Arch. Pol. Fish.	Archives of Polish Fisheries	Vol. 13	Fasc. 1	39-49	2005

THE OCCURRENCE OF M74 SYNDROME IN SEA TROUT (*SALMO TRUTTA* M. *TRUTTA* L.) INDIVIDUALS RETURNING TO SPAWN IN THE POLISH RIVERS OF THE VISTULA CATCHMENT AREA

Bazyli Czeczuga, Ryszard Bartel**, Ewa Czeczuga-Semeniuk*, Przemysław Kosieliński*, Adam Grochowski***

*Department of General Biology, Medical University, Białystok, Poland
**Department of Migratory Fish, The Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland

ABSTRACT. The occurrence of M74 syndrome in female sea trout, *Salmo trutta* m. *trutta*, belonging to two populations returning to spawn in Polish rivers in the fall of 2003 and three from pond cultivation were investigated. Eggs from a total of 250 female specimens were investigated from rivers in northern Poland (10 – Parsęta, 100 – Miastko, 140 – Świbno). The study method applied involved comparing the concentrations in eggs of red (astaxanthin, canthaxanthin) and yellow (lutein, zeaxanthin) carotenoids. The specific carotenoids were determined with column chromatography (CC), thin layer chromatography (TLC), and high performance liquid chromatography (HPLC). The eggs of the investigated female sea trout were divided into three groups according to color: yellow, yellow-orange, and orange. Fifteen carotenoids were identified in the investigated sea trout females. Red carotenoids dominated in orange and yellow-orange eggs, while yellow ones dominated in yellow eggs. M74 syndrome was identified in 35 females, which represented 14.0% of all the investigated sea trout.

Key words: SEA TROUT (*SALMO TRUTTA* M. *TRUTTA*), EGGS, CAROTENOIDS, M74 SYNDROME

INTRODUCTION

The first reported observation of mass mortality in Atlantic salmon, *Salmo salar* L., larvae during the transition period from yolk sac feeding to active feeding was made in 1974 at the Miljö hatchery located on the Mörum River in southern Sweden. These specimens were gray in color due to skin discoloration, their livers had numerous vacuoles and low glycogen levels, and many other histopathological symptoms were noted (Bengtsson et al. 1994). Both eggs and muscles of salmon females with M74 syndrome have low levels of astaxanthin (Pettersson and Lignell 1999), which is a key factor in antioxidation (Kurashige et al. 1990). The eggs of females with M74 syndrome are also yellow in colour due to elevated levels of yellow and reduced levels of red carotenoids.

CORRESPONDING AUTHOR: Prof. dr hab. Bazyli Czeczuga, Akademia Medyczna, Zakład Biologii Ogólnej, ul. Kilińskiego 1, 15-089 Białystok, Tel. +48 (85) 748 54 83; e-mail: czecz@amb.edu.pl

Additionally, the eggs of salmon females with M74 syndrome contain large amounts of long chain highly unsaturated fatty acid (>20:5) (Pickova et al. 1998). The levels of antioxidants such as α-tocoferol and ubiquinone decrease in the livers of larvae (Covey et al. 1985, Lundström et al. 1999). The hepatosomatic index, which is the ratio of liver weight to body weight, also decreases (Nakano et al. 1995). The name of the syndrome, M74, originates from the location and year in which it was first observed. The symptoms of this syndrome are currently seen in the larvae of salmon from rivers in Sweden (Karlström 1999), Finland (Soivio 1996), and Poland (Czeczuga et al. 2002). All studies indicate unequivocally that this syndrome occurs fairly frequently in Baltic salmon, while isolated cases have been noted in sea trout, *Salmo trutta* m. *trutta* L. (Amcoff et al. 1999, Bengtsson et al. 1999, Landergren et al. 1999).

