cdot g^{-1}$ natomiast u troci od 3,762 do 10,473 $\mu g \cdot g^{-1}$ świeżej ikry.

Średnia zawartość astaksantyny wraz z kantaksantyną (czerwone karotenoidy) w żółtej ikrze łososia wahała się od 12,23 do 14,89%, w ikrze żółto-pomarańczowej od 24,65 do 32,15%, a w ikrze pomarańczowej od 37,98 do 56,64% wszystkich karotenoidów. Natomiast w żółtej ikrze troci astaksantyna wraz z kantaksantyną stanowiła średnio od 13,90 do 14,52%, w żółto-pomarańczowej od 25,40 do 34,44%, a w pomarańczowej od 41,63 do 54,51% wszystkich karotenoidów.

Z kolei najwięcej żółtych karotenoidów (luteina wraz z zeaksantyną) wystçpowało w żółtej ikrze obu gatunków, mniej w ikrze żółto-pomarańczowej i najmniej w ikrze pomarańczowej. Kolor ikry u samic łososia i troci nie zależy od ogólnej ilości karotenoidów, lecz od ilości w niej astaksantyny wraz z kantaksantyną z jednej strony i luteiny wraz z zeaksantyną z drugiej. Jeżeli dominuje astaksantyna wraz z kantaksantyną, ikra jest koloru pomarańczowego lub ciemno pomarańczowego, natomiast przy przewadze luteiny wraz z zeaksantyną ikra przyjmuje zabarwienie żółte.

Przyjmując za Petterssonem i Lignellem (1999), że 2,22 $\mu g \cdot g^{-1}$ zawartość astaksantyny wraz z kantaksantyną stanowi wielkość progową dla ikry łososi z syndromem M74, to w naszych badaniach żółta ikra zarówno łososia jak i troci zawierała te karotenoidy poniżej wielkości progowej, w ikrze żółto-pomarańczowej tylko 3 samice łososia, a w obrębie ikry pomarańczowej u 15 samic łososia i 3 samic troci zawierały więcej tych karotenoidów niż wynosi wielkość progowa dla syndromu M74.

Received: 18 September 2001

Author's address:

Bazyli Czeczuga PhD DSc Prof
Department of General Biology
Medical University in Białystok
Kilińskiego 1, 15-230 Białystok, Poland