Journal of Elementology ISSN 1644-2296

Stanek M., Dąbrowski J., Cygan-Szczegielniak D., Roślewska A., Stasiak K., Janicki B. 2017. *Influence of the sex and age on the content of selected minerals in the meat of ruffe (Gymnocephalus cernua* L.*) from the Vistula River, Poland*. J. Elem., 22(4): 1323-1331. DOI: 10.5601/jelem.2017.22.1.1323

ORIGINAL PAPER

INFLUENCE OF THE SEX AND AGE ON THE CONTENT OF SELECTED MINERALS IN THE MEAT OF RUFFE (*GYMNOCEPHALUS CERNUA* L.) FROM THE VISTULA RIVER, POLAND*

Magdalena Stanek[1], Janusz Dąbrowski[2], Dorota Cygan-Szczegielniak[1], Aleksandra Roślewska[1], Karolina Stasiak[1], Bogdan Janicki[1]

[1]Chair of Biochemistry and Toxicology
[2]Department of Ecology
UTP University of Science and Technology in Bydgoszcz, Poland

Abstract

The aim of this study was to determine concentrations of Na, K, Mg, Ca, P, Zn and Fe in the meat of ruffe (*Gymnocephalus cernua* L.) and to investigate the relationship between fish sex or age and the mineral content of fish meat. 90 individuals of ruffe were caught in autumn (from October 9 to November 6) from the Vistula River, near Toruń and he local wastewater treatment plant. The muscle tissues from the large side muscle in a fish body, above the lateral line, were taken for analyses. All frozen meat samples were freeze dried in a freeze dryer and then tissues were mineralized in a microwave mineralizer. Concentrations of minerals were determined by flame atomic absorption spectroscopy. As the analyses indicated, the concentrations of macroelements were arranged in the order: K > Ca > P > Na > Mg and the content of microelements was noted in the following decreasing sequence: Zn > Fe. The ranges of the mean concentrations of Na, K, Mg, Ca and P (g kg^{-1}) were as follows: from 2.38 to 2.68, from 14.74 to 16.16, from 1.47 to 1.60, from 5.63 to 8.06, from 2.18 to 3.59, respectively. The ranges of the mean concentration of microelements (mg kg^{-1}) were from 49 to 74 for Zn and from 26 to 29 for Fe. Statistically significant relationships between the age and the macro- and microelement concentrations were verified, and this tendency was comparable to the data obtained by other authors. Results of these studies showed that the sex of ruffe was not an important factor for the levels of macro- and microelements in this species.

Keywords: macro-, microelements, meat, ruffe (*Gymnocephalus cernua* L.), the Vistula River, Poland.

Magdalena Stanek, PhD, Chair of Biochemistry and Toxicology, Faculty of Animal Breeding and Biology, UTP University of Science and Technology, Mazowiecka Street 28, 85-084 Bydgoszcz, Poland, phone: +48 52 374 9739, e-mail: winiarska@utp.edu.pl
* The study was supported by the department's sources.

INTRODUCTION

Macroelements such as sodium, potassium, calcium, phosphorus or magnesium, and microelements such as zinc or iron, are required for many biochemical processes and are essential for good health (Łuczyńska et al. 2009, Bilandžić et al. 2015). Because fish are located at the top of the aquatic food chain, they may absorb various metals from the surrounding environment (food, water and sediments) (Al-Yousuf et al. 2000, Özparlak et al. 2012, El-Moselhy et al. 2014). Concentrations of metals in the fish body have been extensively analysed in different aspects over a few last decades. Most of these studies concentrated on the edible part of their body, i.e. on the meat, but also on various organs such as the liver, kidneys, gonads, bone or the digestive track. In all of these studies, the impact of biological and environmental factors like the body length and weight, type of tissue, season of catch, geographical location of a water body, water quality such a salinity, water hardness, dissolved oxygen and pollution, were taken into consideration (Dural et al. 2006, Uysal et al. 2008, Akbulut, Akbulut 2010, Mendil et al. 2010, Mortazavi, Sharifian 2012, Özparlak et al. 2012, El-Moselhy et al. 2014). The concentration and distribution of metals in the fish body may depend on the metal's properties and physicochemical factors, and it is commonly known that the effects of toxic substances on organisms depend on their concentration, dose, duration of exposure and route of exposure (Bochenek et al. 2008). Because there is few reports related to the impact of the sex on the metal content in the meat of fish, the authors decided to undertake this research topic.

The aim of this study was to determine the concentrations of Na, K, Mg, Ca, P, Zn and Fe in the meat of ruffe (*Gymnocephalus cernua* L.) and to investigate gender- and age-dependent differences in the mineral content.

