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COMPARATIVE STUDY OF GLUTATHIONE-S-TRANSFERASE ACTIVITY IN TISSUES OF SOME BLACK SEA TELEOSTS

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ABSTRACT
The activity of glutathione-S-transferase (GST) in blood cells, liver, spleen and gonads in five Black Sea fish species horse mackerel *Trachurus mediterraneus* (Staindachner), high body pickarel *Spicara flexuosa* (Rafinesque), shore rockling *Gaidropsarus mediterraneus* (L.), round goby *Neogobius melanostomus* (Pallas), scorpion fish *Scorpaena porcus* (L.) caught in coastal area of Sevastopol (Black Sea, Ukraine) was studied. All the examined tissues had GST activity. The high enzyme activity was shown in spleen and in the liver. The interspecies differences of GST activity associated with the specific fish biology and ecology were demonstrated. The different ways of physiological adaptation for effective xenobiotics biotransformation and detoxification in fish tissues are discussed.

Key words: Black Sea fish, enzymes, detoxification, interspecies differences

INTRODUCTION

Glutathione-S-transferases (GSTs) E.C.2.5.1.18. are a family of intracellular enzymes with the main function in detoxification processes by catalyzing the conjugation of tripeptide glutathione (GSH) with some endogenous toxic metabolites and many environmental contaminants (Egaas *et al*., 1995). The enzymes take part in transport of endogenous hydrophilic compounds, including steroids, haem, pigments, bile acids and their metabolites. Additionally they also play an important role in the detoxification of lipid peroxides and demonstrate the functions such as glutathione peroxidase activity towards reactive oxygen species in the cells in the case of oxidative stress. Induction of GST activity in some aquatic organisms such as mussels has been also found in high polluted marine environments (Hansson *et al*., 2006), after the wreckage and oil spills of the tankers (Martinez-Gomez et al., 2009). The increase of GST activity was demonstrated in some fish species and invertebrates collected in environments impacted by complex discharges of contaminants and accidents (Hamed *et al*., 2003). Thus the enzymes could be used as a biomarker of water and sediments contamination.

Fish are very sensitive to anthropogenic pollution and some of them may be tested as biomonitors for the evaluation of the ecological status of marine environment. The resistance of aquatic organisms to contamination depends on many factors including their phylogenic position, ecological and biological characteristics, physiological status (Hotard & Zou, 2008) and the presence of efficient detoxification mechanisms. Previously we described the variations in blood antioxidant system of some Black Sea elasmobranch and teleosts which reflected adaptive strategy of fish species and their ability to cope with the environment (Rudneva, 1997). The further study of the molecular mechanisms of fish resistance to environmental stress led the anthropogenic pollution is very important for the understanding the different ways of adaptations of aquatic organisms and for risk assessment.

The aim of the present work was to study the potential of GST activity in red blood cells, liver, spleen and gonads of five Black Sea teleost fish species.

MATERIALS AND METHODS

The following fish species were used: horse mackerel *Trachurus mediterraneus* (Staindachner) (n=134), high body pickarel *Spicara flexuosa* (Rafinesque) (n=70), shore rockling *Gaidropsarus mediterraneus* (L.) (n=48), round goby *Neogobius melanostomus* (Pallas) (n=56), scorpion fish *Scorpaena porcus* (L.) (n= 241).

The fish were caught in autumn-winter period of 2000-2008 in Karantinnaya Bay in the Sevastopol region (Black Sea, Ukraine) (Fig. 1). The animals were transported to the laboratory in the containers with marine water and constant aeration. The blood was taken by caudal arteria puncture and serum was separated. The red blood cells were processed as we described previously (Rudneva, 1997). The sediment was washed three times with 0.85% NaCl solution and then lysed by addition of 5 vol of distilled water for 24 h at the refrigerator. The enzyme activity was determined in the lysates immediately after preparation.

