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RESEARCH PAPER

Multi-Marker Study of *Dreissena polymorpha* Populations from Hydropower Plant Reservoir and Natural Lake in Latvia

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Abstract

Hydropower plants (HPPs) are equipped with reservoirs that can accumulate the toxic effluents and disturb the water flow of rivers. The aim of this study was to evaluate the biochemical responses of zebra mussel *Dreissena polymorpha* inhabiting the reservoir of Riga HPP in comparison with the responses of the mussels from the natural lake Kanieris in Latvia. In the reservoir mussels, high Mn-SOD activity and low level of the lipid peroxidation (production of TBARS) in the soft tissues suggested an elevated antioxidant activity. Compared to the lake specimens, the reservoir mussels had low levels of RI GSH while their lactate/pyruvate ratio was 1.4 times higher. In the mussels from the reservoir, high level of protein carbonilation products, as well as lowered caspase-3 and extralysosomal cathepsin D activities (by 1.4 and 1.6 times respectively, compared to the lake specimens) indicated delayed apoptosis. Catalase and glutathione *S*-transferase activities and metallothionein concentration were similar in both groups. Vitellogenin-like proteins (detected as the alkali-labile phosphates) and cholinesterase levels were higher in the mussels from the reservoir. We chose the indexes of the redox capacity as the most suitable markers of the environmental impact on this organism.

Introduction

Hydropower is the most important and most economical of renewable energy sources for electricity generation; its importance in the future is expected to increase (Kaunda, Kimambo, & Nielsen, 2012; Atilgan & Azapagic, 2016). Hydropower could prove especially valuable in the context of climate change due to its low carbon footprint and high power generation efficiency. A comparison of different Hydroelectric Power Plants (HPPs) demonstrated that environmental impacts from large reservoirs were overall lower than that of small reservoirs and run-of-river hydropower (Atilgan & Azapagic, 2016). Additionally, large reservoirs could be utilised for other purposes. For example, the reservoir of Riga HPP is also the source of drinking water for Riga

City. On the other hand, these artifical reservoirs could accumulate toxic effluents, alter temperature regime and cause degradation of aquatic ecosystems (Faria *et al.,* 2010; Warner, 2012; Fu, Wang, Xu, Yan, & Li, 2014; Rambo *et al.,* 2017). The Daugava River alone accounts for approximately 60% of the total riverine load to the Gulf of Riga (Laznik, Stålnacke, Grimvall, & Wittgren, 1999). Therefore, the environmental impact of HPP reservoirs needs to be evaluated.

The majority of ecotoxic studies of the impact of HPPs is mostly focused on the accumulation of toxic metals, persistent organic substances (Lebedynets, Sprynskyy, Kowalkowski, & Buszewski, 2004; Zhu *et al.*, 2012) and genotoxicity caused by the sediment (Rambo *et al.*, 2017). Data on bioindication of ecotoxicity of Riga reservoir is relatively limited. Some information is

410

Turk. J. Fish.& Aquat. Sci. 20(6), 409-420

available on riverine contribution to the eutrophication of the Gulf of Riga and its dependence on the river input (Stålnacke *et al.,* 1999; Agrell *et al.,* 2001). Biochemical markers in the aquatic animals in this area were not evaluated (Agrell *et al.,* 2001).

The selection of relevant bioindicative organisms is a crucial point for an ecotoxicity study (Hook, Gallagher, & Batley, 2014). Bivalve molluscs are excellent indicator organisms to assess the effects of environmental stressors on aquatic ecosystems and human exposure since they have sedentary nature, filter-feeding behavior, ability to accumulate pollutants and sensitivity to environmental temperature (Viarengo, Lowe, Bolognesi, Fabbri, & Koehler, 2007; Dailianis, 2010). In particular, the invasive bivalve zebra mussel is a popular indicator organism due to its ability to accumulate toxic metals and persistent organic substances (Kwan, Chan, & de Lafontaine, 2003; Quinn *et al.,* 2004; Contardo-Jara & Wiegand, 2008; Faria *et al.,* 2010; Riva, Parolini, Binelli, & Provini, 2010; Lepom, Irmer, & Wellmitz, 2012). Stress biomarkers can be used to evaluate the health status affected by both contaminants and non-chemical stressors (Lushchak, 2011; Hook *et al.,* 2014; Kerambrun *et al.,* 2016; Potet *et al.,* 2018a,b). In mussel, the development of a stress syndrome along the pollution gradient was demonstrated using a novel species-specific microarray, both in terms of numbers of expressed genes and level of gene response (Dondero *et al.,* 2006). However, since zebra mussel is an invasive species, it has been debated whether its biomarker responses appropriately reflect the environmental impact compare to the native species (Crooks, Chang, & Ruiz, 2011).

The main objective of this study was to evaluate the environmental impacts in the reservoir of Riga HPP using zebra mussel as a sentinel organism. For this purpose, we aimed to study the responses of stress and toxicity in the populations inhabiting both the artificial Riga reservoir and a natural lake Kanieris in Latvia, which is presumably clean. We selected the widely utilized biochemical markers in molluscs, namely the indices of oxidative stress, metabolic depression, metallothionein levels (as the marker of contamination by toxic metals), and cholinesterase levels (as the marker of organophosphate and thiocarbamate toxicity). Vitellogenin-like proteins, determined as alkali-labile proteins (ALP), were included as components that supply gametogenesis with phosphates and zinc. The indices of apoptosis, caspase-3 and cathepsin D were also evaluated. To the best of our knowledge, such approach was applied for the first time for the evaluation of the ecosystem of Riga reservoir.

