



**HAL**  
open science

## **Toxicity of dispersant application: biomarkers responses in gills of juvenile golden grey mullet (*Liza aurata*)**

Thomas Milinkovitch, Joachim Godefroy, Michaël Théron, Hélène Thomas-Guyon

### ► **To cite this version:**

Thomas Milinkovitch, Joachim Godefroy, Michaël Théron, Hélène Thomas-Guyon. Toxicity of dispersant application: biomarkers responses in gills of juvenile golden grey mullet (*Liza aurata*). *Environmental Pollution*, 2011, 159 (10), pp.2921-2928. 10.1016/j.envpol.2011.04.035 . hal-00647259

**HAL Id: hal-00647259**

**<https://hal.science/hal-00647259>**

Submitted on 1 Dec 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.

1 **Toxicity of dispersant application: biomarkers responses in gills of**  
2 **juvenile golden grey mullet (*Liza aurata*)**

3  
4 Thomas Milinkovitch<sup>1\*</sup>, Joachim Godefroy<sup>1</sup>, Michaël Théron<sup>2</sup>, Hélène Thomas-Guyon<sup>1</sup>

5  
6  
7 **Affiliations**

8  
9 <sup>1</sup>Littoral Environnement et Sociétés (LIENSs), UMR 6250, CNRS-Université de La Rochelle,  
10 2 rue Olympe de Gouges – F-17042 La Rochelle Cedex 01, France.

11 Email: [thomas.milinkovitch01@univ-lr.fr](mailto:thomas.milinkovitch01@univ-lr.fr) ; helene.thomas@univ-lr.fr ; tel: 0546507623

12 <sup>2</sup>Laboratoire ORPHY EA4324, Université de Bretagne Occidentale, 6 Avenue le Gorgeu, CS  
13 93837, 29238, Brest, Cedex 3, France

14 Email: [michael.theron@univ-brest.fr](mailto:michael.theron@univ-brest.fr)

15  
16 **\* Corresponding author:** T. Milinkovitch

17 Littoral Environnement et Sociétés (LIENSs)

18 UMR 6250, CNRS-Université de La Rochelle

19 2 rue Olympe de Gouges

20 F-17042 La Rochelle Cedex 01, France

21 Email : [thomas.milinkovitch01@univ-lr.fr](mailto:thomas.milinkovitch01@univ-lr.fr)

22  
23 tel : +33 (0)5 46 50 76 48

24 fax : +33 (0)5 46 45 82 64

27 **Abstract**

28

29 Dispersant use in nearshore areas is likely to increase the exposure of aquatic organisms to  
30 petroleum. To measure the toxicity of this controversial response technique, golden grey  
31 mullets (*Liza aurata*) were exposed to mechanically dispersed oil, chemically dispersed oil,  
32 dispersant alone in seawater, water-soluble fraction of oil and to seawater as a control  
33 treatment. Several biomarkers were assessed in the gills (enzymatic antioxidant activities,  
34 glutathione content, lipid peroxidation) and in the gallbladder (polycyclic aromatic  
35 hydrocarbons metabolites). The significant differences between chemically dispersed oil and  
36 water soluble fraction of oil highlight the environmental risk to disperse an oil slick when  
37 containment and recovery can be conducted. The lack of significance between chemically and  
38 mechanically dispersed oil suggests that dispersant application is no more toxic than the  
39 natural dispersion of the oil slick. The results of this study are of interest in order to establish  
40 dispersant use policies in nearshore areas.

41

42 *Capsule:*

43 When the meteorological conditions induce the dispersion of the oil slick (e.g. wave), the  
44 application of dispersant does not increase the toxicity of petroleum.

45

46 **Keywords:** dispersed crude oil; oxidative stress; glutathione; PAH biliary metabolites; gills;  
47 *Liza aurata*.

