



HAL
open science

Assessment of pollution in the west black sea coast of turkey using biomarker responses in fish

Azra Bozcaarmutlu, Canan Sapmaz, Zuleyha Aygun, Emel Arinç

► **To cite this version:**

Azra Bozcaarmutlu, Canan Sapmaz, Zuleyha Aygun, Emel Arinç. Assessment of pollution in the west black sea coast of turkey using biomarker responses in fish. *Marine Environmental Research*, 2009, 67 (4-5), pp.167. 10.1016/j.marenvres.2008.12.009 . hal-00563067

HAL Id: hal-00563067

<https://hal.science/hal-00563067>

Submitted on 4 Feb 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Assessment of pollution in the west black sea coast of turkey using biomarker responses in fish

Azra Bozcaarmutlu, Canan Sapmaz, Zuleyha Aygun, Emel Arinç

PII: S0141-1136(09)00002-6

DOI: [10.1016/j.marenvres.2008.12.009](https://doi.org/10.1016/j.marenvres.2008.12.009)

Reference: MERE 3313

To appear in: *Marine Environmental Research*

Received Date: 9 September 2008

Revised Date: 29 December 2008

Accepted Date: 30 December 2008



Please cite this article as: Bozcaarmutlu, A., Sapmaz, C., Aygun, Z., Arinç, E., Assessment of pollution in the west black sea coast of turkey using biomarker responses in fish, *Marine Environmental Research* (2009), doi: [10.1016/j.marenvres.2008.12.009](https://doi.org/10.1016/j.marenvres.2008.12.009)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

ASSESSMENT OF POLLUTION IN THE WEST BLACK SEA COAST OF TURKEY USING BIOMARKER RESPONSES IN FISH

Azra Bozcaarmutlu^{1*}, Canan Sapmaz¹, Zuleyha Aygun¹, Emel Arinç²

¹Department of Chemistry, Abant İzzet Baysal University, Bolu, Turkey

²Biochemistry Graduate Programme and Department of Biological Sciences, Middle East Technical University, Ankara, Turkey

* Corresponding author: bozcaarmutlu_a@ibu.edu.tr (A. Bozcaarmutlu).
Tel: +90-374-2541247 and Fax: +90-374-2534964

A part of this study was presented in poster format at the 14th International Symposium on Pollutant Responses in Marine Organisms (Primo 14) (May 6-9, 2007).

50 **ABSTRACT**

51

52 Aim of this study was to determine the extent of pollution in the West Black Sea Coast of
53 Turkey by measuring CYP1A associated EROD activity, phase II enzyme, glutathione S-
54 transferase and antioxidant enzymes, catalase and glutathione reductase activities and
55 immunochemical detection of CYP1A protein level in the liver of mullet. The fish samples
56 were caught from six locations having a varying degree of pollution in the West Black Sea
57 Region of Turkey in August 2005, 2006 and 2007. Mulletts caught from Zonguldak
58 Harbour, Ereğli Harbour and Güllüç Stream's Mouth displayed 6-9 fold higher EROD, 2-4
59 fold higher glutathione S-transferase and 2-3 fold higher catalase activities than the
60 reference site, Amasra. Total polyaromatic hydrocarbon levels in mullets caught from
61 these locations were also significantly higher (2-4 fold) than Amasra. The results of this
62 study indicate that Zonguldak Harbour, Ereğli Harbour and Güllüç Stream are highly
63 polluted by polycyclic aromatic hydrocarbons and related contaminants.

64

65

66

67

68

69

70 **Keywords:** Black Sea, Biomonitoring, CYP1A, Catalase, Chemical pollution, EROD,
71 Glutathione S-transferase, Mullet.

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89

90

91

92

93

94

95

96

97

98

99

100 1. INTRODUCTION

101

102

103

104

105

106

107

108

109

110

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129

130

131

132

133

134

135

136

137

138

139

140

141

142

143

144

145

146

147

148

149

The Black Sea has been increasingly threatened by pollutants over the past decades as a result of accidental crude oil spills, dumping of toxic industrial wastes, discharge of domestic wastes from coastal settlements and industrial and domestic pollutants carried by rivers. Some industrial areas, rivers and coastal cities in the Black Sea have tendency to create local pollution (Mee, 1992). The Sakarya River, from its source in the West Anatolia, flows through many industrial and agricultural areas and drains into the Black Sea. It carries many pollutants to the Black Sea from inner regions of Turkey (Tuncer et al., 1998; Barlas, 1999). Industrial areas found in Düzce and Adapazarı drain their discharges into Efteni Lake. The Melen Stream originates from this lake and flows through many industrial and agricultural areas. The Gülüç Stream is very close to the Ereğli Harbour and Ereğli Iron and Steel Factory. This small stream was highly polluted primarily by domestic waste discharges from Ereğli and villages around the stream. Zonguldak and Ereğli, with increasing population, shipping activities, uncontrolled discharges from industries and coastal settlements, are among the most polluted regions in the Black Sea. Zonguldak is also an important coal mining area in Turkey. It suffers from particulate discharges from the thermal power plant and coal processing wastes, resulting in permanently elevated turbidity levels along the coast of the city (Tuncer et al., 1998).

Chemical analyses of the sea water and sediment give valuable information about the levels of chemical pollutants. However, organisms are often exposed to complex mixtures of pollutants, including polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polyaromatic hydrocarbons (PAHs), alkyltin compounds, and metals. Chemical analyses do not reveal the impact of these pollutants on organisms. The use of biochemical markers fulfills this purpose. Induction of CYP1A (P4501A) and associated EROD activity are the best characterized and used biochemical markers of exposure of fish to organic pollutants such as PCBs, PAHs and dioxins. CYP1A induction provides a useful “tool” for the assessment of exposure to these types of organic pollutants and acts as an early warning sign for potential harmful effects of many organic molecules (Payne et al., 1987; Goksøyr and Förlin, 1992; Bucheli and Fent, 1995; Stegeman, 1995; Addison, 1996; Arinç and Sen, 1999; Miller et al., 2003; Arinç et al., 2000, 2001). Glutathione-S-transferases (GSTs) catalyse the conjugation of electrophilic compounds (or phase I metabolites) with reduced glutathione (GSH) and are one of the main enzymes involved in xenobiotic phase II metabolism. GSTs detoxify a number of environmental carcinogens and epoxide intermediates (Gallagher et al., 1996). Glutathione S-transferase activity measurement is also used as a marker of oxidative stress (Rodriguez-Ariza et al., 1993; Martínez- Gómez et al., 2006). The induction of antioxidant enzyme activities represents a cellular defense mechanism to neutralize toxic effects of reactive oxygen species. Benzo[*a*]pyrene as well as other PAHs and PCBs have been shown to cause significant alterations in the activities of antioxidant enzymes in laboratory experiments with molluscs and fish (Otto and Moon, 1995, Van der Oost et al., 2003). Changes in the levels of phase II enzymes, glutathione S-transferase and antioxidant enzymes have also been proposed as biomarkers of contaminants in a variety of marine organisms, including invertebrates, mussels, and fish (Otto and Moon, 1995, Livingstone, 2001; Orbea et al., 2002; Sen and Kirikbakan, 2004; Ferreira et al., 2005; Martínez- Gómez et al., 2006).

The studies concerning the effects of pollutants on the Black Sea ecosystem are relatively few (Telli-Karakoç et al., 2001; Bozcaarmutlu et al., 2006, 2008). Monitoring

150 biological effects by using biomarkers and their implementation into environmental
151 monitoring programmes in the Black Sea fall behind the advanced studies taking place in
152 the USA, Canada and most of the Western and Southern Europe. In this study, our aim was
153 to determine and monitor the extent of pollution in the West Black Sea Coast of Turkey by
154 measuring CYP1A associated EROD activity, phase II enzyme, glutathione S-transferase
155 and antioxidant enzymes, catalase and glutathione reductase activities and
156 immunochemical detection of CYP1A protein level in mullet liver. Besides these
157 biochemical parameters, total PAH concentrations were determined in fish liver tissues to
158 show the presence of one of the inducer organic pollutants in fish samples. Mullet is
159 chosen as a test organism. It is an economically important marine fish due to the marketing
160 of their meats and eggs. It is a pelagic fish inhabiting usually inshore regions, enters
161 lagoons and estuaries along the Atlantic coasts to the north of Bay of Biscay, also the
162 whole of the Mediterranean, Black Sea and Sea of Azov. Since mullet is resistant to
163 pollutants, it is widely used in biomonitoring programs of contaminated waters (Arinç and
164 Sen, 1999; Arinç et al., 2000; Ferreira et al., 2004). Biomarker enzyme activities such as
165 components of cytochrome P450 system, glutathione S-transferase and antioxidant
166 enzymes have been well characterized in mullet (Sen and Arinç, 1998a, b; Bozcaarmutlu
167 and Arinç, 2004, 2007, 2008; Ferreira et al., 2004; Sen and Kirikbakan, 2004;
168 Bozcaarmutlu, 2007; Sen and Semiz, 2007).