Materials and Methods

Chemicals

All chemicals were purchased from Sigma Aldrich (St. Louis, USA) and SinbiaS (Ukraine) and were of the Reagent grade or higher.

Sampling

Zebra mussels, *Dreissena polymorpha* (Pallas, 1771), were collected in Autumn (September) 2017 in the north-western part of the Reservoir of Riga HPP
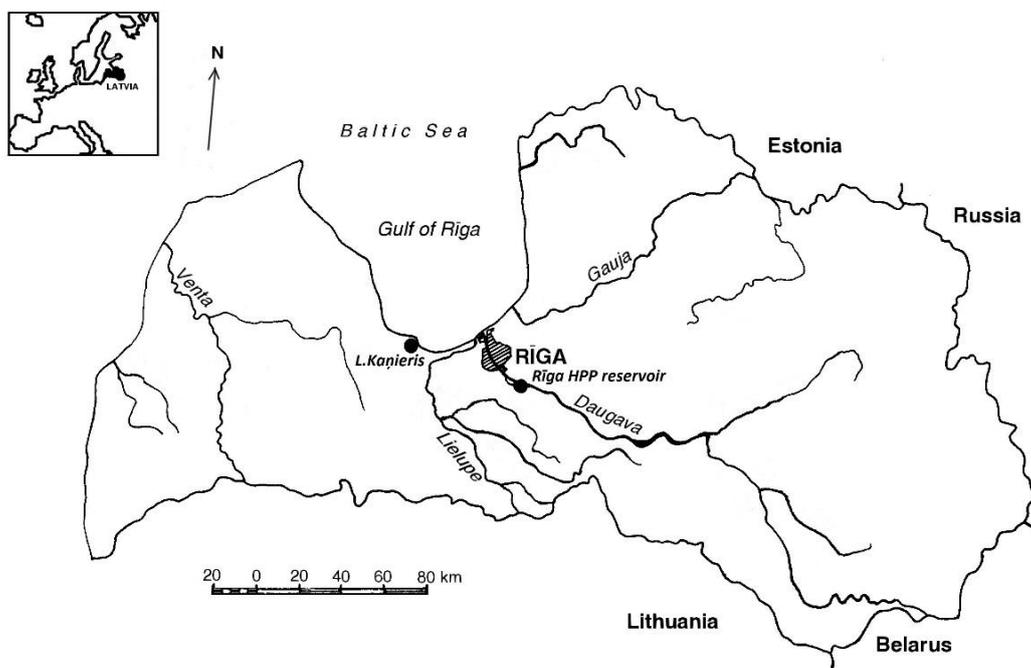

Figure 1. Localization of the sampling sites in Latvian area: Lake Kanieris (57°00'04''N 23o29'20''E) and Reservoir of Riga HPP (56°49'59''N 24°19'59''E). Note. (•) indicate the location of sampling sites

411

Turk. J. Fish.& Aquat. Sci. 20(6), 409-420

(56°49′59′′N 24°19′59′′E) and the natural lake Kanieris (57°00′04′′N 23°29′20′′E). The Riga reservoir is situated on the cross-border River Daugava and is the last one in the cascade of reservoirs on the river (Figure 1). Lake Kanieris is a shallow lagoon clearwater macrophyte lake. It has been a protected site since 1964 as an important place for birds, and is also a part of Kemeri National Park (1997) (https://www.meteo.lv).

The samplings were carried out simultaneously in both sites. The individuals were transported to the cooled boxes filled with lake water and constantly aerated. In each group, the specimens with the average weight 2-4 g and the shell length between 2.4 and 3.0 cm were selected for the purposes of the present study. The molluscs were dissected on ice. For all biochemical traits, total soft tissue from 5 molluscs were combined in each sample. Totally, six such combined sets were used for each biochemical analysis in each experimental group. The samples were stored at -40° C until further analysis. The soluble extracted protein concentration in the samples was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as the protein standard. The absorbance values were measured on an UV/Vis spectrophotometer "Lomo-56" (Russian Federation).

Oxidative Stress Indices

For the oxidative stress indices in the soluble fraction of homogenate, the samples of tissue were homogenized (1/10 w/v) in 0.1 M phosphate buffer, pH 7.4, containing 100 mM KCl and 1 mM EDTA, as well as 0.1 mM phenylmethylsulfonyl fluoride for proteolysis inhibition. Homogenates were centrifuged for 10 min at $6{,}000 \times g$.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured according to the method of Beauchamp and Fridovich (1971) based on the aerobic reduction of nitroblue tetrazolium at 535 nm by superoxide radicals in the darkness and expressed as units·mg^{-1} of soluble protein. In order to assess Mn-SOD activity, the supernatant was preincubated for 60 min at 0 °C in the presence of 5 mM KCN, which produced total inhibition of Cu, Zn-SOD. The latter activity was calculated as the difference between the activities in the absence and the presence of KCN. A unit of SOD activity was defined as the amount of protein causing 50% inhibition of NBT reduction rate.