MATERIAL AND METHODS

The experimental fish were caught from the Vistula River in Toruń, near the local wastewater treatment plant (N 53°00′42″, E 18°31′55″) – Figure 1. The Vistula River is the longest and largest river in Poland, 1,047 kilometres long and with the drainage basin covering about 194,500 square kilometres. The quality of the Vistula's water and its microbiological purity are monitored because this river is the source of drinking water for many cities and it is used in industries (Lenart-Boroń et al. 2015). The main sources of pollution may be the discharge of municipal and industrial wastewater as well as agricultural and storm runoff. The research carried out in 2007-2010 on the river near Toruń indicated that the ecological potential has improved from good to maximum (Dembowska 2014).

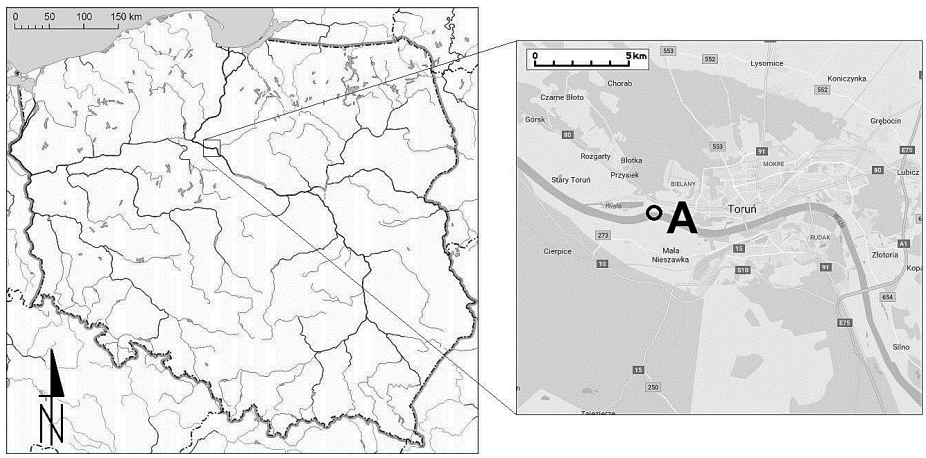

Fig. 1. Map of Poland. A - the place of fish collection (N 53°00′42″, E 18°31′55″)

In total, 90 individuals of ruffe (*Gymnocephalus cernua* L.) were caught in autumn (from October 9 to November 6, 2015) from the Vistula River. The body mass (BW) (± 0.01 g), body length (Lc) (± 0.1cm) and total length, Lt (± 0.1 cm) were measured. Additionally, the age of each fish was calculated on the basis of scales (Heese, 1992) – Table 1. The meat samples for analyses were taken from the large side muscle of fish body, above the lateral line.

The samples were kept in a deep freezer before analyses and were first freeze dried in a freeze dryer (Finn-Aqua Lyovac GT2) for 48 h at the temp. -40°C and pressure $6 \cdot 10^{-2}$ mbar. The samples were then mineralized in a microwave mineralizer (Ethos Plus, Milestone). They was weighed for mineralization (0.1 g) and then a mixture (4:1 *v*/*v*) of HNO_3 and H_2O_2 was added. The temp. was increased to 190°C and was maintained at that level for the

Table 1

Biometric measurements of ruffe (*Gymnocephalus cernua* L.) caught from the Vistula River

Age	Individuals	n	Body lenght (Lt) (cm) min-max (mean)	Body mass (BW) (g) min-max (mean)
2-year-old	females	25	9.6-10.0 (9.83)	17.4-19.9 (19.10)
	males	25	8.5-9.9 (9.45)	14.0-19.6 (17.48)
3-year-old	females	20	10.7-11.7 (11.14)	24.5-28.8 (27.21)
	males	20	10.1-10.8 (10.43)	21.8-26.3 (23.11)

next 7 min. The mineralized meat samples were transferred quantitatively to a measuring flask (with the capacity of 50 cm^3).

Concentrations of minerals were measured by flame atomic absorption spectroscopy (FAAS) using a Thermo Scientific ICE 3000 spectrophotometer. Phosphorus was analyzed by using a spectrophotometric camera Marcel-Media. All determinations were made in triplicate and the data for samples of fish meat were corrected according to the moisture content. Tissue concentrations of minerals were expressed as mg kg^{-1} dry weight (mg kg^{-1} d.w.) for Zn and Fe and g kg^{-1} dry weight (g kg^{-1} d.w.) for Na, K, Mg, Ca and P. The concentrations of the minerals were calculated from linear calibration plots obtained from measurements of the working standard solutions. For the calibration and validation of the standard curves, certified ICP standards (Merck) were used.