169

170

171

172

173

174

175

176

177

178

179

180

181

182

183

184

185

186

187

188

189

190

191

192

193

194

195

196

197

198

199

200 2. MATERIALS AND METHODS

201

202 2.1 Chemicals

203

204 Acrylamide, anti-rabbit IgG alkaline phosphatase antibody, ϵ -amino caproic acid
205 (ϵ -ACA), 7-ethoxyresorufin, N-2-hydroxyethylpiperazine-N'-2-ethansulphonic acid
206 (HEPES), β -mercaptoethanol, N,N'-methylene bisacrylamide, phenylmethanesulfonyl
207 fluoride (PMSF), resorufin sodium salt were purchased from Sigma-Aldrich, Saint Louis,
208 Missouri, USA. Bromophenol blue, N,N-dimethylformamide and nitro blue tetrazolium
209 chloride were purchased from Merck, Darmstadt, Germany. 5-bromo-4-chloro-3-
210 indolylphosphate disodium salt (BCIP), glucose-6-phosphate dehydrogenase, α -D-glucose-
211 6-phosphate monosodium salt, polyethylene sorbitan monolaurate (Tween 20), sodium
212 dodecyl sulfate (SDS) were purchased from Calbiochem, California, USA. Ammonium
213 persulfate, N, N, N', N' tetramethylethylene diamine (TEMED), trans blot transfer medium
214 (pure nitrocellulose membrane) were purchased from Bio-Rad Laboratories, California,
215 USA. β -Nicotinamide adenine dinucleotide phosphate disodium salt (NADP⁺), β -
216 nicotinamide adenine dinucleotide, reduced form (NADPH), phenazine methosulfate
217 (PMS) were purchased from Applichem, Darmstadt, Germany. All other chemicals were
218 of analytical grade and were obtained from commercial sources at the highest grade of
219 purity available.

220

221 2.2 Sampling Sites and Fish

222

223 The fish samples were collected in August 2005, August 2006 and August 2007
224 from six different locations of the West Black Sea Region of Turkey. The fish sampling
225 stations were shown in Figure 1. As indicated in this figure, Amasra was used as the
226 reference site. Three different species of mullet, namely so-iuy mullet (*Mugil soiuy*),
227 flathead mullet (*Mugil cephalus*) and golden grey mullet (*Liza aurata*), each weighing 600-
228 750 g, were captured by fish net.

229

230 Figure 1

231

232 2.3 Preparation of Tissue Samples

233

234 Fish were killed by decapitation and livers were removed immediately by avoiding
235 the gall bladders and flash frozen in liquid nitrogen. Tissues were taken from liquid
236 nitrogen and cut into two pieces without thawing. One of the pieces was wrapped by
237 freezing bags and covered by aluminum foil and then put back into liquid nitrogen. This
238 piece was later used in PAH analysis. The other piece of the liver tissue was used in the
239 preparation of microsomes and thawed on ice. Liver microsomes were prepared as
240 described before (Adali and Arinç, 1990; Arinç and Sen, 1993) except that homogenization
241 solution contained 10 mM EDTA, 0.25 mM PMSF and 0.25 mM ϵ -ACA. The homogenate
242 was centrifuged at 13300 \times g for 40 minutes. The microsomes were sedimented from the
243 supernatant by centrifugation at 70000 \times g for 60 minutes. The supernatant fraction (cytosol)
244 was removed and stored at -80°C until use. The microsomal pellet was resuspended in
245 1.15% KCl solution containing 10 mM EDTA and resedimented by ultracentrifugation at
246 70000 \times g for 50 minutes. The washed microsomes were resuspended in 10% glycerol
247 containing 10 mM EDTA. Aliquots of microsomes were gassed with nitrogen and stored in
248 liquid nitrogen until used. All the procedures involved in the preparation of microsomes
249 and cytosols were performed at 0-4°C.

250 2.4 Biochemical Methods

251

252 Protein concentrations of microsomes and cytosols were determined by the method
253 of Lowry et al. (1951). Bovine serum albumin was used as a standard.

254

255 EROD activity of the mullet liver microsomes was determined by the
256 spectrofluorometric method of Burke and Mayer (1974), with some modifications. Assay
257 conditions optimized for gilthead seabream (*Sparus aurata*) liver microsomes by Arinç and
258 Sen (1994) were also used in this study. The reaction mixture contained 0.1 M potassium
259 phosphate buffer, pH 7.8, 0.1 M NaCl, 2.4 mg bovine serum albumin (BSA), 1.5 μ M 7-
260 ethoxyresorufin, 50-100 μ g of fish liver microsomal protein, and 0.5 mM NADPH
261 generating system in a final volume of 2.0 mL. The reaction was initiated by the addition
262 of substrate and followed for 2 minutes in a spectrofluorometer. Finally, a known amount
263 of resorufin was added as an internal standard to the reaction mixture and the increase in
264 fluorescence was recorded. EROD activity was calculated using the fluorescence increase
265 caused by the addition of resorufin.

266

267 Total glutathione S-transferase (GST) activity of mullet liver cytosols was
268 determined according to the method of Habig et al. (1974). The reaction mixture contained
269 50 mM potassium phosphate buffer, pH 7.5, 1.3 mM GSH and fish liver cytosols. The
270 reaction was initiated by the addition of 1 mM CDNB in a final volume of 3.0 mL. The
271 thioether formation was followed at 340 nm for 2 minutes. Specific total glutathione S-
272 transferase activity was calculated as nmol CDNB conjugate formed $\text{min}^{-1} \text{mg}^{-1}$ protein at
273 25°C, using $9.6 \text{ mM}^{-1} \text{ cm}^{-1}$ as an extinction coefficient (ϵ_{340}).

274

275 Catalase (CAT) activity of mullet liver cytosols was determined according to the
276 method of Aebi (1984). The reaction mixture contained 50 mM potassium phosphate
277 buffer, pH 7.5, 10 mM H_2O_2 and fish liver cytosols. The rate of reaction was followed by
278 measuring the consumption of H_2O_2 spectrophotometrically at 240 nm for 1 minute.
279 Specific catalase activity was calculated as nmol H_2O_2 consumed $\text{min}^{-1} \text{mg}^{-1}$ protein at
280 25°C, using $36.4 \text{ mM}^{-1} \text{ cm}^{-1}$ as an extinction coefficient (ϵ_{240}).

281

282 Glutathione reductase (GR) activity of mullet liver cytosols was determined
283 according to the method of Carlberg and Mannervick (1985). The reaction mixture
284 contained 100 mM potassium phosphate buffer, pH 7.6, 0.5 mM EDTA, pH 7.6, 0.1 mM
285 NADPH and fish liver cytosols in a final volume of 2.0 mL. The reaction was started by
286 adding 1 mM oxidized glutathione (GSSG). Then the GSH formation was followed by
287 measuring the NADPH utilization spectrophotometrically at 340 nm for 5 minutes. One
288 unit of glutathione reductase activity was calculated as nmol NADPH oxidized $\text{min}^{-1} \text{mg}^{-1}$
289 protein at 25°C, using $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ as an extinction coefficient (ϵ_{340}).

290

291 2.5 Western Blot Analysis

292

293 Cytochrome P4501A (CYP1A) protein levels were determined using the polyclonal
294 antibodies produced against purified leaping mullet (*Liza saliens*) liver cytochrome
295 P4501A (Arinç and Sen, 1999). Western blot analysis of fish liver microsomes was carried
296 out essentially as described by Towbin et al. (1979). Sodium dodecyl sulfate-
297 polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli
298 (1970) and proteins were transferred from slab gels to nitrocellulose membrane by use of a
299 Bio-Rad Trans-Blot electrophoretic transfer apparatus. The proteins on membranes were

300 first reacted with 1:5000 diluted leaping mullet CYP1A antibodies and then with the
301 enzyme-linked secondary antibody, and alkaline phosphatase activity was detected by the
302 method of Ey and Ashman (1986). The final images of the blots were photographed using
303 a computer-based gel imaging instrument (Infinity 3000-CN-3000 darkroom, Vilber
304 Lourmat, Marne-la-Vallee Cedex 1, France), and relative peak area (R.P.A.) of the blots
305 was analyzed using the Scion Image software for Windows (Version 4.0.2, Scion
306 Corporation, Maryland, USA) as a quantitative tool to determine CYP1A protein levels.

307

308 **2.6 PAH Analysis**

309

310 Spectrofluorometric measurements of the total PAH concentration in fish liver
311 tissues were performed according to the method described by UNEP, 1992. The principle
312 of the method is based on the extraction of PAHs by suitable pure solvent. Mullet livers
313 were dried in an oven at 40 °C overnight. Dried tissue (0.2 g) was ground and refluxed
314 with 20 mL of ethanol containing 0.75 g of KOH for 120 minutes by constant heating.
315 Then 20 mL of PAH-free hexane was added to the flask and mixed very well and waited
316 until the flask reached to room temperature. When the flask was attained to room
317 temperature, it was poured into a separatory funnel and sufficient amount of distilled water
318 was added. The lower aqueous phase was extracted further two times with 20 mL of PAH-
319 free hexane. The fluorescence intensities of the pooled samples were measured at 310 nm
320 excitation and 360 nm emission wavelengths. The fluorescence intensity of the pooled
321 samples was compared with the fluorescence intensity of known concentrations of
322 chrysene standard.

323

324 **2.7 Statistical Analysis**

325

326 Results were expressed as means \pm standard error of the mean (S.E.M.). Data were
327 analyzed by two-way ANOVA using years and sampling sites as independent variables.
328 When significant differences were observed, Tukey HSD Post Hoc Test was applied for a
329 pair-wise comparison. The analyses were carried out using the SPSS statistical package
330 (SPSS 15.0 for Windows Evaluation Version). No significant sex dependent differences
331 were observed in the fish samples. Therefore the data were pooled.

332

333

334

335

336

337

338

339

340

341

342

343

344

345

346

347

348

349

350 3. RESULTS

351

352

353

354

355

356

357

358

359

360

361

362

363

364

365

366

367

368

369

370

371

372

373

374

375

376

377

378

379

380

381

382

383

384

385

386

387

388

389

390

391

392

393

394

395

396

397

398

399

In this study 147 fish samples were collected from six different locations of the West Black Sea Coast of Turkey. In all samples, EROD, glutathione S-transferase, catalase and glutathione reductase activities were measured. Table 1 illustrates biomarker enzyme activity results. The lowest biomarker activities were measured in fish samples (so-iuy mullet, flathead mullet and golden grey mullet) caught from Amasra. The biomarker enzyme activities of different mullet species were compared with the corresponding mullet species caught from Amasra. This relatively clean site was considered as a reference site to demonstrate the extent of pollution.

