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Biochemical Characterization and Distribution of Glutathione S-Transferases in Leaping Mullet (*Liza saliens*)[1]

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Abstract—In this study, feral leaping mullet (*Liza saliens*) liver cytosolic glutathione S-transferases (GSTs) were investigated and characterized using 1-chloro-2,4-dinitrobenzene (CDNB) and ethacrynic acid (EA) as substrates. The average GST activities towards CDNB and EA were found to be 1365 ± 41 and 140 ± 20 nmol/min per mg protein, respectively. The effects of cytosolic protein amount and temperature ranging from 4 to 70°C on enzyme activities were examined. While both activities towards CDNB and EA showed similar dependence on protein amount, temperature optima were found as 37 and 42°C, respectively. In addition, the effects of pH on GST-CDNB and -EA activities were studied and different pH activity profiles were observed. For both substrates, GST activities were found to obey Michaelis–Menten kinetics with apparent V_{max} and K_m values of 1661 nmol/min per mg protein and 0.24 mM and 157 nmol/min per mg protein and 0.056 mM for CDNB and EA, respectively. Distribution of GST in *Liza saliens* tissues was investigated and compared with other fish species. Very high GST activities were measured in tissues from *Liza saliens* such as liver, kidney, testis, proximal intestine, and gills. Moreover, our results suggested that GST activities from *Liza saliens* would be a valuable biomarker for aquatic pollution.

Key words: phase II, glutathione S-transferases, 1-chloro-2,4-dinitrobenzene, ethacrynic acid, biomonitoring, *Liza saliens*

It is well known that aquatic environments are being contaminated with a great variety of inorganic and organic pollutants, possibly affecting the integrity of ecosystems and physiological functions of individual organisms. The study of biotransformation enzymes such as monooxygenases and glutathione S-transferases in fish is important in many respects including evolutionary, ecological, and toxicological standpoints [1]. In addition, fish represent a vital source of protein for humans. There has been increasing number of studies to characterize these biotransformation enzymes in various aquatic organisms because various biotransformation enzymes are accepted as effective biomarkers for chemical pollution [2-7].

Biotransformation of xenobiotics through phase I (monooxygenase) and phase II (conjugase) enzymes is a requisite for detoxification and excretion [8, 9]. Glutathione S-transferases (GSTs, EC 2.5.1.18) catalyze the conjugation of glutathione (GSH) with wide variety of endogenous and exogenous compounds. Although they are primarily involved in detoxification, in a few instances they play a role in activation of several chemicals including toxic and carcinogenic compounds [10-12]. These enzymes have been found in virtually every living species studied, including plants, animals, and bacteria [13-15].

Together with glutathione (GSH), GSTs are the predominant cytosolic defense system responsible in protecting cellular components against various toxic effects and oxidative stress [16-21]. Five classes of cytosolic GST isoenzymes have been determined and are referred to as α, μ, π, σ, and θ. The individual glutathione S-transferase isoenzymes exhibit different substrate specificity, rendering them suitable for different defense tasks [22]. These enzymes are involved in detoxification of a great variety of compounds in fish and other marine animals. Various isoforms of hepatic GSTs have been purified and partially characterized in different fish species [23-26]. GST isoenzymes appear to be expressed to varying extent in tissues from different species and even in tissues within the same species. Although these enzymes have been mainly found in liver, GST activities have been demon-

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strated in gills, kidney, and intestinal ceca of several fish species [5, 25-27]. The differential expression of GST isoenzymes in different organs is of particular interest as it might be a factor in the differential susceptibility of tissues to the toxic effects of xenobiotics [28]. The GST expression levels in many species can be significantly increased by exposure to foreign compounds, suggesting that they form part of an adaptive response to chemical stress, which is used as effective biomarkers of aquatic contamination [15].

In this study, first, we have characterized the GST activities using 1-chloro-2,4-dinitrobenzene (CDNB) and ethacrynic acid (EA) as substrates in cytosolic fractions of livers from feral leaping mullet (*Liza saliens*). In addition, we have investigated the distribution of these activities in various tissues of *L. saliens* and in fish caught from various sites along the Aegean coasts of Izmir, Turkey; to study the differential susceptibility of various tissues to toxic effects of pollutants and possible importance as a biomarker.

Further studies such as immunochemical localization and *in vitro* effects of various metals and pollutants are underway to better clarify the role of these enzymes in ecotoxicological aspects.