Catalase (CAT, EC 1.11.1.6) activity was measured by monitoring the decomposition of 10 mM H_2O_2 according to Aebi (1974) at 240 nm (ε=40 M^{-1}·cm^{-1}) in a buffer containing 50 mM KH_2PO_4 (pH 7.0) and approximately 150 µg of protein. The results were related to the soluble protein.

Glutathione *S*-transferase (GST, EC 2.5.1.18) activity was measured using 1-chloro-2,4-dinitrobenzene as the substrate (Habig, Pabst, & Jakoby, 1974). The GST activity was expressed as nmol·min^{-1}·mg^{-1} soluble protein.

Lipid peroxidation (LPO) was determined by the production of thiobarbituric acid-reactive substances (TBARS) (Ohkawa, Onishi, & Yagi, K., 1979). A molar extinction coefficient of 1.56·10^5 M^{-1}·cm^{-1} was used.

Protein carbonyl (PC) content, as an index of protein oxidation, was measured by the reaction with 2, 4-dinitrophenylhydazine (DNPH) (Reznick & Packer, 1994). The differences in absorbance between the DNPH- and the HCl-treated samples were determined by spectrophotometry at 375 nm, and the amount of PC was calculated by using a molar extinction coefficient of 2.2·10^4 M^{-1}·cm^{-1}. The results were expressed as nmol PC·per g of fresh weight (FW).

Redox balance and metabolic characteristics

Total glutathione (GSHt) and oxidized glutathione (GSSG) concentrations were quantified in the 1/10 w/v homogenate of tissue by the glutathione reductase recycling assay (Anderson, 1985). To estimate the GSSG level, the protein free sample was treated with 2-vinylpyridine prior to the assay at 2% final concentration. The rate of 5-thionitrobenzoic acid formation from 5,5-dithiobis-2-nitrobenzoate (DTNB) was detected spectrophotometrically at 412 nm. Standards were prepared from reduced glutathione (GSH) and GSSG, and concentrations were expressed as nmol/g wet weight. Redox index of glutathione (RI GSH) was calculated as the ratio of concentrations of GSH/GSSG by dividing the difference between the GSHt and GSSG concentrations by the concentration of GSSG: (GSHt-2GSSG)/2GSSG.

The lactate and pyruvate levels were determined spectrophotometrically. Lactate was assayed by the NAD-dependent enzymatic oxidation of lactate to pyruvate by D-lactate dehydrogenase (D-LDH) (EC 1.1.1.28) from *Lactobacillus leichmannii*. Pyruvate was measured by conversion of pyruvate acid to D-lactate by D-LDH in the presence of NADH. The changes in NADH concentration were monitored by absorbance at 340 nm using the molar extinction coefficient of 6.22·10^6 M^{-1}cm^{-1} (Gawehn, 1988; Lamprecht & Heinz, 1988). The ratio of the concentrations of lactate/pyruvate was calculated.

The concentration of the alkali-labile phosphates (ALP) related to the lipophosphoprotein vitellogenin was measured in the 10 w/v homogenate of gonads in 25 mM Hepes-NaOH buffer, pH 7.4, containing 125 mM NaCl, 1 mM dithiothreitol, and 1 mM EDTA according to the protocol of Gagné *et al.* (2003). The labile phosphates were quantified by the phosphomolybdenum assay. ALP levels were given as µmol phosphates mg^{-1} soluble protein.

Markers of toxicity

The concentration of metallothioneins (MTs) was assessed in the 1/10 w/v homogenates by the concentrations of thiols using DTNB reduction method (Viarengo, Ponzano, Dondero, & Fabbri, 1997) after the ethanol/chloroform extraction and calculated assuming

412

Turk. J. Fish.& Aquat. Sci. 20(6), 409-420

the relationship: 1 mol of MTs corresponds to 20 mol of GSH and expressed as µg of MTs per g of FW.

The cholinesterase (ChE, EC 3.1.1.7) activity was determined according to the colorimetric method of Ellman, Courtney, Andres, and Featherstone (1961) at 25 ºC. The reaction mixture contained 3.0 ml 0.1 M Na-phosphate buffer, pH 8.0, 0.1 mL 0.01 M DTNB in 0.1 M Na-phosphate buffer (pH 7.0, 15 mg sodium bicarbonate per 10 mL of solution) and an aliquot of supernatant (0.02 mL). The reaction was initiated by addition of 0.04 mL 0.075 M acetylcholine iodide to the reaction mixture. The rate of thionitrobenzoate production evaluated during 5 min at 412 nm was used to estimate hydrolysis. The enzyme activity was calculated using a molar extinction coefficient of $13.6 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ and referred to the soluble protein content.

Assays of apoptotic enzymes activities

The cathepsin D (EC 3.4.23.5) activity was determined with 1% hemoglobin as substrate as described by Dingle, Barrett, and Weston (1971). Cathepsin D acts on acid denatured hemoglobin resulting in a soluble colored complex which can be read spectrophotometrically at 280 nm. The free cathepsin D activity (out of lysosomes) was assessed in the homogenate without detergent addition, whereas the total cathepsin D activity was measured after the enzyme release by Triton X100 treatment. The activities were determined using a standard curve with thyrosine, and expressed as nmol tyrosine min^{-1} mg^{-1} of soluble protein.