48

49

50

51

52 **1. Introduction**

53

54 Dispersant application is an oil spill response technique that permits the transfer of the oil  
55 slick from the surface to the water column. When applied on an oil slick, the chemical  
56 formulation of the dispersant (surface active agent) induces the formation of oil–surfactant  
57 micelles. In offshore areas, dispersion shows many advantages since it accelerates the  
58 bacterial degradation of petroleum (Tiehm, 1994; Churchill et al., 1995), reduces the chance  
59 of drifting of the oil slick to the shoreline and also limits the risk of contamination to the  
60 surface occupying organisms (e.g. seabirds, marine mammals). However, when the oil spill  
61 site is in a nearshore area or if an oil slick reaches the coast (as observed recently in the Deep  
62 Water Horizon oil spill), slick dispersion is prohibited (Chapman et al., 2007). This  
63 environmental precautionary principle is based (i) on the low dilution potential of the oil slick  
64 in the shallow waters of nearshore areas and (ii) on the ecological sensitivity of nearshore  
65 areas since they are nurseries for many fish species (Martinho et al., 2007). On the other hand,  
66 a field study conducted by Baca et al. (2006), but only applicable to nearshore mangroves,  
67 seagrass and coral ecosystems, revealed a positive net environmental benefit of dispersant  
68 application in nearshore areas. Thus, with regards to the precautionary principle and the recent  
69 results of field studies, dispersant application in nearshore areas seems to be a controversial  
70 response technique. In this context, in order to contribute to dispersant use policies in  
71 nearshore areas, an on-going project is being conducted: the DISCOBIOL project (DISpersant  
72 and response techniques for COastal areas; BIOLogical assessment and contributions to the  
73 regulation). This study is part of this project and intended to assess the toxicity of a  
74 chemically dispersed oil. For this purpose, most studies have evaluated the toxicity of  
75 dispersant alone in seawater (Adams et al., 1999; George-Ares and Clark, 2000) or the  
76 dispersed oil water-accommodated fraction (Cohen and Nugegoda, 2000; Mitchell and

77 Holdway, 2000; Ramachandran et al., 2004; Perkins et al., 2005; Jung et al., 2009), not taking  
78 into account the presence of oil droplets in the water column. However, many field  
79 observations have shown the presence of oil droplets in the water column. Their formation  
80 can be induced within 2 hours (Cormack, 1977) or during a period of more than 1 week, as  
81 observed during the Braer oil spill (Lunel, 1995). In this context, our experimental approach  
82 was conducted in order to evaluate the actual toxicity of a chemically dispersed oil treatment  
83 containing oil droplets. Toxicity was measured through the assessment of biomarkers in a  
84 target organ of a pelagic fish species.

85 Oxidative stress and antioxidant defences were considered as suitable biomarkers since they  
86 have been shown in many studies to respond to petroleum contamination and especially to the  
87 PAH (polycyclic aromatic hydrocarbons) contained in petroleum (Avci et al., 2005 ; Almroth  
88 et al., 2008; Oliveira et al., 2008; Jung et al., 2009; Kopecka Pylarczyk and Correia, 2009;  
89 Narghang et al., 2009). Moreover, both, oxidative stress and antioxidant defences could give  
90 information on the health of the contaminated organisms: (i) oxidative stress, since it is  
91 considered as a cause of tissue injury (Halliwell, 1999) and (ii) antioxidant defences, since  
92 authors linked modulation of this biological parameter to fish health indicators such as  
93 progression of diseases and/or cellular mortality (Allen and Moore, 2004).

94 In our study, oxidative stress and antioxidant defences were assessed by evaluating lipid  
95 peroxidation (a marker of lipid degradation due to oxidative stress) and evaluating the  
96 response of antioxidant enzymatic activities (catalase, superoxide dismutase and glutathione  
97 peroxidase), respectively.

98 Additionally, total glutathione was measured taking into account the importance of the  
99 cellular status of this molecule for the defence of the organism against xenobiotics (Maracine  
100 and Segner, 1998). Indeed, glutathione is implied in many cellular defence mechanisms such  
101 as (i) antioxidant defences, by its conjugation to reactive oxygen species (Amiard-Triquet and

102 Amiard, 2008); (ii) heavy metals (such as Vanadium and Nickel present in petroleum, Salar  
103 Amoli et al., 2006) chelation, as described in Sies, 1999; and (iii) detoxification processes, by  
104 its conjugation to xenobiotics such as PAH (van der Oost et al., 2003). In our study, we  
105 evaluated the glutathione status through the measurement of the total glutathione content  
106 which is the sum of the oxidized and the reduced form of this molecule.  
107 These biomarkers were assessed in fish gills, taking into account their target organ status:  
108 several studies have shown an effect of petroleum compounds on gills (McKeown, 1981;  
109 Oliveira et al., 2008; Mendonça Duarte et al., 2010). In parallel, PAH biliary metabolites were  
110 measured in order to evaluate the level of exposure to PAH following dispersant application.  
111 The choice of the golden grey mullet (*Liza aurata*) as a biological model was based on the  
112 fact that (i) it represents a relevant biomass in nearshore ecosystems; (ii) it is a commercially  
113 important species especially in Europe and Egypt (Gautier and Hussenot, 2005) and (iii) this  
114 species is present in nearshore areas during its early life stages (Gautier and Hussenot, 2005)  
115 being consequently a target organism for anthropogenic pollutants (Bruslé, 1981).