Statistical analyses

All statistical analysis were performed by using Statistica 8.0 software (StatSoft, USA). The Shapiro-Wilk's test and Levene's test were used to assess the normality of data and the homogeneity of variance, respectively. The two-way analysis of variance ANOVA (the Tukey's test) was used to test significant differences in the average content of minerals between 3- and 2-year-old females and males. T-test was used for the age- and gender-dependent differences. The statistical significance was estimated at $p \leq 0.05$.

RESULTS AND DISCUSSION

As the analyses of ruffe meat samples indicated, the concentrations of macroelements were arranged in the order: K>Ca>P>Na>Mg in 2-year-old fish, and K>Ca>Na>N>P>Mg in 3-year-individuals. The microelement content was noted in the following decreasing sequence: Zn > Fe (Table 2). Łuczyńska et al. (2009) confirmed the same results for microelements and similar for macroelements in the meat of freshwater fish caught from the Mazurian Great Lakes (Poland). Results of these studies indicated that the highest concentrations of the minerals were determined in the meat of 2-year-old individuals: Na, P and Fe in males and K and Ca in females. The lowest values of these minerals were observed in the meat of 3-year-old males (Na, P, Mg and Fe). Our analyses indicated that there were significant differences in the concentrations of K, Ca, Zn and Fe between 2-year-old females and males and in K, Mg, Ca and Zn content between sexes of 3-year-old individuals (Table 2).

Analyses of the minerals contents in the meat of fish in two age groups, regardless of sex (*T*-test) showed statistically significant differences for most of the elements (except Mg and Fe) – Table 2. Concentrations of the mine-

Table 2

Mineral concentrations in the meat of 2- and 3-year-old females and males of ruffe (*Gymnocephalus cernua* L.) caught from the Vistula River

Minerals	2-year-old		3-year-old		♀♂	2+3+
	♀	♂	♀	♂		
Na (g kg^{-1})	$2.66^{a} \pm 0.31$	$2.68^{a} \pm 0.29$	$2.53^{a,b} \pm 0.13$	$2.38^{b} \pm 0.14$	nd	*
K (g kg^{-1})	$16.16^{a} \pm 0.46$	$15.66^{b} \pm 0.50$	$15.65^{b} \pm 0.32$	$14.74^{c} \pm 0.27$	*	*
Mg (g kg^{-1})	$1.47^{a} \pm 0.07$	$1.51^{a,b} \pm 0.04$	$1.60^{b} \pm 0.06$	$1.47^{a} \pm 0.20$	nd	nd
Ca (g kg^{-1})	$8.06^{a} \pm 0.76$	$6.74^{b} \pm 0.28$	$5.63^{c} \pm 0.18$	$6.50^{b} \pm 0.27$	nd	*
P (g kg^{-1})	$3.50^{a} \pm 0.37$	$3.59^{a} \pm 0.36$	$2.18^{b} \pm 0.25$	$2.25^{b} \pm 0.17$	nd	*
Zn (mg kg^{-1})	$49^{a} \pm 4.9$	$57^{b} \pm 3.3$	$74^{c} \pm 2.1$	$65^{d} \pm 2.1$	nd	*
Fe (mg kg^{-1})	$26^{a} \pm 3.4$	$29^{b} \pm 3.2$	$28^{a,b} \pm 3.8$	$26^{a} \pm 2.1$	nd	nd
Ca/P	$2.32^{a} \pm 0.29$	$1.89^{b} \pm 0.20$	$2.61^{c} \pm 0.32$	$2.90^{d} \pm 0.21$	nd	*

The values in the same row marked with different letters differ statistically significantly at $p \leq 0.05$ (the Tukey's test), * statistically significant gender- and age-dependent differences at $p \leq 0.05$ (*T*-test), nd – differences not detected.

