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# Glutathione-S-Transferase Activity in Tissues of Black Sea Fish Species

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***ABSTRACT***

*The activity of glutathione-S-transferase (GST) in blood and liver in two elasmobranch and ten teleost Black Sea fish species belonging to four ecological groups was studied. Enzymatic activity varied independently in blood and liver. The interspecies differences of GST activity were associated with the specific fish taxonomic position, biology and ecology. Hepatic GST level in fish was higher as compared with that in blood which could be explained by high metabolic rate in this organ, its key role in the processes of xenobiotic detoxification and specificity of enzymes composition. High GST activity in the liver of sluggish and slow swimming fish species ( with the exception of scorpion fish) in relation to active forms was observed. The analysis of GST activity in different fish species taking into account their phylogenic position, specific features of ecology and physiology in different marine locations is important for the evaluation of fish abilities to protect against pollutants and keep their life in the impact environments.*

***KEY WORDS****: Black Sea, elasmobranches, teleosts, GST, interspecies differences*

## INTRODUCTION

Glutathione-S-transferases (GSTs) E.C.2.5.1.18) are a group of intracellular enzymes with the main function in detoxification processes by catalysing the conjugation of tripeptide glutathione (GSH) with some endogenous toxic metabolites and many environmental contaminants [1]. Additionally, the enzymes take part in transport of endogenous hydrophilic compounds, including steroids, hem, pigments, bile acids and their metabolites. GSTs also play an important role in phase II detoxification of lipid peroxides and demonstrate the functions such as glutathione peroxidase activity towards reactive oxygen species in the cells under oxidative stress [2]. A number of mechanisms have evolved to defend organism against oxygen deprivation [3]. Together with antioxidant enzymes GSTs protect the organisms from peroxidative damage and have also been used in detoxification of toxicants including pesticides, cyclic hydrocarbons, oil and other xenobiotics because they help to eliminate the oxidative by-products.

GSTs have been investigated in tissues and organs of various aquatic organisms including algae [4], mollusks [5, 6], crustaceans [7, 8], fish [9, 10] and amphibian [11]. GST activity between species was clearly quite different and reflected the peculiarities of their phylogenic position [12, 13] and diverse of ecophysiological characteristics [14]. The interspecies variations of enzyme activity were shown between fish species [15-17], invertebrates [8] and plants [4]. Induction of GST activity in some aquatic organisms such as mussels and fish has been also found in high polluted marine environments [9, 15]. Fish are very sensitive to anthropogenic impact and some of them may be tested as biomonitors for the assessment of the ecological status of marine environment. The comparative studies of biomarkers in croakers and flounder indicated the higher GST activities in fish liver in polluted site as compared with non-polluted [18, 19]. The significant differences of hepatic GST activities in two demersal fish species *Lepidorhombus boscii* and *Callinymus lyra* inhabited impacted and non-impacted areas were also found [17, 20].

Thus GST activity in fish liver could be used as exposure biomarker of water and sediments contamination.

Previously we described the variations in blood antioxidant system of some Black Sea elasmobranch and teleosts which reflected adaptive strategy of fish species to oxidative stress and their ability to cope with the environment [21]. Other researchers reported also that antioxidant enzyme activities can be correlated with phylogenic position and these parameters in primitive life are lower than in mammals and birds [14, 22]. High contents of low molecular weight antioxidants including vitamins E, K, C in organs and glutathione in red blood cells in marine elasmobranch have been found and these substances might compensate for the low level of their enzymatic status [21, 23]. The glutathione of erythrocytes protects the hemoglobin from spontaneous oxidation to metahemoglobin. Erythrocytes depleted of glutathione become very sensitive to oxidative stress [24]. Fish hemoglobin has a higher tendency to oxidation as compared with other vertebrate animals [12, 25]. In this case the investigation of glutathione related enzymes in fish red cells represents the special interest.

The main objectives of the present work were: 1. to study the potential of GST activity in red blood cells and liver in different Black Sea fish species; 2. to compare GST activity in teleosts and elasmobranch; 3. to evaluate the dependence of enzymatic activity from fish ecological characteristics.

