## **ORIGINAL PAPERS**

# LEVEL OF MAGNESIUM IN TISSUES AND ORGANS OF FRESHWATER FISH\*

### Ewa Brucka-Jastrzębska, Dorota Kawczuga

Chair of Physiology University of Szczecin

#### Abstract

The aim of the study has been to estimate the effect of culture conditions and a culture site on magnesium (Mg) concentrations in freshwater fish. The study encompassed tissues (blood) and organs (gills, liver, kidney, dorsal muscles) of five fish species: common carp (*Cyprinus carpio* L.), rainbow trout (*Oncorhynchus mykiss* Walbaum), Siberian sturgeon (*Acipenser baeri* Brandt), northern pike (*Esox lucius* L.) and grass carp (*Ctenopharyngodon idella* Valenciennes).

A total of 125 fish comprised 25 individuals of each species, aged 6, 12, 18 and 24 months. The fish were cultured in privately owned fish breeding ponds (Western Pomerania, Poland). Tissue and organ samples were wet mineralised in concentrated HNO<sub>3</sub> in a CEM MDS 2000 microwave oven. Magnesium concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-MS) in a Jobin Yvon type JY-24 apparatus. The research had an approval of the Polish Local Ethics Committee no 9/05. The magnesium concentration in the tissues and organs ranged from  $26.3 \div 174.2 \text{ mg kg}^{-1}$  w.w. The lowest Mg concentration was found in the gills of rainbow trout ( $26.3 \pm 5.4 \text{ mg kg}^{-1}$  w.w.), and the highest – in the liver of rainbow trout ( $174.2 \pm 27.6 \text{ mg kg}^{-1}$  w.w.). The magnesium concentrations were also significantly affected by the type of feed.

Key words: common carp, rainbow trout, Siberian sturgeon, northern pike, grass carp, magnesium.

dr Ewa Brucka-Jastrzębska, Chair of Physiology, University of Szczecin, av. Piastów 40 B, Szczecin, e-mail: ewabrucka@poczta.onet.pl

<sup>\*</sup>The study has been financially supported by the Ministry of Science and Higher Education, grant no N 304 026 31/0814.

#### POZIOM MAGNEZU W TKANKACH I NARZĄDACH RYB SŁODKOWODNYCH

#### Abstrakt

W procesie rozwoju organizmów zasadniczą rolę odgrywają warunki hodowli. Ryby słodkowodne charakteryzują się dużym zróżnicowaniem pod względem miejsca bytowania w toni wodnej, warunków hydrochemicznych i rodzaju pożywienia.

Celem pracy było zbadanie wpływu wieku ryb, warunków termicznych i zanieczyszczeń antropogenicznych na poziom magnezu w tkankach (krew) i narządach (skrzela, wątroba, nerki i mięśnie grzbietowe) pięciu gatunków ryb: karpia (Common carp L.), pstrąga tęczowego (Oncorhynchus mykiss Walbaum), jesiotra syberyjskiego (Acipenser baeri Brandt), szczupaka pospolitego (Esox lucius L.) i amura białego (Ctenopharyngodon idella Valenciennes). Badania przeprowadzono w 6., 12., 18. i 24. miesiącu życia. Ryby do badań pobierano z prywatnego ośrodka hodowlanego na Pomorzu Zachodnim (Polska). Próbki tkanek i narządów zmineralizowano na mokro w stężonym HNO<sub>3</sub> w piecu mikrofalowym CEM MDS 2000. Mg oznaczono z użyciem emisyjnej spektrometrii atomowej w plazmie indukcyjnie sprzężonej (ICP-AES) w aparacie Jobin Yvon typ JY-24. Na przeprowadzenie badań uzyskano zgodę (nr 9/05) Lokalnej Komisji ds. Etyki. Poziom Mg w tkankach i narządach ryb kształtował się w zakresie 26.3±174.2 mg kg<sup>-1</sup> w.w. Najniższy poziom Mg oznaczono w skrzelach pstrąga tęczowego (26.3±7.4 mg kg<sup>-1</sup> w.w.), a najwyższy w jego wątrobie (174.2±27.6 mg kg<sup>-1</sup> w.w.). Można wnioskować, że stężenie magnezu były zależne od rodzaju spożywanej paszy.

Słowa kluczowe: karp, pstrąg tęczowy, jesiotr syberyjski, szczupak, amur biały, magnez.