The caspase-3-like activity was assayed colorimetrically in 25% w/v homogenate of tissue based on the hydrolysis of peptide acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA) by caspase-3 that produces a colored product p-nitroaniline (pNA). pNA was detected at 405 nm (ε_{mM}=10.5 $mM^{-1} \cdot cm^{-1}$) (Bonomini, Dottori, Amoroso, Arduini, & Sirolli, 2004). The activity of caspase-3 was expressed as nmol pNA min^{-1} mg^{-1} of soluble protein.

Statistical analysis

The data are presented as means ± standard deviation (SD) unless indicated otherwise. Data were tested for normality and homogeneity of variance by using Kolmogorov-Smirnoff and Levene's tests, respectively. Whenever possible, data were normalized by Box-Cox common transforming method. For the data that were not normally distributed even after the transformation, non-parametric tests (Kruskall–Wallis ANOVA and Mann–Whitney U-test) were performed. Pearson's correlation test for the pairs of variables was performed at 0.05 level of significance. All statistical calculations were performed with Statistica v. 10.0 and Excel for Windows-2010. Differences were considered significant if the probability of Type I error was less than 0.05.

Results

Oxidative stress response

The analysis of antioxidant enzymes determined that total SOD activity was similar in the mussels from both sites (Figure 2A). However, the distribution of activity among Cu, Zn- and Mn-forms of SOD was very different. In the samples from the natural lake, each form accounted for the similar proportion of the activity, while in the mussels from the reservoir Mn-SOD activity was dominant (≈ 93.6% of total activity). The CAT and GST activities did not differ significantly between the specimens from both sites (Figure 2B). The level of LPO was higher (TBARS, by 38.5%) in the specimens from the natural lake (Figure 2C). In contrast, protein carbonyl level was lower in these molluscs by 25.4% (Figure 2C).

Cellular low weight thiols

The metallothionein had similar concentrations in both groups (Figure 3A). However, the levels of GSH, GSSG and the RI GSH were substantially different, with higher values for all of them in the mussels from the natural lake, by 1.57, 1.18 and 1.32 times respectively (Figure 3B,C,D).

Markers of metabolic response

The level of lactate did not differ between both groups, whereas pyruvate level was 1.54 times lower in the mussels from the reservoir, resulting in a significantly higher lactate/pyruvate ratio (by 1.4 times) in reservoir mussels (Figure 4A,B,C). The level of ALP was higher in the mussels from the reservoir (Figure 4D).

Markers of toxicity

The intensity of apoptosis, as determined based on the activity of caspase-3, was 1.43 times higher in the molluscs from the natural lake (Figure 5B). The total cathepsin D activity was of the same magnitude in the mussels from both sites, however, the distribution of this activity between the lysosomes and a soluble fraction was different, with 1.62 times higher efflux in the mussels from the natural lake (Figure 5A). The evaluation of ChE activity also detected differences between the mussels from both sites, with values measured in the specimens from the natural lake 1.36 times lower compared to the reservoir (Figure 5B).

Correlation Analysis

The correlation analysis (Table 1) shows the highest number of associations for pyruvate (six positive, two negative), GSH (five positives and one negative), and GSSG (two positive and three negative). For metallothionein, the correlations were absent.
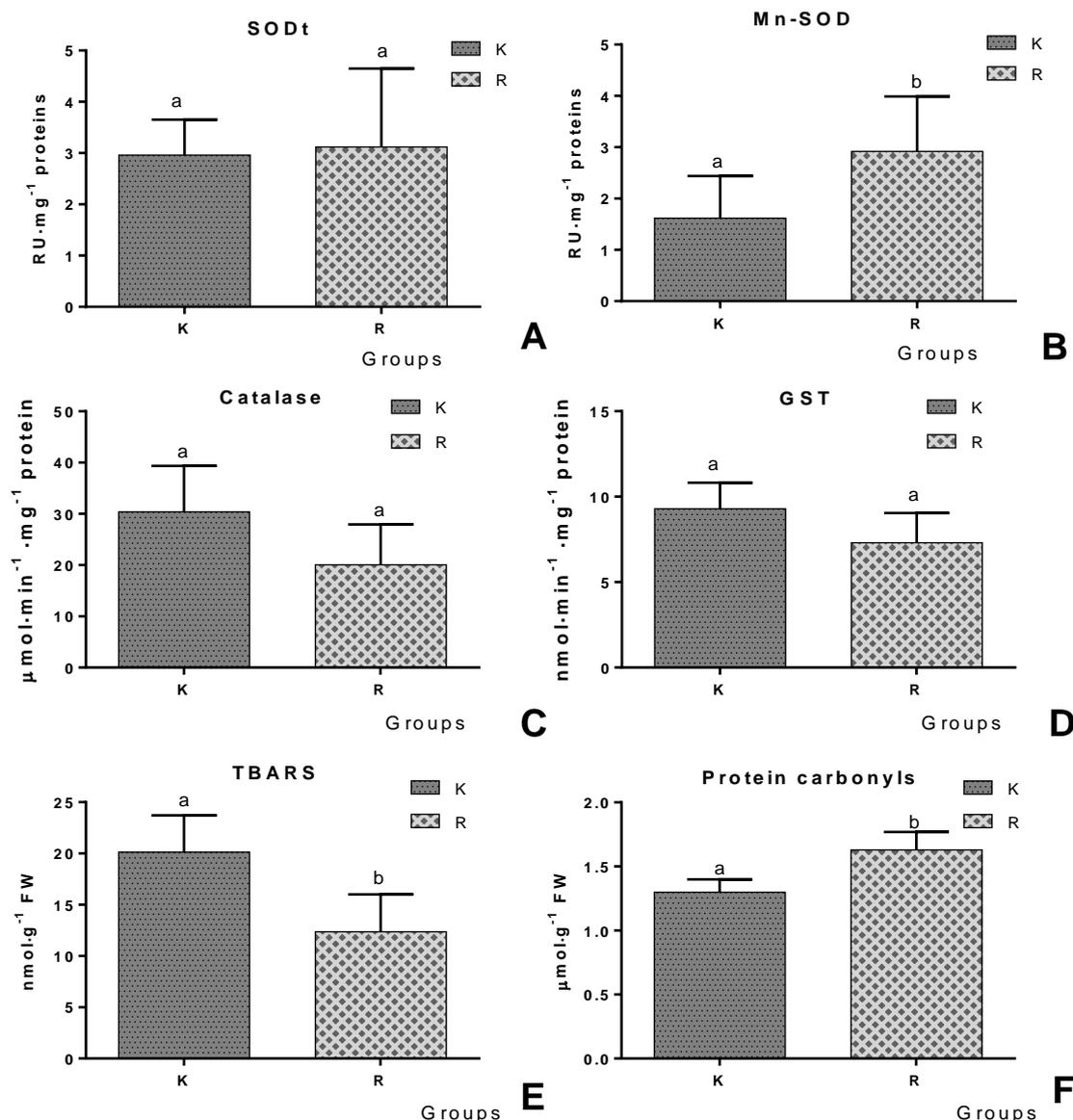
413