## MATERIALS AND METHODS

### Animals

The following fish species were used stingray *Dasyatis pastinaca* (L.) (Dasyatidae) (n=4), buckler skate *Raja clavata* (L.) (Rajidae) (n=5), *Trachurus mediterraneus* (Staindachner) (Carangidae) (n=134), *Spicara flexuosa* (Rafinesque) (Centracanthidae) (n=70), whiting *Merlangus merlangus euxinus* (Nordmann) (Gadidae) (n=132), *Lisa aurata* (Risso) (Mugilidae) (n=65), *Gaidropsarus mediterraneus* (L.) (Gadidae) (n=48), *Symphodus tinca* (L.) (Labridae) (n=3), *Mullus barbatus ponticus* (Essipov) (Mullidae) (n =65), *Neogobius melanostomus* (Pallas) (Gobiidae) (n=56), *Mesogobius batrachocephalus* (Pallas) (Gobiidae) (n= 20), *Scorpaena porcus* (L.) (Scorpaenidae) (n= 241). The fish were carried out in front of the Sevastopol coast (Black Sea, Ukraine) during autumn-winter period 2000-2008 (Fig. 1). The animals were transported to the laboratory in the containers with marine water and constant aeration. For further investigations fish were grouped in four ecological groups according their belonging to different depth habitats and their biological, ecological and physiological features (Table 1).

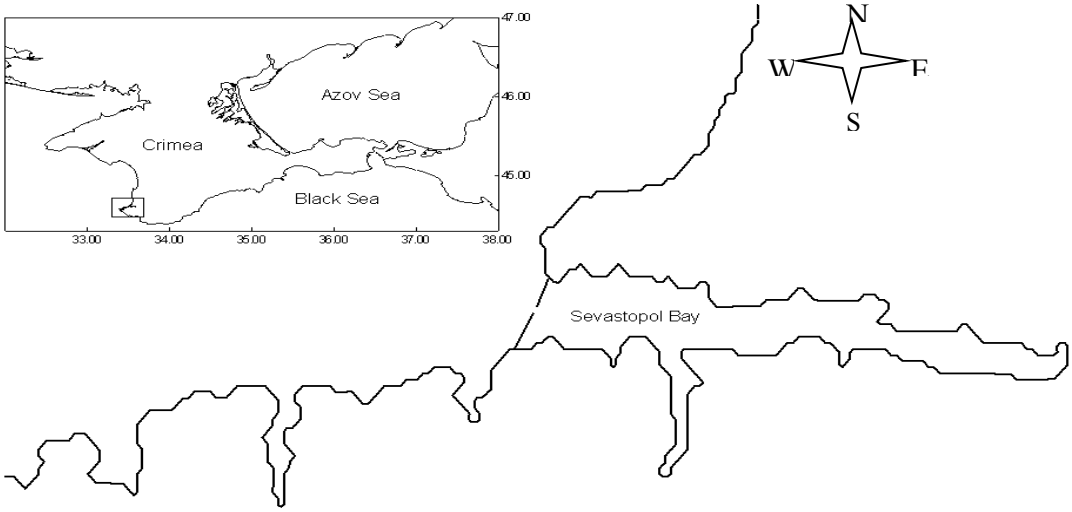


**Fig. 1. Sampling sites of fish specimens in Sevastopol Bay (Sevastopol, Ukraine, Black Sea)**

**Sample preparation**

The blood was taken by caudal arteria puncture and serum was separated. The red blood cells were processed as we described previously [21]. 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 was quickly removed at the ice. The organ was washed in the cold 0.85 % NaCl solution several times and 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*. [27] 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 hepatic homogenates and blood lysates was used as blank

**Table 1. Ecological characteristics of examined Black Sea fish species [26]**

| Fish ecological groups | | | |
|---|---|---|---|
| **Benthic** | **Suprabenthic** | **Suprabenthic/pelagic** | **Pelagic** |
| Stingray *Dasyatis pastinaca* L. (slow swimming, carnivorous) | Shore rockling *Gaidropsarus mediterraneus* L (slow swimming, predator) | Golden grey mullet *Lisa aurata* Risso (fast swimming, omnivorous) | Horse mackerel *Trachurus mediterraneus* Staidachner ( fast swimming, plankton feeder) |
| Bukler skate *Raja clavata* L. (slow swimming, carnivorous) | Peacock wrasse *Symphodus tinca* L. ( sluggish, omnivorous) | High body pickarel *Spicara flexuosa* Rafinesque (less mobile, omnivorous) | |
| Round goby *Neogobius melanostomus* Pallas (slow swimming, carnivorous) | *Mullus barbatus ponticus* Essipov ( sluggish, carnivorous) | Whiting *Merlangus merlangus euxinus* Nordmann ( less mobile, predator) | |
| Toad goby *Mesogobius batrachocephalus* Pallas (slow swimming, carnivorous) | | | |
| Scorpion fish *Scorpaena porcus* L (slow swimming, predator) | | | |

The enzymatic activity was calculated via the formula:

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

where A – enzyme activity, conjugate nmol/mg protein/min, $E_{exp}$ – 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.
The protein concentration in the liver homogenates was estimated by the method of Lowry *et al*. [28] using human serum albumin as the standard protein. Hemoglobin concentration in blood lysates was detected spectrophotomerically, using human hemoglobin as a standard [29].