The fish were dissected and the liver, spleen and gonads were quickly removed at the ice. The organs were washed in the cold 0.85 % NaCl solution several times, then homogenized in the physiological solution using glass

homogenizer. The resulting homogenate was centrifuged at 5000 g for 20 min. The supernatant was used for further enzyme analysis.

Enzyme assays

GST activity was determined by the method of Habig *et al*. (1974) by following the increase in absorbance at 340 nm due to the formation of the conjugate 1-chloro-2,4-dinitrobenzene (CDNB) using as substrate at the presence of reduced glutathione (GSH). The reaction mixture was prepared by mixing 1.5 ml sodium phosphate buffer 0.1 M pH 6.5, 0.2 ml GSH 9.2 mM, 0.02 ml CDNB 0.1 M and 0.1 ml of the sample. The absorbance was measured at 340 nm and at the temperature $+25^0$C spectrophotometrically using Specol-211 (Germany). The increase in absorbance was recorded for a total 3 min. The reaction solution without the fish tissue homogenates and erythrocytes lysates was used as blank. The enzymatic activity was calculated via the formula:

$$A = \frac{1000 \times (E\exp - Econt) \times 1.82}{9.6 \times V \times t \times c}$$

where A – enzyme activity, conjugate nmol/min/mg protein, E_{exp}– increase of the optical density at 340 nm of the sample, E_{cont} – increase of the optical density at 340 nm of the blank, 1000 – coefficient, 1.82 - the total volume of the mixture, ml, 9.6 – molar coefficient of the conjugate formation, V – volume of the sample, ml, t – time, min, c – protein or hemoglobin (Hb) concentration.

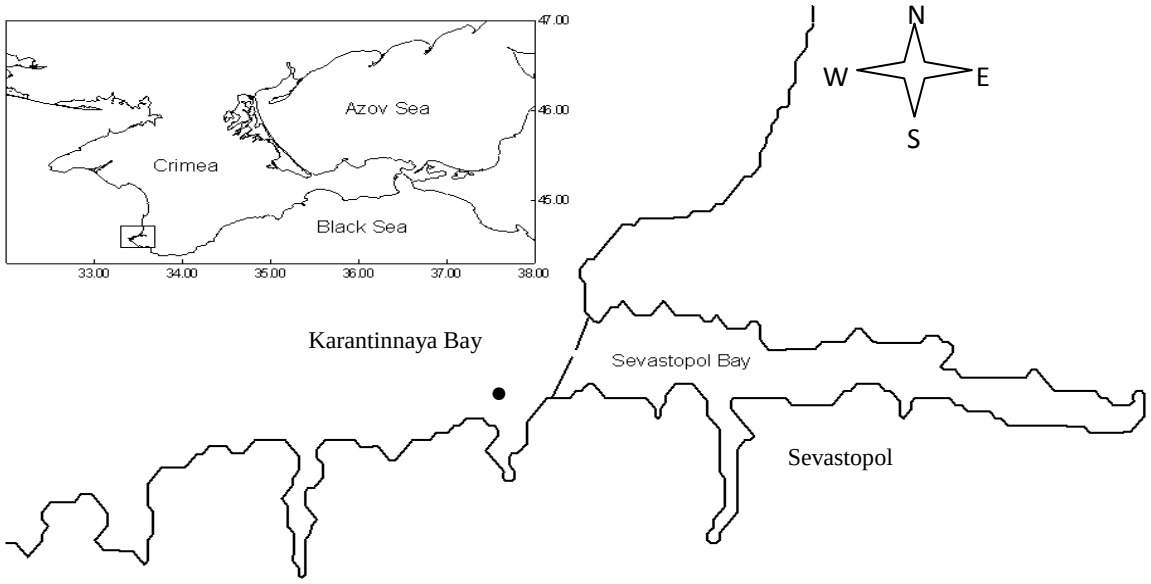

Figure 1. Sampling sites of fish specimens in Karantinnaya Bay (Sevastopol, Black Sea, Ukraine).