During studies of salmon returning to spawn in Polish rivers (Czeczuga et al. 2002), the authors also noted this syndrome among several sea trout females. This provided the impetus for conducting research on the eggs of the abundant sea trout population at three different locations in order to determine if the population in Polish rivers is afflicted by M74 syndrome.

MATERIALS AND METHODS

The eggs for the investigation were collected in late October and early November 2003 from 250 sea trout females returning to spawn in the Parsęta River in the town of Parsęta (10 females) and the Vistula River in the town of Świbno (140 females) and from cultivation ponds at the Miastko hatchery (100 females) supplied with water from the Studnica River tributary of the Wieprz River in northern Poland. According to the four-stage scale prepared by the Swedish Salmon Research Institute SR-81494 Alvkarleby, Sweden (Börjeson et al. 1996), the eggs belonged to three colour groups.

Following a week of frozen storage at a temperature of -4°C, the eggs were delivered to the laboratory of the Medical Academy of Białystok, where, during the subsequent week, the samples were analyzed with chromatography to determine the carotenoid contents.

The occurrence of M74 syndrome in female sea trout was studied in the same manner as in female Atlantic salmon (Czeczuga et al. 2002) by determining the amount of red (astaxanthin, canthaxanthin) and yellow (lutein, zeaxanthin) carotenoids in eggs. If the content of red carotenoids was lower than 2.220 μg g^{-1} raw egg mass (r.e.m.), the female was included in the group with M74 syndrome (Pettersson and Lignell 1999). The various carotenoids in the sea trout eggs were determined with column chromatography (CC), thin layer chromatography (TLC) using various solvent combinations (Czeczuga 1986), and high performance liquid chromatography (HPLC).

Sub-samples of eggs were homogenized, and then hydrolyzed in a 10% methanolic KOH solution in a nitrogen atmosphere, in the dark, at room temperature, for 24 hours. The extract obtained was placed either on a column (Quickfit) filled with Al_2O_3 (CC) or a glass plate coated with silicon gel (Merck Co) (TLC). The various CC and TLC fractions were then rinsed with various solvent mixtures. After the eluents of the various fractions obtained were evaporated and then redissolved in one of four solvents (petroleum benzine, hexane, acetone, ethanol), readings were taken of the maximum absorption in UV and VIS. Details of column chromatography (CC) and thin layer chromatography (TLC) can be found in the paper by Czeczuga (1986).

Some of the carotenoids were determined with high performance liquid chromatography with the two-phase ion-exchange process. Ion-exchange reagent (Shimadzu) was added to the appropriate amount of extract. A Shimadzu SCL-6B gradient programmer and a Rheodyne 7125 injector were used during the HPLC process. A Shimadzu SPD-6A spectrophotometer was used to determine the UV and VIS absorbed by the various carotenoids. The fluorescent properties of some of the pigments were investigated with a Shimadzu RF-535 detector. Details regarding high performance liquid chromatography can be found in the work by Mantoura and Llewellyn (1983).

Specific carotenoids were identified by comparing their data with that of standards: a) general appearance of the column chromatogram; b) spectra in UV and VIS; c) ratio of epi- and hypophases in hexane and 95% ethanol; d) values of R_f from thin layer chromatograms according to Kraus and Koch (1996); e) presence or absence of the allylic OH groups determined with the test with $CHCl_3$; f) epoxide test; g) spectral analyses (cf. Vetter et al. 1971).

The pigment standards used were carotenoids from Hoffman-La Roche, Switzerland, the International Agency for ^{14}C Determinations, Denmark, and Sigma Chemical Co., USA. The quantity of the carotenoids was determined with a spectroscope in UV and VIS according to Davies (Czeczuga 1986). The structure of individual carotenoids is presented according to Straub (1987) and Czeczuga (1988).

RESULTS

According to the scale applied, the eggs of the 250 female sea trout investigated belonged to three pigmentation groups. In 23 females, the eggs were orange (9.2% of all females), while in 192 they were yellow-orange (76.8%), and 35 females had yellow eggs (14.0 %). Fifteen carotenoids were detected in the investigated eggs (Table 1, Fig. 1).