### INTRODUCTION

Magnesium as a metal itself was first obtained in England in 1808, by Sir Humphry Davy, who performed electrolysis of a mixture of magnesia and mercury oxide. Antoine Bussy prepared magnesium in a consistent form in 1831. Davy's first suggestion for the name was magnium, but now the name magnesium is used. Because of some important interaction between phosphate and magnesium ions, the latter are essential to the basic nucleic acid chemistry of life and are crucial for all cells of all known living organisms. Over 300 enzymes require the presence of magnesium ions for their catalytic action, including all enzymes utilizing or synthesizing ATP, or those that use other nucleotides to synthesize DNA and RNA (FLOOR 2006). Magnesium compounds are used medicinally as common laxatives, antacids and in a number of situations where stabilization of abnormal nerve excitation and blood vessel spasm is required. High solubility of magnesium ions in water helps ensure that it is the third most abundant element dissolved in seawater. Magnesium ions are sour in taste, and in low concentrations help to impart natural tartness to fresh mineral waters. The free element (metal) is not found naturally on Earth. The free metal burns with a characteristic brilliant white light, making it a useful ingredient in flares. The metal is now mainly obtained by electrolysis of magnesium salts obtained from brine.

Breeding conditions, e.g. feeding, have a significant effect on the development of fish. In the growth and development of both terrestrial and aquatic animals, culture conditions play a key role. A variety of chemicals occur in the natural environment, most of which, however, do not penetrate inside living organisms in significant amounts despite being in direct contact. Due to human actions, man-made pressure on the environment is increasing. It can interfere, for instance, in the homeostasis of aquatic environments, which mau cause soem disturbance in the fish body balance.

During the evolution, countless relationships have developed between organisms and their environment. When these relationships are disrupted by altered environmental conditions, diseases or even death of organisms may occur. Elements of the environment such as water, air and food deliver essential components for organisms, but at the same time they may be sources of xenobiotics and harmful substances, which can impair life functions of organisms.

The control of levels of micro- and macroelements in fish organisms is a significant diagnostic research tool, as it shows the physiological condition of organisms. Concentration of some mineral elements in fish depends mainly on the culture and water type in which the fish are bred, as well as the season of the year and the feeds the fish receive. All the elements affect the homeostatic behavior of fish, which may vary due to the excess or deficiency of any of the factors. Their surplus or shortage may lead to serious disorders of the biochemical processes, which can result in many diseases.

The aim of this study has been to evaluate the effect of culture conditions and culture site on levels of the selected macroelement (Mg) in five species of freshwater fish: rainbow trout (*Oncorhynchus mykiss* Walbaum), common carp (*Cyprinus carpio* L.), Siberian sturgeon (*Acipenser baeri* Brandt), northern pike (*Esox lucius* L.) and grass carp (*Ctenopharyngodon idella* Valenciennes). The study encompassed tissues (blood) and organs (kidney, liver, gills and dorsal muscles) in fish.

### MATERIAL AND METHODS

The study involved 125 individuals of freshwater fish reared in commercial fish farms in West Pomeranian Province, Poland. The fish were represented by 25 individuals of each of the five species: rainbow trout, common carp, Siberian sturgeon, northern pike and grass carp. The research had an approval of the Polish Local Ethics Committee no 9/05.

The fish aged from 6 to 24 months, weighed from 147.8 to 985.4 g and measured from 18.4 to 39.5 cm (total length). The fish were collected four times: in April (6 month – spring), October (12 month – autumn), April (18 month – spring) and October (24 month – autumn).

The fish were fed Aller Aqua pelleted feeds (Table 1), each species with an appropriate feed type. All the fish feed products were produced by extrusion. The fish feed must cover the basic metabolism of the fish and ensure healthy growth. In order to meet these requirements, the fish feed composition must fulfill all requirements for nutrients, vitamins (A, E and D<sub>3</sub>) and minerals. The daily food ration was  $3.4\pm0.8$  g per kg fish. The fish were fed twice a day.

Table 1

	Examined fish					
Feed parameters	common carp	rainbow trout	Siberian sturgeon	northern pike	grass carp	
Name of feed	Aller classic	Aller 576	Aller M/L	Aller M/L	Aller classic	
Composition						
Size of feed (mm)	5.0	M/L	M/L	M/L	5.0	
Protein (%)	35.0	42.0	45.0	45.0	35.0	
Fat (%)	9.0	30.0	15.0	15.0	9.0	
Carbohydrates (%)	43.0	14.0	21.0	21.0	43.0	
Ash (%)	7.0	7.5	8.0	8.0	7.0	
Fiber (%)	5.0	1.0	2.5	2.5	5.0	
All-out energy (gross energy) (Kcal MJ <sup>-1</sup> )	4325/18.1	5823/2.3	4924/20.5	4924/20.5	4325/18.1	
Digestible energy (Kcal MJ <sup>-1</sup> )	3353/14.0	4833/20.2	38887/16.2	38887/16.2	3353/14.0	
Nitrogen (d.m.* %)	5.2	7.1	7.9	7.9	5.2	
Phosphorus (d.m. %)	1.3	7.0	1.2	1.2	1.3	
Energy in dry matter (Kcal MJ <sup>-1</sup> )	4701/19.6	6162/25.7	5381/022.5	5381/022.5	4701/19.6	

Composition of Aller Aqua feed for individual species of freshwater fish

\* d.m. - dry mass

Table 2 presents chemical and biochemical parameters of water in which the fish were kept. Fish behaviour and appearance were recorded. Intravital examination involved observation of fish behaviour, assessment of rearing conditions, as well as evaluation of the quality and general appearance of fish skin, fins, eyes and gills. *Post mortem* examination involved autopsy to verify if there were any anatomical or pathological changes in internal organs.