Turk. J. Fish.& Aquat. Sci. 20(6), 409-420

Figure 2. The indices of oxidative stress in the soft tissues of zebra mussel from the reference site (K) and Riga reservoir (R). Data for A: total SODt activity; B: Mn-SOD activity; C: catalase activity; D: GST activity; E: TBARS level; F: protein carbonyls level are presented as mean ± SD (n=6). For each endpoint/parameter, different letters above the bars indicate significant differences (*P*<0.05)

Discussion

The Daugava river in its lower portion collects the effluent from a large area. A sizable proportion of the pollution load comes from transboundary sources in Russia and Belarus. It is estimated that 37% of total nitrogen load and 46% of total phosphorus load entering the Baltic Sea via Latvian territory originate from transboundary sources (HELCOM, 2018). Locally, Riga City as well as agricultural lands are likely contributors to the PCBs and DDTs pollution in the reservoir (Agrell *et al.,* 2001). A recent study (Ikkere, Perkons, Sire, Pugajeva, & Bartkevics, 2018) that had measured persistent organic pollutants and emerging pollutants in freshwater molluscs in Latvia found high concentrations

of ibuprofene and polybrominated diphenyl ethers in the samples from Riga HPP contained. Nevertheless, the level of pollution in the lower portions of the Daugava was estimated as comparatively low (Strode, Jansons, Purina, Balode, & Berezina, 2017).

Previous studies utilizing biochemical markers in aquatic animals to assess environmental quality of the aquatic ecosystems in Latvia were mainly focused on the Gulf of Riga (e.g., Putna *et al.,* 2014). There was evidence that the river Daugava pollution affects benthic animals in the Gulf of Riga, since the highest values of Integrated Biomarker Response (IBR index) were found in the *Macoma balthica* sampled near the mouth of the river (Barda, Purina, Rimsa, & Balode, 2014). In a comparative study of biomarkers in the Atlantic salmon *Salmo salar*
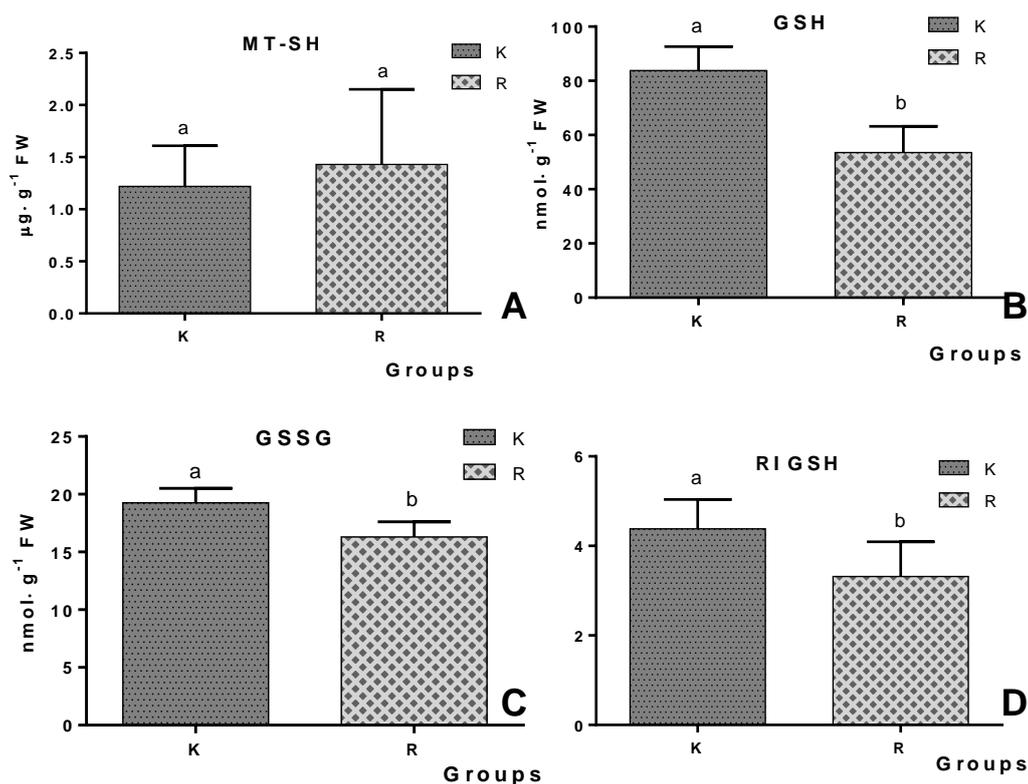
414