116

## 117 **2. Materials and methods**

118

### 119 2.1. Experimental design

120

#### 121 2.1.1. Experimental animals

122

123 Fifty juvenile golden grey mullets (*Liza aurata*), fished in Venice (Italy) lagoons and  
124 provided by Commercio Pesca Novellame Srl (Chioggia, Italy), were used to conduct this  
125 study.

126 For 4 weeks, fish were acclimatized in 300-L flow-through tanks prior to the exposure studies  
127 (dissolved oxygen:  $94 \pm 2\%$ ; salinity:  $35 \pm 0\%$ ; temperature:  $14.9 \pm 0.5$  °C, with a 12 h  
128 light:12 h dark photoperiod in seawater free of nitrate and nitrite). During acclimation, they  
129 were fed daily with fish food (Neosupra AL3, from Le Gouessant aquaculture) which does  
130 not contain additives (also called synthetics) antioxidants authorised by the European Union  
131 (butyl-hydroxy-anisol, butyl-hydroxy-toluene, ethoxyquin, propyl gallate and octyl gallate).  
132 Fish were starved for 48 h prior to bioassays and throughout the exposure period, in order to  
133 avoid bile evacuation from the gallbladder. Prior to bioassay, their average length was  $136.6 \pm$   
134  $0.1$  mm (mean  $\pm$  standard error of the mean) and their average weight was  $32.33 \pm 0.87$  g.

135

#### 136 2.1.2 Chemicals

137

138 A dispersant formulation (Total Fluides) was selected based on its efficiency. The efficiency  
139 was preliminary determined in the CEDRE (CEntre de Documentation de Recherche et  
140 d'Expérimentations sur les pollutions accidentelles des eaux, France) using the method  
141 NF.T.90-345. The dispersant was non-toxic at the concentration recommended by the  
142 manufacturer (preliminary determined using standard toxicity test: method NF.T.90-349) and  
143 biodegradable.

144 A Brut Arabian Light (BAL) crude oil was selected for this study. The oil is composed of  
145 54% saturated hydrocarbons, 10% polar compounds and 36% aromatic hydrocarbons.

146 To simulate the natural behaviour of the oil after it is released at sea (evaporation of light  
147 compounds and natural photodegradation, respectively) the oil was evaporated under  
148 atmospheric conditions and natural UV-sunlight, prior to fish exposure. The resulting  
149 chemical composition of the oil was 54% saturated hydrocarbons, 12% polar compounds and  
150 34% aromatic hydrocarbons. Among aromatic hydrocarbons, concentration of 21 PAH was

151 measured (the 16 PAH listed by the USEPA as priority pollutants and five supplementary  
152 PAH: benzo[*b*]thiophene, biphenyl, dibenzothiophene, benzo[*e*]pyrene, perylene). The sum of  
153 the 21 PAH represents 16.4 mg/g of petroleum (1.64 % of the petroleum). More information  
154 concerning the composition of the petroleum used in this study is available in (Milinkovitch et  
155 al., accepted for publication).

156

### 157 2.1.3. Experimental system (**Figure 1**)

158

159 The experimental system (also described in Milinkovitch et al., 2011) comprised five 300-L  
160 seawater tanks. Each one contains a funnel (**a**, at the surface) linked to a Johnson L450 water  
161 pump (**b**, at the bottom of the tank). After 24 h homogenization, this system was set up to  
162 maintain a mixture of oil and dispersant as a homogenous solution despite the hydrophobic  
163 character of the oil (preliminary tests not shown). The temperature in this static water system  
164 was controlled using two heaters (RENA CAL 300) so that the exposure temperature was  
165  $15.3 \pm 0.3$  °C (mean  $\pm$  standard error mean). Other physico-chemical parameters were also  
166 measured: seawater was free of nitrate and nitrite, pH ( $7.99 \pm 0.03$ ) and dissolved oxygen ( $98$   
167  $\pm 5\%$ ) remained constant throughout the study.