For chemical analysis, samples of tissues (blood) and organs (skin, liver, kidney, dorsal muscles) were collected from each fish. The collected material was stored at -20°C. Prior to analysis, 1-g subsamples of organs and tissues, weighed to the nearest 0.001 g, were wet mineralised in 3 cm<sup>3</sup> HNO<sub>3</sub> in a CEM MDS 2000 microwave oven. The solutions obtained were quantita-

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	April/ Spring	October/ Autumn	April/ Spring	October/ Autumn	Statistically
Water parameters		month of	f fish life	1	significant
1	6	12	18	24	$P \le 0.01$
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	
Temperature (°C)	$11.20 \pm 2.88$	$6.80 \pm 3.40$	$13.80 \pm 3.40$	$7.85 \pm 4.24$	a, b, c
pH	$7.78 \pm 0.55$	$7.38 \pm 0.95$	$7.48 \pm 0.95$	$7.68 \pm 0.91$	-
Dissolved oxygen (mg dm <sup>-3</sup> )	$7.81 \pm 0.35$	$7.98 \pm 0.63$	$7.94 \pm 0.55$	$7.33 \pm 0.73$	-
Oxygen saturation (%)	$78.21 \pm 2.50$	$79.51 \pm 3.48$	78.79 ± 4.28	$76.55 \pm 4.41$	-
Alkalinity (mmol dm <sup>-3</sup> )	$1.75 \pm 0.80$	$1.69 \pm 0.88$	$1.65 \pm 0.68$	$1.78 \pm 0.78$	-
Water hardness (mg dm <sup>-3</sup> )	8.25 ± 1.08	$7.25 \pm 1.18$	$7.75 \pm 1.58$	7.36 ± 1.35	-
ChOD (mg dm <sup>-3</sup> )	$1.67 \pm 1.32$	$1.71 \pm 1.09$	$1.66 \pm 1.12$	$1.62 \pm 1.19$	-
BOD (mg dm <sup>-3</sup> )	$4.34 \pm 1.22$	$4.61 \pm 1.33$	$4.81 \pm 1.32$	$4.75 \pm 1.13$	-
${\rm NH_4^{+-N}} \ ({\rm mg} \ {\rm dm}^{-3})$	$1.18 \pm 0.75$	$1.34 \pm 0.48$	$1.26 \pm 0.29$	$1.39 \pm 0.33$	-
$NO_3^{-}-N \ (mg \ dm^{-3})$	$7.41 \pm 1.05$	$6.11 \pm 1.15$	$6.78 \pm 0.55$	$6.12 \pm 1.45$	<i>a</i> , <i>d</i>
$NO_2^{-}-N \ (mg \ dm^{-3})$	$0.68 \pm 0.16$	$0.48 \pm 0.36$	$0.56 \pm 0.26$	$0.52 \pm 0.58$	a
$PO_4^{3-}-P (mg \ dm^{-3})$	$0.15 \pm 0.07$	$0.14 \pm 0.05$	$0.13 \pm 0.03$	$0.16 \pm 0.03$	-
$Ca^{2+}$ (mg dm <sup>-3</sup> )	$66.51 \pm 4.25$	$59.51 \pm 3.75$	$69.31 \pm 2.79$	$55.54 \pm 3.25$	<i>c</i> , <i>d</i>
$Cd^{2+}$ (mg dm <sup>-3</sup> )	$0.02 \pm 0.01$	$0.01 \pm 0.01$	$0.02 \pm 0.01$	$0.02 \pm 0.01$	-
$Pb^{2+}$ (mg dm <sup>-3</sup> )	$0.03 \pm 0.05$	$0.03 \pm 0.07$	$0.03 \pm 0.07$	$0.04 \pm 0.057$	-
$Mg^{2+}$ (mg dm <sup>-3</sup> )	$16.33 \pm 4.05$	$15.23 \pm 3.33$	$17.44 \pm 4.47$	$13.79 \pm 3.41$	с

Hydrochemical parameters at the fish farm 30 km from Szczecin

a – statistically significant differences in the water parameter between the 6<sup>th</sup> and 12<sup>th</sup> month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter between the 12<sup>th</sup> and 18<sup>th</sup> month of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter between the 18<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ).

tively transferred to polyethylene vials and brought up to 25 g with deionised water. Magnesium was determined with inductively coupled plasma atomic emission spectrometry (ICP-AES) in a JY-24 Jobin Yvon apparatus. Tissue concentrations of metals were reported as mg kg<sup>-1</sup> wet weight (mg kg<sup>-1</sup> w.w.). The results were subjected to statistical treatment with Statistica 6.0 software. Analyses of variance (ANOVA) were performed at the significance levels of P = 0.01.