**Statistical analysis**

The results were processed to statistical evaluation with Student's tests for each paired sample. All numerical data are given as means ± SEM [30]. The significance level was 0.05. The correlation coefficients were calculated by the least-squares method between GST activities in fish blood and liver. Additionally correlation test was employed to analyze the relationship between different values of enzymatic activity in fish belonging to different phylogenic and ecological groups (elasmobranch → teleosts and for paired of ecological groups of teleost species). In all cases the significant level adopted was 95% (p=0.05) [31].

## RESULTS

### GST activity in red blood cells

All the tissues of examined fish species had GST of different specific activity. In blood cells it varied from 4.19±1.71 nmol/mg Hb/min in golden grey mullet to 23.23±4.01 nmol/mg Hb/min in red mullet (Fig.2). Average value of enzymatic activity in elasmobranch blood (5.98±1.98 nmol/mg Hb/min) was lower than in major of teleosts (14.64±1.25 nmol/mg Hb/min) (Fig. 3). At the same time GST activity in skate was approximately similar with the golden grey mullet (4.19±1.17 nmol/mg Hb/min).

Teleost fish species demonstrated wide range of GST activity in red cells. Comparative analysis of enzymatic activities in the blood of the examined fish species belonging to different ecological groups is presented in Fig. 4.

Statistically significant differences were observed between blood GST level in pelagic and suprabenthic-pelagic fish species ($p<0.01$). Average value of GST activity in pelagic horse mackerel (20.03±1.61 nmol/mg Hb/min) was significantly higher ($p<0.01$) than in suprabenthic-pelagic fish species (12.09±1.73 nmol/mg Hb/min). No significant differences were shown between suprabenthic (16.42±3.59 nmol/mg Hb/min) and pelagic fish species. Differences between pelagic and benthic fish enzymatic activities were statistically significant ($p<0.01$). The average level of GST activity in horse mackerel was higher as compared with the benthic fish species (13.64±1.48 nmol/mg Hb/min).

No significant differences were observed in blood GST activity between other ecological fish groups, including suprabenthic-pelagic→suprabenthic, suprabenthic-pelagic→benthic and suprabenthic→benthic.

### Hepatic GST activity

GST activity in liver of the examined fish species varied also (Fig. 5). The lowest enzymatic activity was identified in whiting liver (12.51±5.74 nmol/mg protein/min), while the highest was in peacock wrasse (209.73±131.39 nmol/mg protein/min). The average value of GST level in elasmobranch (46.94±9.88 nmol/mg protein/min) was lower than in teleosts (99.49±25.25 nmol/mg protein/min), but the differences were not significant (Fig. 6).

Comparative analysis of GST activity in the liver showed some differences between examined ecological groups of teleost fish species (Fig. 7). No significant differences between the average value of liver enzymatic activity in pelagic (89.81±61.99 nmol/mg protein/min), suprabenthic-pelagic (45.62±13.32 nmol/mg protein/min), suprabenthic (154.42±52.38 nmol/mg protein/min) and benthic (108.11±35.31 nmol/mg protein/min) fish species were observed. Statistically significant differences were shown between GST activity in suprabenthic-pelagic and suprabenthic fish species. Average value of enzymatic level in the liver of suprabenthic species was significantly higher ($p<0.05$) than in suprabenthic-pelagic group.