MATERIALS AND METHODS

Chemicals. 1-Chloro-2,4-dinitrobenzene (CDNB), butylated hydroxytoluene (BHT), cholic acid, ethacrynic acid (EA), glutathione reduced (GSH), and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma (USA). Ethylenediaminetetraacetic acid disodium salt (EDTA), glycerol, and potassium chloride were from Merck (Germany). All other chemicals were of the highest grade commercially available.

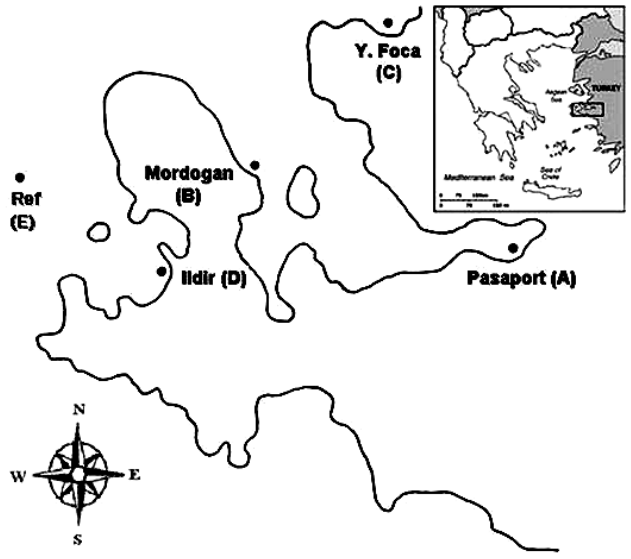

Fig. 1. *Liza saliens* sampling sites around Izmir Bay on the Aegean cost of Turkey.

Fish and sampling sites. Leaping mullet (*Liza saliens*) belongs to the family of Mugilidae of the class Osteichthyes. It is an economically important food source and is used locally for bioindication of organic pollution in the Aegean Sea [29]. Leaping mullet (weighing between 300-600 g) were collected by fish netting along Izmir on the Aegean coast of Turkey at different sites receiving municipal and industrial discharges (Fig. 1). Izmir is located in the Eastern part of the Aegean Sea of the Mediterranean and is one of the heavily contaminated areas in this part of Turkey. *Liza saliens* were captured in April, May, June, and August of 2003.

Preparation of cytosolic fractions of tissues from *Liza saliens*. Fish were killed by decapitation and dissected, and several tissues such as intestine and stomach were rinsed thoroughly and carefully with ice-cold physiological saline. The dissected intestine (proximal intestine (PI) and distal intestine (DI)) and nine other tissues (liver, gills, stomach, kidney, testis, heart, brain, spleen, and muscle) were then immediately frozen in liquid nitrogen. Tissues were transported in liquid nitrogen from sampling sites to laboratories in Pamukkale University, Denizli. Frozen tissues were first thawed on ice and then weighed and minced with scissors. The resulting tissue mince was homogenized in five volumes of 1.15% KCl solution containing 2 mM EDTA, 0.15 mM PMSF, 0.10 mM BHT, and 0.025% cholate by using a Potter–Elvehjem glass homogenizer (packed in crushed ice) coupled with motor (Black and Decker Skill Plus multispeed drill)-driven Teflon pestle at 2600 rpm; ten passes were made for homogenization. The homogenate was then centrifuged at 13,300 rpm using a Sigma 3K30 high-speed refrigerated centrifuge with 12156 rotor for 40 min to obtain post-mitochondrial fraction. Aliquots were removed from post-mitochondrial fractions and remaining fractions were filtered through doubled layers of cheese-cloth and centrifuged at 145,215 rpm using a Sorvall Combi Plus Ultraspeed centrifuge with T880 rotor (DuPont, USA). Calculated volumes of glycerol were added as a stabilizer to the supernatant (cytosol) fractions to obtain 10% final concentration, and pellet (microsomes) was suspended in homogenization solution and re-centrifuged as above. The resulting washed microsomal pellet was resuspended in 10% glycerol containing 2 mM EDTA and homogenized manually using a glass–Teflon homogenizer. All fractions were stored in liquid nitrogen until use.

Protein concentrations of the cytosolic and microsomal fractions of tissues from *L. saliens* were determined by the Bradford method [30] using Sigma Protein Dye Reagent. Crystalline bovine serum albumin was used as standard and measurements were performed on a Shimadzu UV1600 (Japan) spectrophotometer at 595 nm.