The protein concentration in the tissues homogenates was estimated by the method of Lowry *et al*., (1951) using human serum albumin as the standard protein. Hemoglobin concentration in red blood cells lysates was detected spectrophotometrically, using human hemoglobin as a standard (Andreeva, 2001).

Statistical analysis

The results were processed to statistical evaluation with Student's tests for each paired sample. All numerical data are given as means ± SE (Lakin, 1990). The significance level was 0.05. The correlation coefficients were calculated by the least-squares method between GST activities in all examined fish tissues.

RESULTS

All the tissues of examined fish species had GST of different specific activity. In red blood cells it varied from 10.99±0.73 nmol $\cdot$ $min^{-1}mg^{-1}$ Hb in scorpion fish to 20.03±1.61 nmol $\cdot min^{-1}$ mg^{-1} Hb in horse mackerel (Fig.2). The enzyme activity in scorpion fish blood was significant lower ($p< 0.01$) than in other examined fish species. GST activity in blood cells of shore rockling and round goby was the similar. The enzyme activity in horse mackerel was the highest and it was significant differed from the corresponding parameter of high body mackerel and scorpion fish, but showed no significant differences between the other fish species.

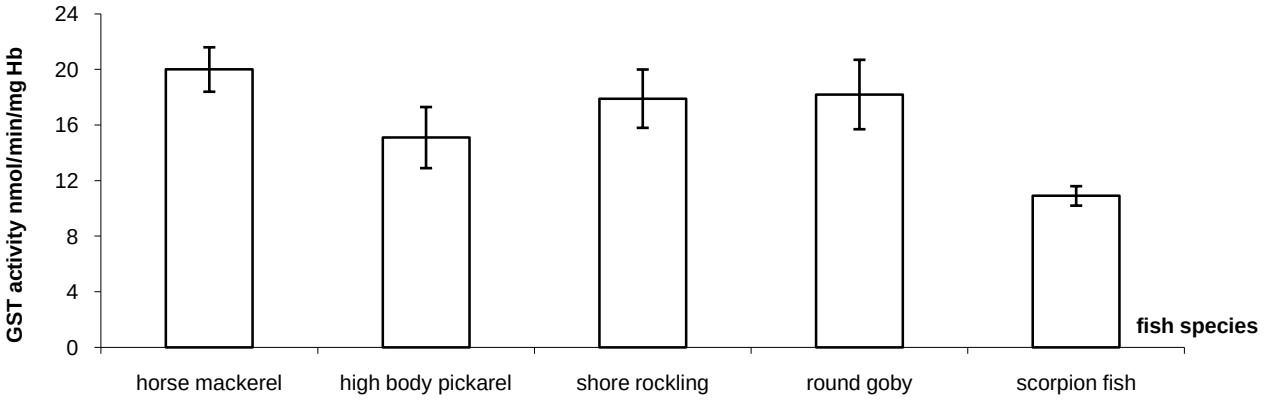

Figure 2. GST activity in the blood cells of Black Sea fish species collected in Karantinnaya Bay (Black Sea, Sevastopol, Ukraine)

The GST activity in fish liver varied from 43.02±16.55 nmol $\cdot min^{-1}$ mg^{-1} protein in shore rockling to 97.99±37.06 nmol $\cdot min^{-1} mg^{-1}$ protein in high body pickarel (Fig.3). In spite of high variations of the parameters there were no significant differences between them in examined fish species.

The GST activity in spleen of examined fish species varied less from 29.93±9.77 nmol $\cdot min^{-1}$ mg^{-1} protein in scorpion fish to 104.78±66.54 nmol $\cdot min^{-1}$ mg^{-1} protein in round goby (Fig 4). Significant differences were no shown between the enzyme activities in the spleen of examined teleosts.