### **RESULTS AND DISCUSSION**

The growth in industrial and agricultural production has resulted in an increasing number of systems impacted by contaminants present in wastewater releases. For example, heavy metals (Cd, Pb, Cu, Zn) are known to accumulate in organs of fish (BALINT et al. 1997). These metals pollute aquatic and terrestrial ecosystems, adversely affecting the environment and inhabiting organisms. High concentration of metals in fish tissues can lead to a redox reaction, generating free radicals, especially reactive oxygen species (DAUTREMEPUITS et al. 2002). These highly reactive compounds may induce tissue alternations and change some physiological responses of fish (PARIS--PALACIOS et al. 2000, VARANKA et al. 2001). Magnesium plays a regulatory role in prooxidant and antioxidant processes (LOPEZ-TORRES et al. 1993, OZMEN et al. 2004). Aquatic organisms are more sensitive to exposure and toxicity compared to terrestrial ones, including mammals, and in this respect they may provide experimental data for evaluation of subtle effects of oxidative stress, mutagenicity and other adverse effects of pollutants (VALAVANIDIS et al. 2006). Moreover, the water parameters can affect water organisms, for example the influence of high temperature on aquatic biocenosis manifests itself as an increase in biological production rate and also shorter lifecycles of aquatic organisms, which die in large numbers due to the lack of synchronisation with climate rhythms. This results in the accumulation of organic matter and an increase in biological oxygen demand, along with a decrease in oxygen solubility and availability.

Nutritional studies have shown that minerals may play a crucial role in preventing oxidative stress. Fluctuations in their concentrations may disrupt internal homeostasis and produce various pathological conditions. Toxic effects of metals on different tissues and organs involve structural damage and functional disorders, which may be reflected by changes in blood composition and levels of ions, proteins, hormones or glucose and its metabolites, as well as by changed enzyme activities.

Intravital and *post mortem* examination showed no changes in fish behaviour, as well as in their external and internal appearance. Comparison of water parameters (Table 2) revealed only slight differences between the dates of taking samples.

The research has shown that Mg concentration changed during the growth of fish. The average magnesium content in blood ranged from 87.7 to 168.2 mg kg<sup>-1</sup> w.w. (Table 3). The highest magnesium levels were detect-

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		Statistically			
TP: 1 .		significant			
Fish species	6	12	18	24	differences
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	<i>I</i> ≥0.01
Common carp	$103.9 \pm 18.3$	$163.6 \pm 35.1$	$126.2 \pm 23.5$	$161.3 \pm 23.9$	a, b, c, d
Rainbow trout	$118.3 \pm 35.4$	$163.5 \pm 25.3$	$165.6 \pm 35.1$	$168.2 \pm 24.4$	a, d
Siberian sturgeon	$88.5 \pm 26.2$	$144.5 \pm 26.3$	$110.8 \pm 32.9$	$141.5 \pm 34.2$	a, b, c, d
Northern pike	$85.3 \pm 24.5$	$91.5 \pm 18.6$	$97.1 \pm 21.7$	$89.1 \pm 25.4$	ns
Grass carp	$105.4 \pm 27.6$	$99.4 \pm 27.4$	$98.5 \pm 25.1$	$104.3 \pm 24.1$	ns

Mg level in blood of five freshwater fish species aged 6, 12, 18 and 24 months

a – statistically significant differences in the water parameter between the 6<sup>th</sup> and 12<sup>th</sup> month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter between the 12<sup>th</sup> and 18<sup>th</sup> month of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter between the 18<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); n – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); n – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); n – no significant differences

ed in blood of rainbow trout (168.2±24.4 mg kg<sup>-1</sup> w.w.). The lowest magnesium levels were found in blood of northern pike (85.3±24.5 mg kg<sup>-1</sup> w.w.). Magnesium was distributed in fish blood according to the following pattern of decreasing concentrations: northern pike > grass carp > Siberian sturgeon > common carp > rainbow trout. Statistically significant differences were also detected in the blood Mg levels during the growth of common carp and rainbow trout (Table 3).

