

GLUTATHIONE REDOX STATUS IN SOME TISSUES AND THE INTESTINAL PARASITE *POMPHORHYNCHUS LAEVIS* (ACANTHOCEPHALA) FROM BARBEL (*BARBUS BARBUS*) (PISCES) FROM THE DANUBE RIVER. Svetlana G. Despotović¹, Branka R. Perendija¹, Tijana B. Kovačević¹, Slavica S. Borković¹, S. Z. Pavlović¹, S. M. Milošević³, Vesna D. Djikanović², P. D. Cakić², Snežana B. Pajović⁴, and Zorica S. Saičić¹. ¹Department of Physiology, Siniša Stanković Institute for Biological Research, 11060 Belgrade, Serbia; ²Laboratory of Hydrobiology, Siniša Stanković Institute for Biological Research, 11060 Belgrade, Serbia; ³Institute of Biology, Faculty of Science, University of Priština, 38220 Kosovska Mitrovica, Serbia; and ⁴Laboratory of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, 11307 Belgrade, Vinča, Serbia

Key words: Barbel, Acanthocephala, glutathione, liver, muscle, parasite, redox status, the Danube, Serbia

UDC 595.133:597.551.2(282.243.7):612

The scope of our experiment was to determine the concentrations of total glutathione (tGSH), reduced glutathione (GSH), and oxidized glutathione (GSSG) in liver and muscle tissue of the freshwater fish species barbel (*Barbus barbus*) and in its intestinal parasite *Pomphorhynchus laevis*. We also estimated glutathione redox index (GSH RI), which is a usable marker of the reciprocal relationship between GSH and GSSG (Benzi et al., 1988).

Barbels (*Barbus barbus*) were caught in the Danube river near the city of Grocka (20 km from Belgrade) during the summer in August of 2007 at a site with an altitude of 71 m above sea-level, and coordinates 44° 40' N, and 20° 43' E. We collected 10 specimens (all males) with average length of 36.20 ± 0.92 cm and average weight of 473.70 ± 33.08 g. The fish were killed by a blow to the head and transported to the laboratory on ice, where tissue samples (liver and abdominal muscle) were taken and intestinal parasites removed. All chemicals were from Sigma (St. Louis, MO, USA).

The samples were minced and homogenized in 5 volumes (Lionetto et al., 2003) of 25 mmol/L sucrose containing 10 mmol/L Tris-HCl (pH 7.5) at 4°C with an Ultra-Turrax homogenizer (Janke and Kunkel, IKA-Werk, Staufen, Germany) (Rossi et al., 1983). The homogenates were sonicated for 30 s at 10 kHz on ice (Takada et al., 1982) and then used for determination of the concentrations of tGSH, GSH, and GSSG. Sonicates were centrifuged at 5000 rpm for 10 min with 10% sulphosalicylic acid (SSA), and the resulting supernatants were stored at -80°C (Griffith, 1980).

Concentrations of tGSH and GSSG were measured in triplicate using a Shimadzu UV-160 spectrophotometer and a temperature controlled cuvette holder. The GSSG concentration was obtained after reaction of GSH with vinyl pyridine. The concentration of reduced GSH was obtained by subtracting oxidized GSH from total GSH. The concentrations of tGSH,

GSH and GSSG were expressed as nmol/g of tissue. GSH RI was calculated by formula and expressed in arbitrary units (Benzi et al., 1988):

$$\text{GSH RI} = ([\text{GSH}] + 2[\text{GSSG}]) / (2[\text{GSSG}] \times 100).$$

The data are expressed as means ± the standard error (SE). Statistical differences were analyzed using the unpaired Student t-test, a level of p<0.05 being considered significant (Hoel, 1966).

Barbel is a suitable test-organism in biomonitoring studies owing to its wide geographic distribution and benthic way of life (Kennedy, 1997). The liver was chosen for the present study because it is responsible for regulation of overall body metabolism (Muriana et al., 1993). Also, the liver is the organ most involved in the detoxification of xenobiotics. Muscle is important for its consumption of energy. The intestinal parasite *Pomphorhynchus laevis* is uncommonly investigated in study of the antioxidative defense system, but published data show that it is very useful for biomonitoring studies in aquatic ecosystems (Sures, 2003).

Table 1 presents data on the concentrations of tGSH, GSH, and GSSG in nmol/g tissue and GSH RI in arbitrary units. Our results show the existence of tissue-specific changes in the investigated parameters. The concentration of tGSH was significantly higher in the liver (p<0.005) and the parasite (p<0.005) than in muscle tissue. Increase of tGSH in the liver of aquatic animals (compared to abdominal muscle) can be attributed to the central role of the liver in metabolism and the detoxification reaction (Dandapat et al., 2000). The concentration of GSH was significantly higher in the parasite (p<0.005) and liver (p<0.005) compared to muscle. Published data indicate that metal levels measured in muscle are lower than in other tissues (Jorhem et al., 1994). These results can be explained in terms of the low metabolic rate in muscle tissue and with low activities of biotransformation enzymes. It should also be noted that the way of variation of GSH concentration in tissues is the

Table 1. Concentrations of total glutathione (tGSH), reduced glutathione (GSH), and oxidized glutathione (GSSG) expressed in nmol/g tissue and the glutathione redox index (GSH RI) expressed in arbitrary units ($\times 10^{-3}$) in the liver (LIV) and muscle (MUS) of barbel (*Barbus barbus*) and in its intestinal parasite (*Pomphorhynchus laevis*) (PAR). Values are means \pm SE.