168

### 169 2.1.4. Experimental treatments

170

171 Each experimental tank contained 300 L seawater provided by Oceanopolis (France). The  
172 control treatment (C) was made up using 300 L seawater. The chemically dispersed (CD) oil  
173 treatment was made by pouring 20 g of petroleum and 1 g of dispersant into the funnel of the  
174 experimental system. The mechanically dispersed (MD) oil treatment was made by pouring  
175 20 g of petroleum into this funnel. The dispersant alone (D) treatment, as an internal control



176 of CD, was made by pouring 1 g of dispersant into the funnel. For the water-soluble fraction  
177 of oil (WSF), a 20 g oil slick was contained using a plastic cylinder (21 cm diameter) placed  
178 on the surface of the seawater (in addition to the funnel and the pump, which were kept to  
179 maintain the same level of agitation of the seawater as for other treatments). Readers must  
180 take into account that the spreading of the oil slick was not prevented by the plastic cylinder  
181 since the oil slick was smaller than the diameter of the plastic cylinder. Thereby the  
182 experimental approach simulates the actual spreading behaviour of oil at sea. During the  
183 entire exposure period, the oil slick remained at the surface without mixing. No droplet was  
184 observed in the water column (visual observations) suggesting that the fish were only exposed  
185 to the soluble fraction of the oil.

186 While the solutions remained homogenous (less than 5 % difference between three TPH  
187 concentration measurements sampled at three different depths in the experimental tanks), no  
188 fish were exposed for 24 hours after making up the solutions. Then, groups of 10 fish were  
189 randomly distributed in the five experimental tanks, each tank containing an exposure media  
190 (described above). The fish were exposed for 48 h (from T=0 h to T=48 h).

191 At the end of the exposure period, the fish in each tank (each treatment) were euthanized  
192 using eugenol (99 %, Sigma Aldrich chemicals, France). The gallbladder was removed from  
193 each fish and stored at  $-80^{\circ}\text{C}$  prior to analysis. Gills were rinsed off by dipping them in PBS  
194 (Phosphate Buffered Sodium 0.01 M, pH=7.4, Sigma) in order to remove blood. Then, the  
195 gills were homogenized in another PBS solution. The homogenates were centrifuged at  
196 10,000 g,  $4^{\circ}\text{C}$ , for 15 min to obtain the post-mitochondrial supernatant. Total protein  
197 concentrations in supernatants were determined using the method of Bradford (1976) with  
198 bovine serum albumin (Sigma-Aldrich Chemicals, France). Then, supernatants were stored at  
199  $-80^{\circ}\text{C}$  prior to biochemical analysis.

200

201

## 202 2.2. Total petroleum hydrocarbon (TPH) seawater concentrations

203

204 The TPH concentration, which is the sum of the dissolved hydrocarbon concentrations plus  
205 the amount of oil droplets, was measured for all treatments at the beginning (T=0 h) and at the  
206 end of fish exposure (T=48 h), using the mean of three replicated measurements for each time  
207 point. The samples were extracted with 10 mL of dichloromethane (Carlo Erba Reactifs,  
208 SDS). After separation of the organic and aqueous phases, water was extracted two additional  
209 times with the same volume of dichloromethane (2 x 10 mL). The combined extracts were  
210 dried on anhydrous sulphate and then analysed using a UV spectrophotometer (UV-Vis  
211 spectrophotometer, Unicam, France) at 390 nm, as described by Fusey and Oudot (1976).  
212 Assays were conducted in collaboration with Cedre (Centre de Documentation de Recherche  
213 et d'Expérimentations sur les Pollutions Accidentelles des Eaux), a laboratory with agreement  
214 ISO 9001 and ISO 14001. In accordance with Cedre, results are not reliable under 1mg/L.

215

## 216 2.3. Biochemical analysis

217

### 218 2.3.1. Fixed wavelength fluorescence analysis of bile

219

220 Bile samples were diluted (1:1000) in absolute ethanol (VWR International, France) and  
221 assessments were conducted for three fixed wavelength fluorescence (FF). FF 290:335 mainly  
222 detects naphthalene-derived metabolites, FF 341:383 mainly detects pyrene-derived  
223 metabolites and FF 380:430 mainly detects benzo[*a*]pyrene-derived metabolites (Aas et al.,  
224 2000). Measurements were performed in quartz cuvettes (Sigma Aldrich, USA) on a  
225 spectrofluorimeter (SAFAS Flx-Xenius, Monaco). The FF values were expressed as arbitrary

226 units of fluorescence and the signal level of pure ethanol was subtracted. Depending on the  
227 spectrofluorimeter, results are not reliable under 0.1 arbitrary units.