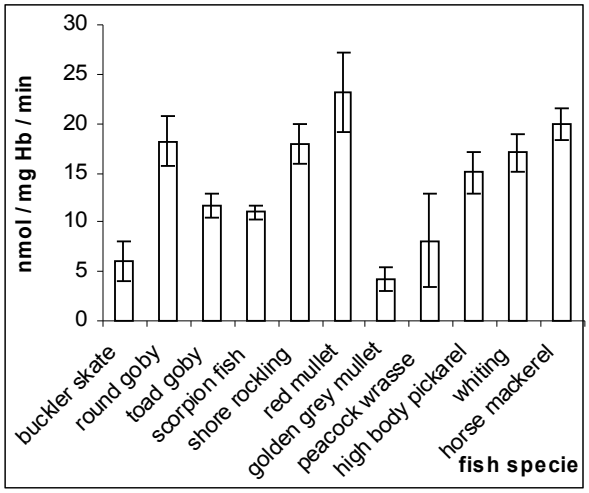


**Fig. 2. GST activity in blood erythrocytes (mean ± SEM ) of Black Sea fish species caught in Sevastopol Bay (Black Sea, Ukraine)**

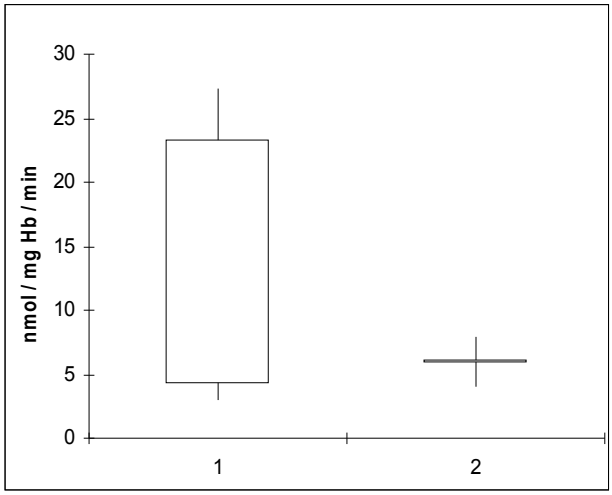


**Fig. 3. Comparative analysis of blood GST activity in Black Sea teleosts (1) and elasmobranch (2). Boxes are interquartile ranges and wisker endpoints are high/low extreams**

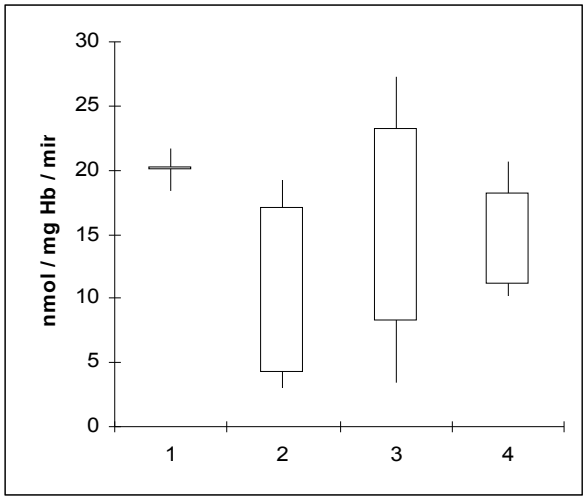


**Fig.4. Comparative analysis of blood GST activity in Black Sea teleosts fish groups: 1 – pelagic group, 2 –suprabenthic/pelagic group; 3 – suprabenthic group, 4 – benthic group. Boxes are interquartile ranges and wisker endpoints are high/low extreams**

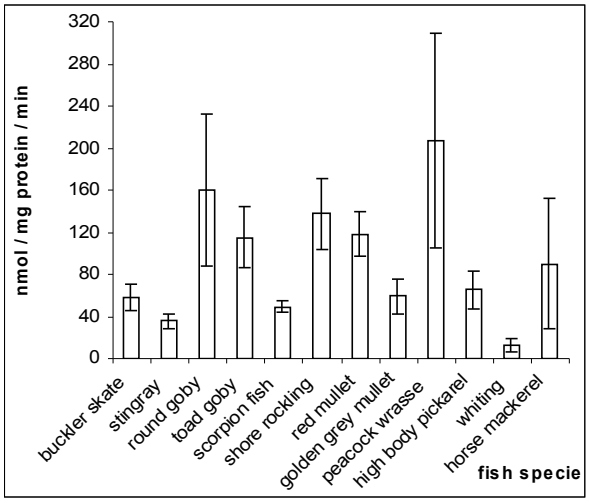


**Fig 5. GST activity in the liver (mean ± SEM) of Black Sea fish species caught in Sevastopol Bay (Black Sea, Ukraine)**