TABLE 1

List of carotenoids from the investigated material

Carotenoid	Summary formula	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 (stereoisomeric)
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

The overall carotenoid content in the investigated eggs ranged from 4.197 to 7.483 μg g^{-1} r.e.m. (mean 5.895 μg g^{-1} r.e.m.). The dominating carotenoid in the yellow eggs was yellow. In the orange eggs the contents of carotenoids ranged from 3.785 to 6.059 μg g^{-1} r.e.m. (mean 4.593 μg g^{-1} r.e.m.). The yellow-orange eggs contained these carotenoids in quantities ranging from 2.118 to 3.018 μg g^{-1} r.e.m. (mean 2.724 μg g^{-1} r.e.m.), while yellow carotenoids occurred in the range of 0.906-2.102 μg g^{-1} r.e.m. (mean 1.682 μg g^{-1} r.e.m.).

The content of red carotenoids in the orange and yellow-orange eggs was higher than the threshold (2.220 μg g^{-1} r.e.m.) for individuals with M74 syndrome, while in the yellow eggs it was below this value. M74 syndrome occurred in 35 females or 14% of the investigated females. No females with M74 syndrome were noted in the population from

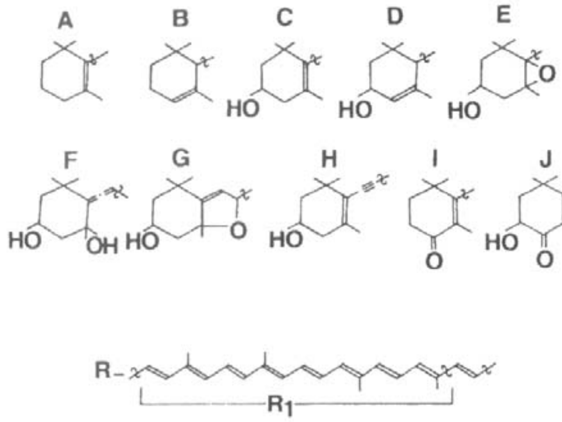

Fig. 1. Structural features of carotenoids from investigated materials.

the Parsęta, while the most were noted in the population from Miastko (9.2%). Only 4.8% of the population that spawned in the Vistula River (Świbno) was affected (Table 2).

TABLE 2

Carotenoid content in sea trout eggs of different color

Specification	Ranged
Number of females	250 (Miastko – 100, Parsęta – 10, Świbno – 140)
Colour of eggs:	
– yellow eggs	35 (Miastko – 23, Parsęta , Świbno – 12)
– yellow – orange eggs	192
– orange eggs	23
Total content of carotenoid, µg g^{-1}	5.895 (4.197-7.483)
Content of red carotenoid (A), µg g^{-1}:	
– yellow eggs	1.682 (0.916-2.103)
– yellow – orange eggs	2.724 (2.118-3.018)
– orange eggs	4.593 (3.785-6.059)
Content of yellow carotenoid (B), µg g^{-1}:	
– yellow eggs	2.275 (2.248-2.286)
– yellow – orange eggs	2.008 (1.804-2.128)
– orange eggs	1.384 (0.912-1.612)
Ratio A/B (red carotenoid /yellow carotenoid):	
– yellow eggs	0.74
– yellow – orange eggs	1.36
– orange eggs	3.32

DISCUSSION

The authors' research (Czeczuga et al. 2002) on the occurrence of M74 syndrome in female salmon returning to spawn in Polish rivers indicated that this syndrome occurred at a higher frequency (75.7%) than in sea trout (14%). The percentage of afflicted female salmon among those returning to Swedish rivers was as high as 95% in some of them (Karlström 1999), while in sea trout this figure was well under twenty percent (Landergren et al. 1999). This might be related to the different feeding biology during migrations of the individuals of these two species. It is known that sea trout feed during spawning migrations while salmon do not (Bartel 2000). However, with regard to salmon it can be assumed that astaxanthin metabolically reduced to zeaxanthin is not supplemented by food since this carotenoid is not consumed, and the quantities of astaxanthin in such specimens decrease rapidly. A similar phenomenon is seen in specimens of chum salmon, *Oncorhynchus keta* (Walb.), that do not feed during spawning migrations (Kitahara 1983, Ando 1986, Ando and Hatano 1987).