Table 1

3.1 7-Ethoxyresorufin O-deethylase (EROD) Activity and CYP1A Protein Level

The highest EROD activities were found in mullets (so-iuy mullets, flathead mullets, and golden grey mullets) caught from Zonguldak Harbour in all sampling years (Table 1). Mulletts caught from Zonguldak Harbour had about 6-9 fold higher EROD activities than those obtained from Amasra. The highly elevated EROD activities were also measured in mullets captured from Ereğli Harbour which were about 7-9 fold higher with respect to the values obtained from Amasra. Gülüç Stream's Mouth was the other highly polluted station in this study. EROD activities of mullets samples caught from this station were about 6 fold higher than those obtained from Amasra. In Figure 2, the data were presented separately for each species. Statistically significant differences were indicated in this figure with different letters ($p < 0.05$). EROD activities of so-iuy mullets caught from Zonguldak Harbour and Gülüç Stream's Mouth were significantly different from the so-iuy mullets caught from Amasra, Melen Stream's and Sakarya River's Mouths. EROD activities of flathead mullets caught from Zonguldak Harbour were significantly different from Amasra. EROD activities of golden grey mullets caught from Zonguldak and Ereğli Harbours were significantly different from Amasra. EROD activities of mullets caught from Melen Stream's Mouth were 2 fold higher than Amasra, however these differences were not statistically significant. Significant differences between years were not found in EROD activities of fish caught from all sampling stations except Sakarya River's Mouth.

Figure 2

CYP1A protein level was also determined in mullet liver microsomes using the polyclonal antibodies produced against leaping mullet cytochrome P4501A. Figure 3 illustrates the western blot analysis results of mullets caught from six different stations in three sampling years. Polyclonal antibody anti-leaping mullet cytochrome P4501A showed strong cross-reactivity with liver microsomes of so-iuy mullets, flathead mullets and golden grey mullets caught from different stations of the West Black Sea. The CYP1A bands of mullets caught from Zonguldak and Ereğli Harbours and Gülüç Stream's Mouth were stained more intensely than those obtained from Amasra. Liver microsomes of mullets caught from Melen Stream's Mouth and Sakarya River's Mouth showed moderate cross reactivity with polyclonal anti-mullet P4501A. Liver microsomes of mullets caught from Amasra showed low cross-reactivity with polyclonal antibody anti-mullet P4501A. The results clearly indicated that mullets caught from the polluted sites had both highly induced EROD activity and elevated CYP1A protein content. The relationship between CYP1A protein levels and EROD activities was also examined with correlation analysis

400 (Figure 3). CYP1A protein levels were highly correlated with the EROD activity results in
 401 the mullet samples caught from different stations of the West Black Sea Coast of Turkey
 402 (R^2 ranged between 0.92-0.96).

403

404 Figure 3

405

406 When EROD activities of different mullet species were analyzed using ANOVA,
 407 species differences were not significant ($p=0.714$). When the species specific cross-
 408 reactivity pattern of anti-leaping mullet CYP1A protein was compared, different mullet
 409 species having similar EROD activity showed similar degree of staining and gave almost
 410 the same EROD activity/CYP1A protein level (R.P.A.) ratio (Figure 4). The induction
 411 responses to organic pollutants, both with respect to EROD activities and CYP1A protein
 412 levels, were not different between these mullet species.

413

414 Figure 4

415

416 3.2 *Glutathione S-transferase Activity*

417

418 The highest total glutathione S-transferase activities were measured in mullets
 419 caught from Zonguldak Harbour in all sampling years which were about 4 fold higher than
 420 those obtained from Amasra (Table 1). Mulletts caught from Güülüç and Melen Streams'
 421 Mouths also displayed highly elevated total glutathione S-transferase activities which were
 422 3-4 fold higher than those obtained from Amasra. In Figure 5, the data were presented
 423 separately for each species. Glutathione S-transferase activities of soiyu mullets caught
 424 from Zonguldak Harbour, Melen and Güülüç Streams' Mouths were not significantly
 425 different from each other. However, they were significantly different from Amasra.
 426 Glutathione S-transferase activities of soiyu mullets caught from Sakarya River's Mouth
 427 (represented by "ab") were not statistically different from Zonguldak Harbour, Güülüç and
 428 Melen Stream's Mouths and Amasra. Glutathione S-transferase activities of golden grey
 429 mullets caught from Ereğli Harbour (represented by "ab") were 2-3 fold higher than those
 430 obtained from Amasra, however these differences were not significantly different from
 431 Amasra and Zonguldak Harbour. Glutathione S-transferase activities of mullets caught
 432 from Ereğli Harbour showed significant difference between years. The highest GST
 433 activities were measured in the fish samples caught in 2005.

434

435 Figure 5

436

437 3.3 *Catalase Activity*

438

439 The highest catalase activities were detected in mullets caught from Sakarya
 440 River's and Melen Stream's Mouths and Zonguldak Harbour in 2005, which were about 3
 441 fold higher than those obtained from Amasra (Table 1). Fish sampled from Güülüç Stream's
 442 Mouth also displayed highly elevated catalase activities which were 2 fold higher than
 443 those obtained from Amasra. In Figure 6, the data were presented separately for each
 444 species. Catalase activities of soiyu mullets caught from Zonguldak Harbour, Melen
 445 Stream's and Sakarya River's Mouths were not statistically different from each other.
 446 However, they were significantly different from Amasra. Catalase activities of soiyu
 447 mullets caught from Güülüç Stream's Mouth (represented by "ab") were not statistically
 448 different from Zonguldak Harbour, Melen Stream's and Sakarya River's Mouths and
 449 Amasra. Catalase activities of flathead mullets caught from Zonguldak Harbour were

450 significantly different from Amasra. Catalase activities of golden grey mullets caught from
 451 Zonguldak and Ereğli Harbours were significantly different from Amasra. Although highly
 452 elevated catalase activities were measured in mullets caught in 2005, this enzyme activity
 453 significantly decreased in the mullets caught from Melen Stream's and Sakarya River's
 454 Mouths and Ereğli Harbour in the following sampling years.

455

456 Figure 6

457

458 3.4 *Glutathione Reductase Activity*

459

460 Highly elevated glutathione reductase activities were measured in mullets caught
 461 from Zonguldak and Ereğli Harbours, Gülüç and Melen Streams' Mouths (Table1). In
 462 2005, mullets caught from Melen Stream's Mouth had the highest glutathione reductase
 463 activities among the other sampling sites which were 1.7 fold higher than those obtained
 464 from Amasra. Glutathione reductase activities of mullets caught from Gülüç Stream's
 465 Mouth were 1.6 fold higher than those obtained from Amasra in 2005 and 2006.
 466 Glutathione reductase activities of mullets captured from Ereğli Harbour were 1.6 fold
 467 higher than those obtained from Amasra. In 2006, glutathione reductase activities of so-iuy
 468 mullets and golden grey mullets captured from Zonguldak Harbour were 1.5 and 1.4 fold
 469 higher than those obtained from Amasra, respectively. In Figure 7, the data were presented
 470 separately for each species. Glutathione reductase activities of soiu y mullets caught from
 471 Melen and Gülüç Streams' Mouths were significantly different from Amasra and Sakarya
 472 River's Mouth. Glutathione reductase activities of soiu y mullets caught from Zonguldak
 473 Harbour (represented by "ab") were not statistically different from Gülüç, Melen Streams',
 474 Sakarya River's Mouths and Amasra. Glutathione reductase activities of golden grey
 475 mullets caught from Zonguldak and Ereğli Harbours were significantly different from
 476 Amasra. Significant differences between years were not found in glutathione reductase
 477 activities of fish caught from all sampling stations except Sakarya River's Mouth.

478

479 Figure 7

480

481

482 3.5 *PAH Analysis*

483

484 Besides biomarker enzyme activity measurements, total PAH concentrations were
 485 determined in mullets caught in 2006 to show the presence and extent of one of the organic
 486 pollutants in the sampling stations. Table 2 illustrates the total PAH concentrations in
 487 mullet liver tissues. The highest total PAH concentrations were measured in mullets caught
 488 from Zonguldak Harbour. Total PAH concentrations of fish caught from Zonguldak and
 489 Ereğli Harbours and Gülüç Stream's Mouth were significantly different from Amasra.
 490 Total PAH concentrations of fish caught from Zonguldak Harbour were significantly
 491 different from fish caught from Ereğli Harbour. However, total PAH concentrations of fish
 492 caught from Ereğli Harbour and Gülüç Stream's Mouth were not significantly different
 493 from each other. The lowest total PAH concentrations were measured in mullets caught
 494 from Amasra. Total PAH concentrations of fish caught from Sakarya River's and Melen
 495 Stream's Mouths (represented as "bc") were not significantly different from Amasra,
 496 Ereğli Harbour and Gülüç Stream's Mouth.

497

498 Table 2

499

500 The relationship between biomarker activities and total PAH concentrations was
501 also examined with correlation analysis (Pearson). The highest positive correlation was
502 found between EROD activities and total PAH concentrations ($r=0.491$, $p<0.01$).
503 Glutathione reductase and glutathione S-transferase activities were slightly correlated with
504 total PAH concentrations ($r=0.392$, $p<0.01$) and ($r=0.283$, $p<0.05$), respectively.
505 Correlation between total PAH concentrations and catalase activities was positive but not
506 significant ($r=0.210$, $p=0.083$).