Turk. J. Fish.& Aquat. Sci. 20(6), 409-420

Figure 3. Low weight thiols in the soft tissues of zebra mussel from the reference site (K) and Riga reservoir (R). Data for A: metallothioneins; B: GSH; C: GSSG; D: RI GSH, presented as mean ± SD (n=6). Different letters above the bars indicates that the values differ significantly (*P*< 0.05).

in the Baltic Sea, the specimens originating from the Daugava and Gauja rivers had lower catalase activity compared to the other stocks (Vuori, Kiljunen, Kanerva, Koljonen, & Nikinmaa, 2012).

In this study we focused on the stress and toxicity responses of a recognized indicator species, the invasive bivalve mollusc zebra mussel. The results reflect substantial differences between the two sampled populations. Most notable differences were related to antioxidant activity and redox level. High Mn-SOD activity in the mussels from the reservoir indicates activation of mitochondrial oxygen-related processes (Santovito, Piccinni, Cassini, Irato, & Albergoni, 2005; Wang, Yuan, Wu, Liu, & Zhao, 2013). Low TBARS level corroborates activation of antioxidant response in the molluscs from the reservoir. On the other hand, protein carbonylation suggests protein damage and aging. This accumulation of protein damage can be the consequence of depressed apoptotic activity (attested by the low caspase-3 activity). Lysosomal cathepsin D activity supports proteoglican breakdown and amino acid utilization for energy metabolism (Dingle *et al.,* 1971; Lamarre *et al.,* 2016; Benes, Vetvicka, & Fusek, 2008). This strategy of resistance to apoptosis is known to assist in long-term survival in some species, particularly invertebrates (Menze, Fortner, Nag, & Hand, 2010; Portt, Norman, Clapp, Greenwood, & Greenwood, 2011).

While HPP reservoir molluscs also had high lactate/pyruvate ratio, it was not caused by the up-regulation of anaerobic conversion of glucose to lactate, given that lactate levels were similar in both populations (Figure 4). Thus, this group did not demonstrate the shift to anaerobiosis, a typical adaptation to a toxic environment or extreme temperature (de Zwaan & Eertman, 1996). In contrast, the comparatively low pyruvate level in the reservoir mussels appears to indicate a higher rate of metabolic source utilization by the mitochondria (Gray, Tompkins, & Taylor, 2014), as confirmed by the elevated Mn-SOD level. The resulting high lactate/pyruvate ratio in the reservoir group favors the high redox potential of NADH, supplying metabolic activity. The elevated level of ALP in the HPP reservoir mussels shows that the rate phosphates and Zn supply to gametogenesis is high (Gagné & André, 2011). These preferential pathways demonstrate that reservoir mussels have developed a tolerance strategy of biochemical adaptation (Somero, 2004). On the other hand, in the natural lake mussels, high TBARS levels suggested oxidative degradation of lipids. Simultaneous cathepsin D efflux from the lysosomes can weaken cathepsin D activity in the lysosomes and stimulate autophagy (Bursch *et al.,* 2008; Man & Kanneganti, 2016). High caspase-3 activity also indicates intensified apoptosis removing injured cells (Romero et al., 2011).

415

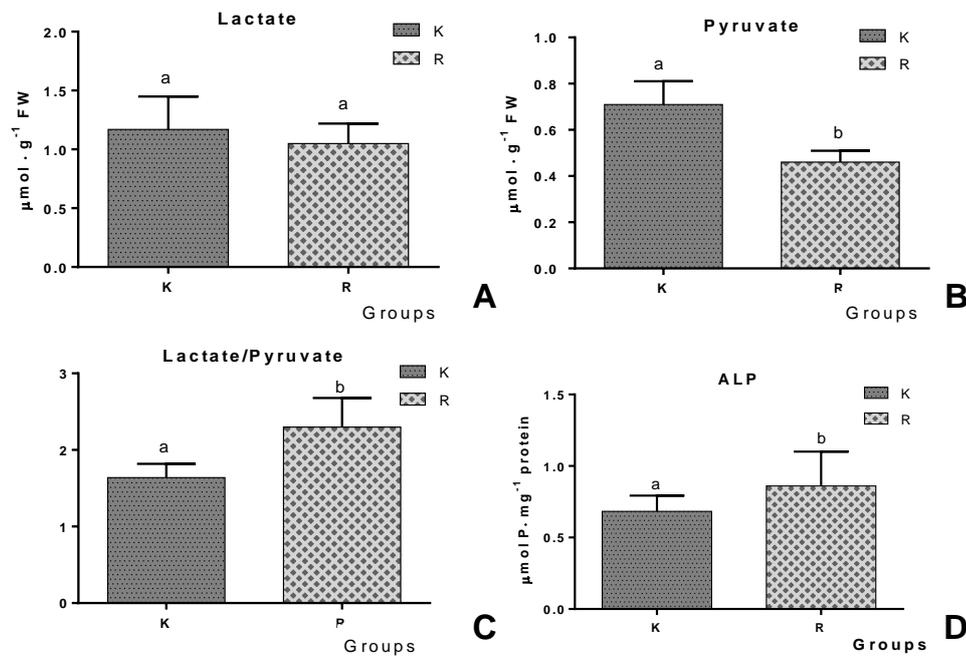*Turk. J. Fish.& Aquat. Sci. 20(6), 409-420*