Enzyme assays. GST activities using CDNB (measured in 0.1 M potassium phosphate buffer, pH 7.5, containing 1 mM CDNB, 1 mM GSH, and 25 μg cytosolic protein) and EA (carried out in 0.1 M Tris-HCl, pH 7.8, containing 0.2 mM EA, 0.5 mM GSH, and 25 μg cytosolic protein) as substrates were determined at room temperature spectrophotometrically by following the change in absorbencies at 340 and 270 nm, respectively [31]. Enzymatic rates were obtained by subtracting the nonenzymatic rate (background rate obtained without sample) from the enzymatic rate (with sample) and enzyme activities towards CDNB and EA were calculated using extinction coefficients of 9.6 and 5.0 $mM^{-1} \cdot cm^{-1}$, respectively [31].

Assuming Michaelis–Menten kinetics, apparent V_{max} and K_m values for the GST activities were calculated using the Graphpad Prism 3.03 graphic software (Graphpad Software Inc., USA). Michaelis–Menten graphs were prepared by applying a nonlinear regression analysis using minimize absolute distances squared method. Finally, V_{max} and K_m values were calculated by using a linear regression curve obtained using the least squares method with testing departures from linearity.

7-Ethoxyresorufin O-deethylase (EROD) activities were determined by fluorometric measurement of resorufin formed using the method of Burke and Mayer [32] optimized by [33].

Statistical analysis. Results are expressed as mean ± SD of at least three determinations for each data point. One-way ANOVA, Tukey, and Dunnett tests were applied for analyzing the significance of difference between and among different groups.

RESULTS AND DISCUSSION

GST activities with CDNB and EA in *L. saliens* liver cytosol were determined by following the appearance of relevant GSH conjugates at 340 and 270 nm, respectively. Standard assay conditions for both substrates were determined and characterized by using the cytosolic fractions from feral leaping mullets caught from Pasaport (sampling site A) due to convenience of obtaining fish from this site. In addition, it was also the choice for environmental aspects because it has been shown to be contaminated with heavy metals, hydrocarbons, organic and nutritive materials, and pathogenic organisms [29, 34, 35]. Some of the standard assay conditions were also determined with the mullets caught from relatively unpolluted waters in order to verify the effects of pollutants on liver enzymes. No significant differences were observed among the assay conditions determined using fish caught from polluted or unpolluted areas.

First, we have determined the subcellular distribution of GST activities in post-mitochondrial (S9), cytosolic, and microsomal fractions from the same sample (Table 1). Since GSTs are mainly cytosolic, predominant activities were detected in cytosol (50.5 and 56.0% for CDNB and EA, respectively). On the other hand, still considerably high activities were observed in post-mitochondrial fractions (38.7 and 31.1% for CDNB and EA, respectively). For both substrates, quite high GST activities were observed in microsomal fractions (287 ± 26.2 and 25.92 ± 2.71 nmol/min per mg protein for CDNB and EA, respectively). These activities are even higher than the cytosolic GST activities reported for various fish species (discussed later in context). Similar subcellular distributions of GSTs in marine and terrestrial animals have been reported [28, 36]. These results suggest that although post-mitochondrial fractions could be used to assess soluble GSTs in tissues from various organisms, it should be better to prepare cytosolic fractions to obtain more reliable and consistent results.

Table 1. Subcellular distribution of GST specific activities towards CDNB and EA in livers from feral *Liza saliens*

Fraction	Enzyme activity, nmol/min per mg protein	
	CDNB	EA
Post-mitochondrial	1340.8 ± 18.5	108.8 ± 6.6
Cytosol	1028.8 ± 8.25	60.0 ± 2.4
Microsomes	287.3 ± 26.2	25.9 ± 2.7

Note: Results are mean ± SD of triplicates measurements of four sets of experiments.

Assay conditions such as reaction time and temperature and protein and substrate concentrations were carefully characterized for hepatic cytosolic GST activities of *L. saliens* towards both CDNB and EA. Under standard assay conditions, GST reaction rates were found to be linear with protein concentrations in the range from 0.005 to 0.05 mg/ml assay mixture followed by slight deviations from linearity in the range of 0.05 to 0.1 mg/ml protein, and finally depicting saturation above protein concentrations of 0.1 mg/ml (data not shown). For this reason, 0.025 mg protein was used throughout the study.