507

508

509

510

511

512

513

514

515

516

517

518

519

520

521

522

523

524

525

526

527

528

529

530

531

532

533

534

535

536

537

538

539

540

541

542

543

544

545

546

547

548

549

550 **4. DISCUSSION**

551

552

553

554

555

556

557

558

559

560

561

562

563

564

565

566

567

568

569

570

571

572

573

574

575

576

577

578

579

580

581

582

583

584

585

586

587

588

589

590

591

592

593

594

595

596

597

598

599

In this study, cytochrome P4501A (CYP1A) associated EROD activity, phase II enzyme, glutathione S-transferase and antioxidant enzymes, catalase and glutathione reductase activities were measured in mullet liver fractions to determine and monitor the extent of pollution in the West Black Sea Coast of Turkey. Highly elevated biomarker enzyme activities were found in the mullets caught from Zonguldak and Ereğli Harbours, and Güllüç Stream's Mouth. Zonguldak is an important coal mining area in Turkey. The harbour of this city is polluted especially with coal processing wastes and discharges of industrial and domestic wastes. Cohen and coworkers (1994) have shown that coal by-products such as coal-tar significantly induce EROD activity in medaka liver. In addition, Vignier and coworkers (1994) have demonstrated that EROD activity significantly increases in winter flounder in coal-tar contaminated estuary. Creosote is a dark liquid made from coal tar. It is a complex mixture of organic compounds, containing about 85% PAHs. Hyötyläinen and Oikari (1999) and Whyte and coworkers (2000) have demonstrated in different studies that the extracts of creosote-contaminated sediments highly induce EROD activities in rainbow trout. In this study, the lowest biomarker enzyme activities were measured in mullets caught from Amasra. The biomarker enzyme activities obtained from the other stations were compared with those obtained from Amasra. 6-9 fold increase in EROD activities and elevated CYP1A protein levels were found in mullets caught from Zonguldak Harbour with respect to Amasra. This result clearly indicates that coal mining activities create pollution in Zonguldak Harbour.

İzmir Bay is one of the most polluted sites in Turkey. Highly elevated EROD activities have been reported in mullets (*Liza saliens* and *Mugil cephalus*) caught from the most polluted site of İzmir Bay. In these studies, EROD activities of *Liza saliens* were reported as 1293 ± 292 pmol min⁻¹ mg⁻¹ protein in 1995 and 1028 ± 287 pmol min⁻¹ mg⁻¹ protein in 1999 (Arinç and Sen, 1999; Arinç et al., 2000). EROD activities of *Mugil cephalus* caught from the same site were 1398 ± 410 pmol min⁻¹ mg⁻¹ protein in 1999 (Arinç et al., 2000). In our study, highly elevated EROD activities were found in mullets caught from Zonguldak and Ereğli Harbours, and Güllüç Stream's Mouth, suggesting the presence of organic pollutants such as PAHs and PCBs in these sampling stations in the West Black Sea Coast of Turkey.

Glutathione S-transferases are involved in the detoxification reactions of xenobiotics. Induction of glutathione S-transferase have been reported in a number of field studies in fish exposed to organic contaminants (Lenartova et al., 1997; Pandey et al., 2003; Martínez- Gómez et al., 2006; Sen and Kirikbakan, 2004). In our study, highly elevated total glutathione S-transferase activities measured in mullets caught from Zonguldak Harbour ($950-1147$ nmol min⁻¹ mg⁻¹ protein), Ereğli Harbour (932 ± 131 nmol min⁻¹ mg⁻¹ protein), Güllüç Stream's Mouth (1352 ± 154 nmol min⁻¹ mg⁻¹ protein) and Melen Stream's Mouth (1192 ± 140 nmol min⁻¹ mg⁻¹ protein) are at the same order of magnitude of that reported by Sen and Kirikbakan (2004) in mullets (*Liza saliens*) caught from highly polluted site of İzmir Bay in Turkey (1183 ± 35.9 nmol min⁻¹ mg⁻¹ protein).

Chronic exposure to organic pollutants increases reactive oxygen species (ROS) formation (Livingstone 2001). The antioxidant enzyme catalase is one of the most responsive enzymes to ROS (Halliwell and Gutteridge, 1999). Elevated catalase enzyme activities have been reported in several fish species in various laboratory and field studies (Otto and Moon, 1995; Orbea et al., 2002; Ferreira et al., 2005). Significantly high catalase

600 activities were measured in mullets caught from Zonguldak and Ereğli Harbours and
601 Melen Stream's and Sakarya River's Mouths, indicating the presence of ROS generating
602 pollutants in these regions. The other biomarker enzyme activity measured in this study
603 was glutathione reductase. Glutathione reductase maintains GSH/GSSG homeostasis under
604 oxidative stress conditions. Induction of glutathione reductase activity have been reported
605 in various field studies in fish exposed to organic pollutants such as PAHs, PCBs and
606 halogenated xenobiotics (Rodriguez-Ariza et al., 1993; Van der Oost et al., 2003;
607 Martínez- Gómez et al., 2006). Significantly high glutathione reductase activities have
608 been found in mullets caught from Zonguldak and Ereğli Harbours, Gülüç and Melen
609 Streams' Mouths, suggesting the presence of PAHs, PCBs and halogenated xenobiotics in
610 these sampling sites.

611

612 Biomarker enzyme activities of fish caught from Sakarya River's Mouth displayed
613 significant differences between sampling years. Sakarya River is the third longest river of
614 Turkey (824 km). It rises from the center of Anatolia and joins with Porsuk, Göksu,
615 Kirmir, Mudurnu and Çark Streams. The river runs through many industrial and
616 agricultural areas before reaching to the Black Sea. The results of this study suggested the
617 existence of qualitative and quantitative differences of contaminants in the sampling
618 station between years. However, it is difficult to determine which chemicals specifically
619 affected the biomarker activities in the environmental monitoring studies. Biomarkers
620 reveal the interactive effects of chemical contaminants on organisms.

621

622 In the Black Sea, total PAH concentrations in fish samples have been determined
623 by Telli-Karakoç and coworkers (2001) in mullet samples caught from Trabzon, Yomra. In
624 our study, the highest total PAH concentrations were measured in so-iuy mullets and
625 golden grey mullets caught from Zonguldak Harbour ($54 \pm 5 \mu\text{g g}^{-1}$ dried liver weight, $n=13$
626 and $47 \pm 6 \mu\text{g g}^{-1}$ dried liver weight, $n=9$, respectively) are at the same order of magnitude
627 of that reported by Telli-Karakoç and coworkers for mullets (*Mugil saliens*) ($55.7 \pm 16.6 \mu\text{g}$
628 g^{-1} dried liver weight) caught from Trabzon, Yomra. In our study, significantly high total
629 PAH concentrations were also measured in mullets caught from Ereğli Harbour and Gülüç
630 Stream's Mouth. The sampling areas arranged in decreasing order of total PAH
631 concentrations are Zonguldak Harbour>Gülüç Stream Mouth>Ereğli Harbour>Melen
632 Stream Mouth>Sakarya River Mouth>Amasra.

633

634 The relationship between biomarker activities and total PAH concentrations was
635 also analyzed. Biomarker activities of fish were moderately related with total PAH
636 concentrations, suggesting the presence of the other organic contaminants such as PCBs
637 and/or dioxins in these regions besides polycyclic aromatic hydrocarbons.

638

639 CONCLUSION

640

641 In this study, the contribution of Turkish rivers and harbours present in the West
642 Black Sea Region of Turkey to the pollution of the Black Sea was assessed using
643 biomarker responses of fish. The elevated biomarker responses found in this study indicate
644 that Zonguldak Harbour, Ereğli Harbour and Gülüç Stream are highly polluted by
645 polycyclic aromatic hydrocarbons and related contaminants. In addition, Melen Stream and
646 Sakarya River also bring some PAHs, PCBs and other type of pollutants from the inner
647 parts of Anatolia and Black Sea Region of Turkey. The results reported here also supported
648 the conclusion of previous field studies that mullet is a suitable fish species for
649 biomonitoring studies.

650 **ACKNOWLEDGMENTS**

651

652

653 The authors thank all the members of the Ministry of Agriculture and Rural Affairs

654 (Tarım ve Köy İşleri Bakanlığı, Turkey) for their help in collecting the fish. They also

655 would like to thank Munire Özlem Çevik and Asiye Kumru for their technical support in

656 the statistical analysis and Serif Akman for his help for providing some laboratory

657 equipments. This research was supported by TÜBİTAK (The Scientific and Technological