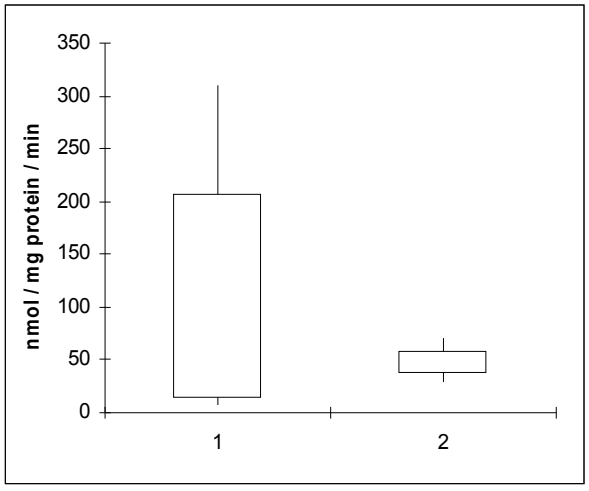


**Fig. 6. Comparative analysis of liver GST activity in Black Sea teleosts (1) and elasmobranch (2). Boxes are interquartile ranges and wisker endpoints are high/low extreams.**

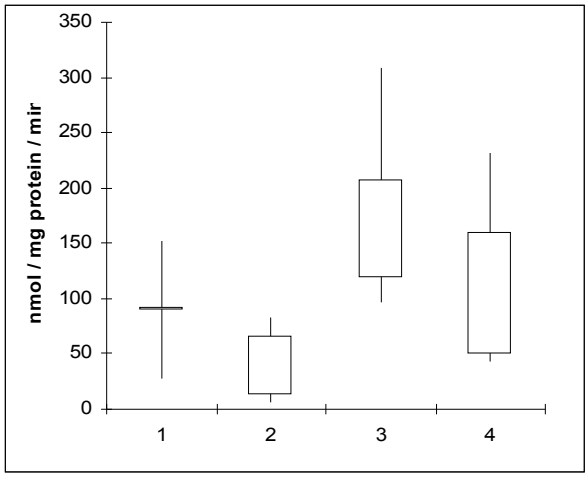


**Fig. 7. Comparative analysis of liver GST activity in Black Sea teleosts fish groups: 1 Pelagic group, 2 – suprabenthic-pelagic group; 3 – suprabenthic group, 4 – benthic group. Boxes are interquartile ranges medians and wisker endpoints are high/low extreams.**

## DISCUSSION

### Comparison of GST activity in fish blood and liver

In our study GST activity in blood of both elasmobranch and teleost fish classes was lower than in liver. Significant interspecies differences were evidenced, but they varied independently in blood and in the liver (Table. 2). No correlation was observed between hepatic and blood enzymatic activities.

GST activity varied in different tissues and organs of aquatic animals [32]. Enzyme was detectable higher level in clam tissues compared to mussel tissues [6]. At the same time in green mussel *Perna viridis* GST responses showed no significance between the gills and soft tissues [5]. The highest GST was detected in hepatopancreas of the prawn *Macrobrachium vollehovenii* while that from the muscle was the lowest [8].

Liver of vertebrates exhibits a high metabolism and oxygen consumption and it is the main organ of xenobiotic detoxification. It is particularly rich source of GST. In the rat GST represents some 10% of soluble hepatic protein and in man about 3% of it [1]. The strong relationships between antioxidant enzyme activities and phase II GST support high level of defense mechanisms against oxidative stress led the pollution. Additionally, fish liver displayed the highest levels of the key antioxidant enzymes SOD and CAT [33-34].

**Table. 2. Differences of GST activity in blood (light field) and liver (dark field) between teleost fish species**

| species | whiting | high body pickerel | golden grey mullet | peacock wrasse | shore rockling | red mullet | toad goby | round goby | Scorpion fish |
|---|---|---|---|---|---|---|---|---|---|
| horse mackerel | n/s | n/s | <0.01 | <0.05 | n/s | n/s | <0.01 | n/s | <0.01 |
| whiting | | n/s | <0.01 | n/s | n/s | n/s | <0.05 | n/s | <0.05 |
| high body pickerel | n/s | | <0.01 | n/s | n/s | <0.01 | n/s | n/s | <0.01 |
| golden grey mullet | n/s | n/s | | n/s | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| peacock wrasse | n/s | n/s | n/s | | n/s | <0.05 | n/s | <0.05 | n/s |
| shore rockling | n/s | n/s | <0.05 | <0.05 | | n/s | n/s | n/s | <0.05 |
| red mullet | n/s | n/s | n/s | n/s | <0.01 | | <0.z05 | n/s | <0.05 |
| toad goby | n/s | n/s | n/s | n/s | n/s | n/s | | <0.05 | n/s |
| round goby | n/s | n/s | n/s | n/s | n/s | n/s | n/s | | <0.01 |
| scorpion fish | n/s | n/s | n/s | n/s | <0.05 | n/s | <0.05 | n/s | |