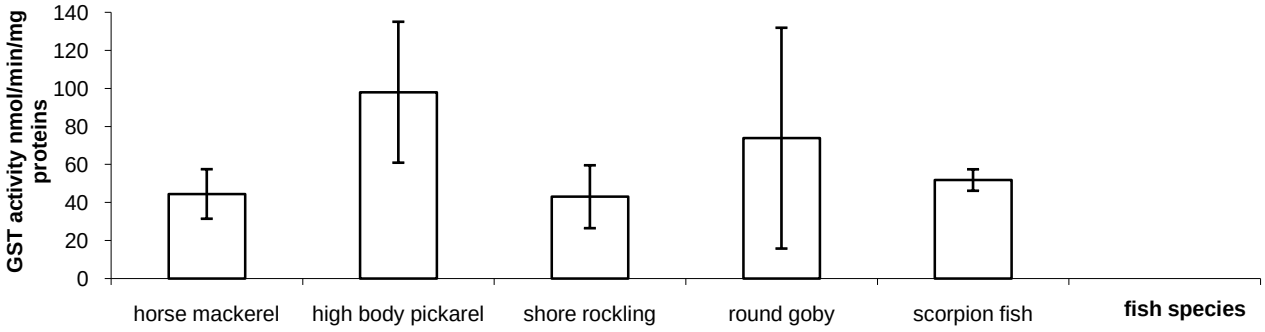

Figure 3. GST activity in the liver of Black Sea fish species collected in Karantinnaya Bay (Black Sea, Sevastopol, Ukraine)

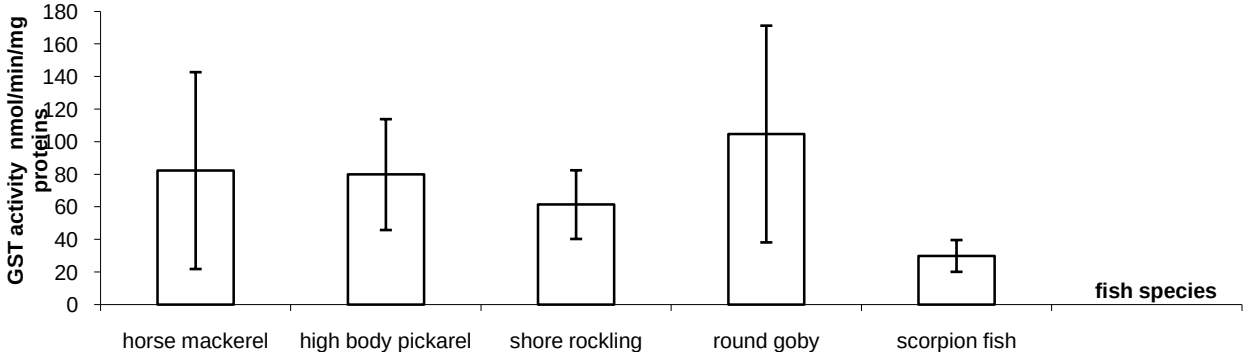

Figure 4. GST activity in the spleen of Black Sea fish species collected in Karantinnaya Bay (Black Sea, Sevastopol, Ukraine)

The highest GST activity was detected in high body pickarel gonads (736.22±303.79 nmol $\cdot min^{-1}$ mg^{-1}protein) while the least was in the round goby (39.86±5.26 nmol $\cdot min^{-1}$ mg^{-1} protein). The enzyme activity in pickarel was significant higher ($p<0.01$) than in other fish species. The GST activity in scorpion fish gonads was significant greater as compared with the parameters of horse mackerel, shore rockling and round goby which were the similar (Fig.5).