The average Mg content in gills ranged from  $26.3\div91.2 \text{ mg kg}^{-1}$  w.w. (Table 4). The highest Mg levels were detected in gills of grass carp (91.2±12.4 mg kg<sup>-1</sup> w.w.) and the lowest ones were found in gills of rainbow trout ( $26.3\pm5.4 \text{ mg kg}^{-1}$  w.w.). Magnesium was distributed in fish gills according to the following pattern of decreasing concentrations: rainbow trout > Siberian sturgeon > northern pike > common carp > grass carp. Statistically significant differences were also found in Mg levels in gills during the growth of common carp, rainbow trout and Siberian sturgeon (Table 4).

The average Mg content in liver ranged from  $35.3 \div 174.2 \text{ mg kg}^{-1}$  w.w. (Table 5). The highest Mg levels were detected in liver of rainbow trout (174.2±27.6 mg kg<sup>-1</sup> w.w.). The lowest Mg levels were found in liver of grass carp ( $35.3\pm9.5 \text{ mg kg}^{-1}$  w.w.). Magnesium was distributed in fish liver according to the following pattern of decreasing concentrations: grass carp > northern pike > Siberian sturgeon > rainbow trout > common carp. We also found statistically significant differences in the liver Mg concentrations during the growth of rainbow trout, Siberian sturgeon and northern pike (Table 5).

		· Statistically significant			
Fish species	6	12	18	24	differences
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	<i>F</i> ≤0.01
Common carp	$43.6 \pm 11.5$	$72.6 \pm 24.4$	$62.2 \pm 13.5$	$74.3 \pm 11.9$	a, b, c, d
Rainbow trout	$26.3 \pm 5.4$	$41.5 \pm 18.3$	$43.6 \pm 22.1$	$62.2 \pm 17.4$	a, c, d
Siberian sturgeon	$43.2 \pm 13.2$	$51.3 \pm 12.3$	$47.8 \pm 11.9$	$58.3 \pm 13.2$	d
Northern pike	$64.3 \pm 17.5$	$68.5 \pm 6.6$	$67.4 \pm 11.3$	$59.8 \pm 19.4$	ns
Grass carp	$85.5 \pm 17.3$	$91.2 \pm 12.1$	$78.3 \pm 15.4$	$84.6 \pm 17.8$	ns

Mg level in gills of five freshwater fish species aged 6, 12, 18 and 24 months

a – statistically significant differences in the water parameter between the 6<sup>th</sup> and 12<sup>th</sup> month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter between the 12<sup>th</sup> and 18<sup>th</sup> month of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter between the 18<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); n – no significant differences

Table 5

		Statistically				
<b>T</b> : 1 · ·		month of fish life				
Fish species	6	12	18	24	differences	
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	1 20.01	
Common carp	$144.6 \pm 35.6$	$161.5 \pm 45.2$	$154.6 \pm 23.2$	$158.1 \pm 26.4$	ns	
Rainbow trout	$102.1 \pm 31.8$	$169.6 \pm 18.5$	$103.1 \pm 38.1$	$174.2 \pm 27.6$	a, b, c, d	
Siberian sturgeon	$41.3 \pm 7.9$	$114.2 \pm 18.1$	$61.2 \pm 28.2$	$102.8 \pm 28.8$	a, b, c, d	
Northern pike	$65.5 \pm 21.3$	$63.7 \pm 16.6$	$79.6 \pm 17.7$	$89.3 \pm 19.6$	<i>b, d</i>	
Grass carp	$35.3 \pm 9.5$	$46.1 \pm 8.4$	$48.5 \pm 18.3$	$43.4 \pm 18.8$	ns	

Mg level in liver of five freshwater fish species in 6, 9, 12 and 24 months

a – statistically significant differences in the water parameter among the 6 and 12 month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter among the 12 and 18 of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter among the 18 and 24 of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter among the 6 and 24 of fish life ( $P \le 0.01$ ); ns – no significant differences

The average Mg content in kidneys ranged from  $66.1 \div 168.7 \text{ mg kg}^{-1}$ w.w. (Table 6). The lowest Mg levels were detected in kidneys of Siberian sturgeon ( $66.1 \pm 17.9 \text{ mg kg}^{-1}$  w.w.). The highest Mg levels were found in kidneys of northern pike ( $168.7 \pm 32.6 \text{ mg kg}^{-1}$  w.w.). Magnesium was distributed in fish kidneys according to the following pattern of decreasing concen-