Dependence of GST activities towards CDNB and EA was investigated by the use of a range of acetate (pH from 4.0 to 5.5), phosphate (6.0-8.5), and glycine-NaOH buffers (from 9.0 to 11.0). Figure 2 shows the effect of pH on GST activities of *L. saliens*. While two pH optima (pH 7.5 and 11.0) were observed for CDNB activity, GST activity towards EA showed a pH optimum plateau between pH 7.8-9.0. Such results suggested the possible presence of multiple GST isoenzymes in hepatic cytosolic fractions from *L. saliens* for CDNB. (In addition, it is

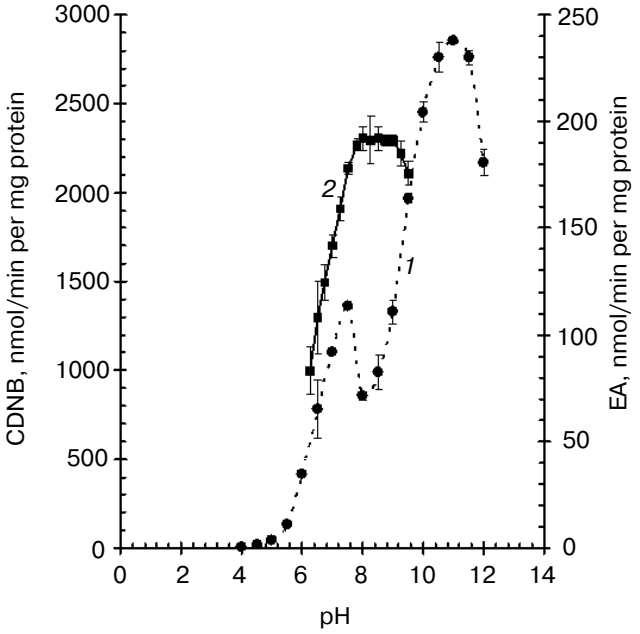

Fig. 2. Effect of pH on *L. saliens* liver cytosolic GST activities with CDNB and EA. The reaction mixture contained 25 μg cytosolic protein and 1 mM CDNB (*1*) or 0.2 mM EA (*2*). The reaction was carried out at room temperature as described in "Materials and Methods". The points are mean ± SD of triplicates of three sets of experiments.

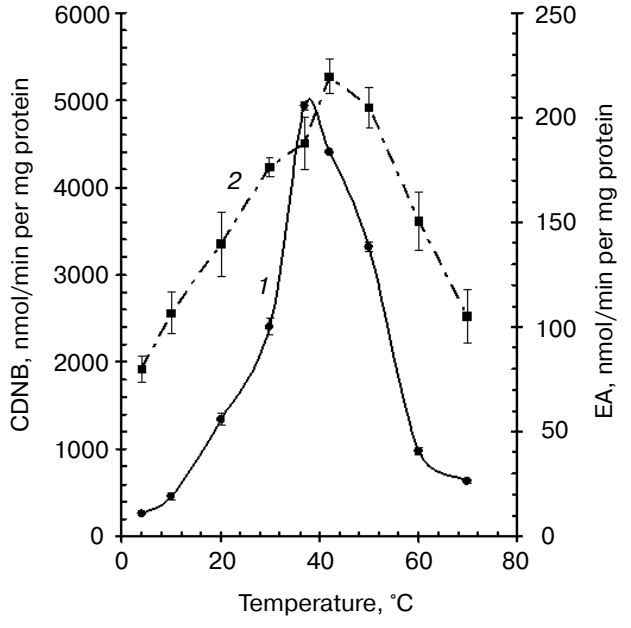

Fig. 3. Influence of temperature on *L. saliens* liver cytosolic GST activities towards CDNB (*1*) and EA (*2*). Reaction conditions are the same as given in Fig. 2. Results are mean ± SD of triplicates of two sets of experiments.

known that the distribution of pH optima for CDNB could be bimodal, indicating the possible existence of several isoenzymes in catalysis of CDNB [36]). Generally, GSTs have pH optima in the range of 6.5 to 9.5 [37]. The pH optimum for both CDNB and EA observed in *L. saliens* are in accordance with the values reported for other fish species such as rainbow trout [23], catfish [25], and butterfly bream [26].