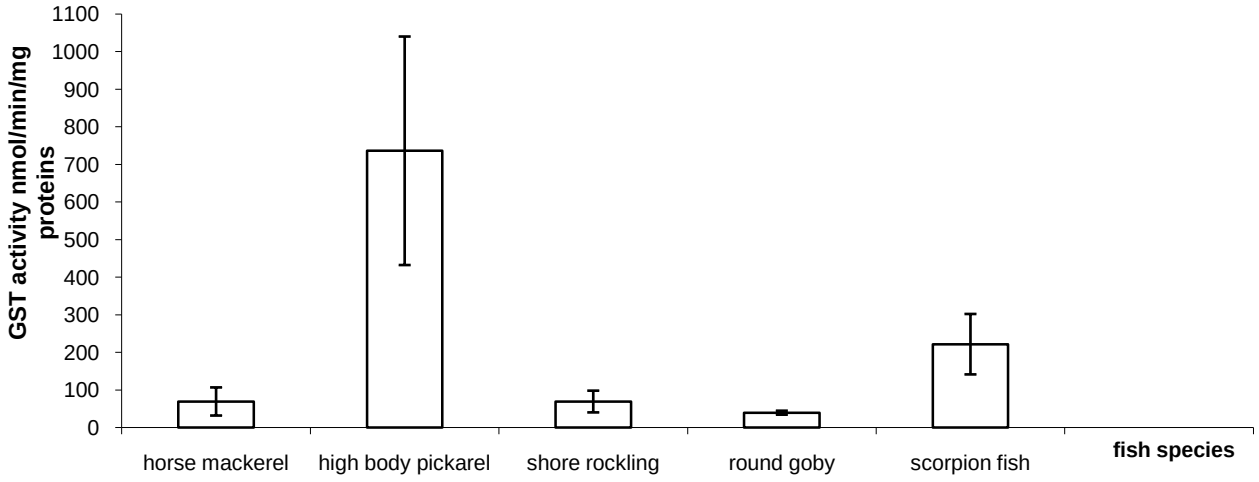

Figure 5. GST activity in the gonads of Black Sea fish species collected in Karantinnaya Bay (Black Sea, Sevastopol, Ukraine)

The correlation coefficients between the GST activities in examined fish tissues are presented in the Table-1

Table 1. Correlations between GST activities of fish blood and tissues

Fish Tissues	Correlation coefficient. r
Blood → liver	-0.28
Blood → spleen	0.43
Blood → gonads	-0.08
Liver → spleen	-0.09
Liver → gonads	0.51
Spleen → gonads	-0.33

The correlations were not shown between GST activities in blood → gonads and in liver → spleen. Strong correlation was noted between the examined parameters in blood → spleen and in liver → gonads. The correlation of enzyme activities between blood → liver and spleen → gonads were lower.

Thus, all examined fish tissues showed different GST activity which depended of tissue and fish species.

DISCUSSION

A number of environment pollutants can cause oxidative stress in aquatic organisms. Fish living in marine locations contaminated with different xenobiotics may be exposed an oxidative stressors caused be a variety of oxiradicals. To protect to this chemical compounds aquatic organisms have developed different mechanisms such as the induction of antioxidant enzymes including superoxide dismutase, catalase, peroxidase and glutathione (GSH) related enzymes (glutathione reductase (GR), glutathione peroxidase (GP) and glutathione-S-transferase (GST). GST is a phase II biotransformation enzyme which plays an important role in the removal of xenobiotics. It conjugates harmful electrophilic compounds with endogenous reduced glutathione to protect the nucleophilic molecules such as proteins and nucleic acids against oxidative damage (Di Giulio *et al*., 1989; Winston & Di Giulio, 1991). Alterations in the antioxidant enzyme activities of aquatic organisms in response to pollutants are used to indicate the potential for more severe hazards. GST activity in hepatopancreas of crustacean and mollusks and in fish liver has been suggested as biomarker of organic pollution of water environments (Filho *et al*., 2001; Ahmad *et al*., 2004; Farombi *et al*., 2007).