As previously noted, the most sea trout with M74 syndrome occurred in the populations from Miastko and those returning to spawn in the Vistula River (Świbno), while the small population from the Parsęta was comprised exclusively of healthy females. The higher percentage of afflicted specimens in Świbno was probably related to the higher concentrations of chloroorganic compounds in the Vistula River due to its large catchment area (Backlund et al. 1993). Does the same apply to the water in the ponds where sea trout were cultivated in the town of Miastko? Mycological studies of the fungal development on the eggs of these three sea trout populations indicated that the eggs of females returning to the Parsęta River are also the most resistant to fungal infections. While 40 species of fungus developed on the eggs of sea trout from Świbno and 34 on those from females from Miastko, only 25 zoospore aquatic fungi occurred on the eggs of females returning to spawn in the Parsęta River (Czeczuga et al. 2005).

Neither animals nor fish are able to synthesize carotenoids *de novo*; the only organisms able to do this are bacteria, cyanobacteria, algae, fungi, and vascular and non-vascular plants (Goodwin 1981). In healthy salmon and sea trout specimens, the main carotenoids are the red ones such as astaxanthin and canthaxanthin (Czeczuga and Chełkowski 1984, Czeczuga and Bartel 1989). Astaxanthin and canthaxanthin are absorbed along the full length of the alimentary tract (Hardy et al. 1990) from ten to twenty times more effectively than lutein and zeaxanthin (Schiedt et al. 1985). Part of the astaxanthin is metabolically reduced to zeaxanthin, especially during anadromous migrations of Pacific salmon that do not feed (Kitahara 1983). Carotenoids, including red ones, move from the alimentary tract and are deposited in the liver, skin, muscle tissues, and ovaries, and metabolic reduction occurs in the skin and muscle tissue. Carotenoids are deposited in the ovaries in the form of the lipovitellin complex and the corresponding carotenoid. Serum astaxanthin and canthaxanthin are combined with the protein deposited in the liver and create lipoproteins (HDL), and some of them are oxidized in the liver (Nakamura et al. 1985, Schiedt et al. 1985, Ando 1986). As Ando (1986) reported, in salmonid fish vitellogenin (a precursor of egg yolk protein) participates in the transfer of astaxanthin from the muscles to the gonads of adult females. Carotenoid metabolism in salmonids occurs primarily in the skin and muscle tissues of adult specimens, which retain approximately 90% of the astaxanthin (Schiedt et al. 1985, Ando 1986, Torrissen et al. 1989). Astaxanthin and canthaxanthin are only partially degraded in the alimentary tracts of salmonids (Foss et al. 1987, Storebakken et al. 1987).

The eggs of females with M74 syndrome, as noted earlier in this paper, have low levels of carotenoids, especially astaxanthin, with a simultaneous increase of yellow carotenoids, especially zeaxanthin (Pettersson and Lignell 1999, Czeczuga et al. 2002). The primary carotenoid in all parts of the bodies of healthy specimens of species from the genus Salmo is astaxanthin. However, how should this phenomenon be interpreted – when the level of one carotenoid decreases the other increases, at nearly the same total level of carotenoids in the eggs of healthy and M74-afflicted females (Czeczuga et al. 2002). The most likely hypothesis is transformation through the reduction of astaxanthin into zeaxanthin. The metabolism of astaxanthin into zeaxanthin in fish is known (Torrissen et al. 1989). The phenomenon of the reduction of astaxanthin into zeaxanthin was determined in specimens of chum salmon during anadromous migration (Kitahara 1983). This was confirmed next by Ando (1986) and Ando and Hatano (1987). Studies conducted on rainbow trout, *Oncorhynchus mykiss* (Walb.), indicated that astaxanthin is metabolically reduced to zeaxanthin in both wild and farm-raised specimens of this species (Schiedt et al. 1986, Al-Khalifa and Simpson 1988). Additionally, the metabolic reduction of astaxanthin to zeaxanthin also occurs in other non-salmonid marine species such as mackerel, *Gneumathoprus japonicus* Hat., or yellowtail, *Seriola quinqueradiata* Temminck & Schlegel (Matsuno et al. 1985).