658 Research Council of Turkey) (Project No:104Y083).

659

660

661

662

663

664

665

666

667

668

669

670

671

672

673

674

675

676

677

678

679

680

681

682

683

684

685

686

687

688

689

690

691

692

693

694

695

696

697

698

699

REFERENCES

- 700
701
702 Adali, O., Arinç, E., 1990. Electrophoretic, spectral, catalytic and immunochemical
703 properties of highly purified cytochrome-P450 from sheep lung. *International Journal of*
704 *Biochemistry* 22, 1433-1444.
705
706 Addison, R.F., 1996. The use of biological effects monitoring in studies of marine
707 pollution. *Environmental Reviews* 4, 225-237.
708
709 Aebi, H., 1984. Catalase in vitro. *Methods in Enzymology* 105, 121-126.
710
711 Arinç, E., Sen, A., 1993. Characterization of cytochrome P450 dependent mixed function
712 oxidase system of gilthead seabream (*Sparus aurata*; *Sparidae*) liver. *Comparative*
713 *Biochemistry and Physiology* 104B, 133-138.
714
715 Arinç, E., Sen, A., 1994. Effects of in vivo benzo[a]pyrene treatment on liver microsomal
716 mixed-function oxidase activities of gilthead seabream (*Sparus aurata*). *Comparative*
717 *Biochemistry and Physiology* 107C, 405- 414.
718
719 Arinç, E., Sen, A., 1999. Hepatic cytochrome P4501A and 7-ethoxyresorufin O-deethylase
720 induction in mullet and common sole as an indicator of toxic organic pollution in İzmir
721 Bay, Turkey. *Marine Environmental Research* 48, 147-160.
722
723 Arinç, E., Sen, A., Bozcaarmutlu, A., 2000. Cytochrome P4501A and associated MFO
724 induction in fish as a biomarker for toxic, carcinogenic pollutants in the aquatic
725 environment. *Pure and Applied Chemistry* 72, 985-994.
726
727 Arinç, E., Kocabiyik, S., Su, E., 2001. Induced CYP1A mRNA, protein and catalytic
728 activity in the liver of feral fish, leaping mullet, *Liza saliens*. *Comparative Biochemistry*
729 *and Physiology* 128C, 281-290.
730
731 Barlas, N., 1999. A pilot study of heavy metal concentration in various environments and
732 fishes in the upper Sakarya River basin, Turkey. *Environmental Toxicology* 14, 367-373.
733
734 Bozcaarmutlu, A., Arinç, E., 2004. Inhibitory effects of divalent metal ions on liver
735 microsomal 7-ethoxyresorufin O-deethylase (EROD) activity of leaping mullet. *Marine*
736 *Environmental Research* 58, 521-524.
737
738 Bozcaarmutlu, A., Aygun, Z., Sapmaz, C., Arinç, E., 2006. Biomonitoring of the toxic
739 pollutants by using mullet liver enzymes in the West Black Sea Region of Turkey. *The*
740 *FEBS Journal* 273, 291-292.
741
742 Bozcaarmutlu, A., 2007. Mechanism of inhibition of purified leaping mullet (*Liza saliens*)
743 NADPH-cytochrome P450 reductase by toxic metals: aluminum and thallium. *Journal of*
744 *Biochemical and Molecular Toxicology* 21, 340-347.
745
746 Bozcaarmutlu, A., Arinç, E., 2007. Effect of mercury, cadmium, nickel, chromium and
747 zinc on kinetic properties of NADPH-cytochrome P450 reductase purified from leaping
748 mullet (*Liza saliens*). *Toxicology in Vitro* 21, 408-416.
749

- 750 Bozcaarmutlu, A., Sapmaz, C., Aygun, Z., Arinç, E., 2008. Biomarker responses in so-iuy
751 mullet (*Mugil so-iuy*) to toxic organic pollutants in the West Black Sea Region of Turkey.
752 Marine Environmental Research 66, 172.
753
- 754 Bozcaarmutlu, A., Arinç, E., 2008. Purification of CYP2B-like protein from feral leaping
755 mullet (*Liza saliens*) liver microsomes and its biocatalytic, molecular, and immunological
756 characterization. Journal of Biochemical and Molecular Toxicology 22, 284-298.
757
- 758 Bucheli, T.D., Fent, K., 1995. Induction of cytochrome P450 as a biomarker for
759 environmental contamination in aquatic ecosystem. Critical Review in Environmental
760 Science and Technology 25, 201-268.
761
- 762 Burke, M.D., Mayer, R.T., 1974. Ethoxyresorufin: Direct fluorometric assay of
763 microsomal O-dealkylation which is preferentially induced by 3-methylcholanthrene. Drug
764 Metabolism and Disposition 2, 583-588.
765
- 766 Carlberg, I., Mannervik, B., 1985. Glutathione reductase. Methods in Enzymology 113,
767 484-490.
768
- 769 Cohen, C., Stiller, A., Miller, M.R., 1994. Characterization of cytochrome P4501A
770 induction in medaka (*Oryzias latipes*) by samples generated from the extraction and
771 processing of coal. Archives of Environmental Contamination and Toxicology 27, 400-
772 405.
773
- 774 Ey, P.L., Ashman, L.K., 1986. The use of alkaline phosphatase-conjugated
775 antiimmunoglobulin with immunoblots for determining the specificity of monoclonal
776 antibodies to protein mixture. Methods in Enzymology 121, 497-509.
777
- 778 Ferreira, M., Antunes, P., Gil, O., Vale, C., Reis-Henriques, M.A., 2004. Organochlorine
779 contaminants in flounder (*Platichthys flesus*) and mullet (*Mugil cephalus*) from Douro
780 estuary, and their use as sentinel species for environment monitoring. Aquatic Toxicology
781 69, 347-349.
782
- 783 Ferreira, M., Moradas-Ferreira, P., Reis-Henriques, M.A., 2005. Oxidative stress
784 biomarkers in two resident species, mullet (*Mugil cephalus*) and flounder (*Platichthys*
785 *flesus*), from a polluted site in River Douro Estuary, Portugal. Aquatic Toxicology 71, 39-
786 48.
787
- 788 Gallagher, E.P., Stapleton, P.L., Slone, D.H., Schlenk, D., Eaton, D.L., 1996. Channel
789 catfish glutathione S-transferase isoenzyme activity toward (\pm)-anti-benzo[a]pyrene-trans-
790 7,8-dihydrodiol-9,10-epoxide. Aquatic Toxicology 34, 135-150.
791
- 792 Goksøyr, A., Förlin, L., 1992. The cytochrome P450 in fish, aquatic toxicology and
793 environmental monitoring. Aquatic Toxicology 22, 287-312.
794
- 795 Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases: the first
796 enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 249, 7130-
797 7131.
798

- 799 Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine. Oxford
800 University Press, New York, pp. 105-245.
801
- 802 Hyötyläinen, T., Oikari, A., 1999. Assessment of the bioactivity of creosote-contaminated
803 sediment by liver biotransformation system of rainbow trout. *Ecotoxicology and*
804 *Environmental Safety* 44, 253-258.
805
- 806 Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of
807 bacteriophage T4. *Nature* 227, 680-684.
808
- 809 Lenártová, V., Holovská, K., Pedrajas, J., Martínez-Lara, E., Peinado, J., López-Barea, J.,
810 Rosival, I., Košuth, P., 1997. Antioxidant and detoxifying fish enzymes as biomarkers of
811 river pollution. *Biomarkers* 2, 247-252.
812
- 813 Livingstone, D.R., 2001. Contaminant reactive oxygen species production and oxidative
814 damage in aquatic organisms. *Marine Pollution Bulletin* 42, 656-666.
815
- 816 Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with
817 the Folin Phenol reagent. *Journal of Biological Chemistry* 193, 265-275.
818
- 819 Martínez- Gómez, C., Campillo, J.A., Benedicto, J., Fernández, B., Valdés, J., García, I.,
820 Sánchez, F., 2006. Monitoring biomarkers in fish (*Lepidorhombus boscii* and *Callionymus*
821 *lyra*) from the northern Iberian shelf after the Prestige oil spill. *Marine Pollution Bulletin*
822 53, 305-314.
823
- 824 Mee, L.D., 1992. The Black-Sea in crisis- A need for concerted international action.
825 *Ambio* 21, 278-286.
826
- 827 Miller, K.A., Addison, R.F., Bandiera, S.M., 2003. Hepatic CYP1A levels and EROD
828 activity in English sole: biomonitoring of marine contaminants in Vancouver Harbour.
829 *Marine Environmental Research* 57, 37-54.
830
- 831 Orbea, A., Ortiz-Zarragoitia, M., Sol'e, M., Porte, C., Cajaraville, M.P., 2002. Antioxidant
832 enzymes and peroxisome proliferation in relation to contaminant body burdens of PAHs
833 and PCBs in bivalve molluscs, crabs and fish from the Urdaibai and Plentzia estuarios
834 (Bay of Biscay). *Aquatic Toxicology* 58, 75-98.
835
- 836 Otto, D.M.E., Moon, T.W., 1995. 3,3',4,4'-tetrachlorobiphenyl effects on antioxidant
837 enzymes and glutathione status in different tissues of rainbow trout. *Pharmacology and*
838 *Toxicology* 77, 281-287.
839
- 840 Pandey, S., Parvez, S., Sayeed, I., Haque, R., Bin-Hafeez, B., Raisuddin, S., 2003.
841 Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl.
842 & Schn.). *The Science of the Total Environment* 309, 105-115.
843
- 844 Payne, J.F., Fancey, L.L., Rahimtula, A.D., Porter, E.L., 1987. Review and perspective on
845 the use of mixed function oxygenase enzymes in biological monitoring. *Comparative*
846 *Biochemistry and Physiology* 86C, 233-245.
847