		Statistically significant			
<b>D</b> . 1					
Fish species	6	12	18	24	differences
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	<i>I</i> ≤0.01
Common carp	$73.6 \pm 25.6$	$141.8 \pm 25.2$	$65.3 \pm 38.6$	$145.3 \pm 34.4$	a, b, c, d
Rainbow trout	$83.4 \pm 22.9$	$147.3 \pm 37.9$	$87.4 \pm 21.7$	$110.3 \pm 31.4$	a, b, c, d
Siberian sturgeon	$66.1 \pm 17.9$	$74.1 \pm 21.8$	$73.2 \pm 23.2$	$68.8 \pm 15.2$	ns
Northern pike	$155.3 \pm 31.7$	$168.7 \pm 32.6$	$163.5 \pm 26.6$	$151.9 \pm 26.4$	ns
Grass carp	$85.4 \pm 24.6$	$91.2 \pm 16.7$	$93.7 \pm 25.4$	$128.9 \pm 28.8$	<i>c</i> , <i>d</i>

Mg level in kidney of five freshwater fish species aged 6, 12, 18 and 24 months

a – statistically significant differences in the water parameter between the 6<sup>th</sup> and 12<sup>th</sup> month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter between the 12<sup>th</sup> and 18<sup>th</sup> month of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter between the 18<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ );  $n = -10^{10}$  month of fish life ( $P \le 0.01$ ); n = -

trations: Siberian sturgeon > grass carp > common carp > rainbow trout > northern pike. Statistically significant differences were also found in Mg levels in kidneys during the growth of common carp, rainbow trout and grass carp (Table 6).

The average Mg content in dorsal muscles ranged from  $46.6\div 143.1$  mg kg<sup>-1</sup> w.w. (Table 7). The highest Mg levels were detected in dorsal muscles of northern pike ( $143.1\pm 28.4$  mg kg<sup>-1</sup> w.w.). The lowest Mg levels were found in dorsal muscles of common carp ( $46.6\pm 8.9$  mg kg<sup>-1</sup> w.w.). Magnesium was distributed in fish dorsal muscles according to the following pattern of decreasing concentrations: common carp > Siberian sturgeon > grass carp > rainbow trout > northern pike. Statistically significant differences were also found in Mg levels in dorsal muscles during the growth of common carp, rainbow trout and Siberian sturgeon (Table 7).

It was found that the breeding site significantly affected the Mg concentration in the tissues and organs among individual freshwater fish species. The magnesium concentration were also significantly affected by the type of feed. All the applied pellet feeds were different in the concentration of fat (9-15%) and protein (35-45%) – Table 1. It was noticed that in common carp and grass carp fed Aller Aqua Aller classic pellet pasture, or in northern pike and Siberian sturgeon fed Aller Aqua Aller M/L, the Mg concentrations were higher compared to rainbow trout fed Aller Aqua Aller 576 pellet pasture. The results allow us to state that the culture site, culture conditions and the feeding type have a significant effect on the Mg level in tissues and organs of the examined fish.

		Statistically significant			
TP: 1 .					
Fish species	6	12	18	24	differences
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	<i>I</i> ≤0.01
Common carp	85.4± 24.1	$57.6 \pm 15.5$	$46.6 \pm 8.9$	$82.6 \pm 21.7$	а, с
Rainbow trout	87.2 ±33.5	$61.3 \pm 14.6$	$99.3 \pm 27.2$	$59.5 \pm 25.4$	a, b, c, d
Siberian sturgeon	$63.6 \pm 16.2$	$89.7 \pm 24.4$	$47.2 \pm 15.2$	$46.7 \pm 14.8$	a, b, d
Northern pike	$125.7 \pm 32.7$	$138.1 \pm 24.6$	$138.3 \pm 24.7$	$143.1 \pm 28.4$	ns
Grass carp	$72.4 \pm 24.3$	$68.7 \pm 27.3$	$71.5 \pm 24.7$	$69.5 \pm 11.3$	ns

Mg level in dorsal muscles of five freshwater fish species aged 6, 12, 18 and 24 months

a – statistically significant differences in the water parameter between the 6<sup>th</sup> and 12<sup>th</sup> month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter between the 12<sup>th</sup> and 18<sup>th</sup> month of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter between the 18<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ );  $n = -10^{10}$  statistically significant differences the 0<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ );  $n \le -10^{10}$  statistically significant differences the 0<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ );  $n \le -10^{10}$  statistically statistically statistically significant differences the 0<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ );  $n \le -10^{10}$  statistically statistic

Many authors reported considerably higher levels of this element in muscles of freshwater and marine fish (PUJIN et al. 1990, KARGIN 1996, GRO-SHEVA et al. 2000, JURKIEWICZ-KARNAKOWSKA 2001). Magnesium is weakly accumulated in fish tissues, as it is retained by gills, where the metal is deposited in large amounts (WITESKA 2003). This may be explained by the fact that Mg penetrates to blood less easily than other metals (Ni, Cd, Pb). Levels of some bioelements in fish bodies depend on culture methods, season of the year, feed quality and water chemistry. All these factors together influence the physiological condition of fish, which can be disturbed by excess or deficiency of minerals. Surplus or shortage of minerals may seriously disturb biochemical processes and upset internal homeostasis, leading in consequence to various diseases. TACON (1992) reported that disorders occurred in organisms of various fish species due to deficiency or excess of macroelements, which were caused by avitaminosis or poisoning and improper nutrition. It is therefore important to monitor levels of macroelements in fish organisms and tissues.