As many studies have indicated (Schiedt et al. 1985, Hardy et al. 1990, Marck et al. 1990, Choubert et al. 1994), astaxanthin and canthaxanthin, along with other carotenoids, are absorbed by the alimentary tract and then transferred to the liver, where some may undergo oxidation. As was mentioned earlier in this paper, in healthy specimens carotenoids are linked with protein and transported by the serum to the skin, muscle tissues, and, during spawning, to the female gonads. In specimens from the genus Salmo, this refers to red carotenoids, especially astaxanthin. Adult specimens deposit primarily astaxanthin and canthaxanthin in the muscle tissues (Schiedt et al. 1986), where both of these carotenoids form complexes with actiomyosin (Heumo et al. 1989). Metabolic reduction occurs in the skin and partially in the muscles (Schiedt et al. 1985, Ando 1986). In specimens afflicted with M74 syndrome, it can be supposed that the reduction processes of astaxanthin to zeaxanthin are activated. As a result of this, two oxygen atoms split off from the astaxanthin molecule and, through adonixanthin, a zeaxanthin molecule is formed. Adonixanthin is noted in sea trout eggs (Czeczuga et al. 2002). Perhaps the decrease in red carotenoid concentration should be explained by their usage as antioxidants. In specimens with M74 syndrome, not only do the number of cells in the liver decrease and numerous vacuoles form (Lundström et al. 1996), above all else, liver function is significantly affected. The level of antioxidants decreases significantly, lipid peroxidation increases, enzymes are activated that increase antioxidation protection, and, above all, the level of thiamine decreases (vitamin B_1) (Cowey et al. 1985, Börjeson et al. 1996, Amcoff et al. 1998, Lundström et al. 1999).

Anthropogenic pollution in the Baltic Sea, especially of chloroorganic compounds, also causes reproductive disturbances in other fish species. This affects both the open and coastal waters of the Baltic (Bengtsson et al. 1999). The mortality of cod, *Gadus morhua* L., eggs is high as is larval deformation. Further, high mortality has been noted in herring, *Clupea harengus* L., eggs from the northern part of the Baltic. High mortality has also been observed in European perch, *Perca fluviatilis* L., fry, as have gonad deformations in burbot, *Lota lota* (L.), from the Bay of Bothnia and in roach, *Rutilis rutilis* (L.), from the southwest coast of Finland. This is one of many causes and it might be a fundamental cause of declining population numbers of many marine species of fish in the Baltic Sea. Yolk sac fry mortality syndrome, which is similar to M74, has also been described in coho salmon, *Oncorhynchus kisutch* (Walb.), chinook salmon, *Oncorhynchus tshawytscha* (Walb.), rainbow trout, brown trout, *Salmo trutta* m. *fario* L., and lake trout, *Salvelinus namaycush* (Walb.), from the Laurentian Great Lakes in North America. This is known as Early Mortality Syndrome (EMS) in the literature (Brouwer et al. 1989).

REFERENCES

Al-Khalifa A. S., Simpson K. 1988 – Metabolism of astaxanthin in the rainbow trout (*Salmo gairdneri*) – Comp. Biochem. Physiol. 91B: 563-568.