	Liver	Muscle	Parasite
tGSH	502.33 \pm 43.89	81.72 \pm 6.71	355.30 \pm 56.08
GSH	359.96 \pm 37.15	38.80 \pm 4.45	306.17 \pm 57.31
GSSG	142.37 \pm 12.61	42.92 \pm 3.39	49.13 \pm 9.22
GSH RI ($\times 10^{-3}$)	10.0198 \pm 0.0068	10.0045 \pm 0.0005	10.0402 \pm 0.0117

tGSH: LIV vs MUS: $p < 0.005$, MUS vs PAR: $p < 0.005$.

GSH: LIV vs MUS: $p < 0.005$, MUS vs PAR: $p < 0.005$.

GSSG: LIV vs MUS: $p < 0.005$, LIV vs PAR: $p < 0.005$.

GSH RI: LIV vs MUS: $p < 0.05$, MUS vs PAR: $p < 0.02$.

same as variation of tGSH. This is comprehensible because GSH represents 9/10 of tGSH. Concentrations of GSSG were significantly higher in the liver ($p < 0.005$) than in muscle tissue and the parasite. High oxidative stress level essentially lowers the level of GSH in favor of GSSG (Tanaka and Paglia, 1995), so a high GSSG level represents an oxidative stress marker. GSH RI was significantly decreased in muscle in comparison with the liver ($p < 0.05$) and the parasite ($p < 0.02$). Benzi et al. (1988) demonstrated that the GSH RI profile is age-linked, decreases with time and represents a marker of the GSH/GSSG ratio, i.e., it defines the glutathione redox status of the cell. The concentrations of tGSH and GSH in the intestinal parasite *Pomphorhynchus laevis* were approximately the same as ones in the liver, but statistically higher than those in muscle tissue. These results can be explained by the fact that we used the whole body of the intestinal parasite in our study.

In this study, we conclude that certain parasites, especially intestinal acanthocephalans of fish, can accumulate heavy metals in concentrations much higher than those in tissues of the host or in the aquatic environment (Sures and Siddall, 1999). The greatest measured Pb and Cd concentrations in the intestinal parasites *Pomphorhynchus laevis* were 2700 and 400 times higher than in muscle of the host *Leuciscus cephalus*, and 11000 and 27000 times higher than in the water (Sures et al., 1994; Sures and Taraschewski, 1995). Earlier investigations support the idea that acanthocephalans are very useful organisms in biomonitoring of metal in aquatic ecosystems (Thielen et al., 2004). Kennedy (1997) suggest the conditions which a parasite must satisfy in order to be used as an indicator species: its host should be widely distributed and readily accessible. Abundance of the parasite in the fish host population should be high and the parasite should be easy to identify. *Pomphorhynchus laevis* satisfies all these requirements: its hosts are very wide spread in European rivers and presence of this parasite in their hosts is usually very high.

Our study represents the first evaluation of tGSH, GSH, and GSSG concentrations, and GSH RI values in the investigated tissues (liver and abdominal muscle) of barbel (*Barbus*

barbus) and its intestinal parasite *Pomphorhynchus laevis* collected from the Danube River. The obtained results indicate significant influence of tissue-specificity on the cellular glutathione redox status of this fish. It is especially important to emphasize that the present study was performed on the intestinal parasite *Pomphorhynchus laevis*, which according to published data is very useful in studies involving biomonitoring of aquatic ecosystems.

Acknowledgment - This study was supported by the Ministry of Science of the Republic of Serbia Grants (Nos. 143035B and 143023B).

References: Benzi, G., Pastoris, O., Marzatico, F., and R. F. Villa (1988). *Neurobiol. Aging* **9**, 371-375. - Dandapat, J., Chainy, G. B. N., and J. K. Rao (2000). *Comp. Biochem. Physiol.* **127C**, 101-115. - Deneke, S. M., and B. L. Fanburg (1989). *Am. J. Physiol.* **257**, L163-L173. - Griffith, O. W. (1980). *Anal. Biochem.* **106**, 207-212. - Hoel, P. G. (1966). *Introduction to Mathematical Statistics*, John Wiley and Sons, New York, 402-403. - Jorhem, L., Engman, J., Sundstrom, B., and A. M. Thim (1994). *Arch. Environ. Contam. Toxicol.* **26**, 137-142. - Kennedy, C. R. (1997). *Parassitologia*, **39**, 249-254. - Lionetto, M. G., Caricato, R., Giordano, M. E., Pascariello, M. F., Marinosci, L., and T. Schettino (2003). *Mar. Poll. Bull.* **46**, 324-330. - Muriana, F. J., Ruiz-Gutierrez, V., and J. Bolufer (1993). *J. Biochem. (Tokyo)* **114**, 404-407. - Poulin, R. (1992). *Parasitol. Today* **8**, 58-61. - Sures, B., and R. Siddall (1999). *Exper. Parasitol.* **93**, 66-72. - Rossi, M. A., Cecchini, G., and M. M. Dianzani (1983). *IRCS Med. Sci. Biochem.* **11**, 805. - Sies, H. (1999). *Free. Rad. Biol. Med.* **27**, 916-921. - Sures, B. (2003). *Parasitology*, **126**, 53-60. - Sures, B., and H. Taraschewski (1995). *Parasitol. Res.* **81**, 494-497. - Sures, B., Taraschewski, H., and E. Jackwerth (1994). *J. Parasitol.* **80**, 335-357. - Takada, Y., Noguchit, T., and M. Kayiyama (1982). *Cancer Res.* **42**, 4233-4235. - Tanaka, K. R. and D. E. Paglia (1995). Pyruvate kinase and other enzymopathies of the erythrocyte. In: (Eds. C. R. Scriver et al.) *The Metabolic Basis of Inherited Disease*, 3485-3512, McGraw-Hill, New York - Thielen, F., Zimmermann, S., Baska, F., Taraschewski, H., and B. Sures (2004). *Environ. Poll.* **129**, 421-429.