In order to study the effect of reaction temperature on enzyme activities, a range of incubation temperatures from 4 to 70°C was assessed. Figure 3 illustrates the dependence of GST activities with CDNB and EA on reaction temperature. Reaction rate, using the CDNB as a substrate, increased relatively more steeply than EA activity and showed temperature optimum at 37°C. On the other hand, GST activity with EA showed a wider temperature activity curve with temperature optimum at 42°C. Whatever the substrate was used no absolute denaturation was detected at both extreme temperatures. *In vitro* metabolism of xenobiotics has been shown to possess considerably varying optimum temperature depending on the type of various factors such as substrates and species. In general, hepatic phase II conjugating reactions represented peak activities at temperatures above 35°C [23, 24, 26]. Optimum temperatures obtained in this study exhibited similar temperature optima with rainbow trout, sturgeon, and bream [23, 24, 26].

Figures 4a and 4b display the substrate saturation kinetics of hepatic cytosolic GSTs from feral leaping mullet with CDNB and EA, respectively. Enzyme activities were found to be saturated at and above 2 mM CDNB and 0.1 mM EA concentrations. For both substrates, reaction rate vs. substrate concentration curves fitted the Michaelis–Menten kinetics with a regression coefficients of 0.9725 for CDNB and 0.9002 for EA. Apparent V_{max} and K_m values for CDNB and EA using Lineweaver–Burk graph were calculated and found to be 1661 nmol/min per mg protein and 0.24 mM and 156 nmol/min per mg protein and 0.056 mM, respectively. The K_m values using CDNB as a substrate reported for fish GSTs have shown great variations ranging from 0.4 to 6.15 mM. Therefore, it is very difficult to compare these apparent kinetic parameters with those found in the literature. These differences may reflect species variations in metabolism of exogenous and/or endogenous compounds. Nonetheless, as reported for other fish species, very high GST activities towards CDNB and much lesser extend towards EA were observed with *L. saliens* liver cytosolic fractions. This might be due to presence of several isoenzymes and few or single isoenzymes catalyzing CDNB and EA, respectively.

It is well documented that multiple GST isoenzymes occur in different tissues of organisms and play important roles in species and tissue specific metabolism of reactive xenobiotics [37, 38]. They catalyze the conjugation of GSH with both phase I and non-metabolized xenobiotics