Figure 4. The metabolic characteristics in the soft tissues of zebra mussel from the reference site (K) and Riga reservoir (R). Data for A: lactate; B: pyruvate; C: lactate/pyruvate concentration ratio, D: alkali-labile proteins; presented as mean ± SD (n=6). For each endpoint/parameter, different letters above the bars indicate significant differences ($P< 0.05$).
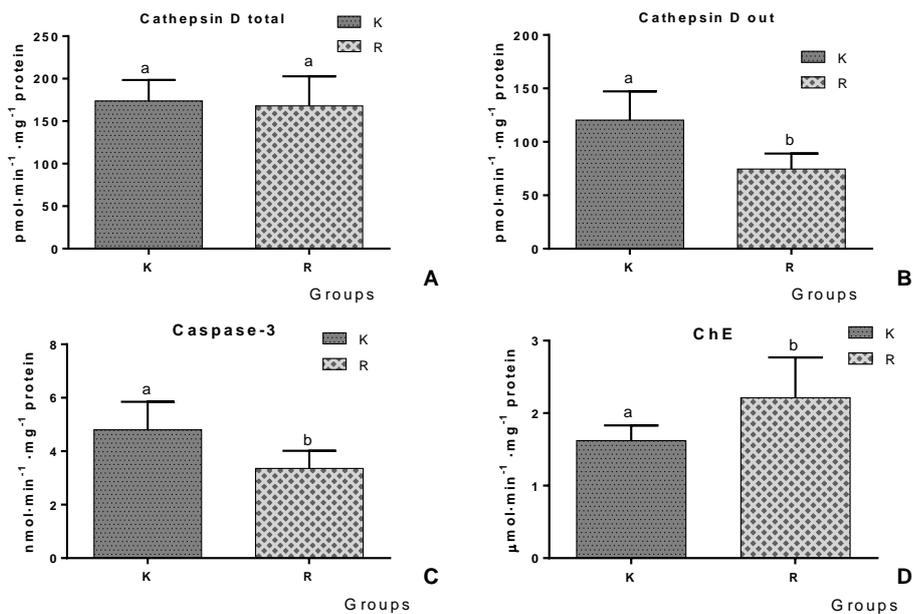

Figure 5. The indices of toxicity in the soft tissues of zebra mussel from the reference site (K) and Riga reservoir (R). Data for A: total cathepsine D activity; B: lysosome-out cathepsine D activity; C: caspase-3 activity; D: cholinesterase activity, presented as mean ± SD (n=6). Different letters above the bars indicates that the values differ significantly ($P< 0.05$).

At the same time, the natural lake mussels demonstrated high GSH and GSSG levels and the redox state of GSH. Moreover, of all studied markers, the GSH-related indices showed the highest difference between the two populations (Figure 3). GSH provides the first line of defence as a ROS scavenger (Circu & Aw, 2008). The GSH level differences were the main distinguishing criteria for gastropods from a lake near the place of Chernobyl nuclear disaster (Gnatyshyna *et al.,* 2012) and

zebra mussels, transplanted to different sites for 14 - 120 days (Falfushynska, Gnatyshyna, Stoliar, Dedourge-Geffard, & Geffard, 2010).

In our study two pairs of redox suppliers, RI GSH and lactate/pyruvate were highly intercorrelated (Table 1) attesting the redox balance as an important link in the chain of mussel's adaptive responses (Dailianis, 2010; Hellou, Ross, & Moon, 2012). The level of GSH correlated with the caspase-3 activity (Table 1). The activity and

Table 1. The Pearson coefficient of correlation *r* for the studied indices in the *D. polymorpha* from two populations; *, correlation significant (*P<0.05*) at *r>0.576*