Amcoff P., Börjeson H., Landergren P., Vallin L., Norrgren L. 1999 – Thiamine (vitamin B_1) concentrations in salmon (*Salmo salar*), brown trout (*Salmo trutta*) and cod (*Gadus morhus*) from the Baltic Sea – Ambio 28: 48-54.

Amcoff P., Börjeson H., Eriksson R., Norrgren L. 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 (Eds) G. McDonald, J. D. Fitzimons and D. C. Honeyfield, American Fisheries Society Symposium 21, Bethesda, Maryland, pp. 26-30.

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

Ando S., Hatano M. 1987 – Metabolic pathways of carotenoids in chum salmon *Oncorhynchus keta* during spawning migration – Comp. Biochem. Physiol. 87B: 411-416.

Backlund P., Holmbom B., Leppäkoski E. 1993 – The Baltic Sea Environment. 5. Industrial Emissions and Toxic Pollutants – Uppsala Univ. Press, Uppsala, 36 pp.

Bartel R. 2000 – Sea trout *Salmo trutta* Linnaeus, 1758 – In: Polish Freshwater Fish (Ed.) M. Brylińska, Wydawnictwo Naukowe PWN, Warszawa, pp. 415-427 (in Polish).

Bengtsson B.-E., Bergman A., Brandt J., Hill C., Johansson N., Sodergren A., Thulin J. 1994 – Reproductive disturbances in Baltic fish – Swedish Environ. Protect. Agen., Stockholm, 24 pp.

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

Börjeson H., Förlin L., Norrgren L. 1996 – Investigation of oxidants and prooxidants in salmon affected by the M74 syndrome – In: Report from the Second Workshop on Reproduction Disturbances in Fish 20-23 Nov. 1995 (Eds.) B.-E. Bengtsson, C. Hill and S. Nellbring, Swedish Environ. Protect. Agen., Report No. 4534, Stockholm, pp. 95-96.

Brouwer A., Reijnders P.J.H., Koeman J.H. 1989 – Polychlorinated biphenyl (PCB) – contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (*Phoca vitulina*) – Aquat. Toxicol. 15: 99-106.

Choubert G., Milicua J.–C.G., Gomez R. 1994 – The transport of astaxanthin in immature rainbow trout *Oncorhynchus mykiss* serum – Comp. Biochem. Physiol. 108A: 1001-1006.

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

Czeczuga B. 1986 – The presence of carotenoids in various species of Lepidoptera – Biochem. System. Ecol. 14: 345-351.

Czeczuga B. 1988 – Carotenoids – In: CRC Handbook of Lichenology (Ed.) M. Galum, CRC Press, Boca Raton, Florida: 25-34.

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

Czeczuga B., Bartel R., Czeczuga-Semeniuk E. 2002 – 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 – Acta Ichth. Piscat. 32: 3-21.

Czeczuga B., Bartel R., Kiziewicz B., Godlewska A., Muszyńska E. 2005 – Zoosporic fungi growing on the eggs of sea trout (*Salmo trutta* m. *trutta* L.) in river water of varied trophicity – Pol. J. Environ. Stud. 14: (in press).

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

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

Foss P., Storebakken T., Austreng E., Liaaen-Jensen S. 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.

Goodwin T.W. 1981 – The Biochemistry of Carotenoids – Plants. Chapman and Hall, London and New York, 377 pp.

Hardy R.W., Torrissen O.J., Scott T.M. 1990 – Absorption and distribution of 14C-labbeled canthaxanthin in rainbow trout (*Oncorhynchus mykiss*) – Aquaculture 87: 331-340.

Heumo H., Hata M., Hata M. 1989 – Astaxanthin and (or) canthaxanthin-actiomyosin complex in salmon muscle – Nippon Suison Gakkaishi 55: 1583-1589.

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 – Behavior of carotenoids in the chum salmon (*Oncorhynchus keta*) during anadromous migration – Comp. Biochem. Physiol. 76B: 97-101.