228

### 229 2.3.2. Total glutathione (GSH)

230

231 Total (reduced plus oxidized) glutathione was determined spectrophotometrically in gills,  
232 according to the procedure of Akerboom and Sies (1981) and using a glutathione assay kit  
233 (SIGMA CS0260, Sigma Aldrich, USA). The samples were first deproteinized with 5% 5-  
234 sulfosalicylic acid solution. The glutathione content of the sample was then assayed using a  
235 kinetic assay in which amounts of glutathione cause a continuous reduction of 5,5'-dithiobis-  
236 (2-nitrobenzoic) acid (DTNB) to TNB. The oxidized glutathione formed was recycled by  
237 glutathione reductase and NADPH. The product, TNB, was assayed colorimetrically at 412  
238 nm in UV microplates (Greiner Bio One), using a spectrophotometer (SAFAS Flx-Xenius,  
239 Monaco). The results are presented in  $\mu\text{mol}$  of GSH/g of protein.

240

### 241 2.3.3. Antioxidant enzymes and lipid peroxidation

242

243 Glutathione peroxidase (GPx) activity was determined according to the method of Paglia and  
244 Valentine (1967), using a glutathione peroxidase assay kit (RS504/RS 505, RANDOX,  
245 France). GPx catalyses the oxidation of reduced glutathione by cumene hydroperoxide. In the  
246 presence of glutathione reductase and NADPH the oxidized glutathione (GSSG) is  
247 immediately converted to the reduced form with concomitant oxidation of NADPH to  
248  $\text{NADP}^+$ . The decrease in absorbance was measured at 340 nm.

249 Superoxide dismutase (SOD) activity was determined according to the method of Woolliams  
250 et al. (1983) and using a superoxide dismutase assay kit (SD125, RANDOX, France). This

251 method employs xanthine and xanthine oxidase to generate superoxide radicals which react  
252 with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red  
253 formazan dye, assessed at 505 nm. The superoxide dismutase activity was then measured by  
254 the degree of inhibition of this reaction. One unit of SOD was that which causes a 50%  
255 inhibition of the rate of reduction of INT. The results are presented in units of SOD/mg of  
256 protein.

257 Catalase (CAT) activity was determined according to the method of Deisseroth and Dounce  
258 (1970) and using a catalase assay kit (CAT 100, Sigma Aldrich, USA). Samples were mixed  
259 (v:v) with hydrogen peroxide. The kinetics of hydrogen peroxide degradation were assessed  
260 at 280 nm. The results are expressed in units of CAT/mg of protein.

261 Lipid peroxidation levels were assessed via malondialdehyde (MDA) contents determined  
262 using a commercially available MDA assay kit (MDA assay kit, Oxis International, USA).  
263 The method was based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole,  
264 with MDA. The blue product was quantified by measuring absorbance at 586 nm (Gérard-  
265 Monnier et al., 1998). The results are presented in nmol of MDA/g of tissue.

266 Materials used to measure spectrophotometrically antioxidant enzymes activity and lipid  
267 peroxidation were UV microplates (Greiner Bio One, Germany) and a spectrophotometer  
268 (SAFAS Flx-Xenius, Monaco).

269

#### 270 2.4. Statistical analysis

271

272 The statistical analysis was carried out using XLstat 2007 software. The assumptions of  
273 normality and homoscedasticity were verified using the Kolmogorov-Smirnov and Cochran  
274 tests, respectively. When homoscedasticity and normality were not respected, a Kruskal  
275 Wallis test was conducted to highlight significant differences between treatments. When

276 homoscedasticity and normality were respected, a factorial analysis of variance (one-way  
277 ANOVA) was performed in order to assess the effects of the different treatments. This  
278 statistical analysis was followed by the Tukey post-hoc test to detect significant differences  
279 between groups. Correlations between fixed wavelength fluorescence intensity and other  
280 variables (GSH, GPx, SOD, CAT and LPO) were conducted using the Spearman test. The  
281 significance of the results was ascertained at  $\alpha=0.05$ . The results are expressed as means  $\pm$   
282 s.e.m. (standard error of the mean) corresponding to groups of 10 fish (n=10).

283

284

### 285 **3. Results**

286

287 No fish died during the acclimation and exposure period. TPH were not detected in the  
288 Control (C) and Dispersant (D) treatments. Moreover no oil slick was observed in the  
289 Chemically Dispersed oil (CD) and the Mechanically Dispersed oil (MD) treatments. In the  
290 Water Soluble Fraction of oil (WSF) treatment, the oil slick remained at the surface  
291 throughout the exposure period and no droplets were observed (visual observations) in the  
292 water column.