GST activity was identified in tissues and organs of different aquatic organisms such as algae (Cairrao *et al*., 2004), mollusks (Lau & Wong, 2003; Luca-Abbot *et al*., 2005), crustaceans (Hotard & Zou, 2008; Adewale & Afolayan, 2005), fish (Hansson T. *et al*., 2006; Sun *et al*., 2006). GST response between species was clearly quite different. It depends on the peculiarities of their phylogenetic position, biology, physiology and ecological status of the locations (Martinez-Alvarez *et al*., 2005). The interspecies variations of enzyme activity were shown between fish species (Hamed *et al*., 2003; 2004; Martinez-Gomez *et al*, 2005), invertebrates (Adewale & Afolayan, 2005) and plants (Cairrao *et al*., 2004). GST activity varied in different tissues and organs of aquatic animals (Farombi *et al*., 2007). Enzyme was detectable higher level in clam tissues compared to mussel tissues (Luca-Abbot *et al*., 2005). At the same time in green mussel *Perna viridis*

GST responses showed no significance between the gills and soft tissues (Lau & Wong, 2003). The highest GST was detected in hepatopancreas of the prawn *Macrobrachium vollehovenii* while that from the muscle was the lowest (Adewale & Afolayan, 2005). In the present study the low GST activity was determined in fish red blood cells as compared to other tissues. The enzyme activity in red blood cells of all examined fish species was the similar with the exception of scorpion fish in which that was significant lower. A significant interaction between GST activity in blood and spleen was estimated which could be connected the production of the blood cells in the spleen.

GST activity showed no differences between examined fish liver. High variability of the values could be associated with the individual responses of fish species to environmental stressors (Martinez-Gomez *et al*., 2006). At the same time the lowest GST activity in blood and in the spleen was indicated in scorpion fish as compared to other fish species.

The highest GST activity was detected in pickerel gonads which was more than 7-fold greater than in other examined fish species. The differences between enzyme activities were not significant in the gonads of the other fish species. Strong correlation was shown between GST activity in the liver and in gonad tissues which could be linked with the interactions between two organs. Yolk proteins and lipids are produced in the liver hepatocytes and then transferred to the gonads for egg formation in fish (Weigand, 1996).

Thus the study of GST activity in six Black Sea fish species allowed us to identify differences between them. We could not suggest that the ecological status influences on the GST activity in liver and spleen because pelagic horse mackerel, benthic-pelagic high body pickarel and benthic shore rockling, round goby and scorpion fish showed no significant differences of enzyme activity. We could not demonstrate the correlation between fish ecological status and GST activity in gonads and in red blood cells also. However, we could be proposed that the differences in the GST activities in examined fish tissues probably indicated that different isoenzymes are involved, as had been reported for the mammals GST (Egaas *et al*., 1995) and birds (Dai *et al*., 1996).

At the other hand the interspecies variations of examined Black Sea fish species may be the result of the different fish sensitivity to organic pollution. Many investigators demonstrated the highest GST activities in hepatopancreas in aquatic invertebrates and in the fish liver. Thus the liver is the main organ in the detoxification processes for xenobiotics and endogenously generated metabolites that could not be metabolized by the other organs. In this case the authors showed that the suitable biomarker is hepatic GST. At the same time in birds the highest GST activity was identified in the kidney followed closely by that in the liver (Dai *et al*., 1996). The tissue specific damage corresponded to the differences in the GST activity potentials of the tissues for their adaptation to environmental stress (Ahmad *et al*., 2004). Previously we described the high anthropogenic impact in Sevastopol Bay (Black Sea) and its negative consequences in fish health (Rudneva & Petzold-Bradley, 2001; Rudneva *et al*., 2008). The interspecies variations of GST activity in fish tissues may reflect the specific adaptations to the oxidative stress and protective mechanisms against oxidative damage. Thus, the analysis of GST activity in fish organs and tissues is important tool for the evaluation of fish abilities to protect against organic pollution and keep their life in the pollute environments.

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