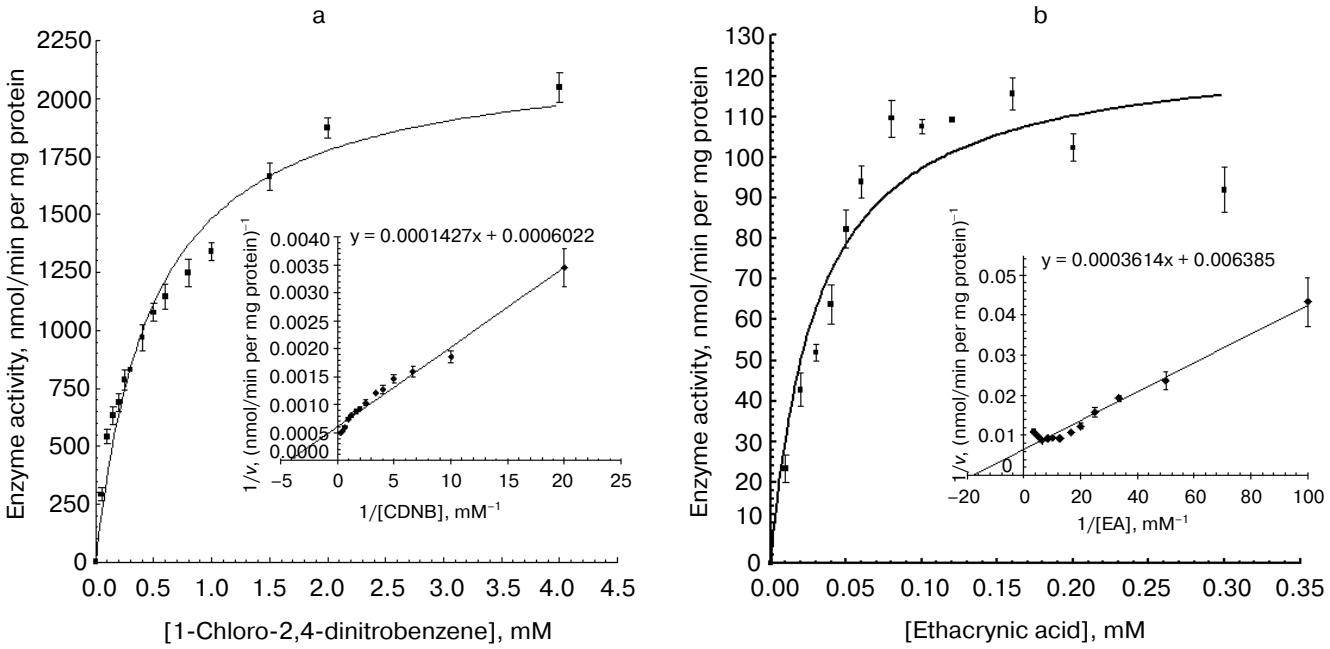

Fig. 4. Substrate saturation curve of *L. saliens* liver cytosolic GSTs for CDNB (a) and EA (b). Substrate concentrations ranging from 0.05 to 4 mM and 0.005 to 0.3 mM were used for CDNB and EA, respectively. Lineweaver–Burk plots showing the effect of substrate concentrations on enzyme activities were depicted in each embedded subfigure in squares. Results are mean ± SD of triplicates of four sets of experiments.

and their expressions are known to be species and tissue specific. Figure 5 shows the distribution of GSTs assessed by measuring the conjugation of GSH with CDNB and EA in various *L. saliens* tissues. For both substrates, the maximum activity was found to be in liver followed by kidney, testis, and proximal intestine. Similar to liver tissues from many mammals and other fish, *L. saliens* liver had a very high GST activity with both CDNB and EA. However, GST activities in several extrahepatic tissues such as kidney and testis of *L. saliens* are high enough to be almost equal to hepatic GST activities. The distribution of GST activity with CDNB showed a similar pattern with EA suggesting the involvement of same isoenzymes(s) in catalysis of both substrates in *L. saliens* tissues. The presence and functions of GSTs in kidney is well establishes and renal GSTs from various species have been purified and characterized [39, 40]. Like many other species, we have observed high GST-CDNB and -EA activities in *L. saliens*. It is interesting that the GST activities detected in testis and proximal intestine are higher than the activities observed in gills. The possible role of GSTs in intestinal first pass metabolism of electrophilic and other xenobiotics has been shown in rat and some fish species [5]. Our results also suggest that intestinal GSTs are important in protecting *L. saliens* against toxic chemicals and potential carcinogens available to fish through the digestive system by which fish acquire resistance to those xenobiotics and their adverse effects. This might be advantageous for *L. saliens* because it usually inhabits polluted areas fed with municipal discharges along seashores.

In the literature, there is not much data on testis GSTs from fish species. It is interesting that the testicular

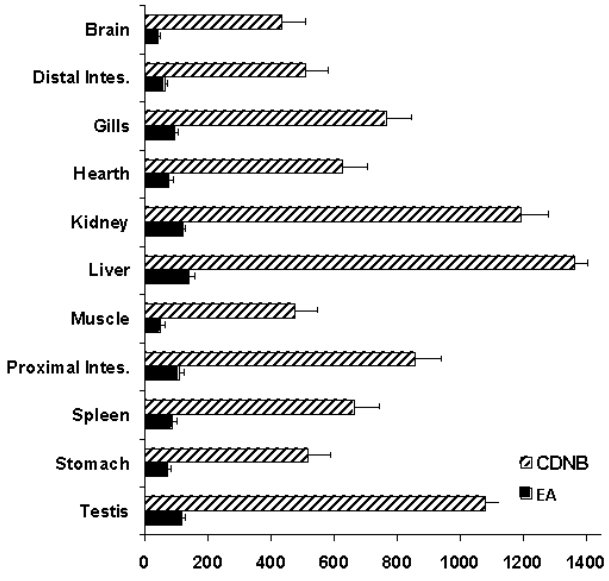

Fig. 5. Distribution of GSTs activities towards CDNB and EA in various tissues from *L. saliens*. Enzyme activities are measured as described in "Materials and Methods". Results are expressed as mean ± SD of triplicates of two sets of experiments.