293

#### 294 3.1. Total petroleum hydrocarbons (TPH)

295

296 The concentration of TPH (**Table 1**) was slightly higher in the CD than in the MD treatment  
297 at T=0 h and at T=48 h. A 68% decrease was observed in the CD treatment (from 46.4 to 14.9  
298 mg/L) and a 73% decrease was observed in the MD treatment (from 39.4 to 10.7 mg/L)  
299 during the 48 h exposure period. No TPH were detected in the WSF treatment, probably due  
300 to the detection limit of the method.

301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325

### 3.2. Fixed wavelength fluorescence analysis of bile

Whatever the fixed wavelength employed (**Figure 2**), no significant difference was found between the fluorescence intensity of the WSF, D and C treatments.

Whatever the fixed wavelength employed, the fluorescence intensity was significantly higher in the CD treatment than in the C, D and WSF treatments.

At FF 380:430 and FF 343:383, the fluorescence intensity was higher in the MD treatment than in the C, D and WSF treatments whereas no significant difference was found at FF 290:335.

At FF 290:335 and FF 343:383, the fluorescence intensity was lower in the MD treatment than in the CD treatment whereas no significant difference was observed at FF 380:430.

### 3.3. Total glutathione (GSH)

Gill GSH content (**Figure 3**) was significantly lower in the CD than in the C, D and WSF treatments, whereas no significant difference was observed between the CD and MD treatments. No significant difference was observed between MD and the other treatments (C, D and WSF).

Significant correlations were found between the fluorescence intensities FF 343:383 and FF 380:430 with GSH content ( $P= 0.001$  and  $P=0.002$  respectively) whereas there was no correlation between the fluorescence intensity at 290:335 with GSH content ( $P>0.05$ ).

### 3.4. Antioxidant enzymatic activity and lipid peroxidation (LPO)

326 No significant difference was found between the five treatments ( $P>0.05$ ), in terms of  
327 antioxidant enzymatic (SOD, CAT, GPx) activities (**Figure 4**). With regards to SOD activity,  
328 the lack of significance could be due to the high intragroup variability. With regards to GPx,  
329 the enzymatic activity seemed to be higher in the CD treatment than in the other treatments.  
330 No correlation was found between the enzymatic activities and fixed wavelength fluorescence  
331 intensity ( $P>0.05$ ). There was no significant difference in LPO (**Figure 5**) between the five  
332 treatments ( $P>0.05$ ) and no correlation was found between LPO and the fixed wavelength  
333 fluorescence intensities ( $P>0.05$ ).

334

#### 335 **4. Discussion**

336

337 The aim of this study was to evaluate the toxicity of dispersant application. Through an  
338 experimental approach, several scenarios occurring during an oil spill were considered and  
339 their toxicity was evaluated. Five exposure treatments were conducted: (i) a control treatment  
340 with only seawater, (ii) a chemically dispersed oil treatment simulating, *in situ*, dispersant  
341 application on an oil slick under mixing processes, (iii) dispersant alone in seawater as an  
342 internal control of CD, (iv) a mechanically dispersed oil simulating only the effect of mixing  
343 processes on the oil slick and (v) a water-soluble fraction of oil simulating contamination due  
344 to an undispersed oil slick.

345 Given observations at oil spill sites (such as during the Braer oil spill, Lunel et al., 1995) and  
346 the natural mixing processes in nearshore areas (e.g. waves), the presence of oil droplets in  
347 the water column seems to be relevant when evaluating the toxicity of dispersant application  
348 in nearshore areas. Thus, the experimental system was devised to maintain oil droplets in the  
349 water column throughout the course of exposure.

350

351 4.1. Total petroleum hydrocarbons (TPH)

352

353 TPH concentrations vary from 46.4 to 14.9 mg/L for CD treatment and from 39.4 to 10.7  
354 mg/L for MD treatment. The concentrations observed at T = 0 h are inferior to the nominal  
355 concentrations (66.6 mg/L). This is probably due to the petroleum adherence to the  
356 experimental system during the 24 h period of homogenisation (prior to the bioassays,  
357 described in **2.1.5.**). The concentrations of TPH, measured in this experimental approach, are  
358 consistent with those observed at oil spill sites. Indeed, Spooner (1970) observed 50 mg/L of  
359 TPH after an oil spill in Tarut Bay (Saudi Arabia) due to a pipeline fracture. This observed  
360 concentration was due to the natural dispersion of 16 000 t of light Arabian crude oil in  
361 nearshore areas (less than 2 km from the shoreline). In the same way, Lunel (1995) observed  
362 concentrations varying between 1 and 100 mg/L during the wreck of the Braer on the  
363 Scotland coast. The cargo released 86 000 t of Gullfaks crude oil which were naturally  
364 dispersed due to severe wind conditions (Force 6 to 10).