- 848 Rodriguez-Ariza, A., Peinado, J., Pueyo, C., Lopez-Barea, J., 1993. Biochemical indicators
849 of oxidative stress in fish from polluted Littoral Areas. *Canadian Journal of Fisheries and*
850 *Aquatic Sciences* 50, 2568-2573.
- 851
- 852 Sen, A., Arinç, E., 1998a. Preparation of highly purified cytochrome P450 1A1 from
853 leaping mullet (*Liza saliens*) liver microsomes and its biocatalytic, molecular and
854 immunological properties. *Comparative Biochemistry and Physiology* 121C, 249-265.
- 855
- 856 Sen A, Arinç E., 1998b. Purification and characterization of cytochrome P450 reductase
857 from feral leaping mullet (*Liza saliens*) liver microsomes. *Journal of Biochemical and*
858 *Molecular Toxicology* 12, 103-113.
- 859
- 860 Sen, A., Kirikbakan, A., 2004. Biochemical characterization and distribution of glutathione
861 S-transferases in leaping mullet (*Liza saliens*). *Biochemistry (Moscow)* 69, 993-1000.
- 862
- 863 Sen, A., Semiz, A., 2007. Effects of metals and detergents on biotransformation and
864 detoxification enzymes of leaping mullet (*Liza saliens*). *Ecotoxicology and Environmental*
865 *Safety* 68, 405-411.
- 866
- 867 Stegeman, J.J., 1995. Diversity and regulation of cytochrome P450 in aquatic species, in:
868 Arinç, E., Schenkman, J.B., Hodgson, E. (Eds.), *Molecular Aspects of Oxidative Drug*
869 *Metabolizing Enzymes: Their Significance in Environmental Toxicology, Chemical*
870 *Carcinogenesis and Health*. Springer: Heidelberg, pp. 135-158.
- 871
- 872 Telli Karakoç, F., Gaines, A.F., Hewer, A., Philips, D., 2001. Differences between blood
873 and liver aromatic DNA adduct formation. *Environment International* 26, 143-148.
- 874
- 875 Towbin, H., Staehelin, T., Gordon, J., 1979. Electrophoretic transfer of proteins from
876 polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings*
877 *of the National Academy of Sciences of the United States of America* 76, 4350-4354.
- 878
- 879 Tuncer, G., Karakaş, T., Balkas, T.I., Gökçay, C.F., Aygün, S., Yurteri, C., Tuncel, G.,
880 1998. Land-based sources of pollution along the Black Sea coast of Turkey: concentrations
881 and annual loads to the Black Sea. *Marine Pollution Bulletin* 36, 409-423.
- 882
- 883 UNEP, 1992. Determination of petroleum hydrocarbons in sediments. Reference methods
884 for marine pollution studies. *United Nations Environment Programme, Geneva*, 20, 36-40.
- 885
- 886 Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and
887 biomarkers in environmental risk assessment: a review. *Environmental Toxicology and*
888 *Pharmacology* 13, 57-149.
- 889
- 890 Vignier, V., Vandermeulen, J.H., Singh, J., Mossman, D., 1994. Interannual mixed
891 function oxidase (MFO) activity in winter flounder (*Pleuronectes americanus*) from a coal
892 tar contaminated estuary. *Canadian Journal of Fisheries and Aquatic Sciences* 51, 1368-
893 1375.
- 894
- 895 Whyte, J.J., Karrow, N.A., Boermans, H.J., Dixon, D.G., Bols, N.C., 2000. Combined
896 methodologies for measuring exposure of rainbow trout (*Oncorhynchus mykiss*) to

- 897 polycyclic aromatic hydrocarbons (PAHs) in creosote contaminated microcosmes.
898 Polycyclic Aromatic Compounds 18, 71-98.

ACCEPTED MANUSCRIPT

Table 1. Mean values of biomarker enzyme activities and Standard Error of Mean (SEM)

Zone	Year	N	Fish Species	EROD ^a	GST ^b	CAT ^b	GR ^b
Zonguldak Harbour	2005	7	<i>Mugil soiuy</i>	3237±539	1260±207	322±34	14.9±1.9
	2006	13	<i>Mugil soiuy</i>	2559±315	950±135	283±46	20.4±0.8
	2006	9	<i>Liza aurata</i>	2302±240	1067±208	163±16	16.2±1.6
	2007	5	<i>Mugil cephalus</i>	3017±565	1017±231	171±12	11.5±2.1
Ereğli Harbour	2005	16	<i>Liza aurata</i>	1871±391	932±131	195±18	17.4±0.7
	2006	12	<i>Liza aurata</i>	2037±280	585±64	138±12	18.1±0.8
	2007	5	<i>Liza aurata</i>	2233±464	406±29	121±12	17.9±1.3
Gülüç Stream Mouth	2005	13	<i>Mugil soiuy</i>	2402±261	1352±154	281±32	20.8±0.9
	2006	11	<i>Mugil soiuy</i>	2415±426	830±154	224±30	20.7±1.3
Melen Stream Mouth	2005	11	<i>Mugil soiuy</i>	1065±176	851±129	349±32	23.1±1.9
	2006	9	<i>Mugil soiuy</i>	818±217	1192±140	200±20	18.7±1.8
Sakarya River Mouth	2005	10	<i>Mugil soiuy</i>	904±216	810±159	352±17	19.7±1.7
	2006	10	<i>Mugil soiuy</i>	438±62	735±134	257±32	9.6±0.7
	2007	4	<i>Mugil soiuy</i>	1642±470	1016±349	187±19	11.3±0.8
Amasra	2006	3	<i>Mugil soiuy</i>	425±18	288±165	123±20	13.4±0.8
	2006	4	<i>Mugil cephalus</i>	541±22	263±62	117±21	11.3±0.5
	2007	5	<i>Liza aurata</i>	259±72	285±37	100±11	11.2±1.7

^a pmol min⁻¹ mg⁻¹ protein^b nmol min⁻¹ mg⁻¹ protein

Table 2 Total PAH concentrations in the livers of mullets collected from six sampling stations of the West Black Sea Coast of Turkey in 2006. Homogenous subsets (groups do not differ significantly) were indicated by the same letters ($p < 0.05$).

Zone	N	Fish Species	PAH Concentration *
Zonguldak Harbour	13	<i>Mugil soiuy</i>	54 ± 5 (a)
	9	<i>Liza aurata</i>	47 ± 6 (a)
Ereğli Harbour	12	<i>Liza aurata</i>	28 ± 3 (b)
Gülüç Stream Mouth	11	<i>Mugil soiuy</i>	35 ± 3 (ab)
Melen Stream Mouth	9	<i>Mugil soiuy</i>	25 ± 3 (bc)
Sakarya River Mouth	8	<i>Mugil soiuy</i>	21 ± 3 (bc)
Amasra	3	<i>Mugil soiuy</i>	12 ± 2 (c)
	4	<i>Mugil cephalus</i>	11 ± 2 (c)

* $\mu\text{g g}^{-1}$ dry weight

FIGURE LEGENDS

Figure 1 Fish sampling stations in the West Black Sea Coast of Turkey. * Reference site.

Figure 2 Ethoxyresorufin O-deethylase (EROD) activities of liver microsomes of mullets caught from six sampling stations of the West Black Sea Coast of Turkey in 2005, 2006, and 2007. Homogenous subsets (groups do not differ significantly) were indicated by the same letters ($p < 0.05$).

Figure 3 Western blot analysis of CYP1A protein contents and correlation between microsomal EROD activities and CYP1A protein levels of mullets caught from different stations of the West Black Sea Coast of Turkey in 2005 (A), 2006 (B), and 2007 (C). 80 μ g microsomal protein was applied to each slot. (A) Line 1-9 (*Mugil so-iuy*) and Line 10-12 (*Liza aurata*); (B) Line 1-7 (*Mugil so-iuy*) and Line 8-12 (*Liza aurata*); (C) Line 1-4 (*Liza aurata*), Line 5-8 (*Mugil so-iuy*), Line 9-12 (*Mugil cephalus*).

* Sampled in 2006

** Sampled in 2007

Figure 4 Comparison of anti-leaping mullet CYP1A cross reactivity pattern of different mullet species. The intensities of CYP1A protein bands of different mullet species present in the same western blot result (Figure 3C) were compared together with corresponding EROD activities and EROD activity/CYP1A protein level (R.P.A.) ratios. Different mullet species having similar EROD activity showed similar degree of staining and gave almost the same EROD activity/CYP1A protein level (R.P.A.) ratio.

Figure 5 Glutathione S-transferase (GST) activities of mullets caught from six sampling stations of the West Black Sea Coast of Turkey in 2005, 2006, and 2007. Homogenous subsets (groups do not differ significantly) were indicated by the same letters ($p < 0.05$).

Figure 6 Catalase activities of mullets caught from six sampling stations of the West Black Sea Coast of Turkey in 2005, 2006, and 2007. Homogenous subsets (groups do not differ significantly) were indicated by the same letters ($p < 0.05$).

Figure 7 Glutathione reductase activities of mullets caught from six sampling stations of the West Black Sea Coast of Turkey in 2005, 2006, and 2007. Homogenous subsets (groups do not differ significantly) were indicated by the same letters ($p < 0.05$).