However, we could be proposed that the differences in the GST activities in examined fish blood and liver probably indicated that different isoenzymes are involved, as had been reported for the mammals GST [35], birds [36] and fish species [1, 37]. Research the different classes of izoenzymes and the sorts of substrates for GSTs in fish is important for understanding the conjugation mechanisms of xenobiotics of ecological interest and comparison them with the other taxonomic groups because hepatic GST activity has been widely used as biomarker of pollution response. Its induction associates with the metabolic function of the liver and its ability to eliminate the oxidative by-products. Differences in hepatic GST isoenzymes in marine fish from areas with different levels of contamination were reported [9, 37]. The induction of hepatic GST

activity evidences that fish from polluted sites are under oxidative stress and enzyme is involved in defense mechanism against peroxidative products [18, 38].

Thus we could conclude that in our study the hepatic GST level in fish was higher compared with that in blood which agrees with the data of other researchers. It could be connected with high metabolic rate in this organ, its key role in the processes of xenobiotic detoxification and specificity of isoenzymes composition

**Comparison of GST activity in elasmobransh and teleosts**

GST activity was detected in blood and liver both in elasmobranch and teleosts. We've found that enzymatic activity in elasmobranch blood was significantly lower than in teleosts with the exception of golden grey mullet and peacock wrasse. In our previous study we also showed the lack of CAT activity and decrease SOD activity in red blood cells of Black Sea elasmobranch dogfish *Squalus acanthias* as compared with teleosts [21]. At the same time CAT and SOD were presented at high level in blood of some marine fish species [33].

GST activity has been detected in the liver of many elasmobranch and teleosts fish species. In fish as in mammals and birds GST account for an appreciable proportion of soluble hepatic proteins. However, the information of its rations is differed and sometimes it's a problem to compare the activities between species because of differences in assay conditions and substrates. Additionally, the enzyme does not conjugate with some substrates and in this case it will not be detected. The most convenient substrate is 1-chloro-2, 4-dinitrobenzene (CDNB) because it usually conjugates in high rate [1] and we used it in our investigations. But the catalytic GST activity towards CDNB is also considered to represent an integration of the activities of most GST classes, and the induction of specific GST isoenzymes may be masked [37].

A comparative study of hepatic antioxidant defense enzymes in teleosts and elasmobranchs was shown that their content in primitive fish species was lower than that of teleosts and seems to follow the overall metabolic oxygen consumption or activity level from each fish major taxonomic group [34]. Taking into account the peculiarities of metabolic pathways in elasmobranch as compared with teleosts we could propose the presence of specific GST activity in them [12]. But we could not postulate that hepatic GST activity in elasmobranch significantly differed related to teleosts. No differences were observed between both examined elasmobranches also. Enzymatic level was higher than in whiting, approximately similar as compared with most of teleost fish species and significantly lower than in gobies, shore rockling and peacock wrasse. Thus we could suggest that ecological peculiarities of fish species play more important role in GST induction.

**Comparison of GST activity in different fish species**

The present study supports the greater interspecies differences in hepatic and blood GST activity between examined teleosts, which agree with the results of other researchers, who reported about the high variability of antioxidant enzyme and phase II GST contents in fish [14, 34]. In our study interspecies differences in blood were more significantly than in liver which showed more homogenous response (Table 2).

No clear relationships between fish swimming capacity and blood GST activity could be established for the totality of forms investigated. The examined species were classed on their swimming activity as fast swimming (horse mackerel and golden grey mullet), less mobile pickerel and whiting, sluggish red mullet and peacock wrasse and slow swimming gobies, shore rockling and scorpion fish (Table 1). In spite of horse mackerel and golden grey mullet are the active forms their blood GST activity was differed in 5-fold from each to other. Significant difference was detected between sluggish red mullet and peacock wrasse. At the same time less mobile pickerel and whiting showed the similar activity and the values in slow swimming fish varied lower. Contrary, the activity of some antioxidant enzymes in fish blood correlated with their swimming capacity. In our previous study we found that SOD and CAT activities in horse mackerel and pickerel were significant higher than in the blood of slow swimming gobies, scorpion fish and flounder [21]. Other researchers reported also that CAT and SOD contents in

blood of more active forms were higher as compared to more sluggish species [33]. At the same time the changes in the sturgeon blood prooxidant-antioxidant status, as a consequence of adaptation to marine conditions, were not reflected in the liver and other tissues [25].