Indices	GSH	GSSG	RI GSH	GST	SODt	Mn-SOD	CAT	TBARS	PC	LDH	Lac	Pyr	Lac/Pyr	Cas-3	CatDt	CatDe	ALP	ChE	Prot
MT	-0.276	-0.171	-0.243	-0.169	0.208	0.203	0.044	0.067	0.196	0.240	0.063	0.140	-0.145	0.000	-0.022	0.043	-0.124	-0.067	-0.096
GSH		0.532	0.903*	0.686*	0.010	-0.398	0.366	0.543	-0.698*	0.068	0.466	0.783*	-0.498	0.607*	0.398	0.745	-0.294	0.149	0.182
GSSG			0.122	0.128	0.067	-0.328	0.667*	0.506	-0.763*	0.051	0.136	0.643	-0.663*	0.021	-0.038	0.465	-0.661*	0.033	0.619
RI GSH				0.714*	-0.047	-0.314	0.142	0.394	-0.426	0.027	0.465	0.590*	-0.255	0.703*	0.464	0.622*	-0.027	0.126	-0.061
GST					0.010	-0.292	-0.067	0.504	-0.502	0.088	0.250	0.451	-0.280	0.596*	0.586*	0.485	0.094	0.362	-0.322
SODt						0.646	-0.051	0.098	-0.319	-0.115	0.585*	0.081	0.433	-0.361	0.220	-0.026	0.222	0.233	-0.200
Mn-SOD							-0.320	-0.420	0.319	0.130	0.114	-0.473	0.648*	-0.361	0.067	-0.510	0.465	0.439	-0.405
CAT								0.538	-0.520	-0.148	0.091	0.469	-0.466	0.108	-0.063	0.190	-0.518	-0.278	0.683*
TBARS									-0.704*	-0.486	0.172	0.648*	-0.598*	0.273	-0.025	0.529	-0.527	-0.073	0.432
PC										0.097	-0.270	-0.620*	0.504	-0.140	-0.200	-0.585*	0.414	-0.031	-0.357
LDH											-0.154	-0.052	-0.111	0.378	0.524	0.161	0.264	0.487	-0.444
Lac												0.608*	0.220	-0.023	0.287	0.232	-0.005	-0.086	-0.007
Pyr													-0.636*	0.335	0.13	0.720*	-0.609*	-0.125	0.415
Lac/Pyr														-0.383	0.140	-0.658*	0.763	0.080	-0.518
Cas-3															0.571	0.609	0.113	0.432	-0.251
CatDt																0.362	0.632*	0.629*	-0.702*
CatDe																	-0.302	0.202	0.073
ALP																		0.485	-0.888*
ChE																			-0.616*

417

Turk. J. Fish.& Aquat. Sci. 20(6), 409-420

efflux of lysosomal digestion enzyme cathepsin D also correlated with GSH, GST and metabolic characteristics (Table 1). Initiation of apoptosis can be triggered by a loss of cellular redox balance independently of ROS production via decreased NADPH availability for GSSG reduction (Circu & Aw, 2008). While this study cannot provide definitive conclusions, the importance of the observed correlations between the indices of redox state is evident.

We were unable to detect specific metal toxicity in the HPP reservoir: the levels of metallothionein, which indicates toxic metal, primarily cadmium pollution (Amiard, Amiard-Triquet, Barka, Pellerin, & Rainbow, 2006), did not show significant difference between the populations. This finding is corroborated by the similar Cu and Zn concentrations at both sites (National monitoring data: https://www.meteo.lv). A study of two Unionidae species from six lakes and watercourses of Latvia with different anthropogenic pressures also did not find any inter-site differences in the metallothionein concentrations (Purina, Barda, Rimsa, Poikane, and Jansons 2013). This finding was explained by the fact that toxic metals (Cd, Pb, Hg, Cu) accumulate in the deeper sediment layers, while the molluscs inhabit higher layers (Purina *et al.,* 2013). The ChE activity was lower in the natural lake mussels. Such finding would typically indicate acute toxicity of thiocarbamates and phosphoorganic compounds (Nunes, Carvalho, & Guilhermino, 2006; Dailianis, 2010). However, there is a debate on the meaning of this index in molluscs, i.e. low sensitivity or rapid recovery (Rickwood & Galloway, 2004; Gagnaire, Geffard, Xuereb, Margoum, & Garric, 2008; Bonacci, Corsi, & Focardi, 2009). Thus, we could not definitively prove neurotoxic substances pollution in the artificial reservoir of Riga HPP.

Invasive species are known to successfully withstand polluted or degraded areas. The difficulty in evaluating the adverse impact both of field and lab exposures on zebra mussels were reported (Potet *et al.,* 2018a,b). For example, the expression of stress response genes was elevated in the mussels from a less polluted area, and negatively correlated with the level of toxic metals (Kerambrun *et al.,* 2016). Zebra mussels from the large deep perialpine lakes Maggiore and Lugano had low accumulation of toxicants (DDx, PCBs, and Hg), unlike in the fish from the same sites (Guzzella *et al.,* 2018). Results of our study confirm that it is difficult to definitively evaluate site-related responses in the zebra mussel. Some of the markers we have detected might indicate site preferences of the invasive species adapted to a specific environment using their genetic variability, metabolic plasticity and stress response (Crooks *et al.,* 2011; Tarnowska, Daguin-Thiebaut, Pain-Devin, & Viard, 2013; Farkas *et al.,* 2017).

To summarize, the biomarker response in zebra mussels of the Riga HPP reservoir supports the evidence of comparatively low environmental impact of such reservoirs (Atilgan & Azapagic, 2016; Strode *et al.,* 2017).

Conclusion and Significance

The data suggests that Riga HPP reservoir can be qualified of as overall environmentally sustainable. The indexes of the redox capacity can serve as appropriate markers of the environmental impact on this organism.

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418

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419

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420

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