Figure 1

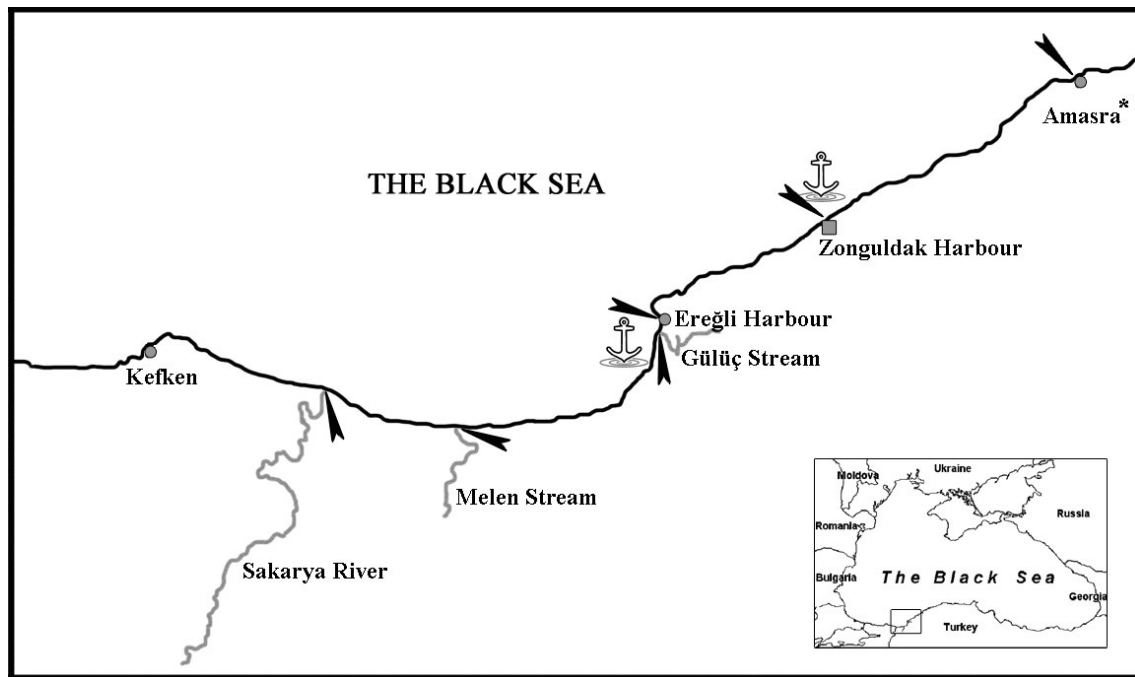


Figure 2

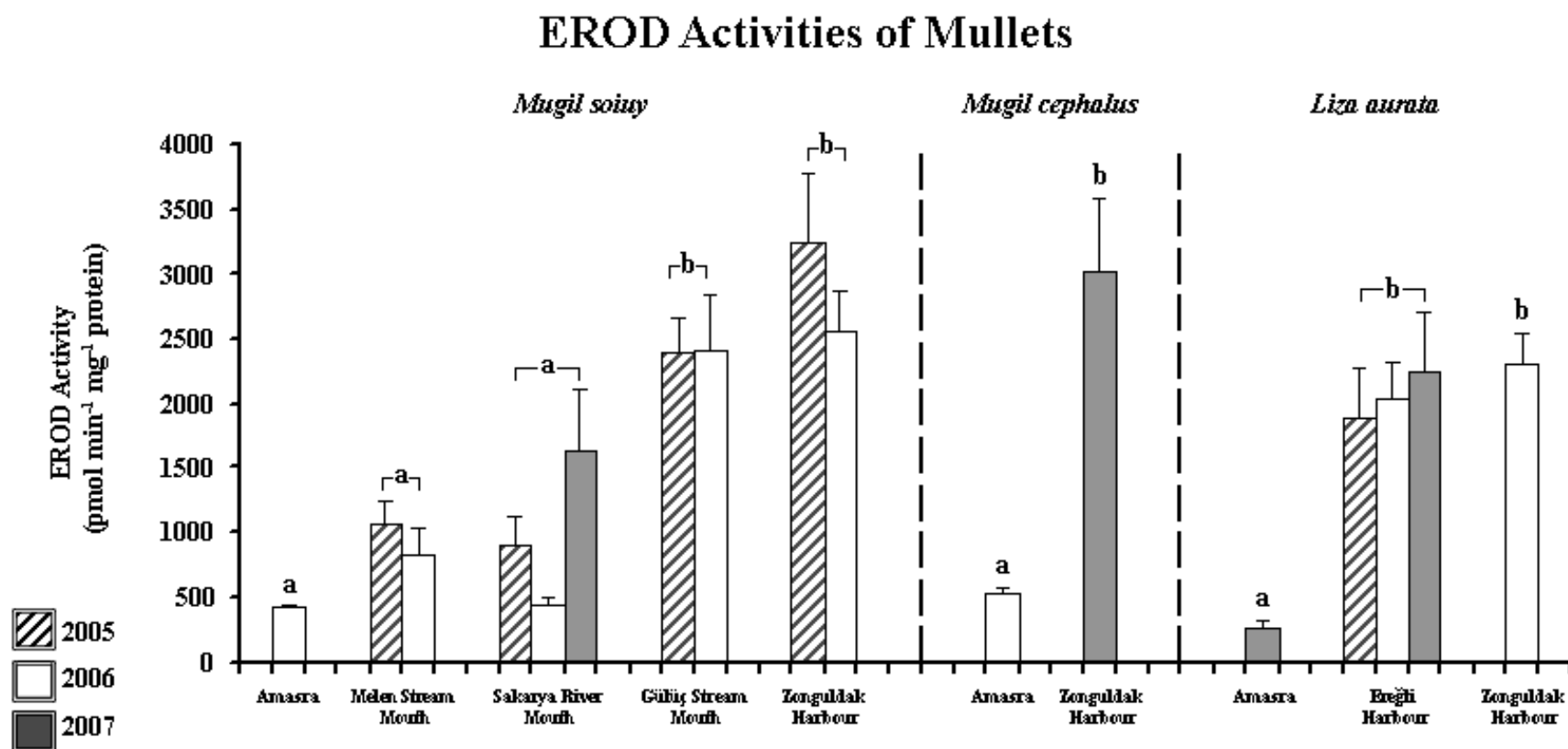


Figure 3

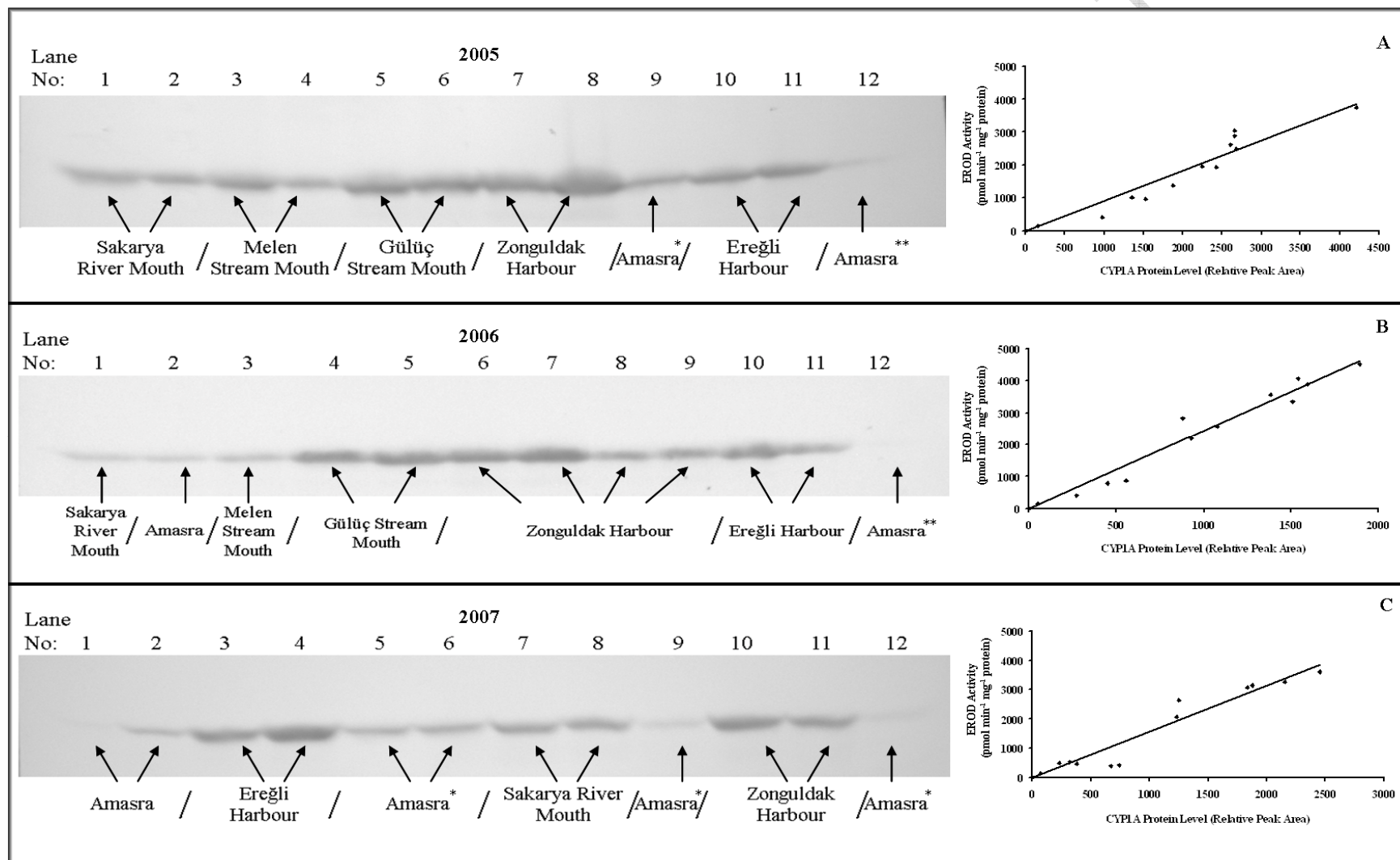


Figure 4

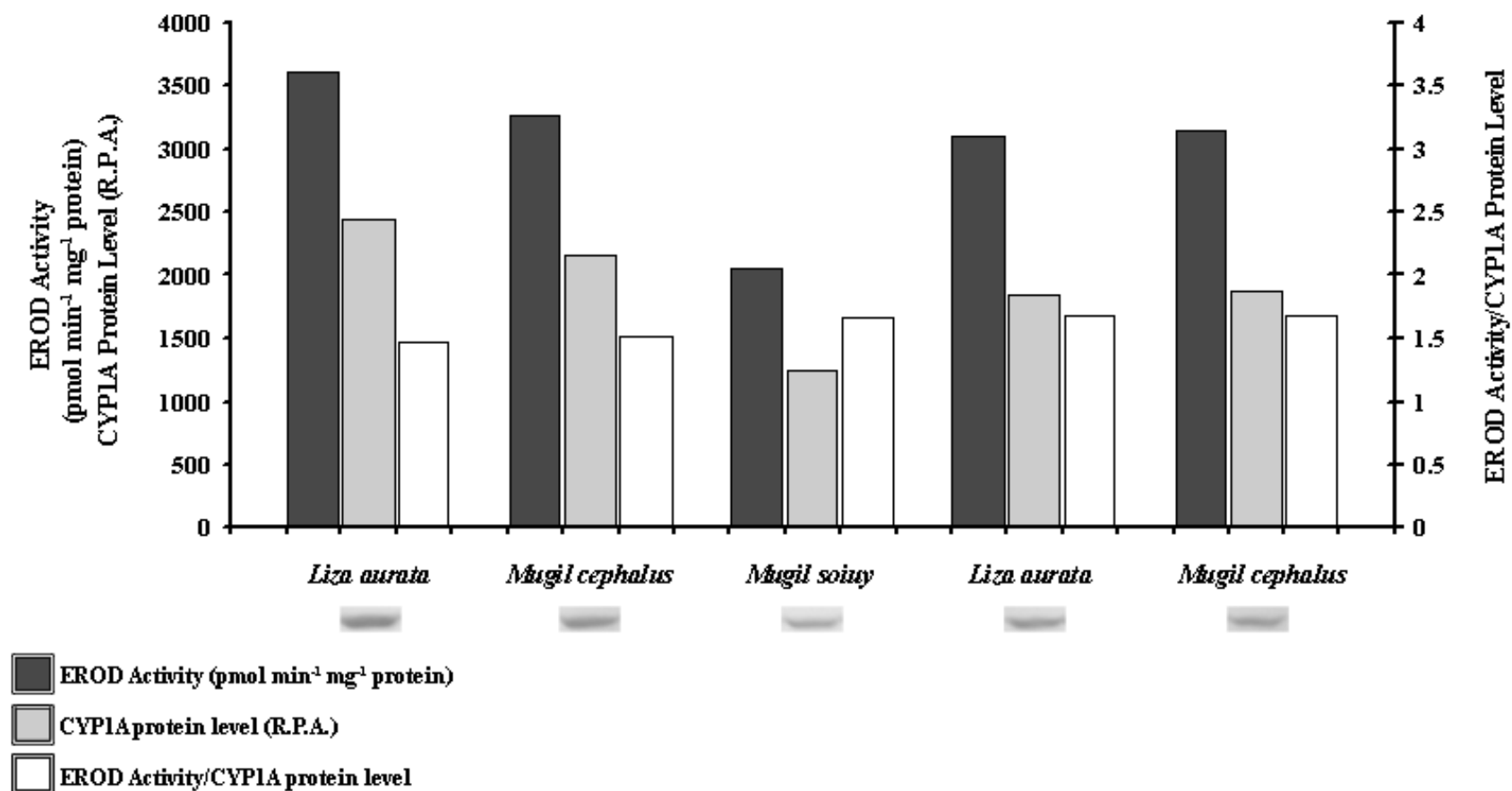