As for results obtained at present study we could mark low GST activity in fish red cells and its independent variations from swimming capacity. We could propose that GST plays an additional role in the detoxification process of scanty xenobiotcs in erythrocytes while the main mechanism is provided by passive gill excretion of pollutants. Such a mechanism was described by Filho *et al*. [33] for the explanations of the absence or relatively low concentration of glutathione peroxidase in some fish species which could rid of $H_2O_2$ by using passive gill excretion also.

The obtained results demonstrated the higher GST activity in the liver of sluggish and slow swimming fish species ( with the exception of scorpion fish) in relation to active forms. The data contradict the results obtained other researchers for hepatic antioxidant enzyme activities in fish with different swimming capacity. The SOD and CAT levels in liver appear to indicate that the most active species both teleosts and elasmobranches had greater enzyme activity compared with low mobile forms [33, 34]. The higher activity of antioxidant enzymes in liver of active fish correlated with the higher oxygen consumption in fast swimming species and their high metabolic rate [12, 25]. Animals with high metabolic rate exhibits the high rates of free radical production and caused the induction of antioxidant defense mechanisms [39].

In contrast, taking into account that the most of sluggish fish species belonging to benthic and suprabenthic groups we could propose that they live in more contaminant environment because many pollutants accumulate in bottom sediments and low water layers. Thus, hard pressing of chemicals induces hepatic GST activity especially in benthic and suprabenthic forms.

Fish trophic level, feeding behavior and nutrition factors also may affect biomarkers including GST [14, 25]. Our species were classed as pelagic plankton feeders (horse mackerel), omnivorous (golden grey mullet, peacock wrasse and pickerel), carnivorous (red mullet and both species of goby) and predators (shore rockling, whiting and scorpion fish) (Table 1). No clear relationships between feeding groups and GST activities in blood and liver could be detected also. However, carnivorous fish species demonstrated approximately similar enzymatic activity in the liver. It could be explained that benthic invertebrates (mollusks, crustacean and worms) and fish which are the preferable prey for such a group might accumulate xenobiotics from the bottom sediments and transfer them via trophic nets to fish with the effect of concentration. Hepatic GST activity in pelagic plankton feeder horse mackerel and omnivorous suprabenthic/pelagic pickerel and golden grey mullet with the exception of peacock wrasse was approximately similar and lower than in carnivorous, which was explained the similarity of their high metabolic rate and diet consumption. High variability of hepatic GST activity we found among predators, but in blood it varied less.

Obtained results demonstrated that the various characteristics of fish biology are reflected on GST level in blood and liver. We could conclude that the complex of specific phylogenic, physiological and ecological features of fish specie may influence on the phase II GST activity and it is important to understand for development of monitoring programs. As we see biomarkers of benthic forms are more convenient for monitoring studies, but benthic fish species are differed each from other, suprabenthic forms were more homogeneous as compared with suprabenthic/pelagic and benthic fish species. However, among suprabenthic species we found the forms with different swimming capacity and type of feeding. Present study indicates that the complex of biotic and abiotic factors including anthropogenic impact may be attributed to GST activity in fish blood and liver.

## CONCLUSION

Thus the study of GST activity in ten Black Sea teleost fish species and two elasmobranch allowed us to identify differences between them. We could not suggest that the phylogenetic position and ecological status influence on the GST activity in liver and blood significantly.

At the other side the interspecies variations of examined Black Sea fish species may be the result of the different fish sensitivity to organic pollution. The tissue specific damage corresponded to the differences in the GST activity potentials of the species for their adaptation to environmental stress [40]. Previously we described the high anthropogenic impact in Sevastopol Bay (Black Sea) and its negative consequences in fish health [41]. The interspecies variations of GST activity may reflect the specific adaptations to the oxidative stress and protective mechanisms against oxidative damage. Thus, the analysis of GST activity in different fish species taking into account their phylogenic position, specific features of ecology and physiology is important for the evaluation of fish abilities to protect against pollutants and keep their life and biodiversity in the impact environments.

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