rals were statistically significantly higher in the meat of younger individuals (except Zn) than in older ones. The lack of a relationship between the Zn content and the fish's size was demonstrated by Al-Yousuf et al. (2000) for *Lethrinus lentjan*. Investigations carried out by Canli, Atli (2003) showed a negative correlation between the concentration of heavy metals and the body length of the Mediterranean fish species. The same results were observed by Farkas et al. (2003), Klavins et al. (2009) and Merciai et al. (2014). This negative correlation may be caused by a higher dilution of elements with the increase in the size of a fish body, increased excretion of the metals by gills, skin and the mouth, and a lower rate of food among older individuals. Canli, Atli (2003) and Mortazavi and Sharifian (2012) confirmed that one of the most important factors in the metal accumulation in bodies of marine animals is their metabolic activities. As Kljakovič Gašpić et al. (2002) and Merciai et al. (2014) investigated, younger fish may accumulate larger amounts of minerals in their bodies because they invest more energy in the growth and less in detoxification of metals, and therefore the mechanism of neutralization of metals is not developed sufficiently in their organisms. Statistically significant and negative correlations between the content of metals and the age of fish were observed by Dobicki and Polechoński (2003) for sander and perch, by Farkas et al. (2003) for common bream, by Canli, Atli (2003) for *Labeo umbratus* and by Drąg-Kozak et al. (2011) for rainbow trout (*Oncorhynchus mykiss*). Previous analyses carried out by Stanek et al. (2013) for Prussian carp (*Carassius auratus gibelio* Bloch) indicated that there were a negative and statistically significant correlations between the body length or the body mass and calcium and phosphorus concentrations.

With respect to the analyses related to the sex of fish, regardless of their age (*T*-test), statistically significant differences were determined only for

potassium (Table 2). Analyses carried out by Protasowicki (1991) on herring (*Clupea harengus*), bream (*Abramis brama* L.) and cod (*Gadus morhua*) confirmed that in 61% of the examined cases, the sex of fish determined the metal content in meat. Mortazavi and Sharifian (2012) confirmed that the main reason for the differences in the metal content between females and males may be the different metabolic activity between the two sexes, as in the case of age-dependent differences. Szefer et al. (2003) and Klavins et al. (2009) reported that a higher content of metals in females' bodies may be influenced by differences in the feeding habits between individuals of the different sexes. Another explanation of the gender-dependent differences in the metal concentrations may be the fact that females are sometimes older than males of the same length and the food composition of younger and older individuals is different. These hypotheses were confirmed by the research conducted by Al-Yousuf et al. (2000) for *Lethrinus lentjan*. Analyses concerning the metal content in bream caught in the Odra estuary indicated no statistically significant differences in the Zn concentration between females and males (Hłyńczak et al. 1998). Previous studies concerning the impact of sexes on the Ca and P content in the meat of roach (*Rutils rutilus* L.) collected form the Brda River showed no statistically significant differences between females and males in samples collected in spring and autumn (Stanek, Janicki 2011). Similarly, analyses of riverine lamprey (*Lampetra fluviatilis*) showed no statistically significant relationship between the level of the analyzed metals and the sex in most of the organs (Protasowicki et al. 2011).

As analyses indicated, the Ca/P ratio in the meat of ruffe ranged from 1.89 to 2.90 and there were statistically significant gender- and age-dependent differences (Table 2). Ian increased calcium content in relation to phosphorus causes some inhibition of phosphorus absorption and formation of biologically inactive calcium phosphates (Chavez-Sanchez et al. 2000). On the other hand, excess of phosphorus in the body causes reduced absorption of calcium, which can lead to decalcification of the bones (Nakamura 1982). As numerous studies show, a proper value of this ratio in food products should be 1:1. Previous investigations showed that the Ca/P ratio was similar to the reference values and ranged from 0.43:1 to 0.82:1 in the meat of wild roach from the Brda River (Stanek, Janicki 2011), 2.37:1 in the meat of Prussian carp (Stanek et al. 2013) and 1.174:1, 0.254:1 and 0.888:1 in the meat of fish caught from Lake Góreckie, Wędromierz and Strzeszyńskie, respectively (Stanek et al. 2014).

CONCLUSIONS

The results obtained from this study showed that the mineral concentrations determined in the meat of ruffe are comparable to the levels achieved by other authors for different fish species. As numerous studies have con-

firmed, the main factors affecting the level of metals in fish are the fishing season, location of a water body, age and length of the fish, and the fish tissues analyzed. The research results showed that the sex of ruffe was not an important factor in determination of the concentrations of macro- and microelements in this species.

Statistically significantly higher concentrations of macro- and microelements were calculated in the meat of younger individuals (except Zn) than in older ones. A similar tendency for the accumulation of heavy metals has been confirmed by other authors. Based on the results achieved in the present work, it could be concluded that smaller (younger) fish could be sometimes more valuable for human consumption than larger individuals. Despite the fact that ruffe is a small fish, classified into a low-value fish group, like roach (*Rutilus rutilus* L.) or common bleak (*Alburnus alburnus* L.), it can be an inexpensive and valuable source of macro- and microelements and may be an interesting subject of further research on the content of minerals, proteins or vitamins.

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