Among the examined freshwater fish species, statistically significant differences in the levels of the macroelement were observed. The analysed bioelement (Mg), which by some is regarded as one of the most important macronutrients, was reported to accumulate in excess during diseases caused by viral or bacterial conditions, and during increased activity of hepatocytes (POURAMAHAD, O'BRIEN 2000, LUSHCHAK et al. 2005). Levels of magnesium recorded in this study were not high (Tables 3, 4, 5, 6, 7) and remained within the normal range for salmonids (*Salmonidae*) and cyprinids (*Cyprinidae*). For the sturgeon family (*Acipenseridae*), northern pike (*Esox lucius* L.) and grass carp (*Ctenopharyngodon idella* Valenciennes), an accurate normal range of magnesium remains undetermined.

In our research, we have observed that magnesium concentration in fish blood of common carp and rainbow trout (Table 3), in gills of common carp, rainbow trout and Siberian surgeon (Table 4), in liver of rainbow trout and northern pike (Table 5) in kidney of grass carp (Table 6) and in muscles of northern pike decreased with the age of fish. Another observation was that the magnesium concentration in kidneys of northern pike increased (Table 6).

OIKARI et al. (1985) have shown that an infusion of magnesium salt into the body cavity of freshwater-adapted fish (rainbow trout) affects the magnesium concentration in plasma. Magnesium could either be reabsorbed or secreted in control freshwater-adapted trout, apparently as a function of nutritional status. Fish could switch from reabsorption to secretion in response to magnesium loading. It is suggested that freshwater fish eliminate excess dietary magnesium renally (OIKARI et al. 1985). Variability of metal concentrations in freshwater fish must be seen from a wider perspective of other variables such as habitat, seasonal variations, age of fish, Fulton's condition factor and individual ability for metal uptake (ALLEN 1993, CANLI, ATLI 2003, LOPEZ-TORRES et al. 1993, MARTINEZ-ALVAREZ et al. 2005, RITOLA et al. 2002, SVOBODOVA et al. 1997).

Fish are characterized by species-specific and seasonal changeability of macroelements. STOSIK and DEPTULA (2000) found changes in magnesium concentrations due to the season of the year and the change in the level of lymphocytes in the examined fish. According to these authors, the above changes resulted from a close relationship of the season and the solar exposure. In many other research projects, it has been shown that Mg levels were different depending on the temperature, season, sex, feeding type and culture type. (THOMAS et al. 1999). We have found that feeding common carp, Siberian sturgeon and rainbow trout Aller Aqua pellet pasture affected the concentration of the analyzed element. The results have confirmed that the content of magnesium was within the physiological reference ranges of fish. The differences found in the bio-element levels resulted from individual and seasonal variability typical of fish. Due to their environmental requirements, fish may be regarded as indicators, which supply information on the degree of pollution of the aquatic environment.

### CONCLUSIONS

1. The culture site was found to have statistically significant influence on the magnesium concentrations in organs and tissues in the examined freshwater fish species.

2. We have found that feeding common carp, Siberian sturgeon, rainbow trout, northern pike and grass carp (Aller Aqua pellet pasture) affected the concentration of the analyzed element.