365 Braer oil spill shows that, in nearshore areas, meteorological conditions could induce  
366 dispersion of the oil slick during a period of more than one week. However, at most oil spill  
367 sites in offshore areas, a decrease in concentration is observed over a 2 to 5 h period (Lessard  
368 and Demarco, 2000). Situated between these two scenarios, our experimental approach  
369 showed a decrease in TPH concentration over a 48 h period. Our observations suggest that  
370 this decrease is mainly due to petroleum adherence to the experimental system. This  
371 phenomenon of adherence to the experimental system simulates the adherence to the substrate  
372 observed in field studies (Baca et al., 2006). In this study, adhered petroleum represents  
373 approximately the nominal concentration of the petroleum minus the concentration of  
374 petroleum assessed in the water column. Even if adhered petroleum represents a relevant  
375 proportion of the petroleum (in particular at T = 48 h), fish were not directly exposed to this



376 fraction of the petroleum since (i) pelagic fish species, such as golden grey mullets, should  
377 only be exposed to petroleum present in the water column; (ii) in our study, most of the  
378 adhered petroleum was present in the funnel, for which fish do not have access.

379

#### 380 4.2. PAH biliary metabolites

381

382 The relative concentration of PAH biliary metabolites (evaluated through fixed wavelength  
383 fluorescence analysis) has often been used as an exposure biomarker (Camus et al., 1998; Aas  
384 et al., 2000; Jung et al., 2009). PAH are well studied since they are considered to be the most  
385 toxic compounds of petroleum. In our study we measured the biliary-derived metabolites  
386 corresponding to PAH (alkylated and parents) of three different weights (naphthalene: 128.2  
387  $\text{g}\cdot\text{mol}^{-1}$ , pyrene: 202.3  $\text{g}\cdot\text{mol}^{-1}$ , benzo[a]pyrene: 252.3  $\text{g}\cdot\text{mol}^{-1}$ ). The results showed a  
388 significant increase in the three PAH metabolites following the CD treatment, when compared  
389 to WSF. This result is in accordance with many studies (Perkins et al., 1973; Cohen and  
390 Nugegoda, 2000; Ramachandran et al., 2004; Lin et al., 2009) since it shows that the  
391 application of dispersant on an undispersed oil slick increases PAH exposure. The same is  
392 true of the MD treatment, when compared to WSF: mechanical dispersion increased pyrene  
393 and benzo[a]pyrene exposure (however no significant difference was observed for  
394 naphthalene-derived metabolites). This increase in PAH exposure, due to the dispersion  
395 (chemical or mechanical), suggests an increase of toxicity for tested organisms. Indeed, PAH  
396 are considered as carcinogenic and mutagenic (Eisler, 1987). Moreover, studies revealed that  
397 PAH induce histopathological effects (Stentiford et al., 2003 ; Ortiz-Delgado et al., 2007),  
398 inflammatory responses (Stentiford et al., 2003), oxidative stress (Sun et al., 2006 ; Oliveira et  
399 al., 2008) and alterations of DNA integrity (Oliveira et al., 2007 ; Maria et al., 2002) in teleost  
400 fish.

401 With regards to the MD and CD treatment, our results show that the differences in the relative  
402 concentration of the metabolites seem to be linked to PAH toxicity: the more toxic a PAH, the  
403 lower the difference, in metabolite concentration, between the two treatments. Indeed,  
404 naphthalene-derived metabolites (described as low toxicity PAH in Petry et al., 1996 and  
405 Bosveld et al., 2002) showed a 40% increase with CD treatment (when compared to MD  
406 treatment). Pyrene-derived metabolites showed a 13% increase. No significant difference was  
407 observed for benzo[*a*]pyrene-derived metabolites, which is considered as a carcinogenic PAH  
408 and induces reactive oxygen species (Lemaire-Gony and Lemaire, 1993).