Kraus L., Koch A. 1996 – Dünnschichtchromatographic – Springer, Berlin, 205 pp.

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

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

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

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

Mantoura R.F.C., Llewellyn C.A. 1983 – The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural water by reverse-phase high-performance liquid chromatography – Anal. Chim. Acta 151: 297-314.

March B.E., Hajen W.E., Deacon G., MacMillan C., Walsh M. G. 1990 – Intestinal absorption of astaxanthin, plasma astaxanthin concentration, body weight, and metabolic rate as determinants of flesh pigmentation in salmonid fish – Aquaculture 90: 313-322.

Matsuno T., Katsuama M., Maoka T., Hirono T., Komori T. 1985 – Reductive metabolic pathways of carotenoids in fish (3S, 3'S)-astaxanthin to tunaxanthin A, B and C – Comp. Biochem. Physiol. 80B: 779--789.

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

Nakano T., Tosa M., Takeuchi M. 1995 – Improvement of biochemical features in fish health by red yeast and synthetic astaxanthin – J. Agric. Food Chem. 43: 1570-1573.

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

Pickova J. Kiessling A., Pettersson A., Dutta P.C. 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. 120A: 256-261.

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

Schiedt K., Vecchi M., Glinz E. 1986 – Astaxanthin and its metabolites in wild rainbow trout (*Salmo gairdneri* R.) – Comp. Biochem. Physiol. 83B: 9-12.

Soivio A. 1996 – M74 in Finland – In : Report from the Second Workshop on Reproduction Disturbances in Fish (Eds.) B.-E. Bengtsson, C. Hill and S. Nellbring, Swedish Environ. Protect. Agen., Stockholm, Raport No. 4534: 42-43.

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

Straub O. 1987 – Key to Carotenoids – Birkhäuser Verlag, Basel-Boston, 296 pp.

Torrissen O.J., Hardy R.W., Shearer K. D. 1989 – Pigmentation of salmonids - carotenoid deposition and metabolism – Crit. Rev. Aquat. Sci. 1(2): 209-225.

Vetter W., Englert G., Rigassi N., Schwieter U. 1971 – Spectroskopic methods – In : Carotenoids (Ed.) O. Isler, Birghauser Verlag, Basel-Boston: 189-229.

Received – 12 November 2004

Accepted – 23 February 2005

STRESZCZENIE

WYSTĘPOWANIE SYNDROMU M74 U TROCI (*SALMO TRUTTA* M. *TRUTTA* L.) WCHODZĄCYCH NA TARŁO DO RZEK POLSKI W DORZECZU WISŁY

Badano występowanie syndromu M74 u samic troci, *Salmo trutta* m. *trutta,* należących do dwóch populacji wchodzących na tarło do rzek polskich jesienią 2003 i trzeciej hodowanej w stawach. Przebadano razem ikrę 250 samic, w tym 10 z Parsęty, 100 z Miastka i 140 ze Świbna (rzeka Wisła, północna Polska). W badaniach tych porównywano koncentrację w ikrze czerwonych (astaksantyna, kantaksantyna) oraz żółtych karotenoidów (luteina, zeaksantyna). Poszczególne karotenoidy oznaczano stosując chromatografię kolumnową (CC), cienkowarstwową (TLC) oraz wysokosprawną chromatografię cieczową (HPLC). Pod względem zabarwienia ikra badanych samic troci należała do trzech grup: żółta, żółto-pomarańczowa i pomarańczowa. W ikrze badanych samic troci ustalono obecność 15 karotenoidów, wśród których w ikrze pomarańczowej i żółto-pomarańczowej dominowały karotenoidy czerwone, w ikrze żółtej – żółte (rys. 1, tab. 1 i 2). Ustalono występowanie syndromu M74 u 35 samic, co stanowi 14,0% wszystkich przebadanych samic troci.