Figure 5

Total Glutathione S-Transferase Activities of Mullet

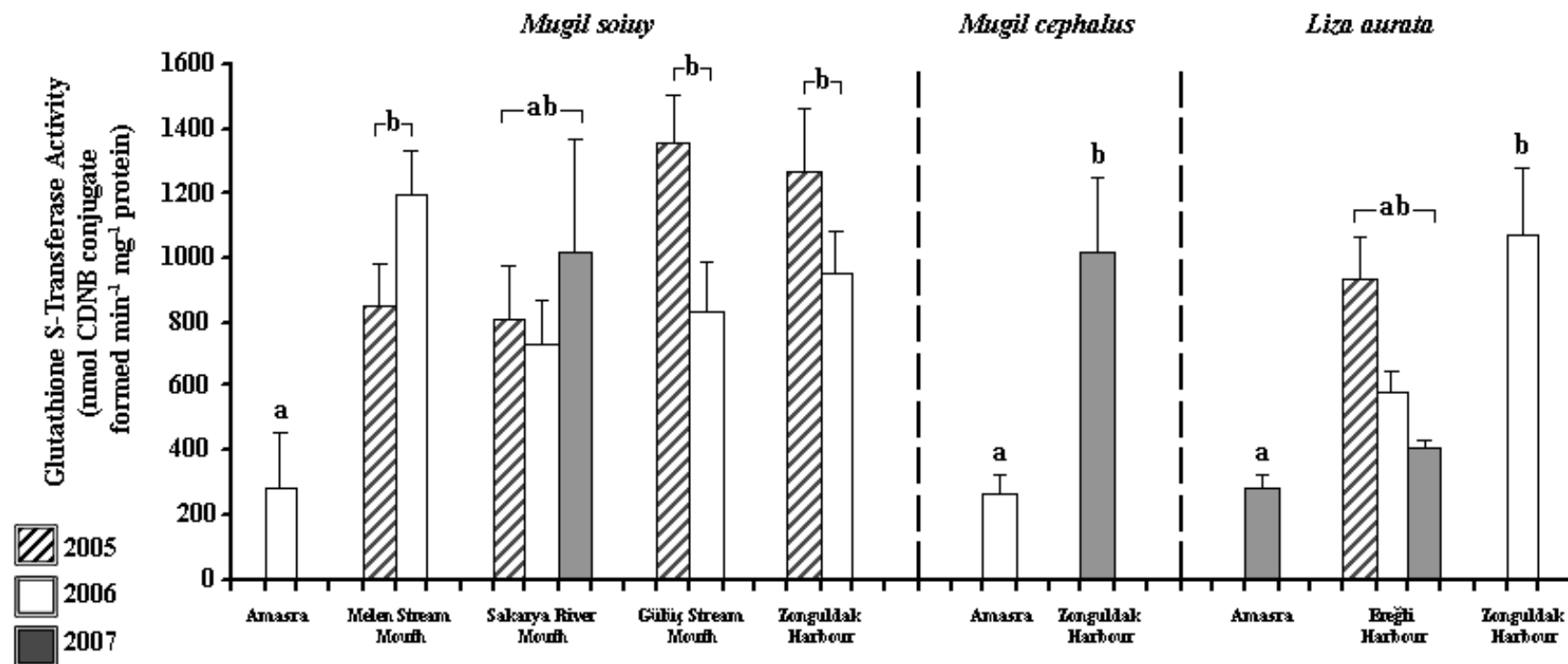


Figure 6

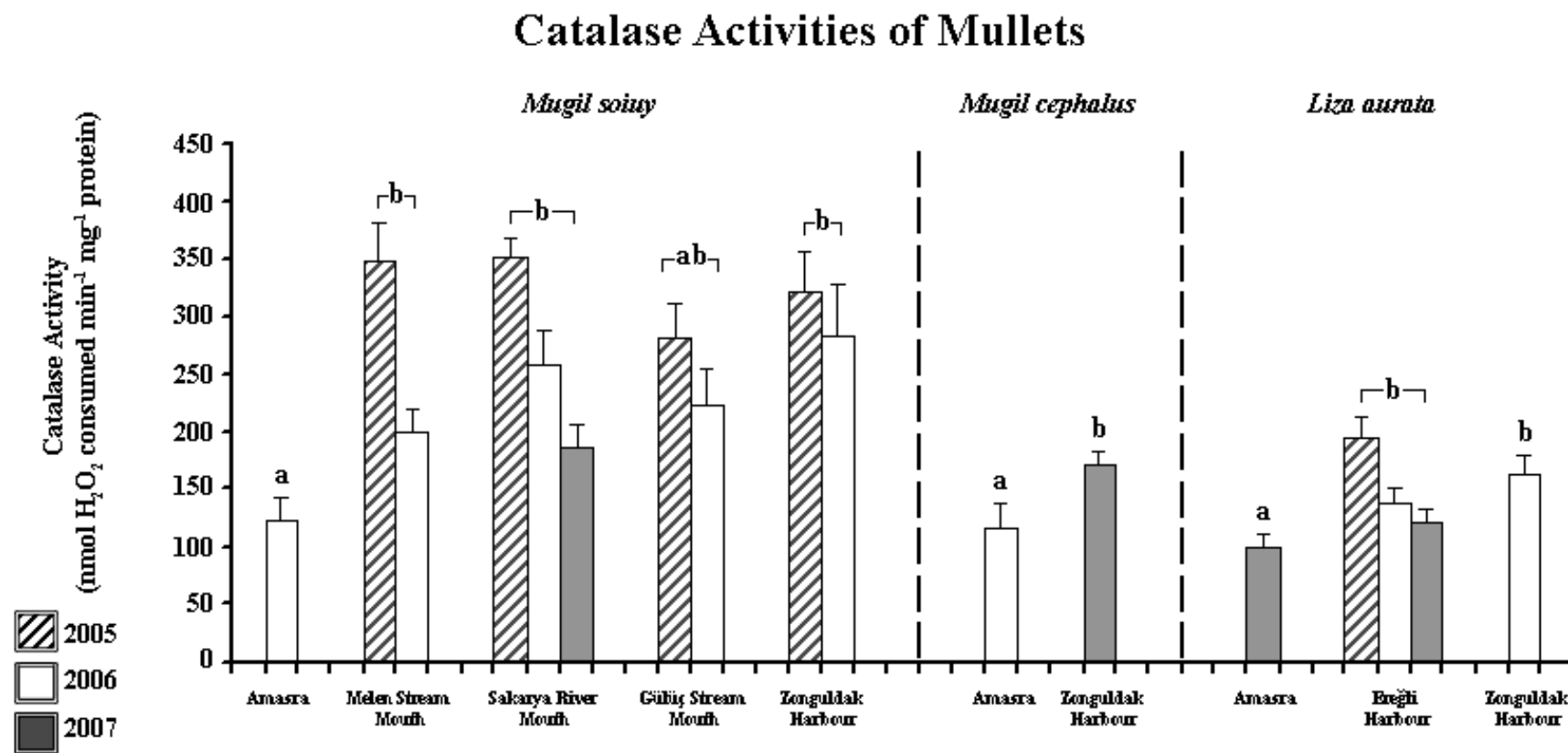


Figure 7

Glutathione Reductase Activities of Mullet