3. The differences in the concentration of magnesium are a result of individual species differences.

4. Mg concentration in the analyzed organs and tissues of freshwater fish were significantly varied.

#### REFERENCES

- ALLAN P. 1993. Effects of acute exposure to cadmium (II) chloride and lead (II) chloride on the hematological profile of Oreochromis aureus (Steindachner). Comp. Biochem. Physiol. C, 105: 213-217.
- BÁLINT T., FERENCZY J., KÁTIA F. 1997. Similarities and differences between the massive eel (Anguilla anguilla L.) devastations that occurred on Lake Balaton in 1995 and 1995. Ecotoxicol. Environ. Safety, 37: 17-23.
- CANLI M., ATLI G. 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ. Pollut., 121: 129-136.
- DAUTREMEPUITS C., BETOULLE S., VERNET G. 2002. Antioxidant response modulated by copper in healthy or parasitized carp (Cyprinus carpio L.) by Ptychobothrium (Cestoda). Bioch. Bioph. A, 1573: 4-8.
- FLOOR A.J. 2006. The chemical composition of seawater. http://www.seafriends.org.nz/oceano/seawater.htm#composition.
- GROSHEVA E.I., VORONSKAYA G.N., PASTUKHOVE M.V. 2000. Trace element biovailability in Lake Baikal. Aquat. Ecosyst. Health Mange., 3: 229-234.
- JURKIEWICZ-KARNAKOWSKA E. 2001. Heavy metals (Cu, Zn, Mn, Fe, Pb and Cd) in short food chains in the lowland dam reservoir (Zegrzyński Reservoir, Central Poland). Ecohydrol. Hydrobiol., 1(4): 449-456.
- KARGIN F. 1996. Seasonal changes of levels of heavy metals in tissues of Mullus barbatus and Sparus aurata collected from Iskenderun gulf (Turkey). Water, Air Soil Pollut., 90: 557-562.
- LOPEZ-TORRES M., PEREZ-CAMPO R., CADENAS S., ROJAS C., BARJA G.A. 1993. Comparative study of free radicals in vertebrate. Non-enzymatics, antioxidants and oxidative stress. Comp. Biochem. Physiol. B, 105: 757-763.
- LUSHCHAK V.I., BAGNYUKOVA T.V., LUSHCHAK O.V., STOREY J.M., STOREY K.B. 2005. Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (Cyprinus carpio) tissues. Int. J. Bioch. Cell Biol., 37: 1319-1330.
- MARTINEZ-ALVAREZ R.M., MORALES A.E., SANZ A. 2005. Antioxidant defenses in fish and abiotic factors. Rev. Fish Biol. Fish, 15: 75-88.
- OIKARI A.O., RANKIN J.C. 1985. Renal excretion of magnesium in a freshwater teleost, Salmo gairdneri. J. Exp. Biol. Jul., 117: 319-333.

- OZMEN I., BAYIR A., CENGIZ M., SIRKECIOGLU A.N., ATAMANALP M. 2004. Effects of water reuse system on antioxidant enzymes in rainbow trout (Oncorhynchus mykiss W., 1792). Vet. Med.-Czech., 49(10): 373-378.
- PARIS-PALACIOS S., BIAGANNTI-RISBOURG S., VERNET G. 2000. Biochemical and (ultra)structural hepatic perturbation of Brachydanio rerio (Teleostei, Cyprinidae) exposed to two suble-thal concentration of copper sulfate. Aquat. Toxicol., 50: 109-124.
- POURAMAHAD J. O'BRIEN, P.J. 2000. A comparison of hepatocyte cytotoxic mechanisms for  $Cu^{2+}$ and  $Cd^{2+}$ . Toxicology., 143: 263-273.
- PUJIN V., DJUKIĆ N., MALETIN S., OBRADOWIĆ S. 1990. Content of heavy metals in some fish species in the section of the Danube flowing through Vojvodina. Wat. Sci. Tech., 22 (5): 79-86.
- RITOLA O., LIVINGSTONE D.R., PETERS L.D., LINDSTRÖM-SEPPÄ P. 2002. Antioxidant processes are affected in juvenile rainbow trout (Oncorhynchus mykiss) exposed to ozone and oxygen-supersaturated water. Aquaculture, 210: 1-19.
- STOSIK M., DEPTUŁA W. 2000. Studies on selected protective functions of thrombocytes and nutrophilic granulocytes in healthy and sick carp. Pol. J. Vet. Sci., 3: 219-225.
- SVOBODOVA Z., GROCH L., FLAJSHANS M., VYKUSOVA B., MACHOVA J. 1997. The effect of longterm therapeutic bath of malachite green on common carp (Cyprinus carpio L.). Acta Vet. Brno, 66: 111-117.
- TACON A.G.J. 1992. Nutritional fish pathology. Morphological signs of nutrient deficiency and toxicity in farmer fish. FAO Fisheries Technical Paper, 330: 75.
- THOMAS M.B., THOMAS W., HORNSTEIN T.W., HEDMAN S.C. 1999. Seasonal leukocytes and erythrocyte counts in fathead minnows. J. Fish Biol., 54: 1116-1178.
- VALAVANIDIS A., VLAHOGIANNI T., DASSENAKIS M., SCOULLOS M. 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotox. Environ. Safety., 64: 178-189.
- VARANKA Z., ROJIK I., NEMCSÓK J., ÁBRAHÁM M. 2001. Biochemical and morphological changes in carp (Cyprinus carpio L.) liver following exposure to copper sulfate and tannic acid. Comp. Biochem. Physiol. C., 128: 467-478.
- WITESKA M. 2003. Wpływ metali (Pb, Cu, Cd i Zn) na parametry hematologiczne i morfologię krwi karpi [Effect of metals (Pb, Cu, Cd and Zn) on hematological parameters and blood morphology of carps]. Wyd. Akademii Podlaskiej, Siedlce, Rozpr. Nauk., 72. (in Polish)