Table 2. GST activities towards CDNB and EA in various tissues of *Liza saliens* and several other fish species together with rat and human

Species	Enzyme activity, nmol/min per mg protein				References
	CDNB		EA		
	liver	kidney	liver	kidney	
Liza saliens	1183.9 ± 35.9	1194.5 ± 86.9	157.1 ± 13.2	122.4 ± 6.1	[27]
Channa punctatus	302.1 ± 38.1	380.9 ± 8.6	—	—	[45]
Clarias lazera	220 ± 20	110 ± 20	—	—	[44]
Onchorynchus mykiss	630 ± 60	—	20 ± 10	—	[27]
Platichthys stellatus	190	—	1	—	[43]
Pleuronectes vetulus	48	—	0.3	—	[43]
Salmo salar	290 ± 78	200 ± 18	70 ± 32	60 ± 20	[7]
Salmo trutta	650 ± 48	390 ± 109	90 ± 21	70 ± 16	[7]
Scolopsis bimaculatus	—	134.7 ± 6.6	—	—	[26]
Siganus canaliculatus	271.2 ± 9.8	86.0 ± 2.9	—	—	[42]
Sparus sarba	65.5 ± 3.4	36.8 ± 2.7	—	—	[42]
Rattus norvegicus	1165.9 ± 90.8	323.5 ± 25.2	158.6 ± 10.8	82.9 ± 6.3	[28]
Homo sapiens	639.2 ± 90.9	—	24.7 ± 4.2	—	[28]

GST-CDNB and -EA activities are higher than those in many other extrahepatic tissues. Thus, male fish might require additional expressions for endogenous metabolism and/or protection of testicular functions. *L. saliens* testicular GST specific activities are almost equal to kidney and liver specific activities. No physiological activities for testicular GSTs have been established in fish but they might be important in protection against DNA damage due to oxidative stress observed in highly proliferating cells [41].

Table 2 shows the cytosolic GST activities towards CDNB and EA from various tissues of some fish species together with rat and human reported by different laboratories. As it is seen from Table 2, there are great variations among the fish species. Our results showed that cytosolic GST activities from leaping mullet liver and several extrahepatic tissues are significantly higher than those reported for most fish species. For instance, GST activity with EA as a substrate is even higher than the total GST activities measured with CDNB for *Sparus sarba* and some other fish species [7, 26-28, 42-45]. These high activities of GSTs of *L. saliens* may contribute to the detoxification of xenobiotics in *L. saliens* and enable this fish to live in highly polluted areas.

We have also determined the GST activities of feral *L. saliens* caught from four different sites around Izmir Bay in order to investigate the possible use of GSTs from this fish as a biomarker for detection of organic pollution. Liver cytosolic GST activities towards CDNB and EA of feral *L. saliens* caught from five different sites around Izmir are presented in Fig. 6. Sampling sites are named from A to E in increasing distance from the closest point to Izmir city center, i.e., Pasaport area (site A) in Inner Izmir Bay where the port of Izmir city and several industries are located. Besides being polluted by industrial wastes, it is also contaminated by discharges from ships and domestic drainages. Site B (Mordogan) is in Outer Izmir Bay and highly urbanized but less industrialized area. The sampling sites C (Y. Foca) and D (Ildir) are located about 100 km away from city center in North and South directions, respectively. They are both outside the Izmir Bay region. Site D is the least urbanized among them. Site E (Ref) was a relatively clean reference site in high seas away from site D. Sampling site E appeared to be a good reference point since the fish caught there showed very low enzyme activities (78.1 ± 11.8 and 27.6 ± 8.9 nmol/min per mg protein for CDNB and EA, respectively, $n = 3$). It would have been better if the number of fish caught from the reference site had been larger for both experimental and statistical view, but it was very difficult. Fish from site A showed highly elevated GST activities (1183.9 ± 352.9 and 157.1 ± 13.2 nmol/min per mg protein for CDNB and EA, respectively, $n = 27$), which were 15.2 and 5.6 times higher with respect to the reference site. Mullet caught from Mordogan (site B) displayed GST activities (573.9 ± 187.4 and 112.6 ± 18.3 nmol/min per mg protein for CDNB and EA, respectively, $n = 8$) in accordance with expected decreasing pollution gradient.

On the other hand, site C (931.7 ± 176.3 and 103.9 ± 12.2 nmol/min per mg protein for CDNB and EA,

respectively, $n = 7$) still showed elevated GST activities. It was found that sampling site C is close to the discharge point of the Gediz River, which brings urban and agricultural run-off waters from fertile agricultural area treated with herbicides and pesticides in the Aegean region of Turkey. Thus, high GST activities observed in fish caught at site C might have resulted from the organic pollutants brought by Gediz River. Mullets captured from a small bay at sampling site D also showed high GST activities for both substrates (754.6 ± 141.8 and 117.7 ± 11.8 nmol/min per mg protein for CDNB and EA, respectively, $n = 10$). This induction in enzyme activities may be attributed to the increased urbanization and related pollution during summer season in addition to big fish farm facilities located in a nearby region.

In addition to GST activities, we have also measured microsomal EROD activities using the same fish samples caught from the sampling sites because many authors have shown that EROD activity is a sensitive and useful indicator of chemical pollution (for reviews see [46-48]). EROD activities of the mullets caught from the same sampling sites showed similar pattern with respect to GST activities towards CDNB and EA (Fig. 6). Despite the difficulty in characterizing the pollution at site D, Fig. 6 provides evidence that the GST values obtained from such fields (sites A-D) are strongly affected and statistically significantly different (4-15 times higher) from reference values. It has been shown that ecological and biological factors cause variations in enzymatic biotransformation activities in fish [49, 50]. In spite of wide naturally occurring variations, *Liza saliens* GST activities might be an efficient tool for monitoring pollutants in aquatic ecosystems. In fact, it is suitable for environmental monitoring [4]. *Liza saliens* belongs to the Mugilidae of the class osteichthyes. It is an economically important marine fish due to marketing of both their meat and eggs, and inhabiting usually inshore, entering lagoons and estuaries along the whole Mediterranean, Black Sea, and Sea of Azov. *Liza saliens* provide advantages for environmental biomonitoring because they can be easily captured and because of their ability to withstand the conditions of highly polluted areas such as Izmir Bay.

In the literature, there are controversial reports on the *in situ* value of GSTs as a biomarker; while some studies have clearly demonstrated that the GST activities were increased with exposure to pollutants [51], others reported no increase in GST activity of fish taken from different exposure sites [52]. In general, the efficiency of GSTs as a biomarker for field studies is less specific than the monooxygenase biomarkers and inductions are considerably species specific and variable.

In conclusion, this study proposes that GSTs are ubiquitously expressed cytosolic enzymes in *Liza saliens*. However, the level of GSTs measured in different tissues might be tissue-specific relating to the functional requirements of the tissues. The magnitude of GST activities

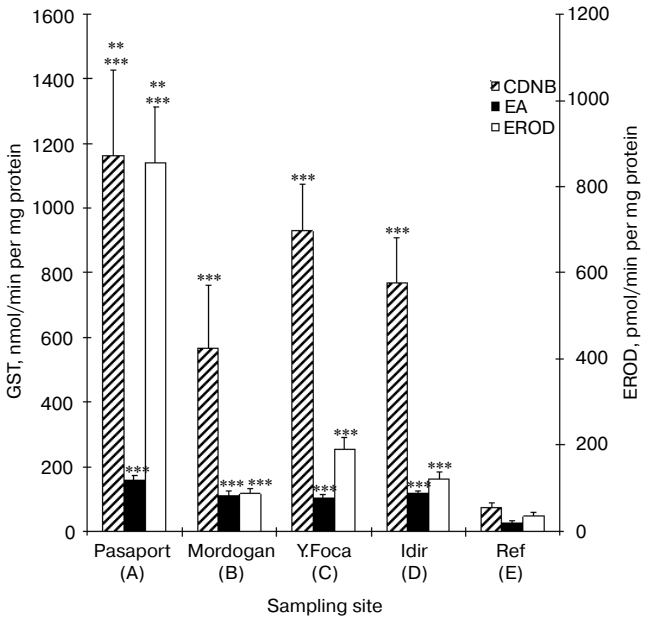

Fig. 6. Hepatic cytosolic GST activities towards CDNB and EA from *Liza saliens* caught at five different sampling sites around Izmir Bay. The sample sizes are 17, 8, 7, 10, and 3 for sampling sites A, B, C, D, and E, respectively. Results are given as mean ± SD of triplicates of three sets of experiment; $^{**}p < 0.01$, significantly different from other groups (B-D); $^{***}p < 0.001$, significantly different from the reference.

reported in this study are far higher than for most of the fish species reported in the literature and even higher than in rat and humans, perhaps forming an adaptive response mechanism to toxic effects of xenobiotics in *Liza saliens*. The results also pointed out that in addition to liver and kidney, testis and intestine play an important role in GST-mediated metabolism of xenobiotics in *Liza saliens*. This study also emphasizes interest in *Liza saliens* GST activities as a monitoring tool for estimating pollution in aquatic ecosystems.

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