409

#### 410 4.3. Total glutathione content (GSH)

411

412 When compared to the WSF treatment, the CD treatment induced a significant decrease in  
413 total glutathione content in the gills. Several hypotheses may explain the decrease in GSH  
414 content, such as the conjugation of glutathione to PAH through the increase in GST activity as  
415 observed in Yin et al. (2007) or the decrease in GSH synthesis due to contaminant exposure  
416 as described in Canesi et al. (1999). Whatever the physiological mechanism implicated, this  
417 study shows that dispersant application induced a depletion of glutathione, which is the first  
418 line cellular defence involved in many detoxification processes (Maracine and Segner, 1998).  
419 Thereby, the chemical dispersion of an oil slick decreases the potential of fish to cope with  
420 contaminated environments.

421 On the contrary, when compared to the MD treatment, the CD treatment did not induce a  
422 significant decrease in the total glutathione content in the gills, suggesting that, even when the  
423 oil slick is mechanically dispersed (e.g. due to meteorological conditions), the application of  
424 dispersant does not significantly decrease the potential of the organism to cope with its  
425 environment.

426 Benzo[*a*]pyrene- and pyrene-derived metabolite concentrations were correlated with the total  
427 glutathione content in the gills. However no correlation was found between naphthalene-  
428 derived metabolites and total glutathione content. Taken together, these results suggest that  
429 glutathione depletion arises due to exposure to heavy PAH whereas light PAH would not be  
430 involved in the observed decrease in glutathione.

431 In Milinkovitch et al. (2011), a similar experimental approach was conducted with the same  
432 exposure treatments as described in this study (C, CD, MD, WSF, D). The total glutathione  
433 content in fish liver was evaluated and appeared to follow the same pattern as the total  
434 glutathione content in gills (exposed in this study): CD treatment induced a significant  
435 decrease of total glutathione when compared to control treatment; and no significant  
436 difference was observed between CD and MD treatments. However, no significant difference  
437 was observed concerning the liver total glutathione content between WSF and CD exposure  
438 whereas, in the present study, when studying the fish gills, a significant difference was  
439 observed between these both conditions. This finding shows that, evaluating dispersant  
440 application toxicity, gill seems to be a more sensitive target organ than liver. This relevant  
441 sensitivity of gills could be due to the fact that gills are target organs immediately in contact  
442 with the external environment and thereby immediately in contact with pollutants presents in  
443 the water column.

444

#### 445 4.4. Oxidative stress

446

447 PAH, when incorporated by the organism, are bound to a cellular aryl hydrocarbon receptor  
448 (AhR). This binding induces the formation of a complex, the aryl hydrocarbon receptor  
449 nuclear translocator (ARNT), which is delocalized in the nucleus of the cell and bound to the  
450 xenobiotic regulatory element (XRE). This phenomenon increases the transcription rate of the

451 P4501A cytochrome genes (CYP1A) and by the way increases the synthesis *de novo* of the  
452 cytochrome P450 enzymes and the catalytic activity of these enzymes (Stegeman, 1987). This  
453 increasing activity enhances the cellular production of reactive oxygen species (Livingstone,  
454 2001), which is counteracted by the antioxidant response (especially through enzymatic  
455 antioxidant activities). When the production of ROS overwhelms the antioxidant response,  
456 free reactive oxygen species can interact deleteriously with cellular components. Lipid  
457 peroxidation is a marker of this impairment.

458 Our results showed no modulation of lipid peroxidation, suggesting a lack of free radical  
459 attack due to PAH exposure. Moreover, no antioxidant response was observed. The absence  
460 of oxidative stress could be due to the composition of the fish food. Indeed, even if fish were  
461 fed during four weeks with a fish food free of additives (also called synthetics) antioxidants,  
462 natural antioxidants (such as vitamins A, C and E) are presents in the food composition. This  
463 consumption of antioxidants could have prevented fish against oxidative stress.

464 Another explanation concerning this lack of significance could also be due to the fact that the  
465 exposure period was too short to induce ROS production. Indeed, although some studies have  
466 shown some effects of PAH following a short exposure period ( $\leq 48$  h, Sun et al., 2006;  
467 Oliveira et al., 2008), many studies investigated the effects of contaminants on oxidative  
468 stress by exposing animals to longer periods (Kopecka-Pilarczyk and Correia, 2009; Jung et  
469 al., 2009; Narghang et al., 2009; Hannam et al., 2010).

470

## 471 **5. Conclusion**

472

473 With regards to gill glutathione content and the relative concentration of PAH biliary  
474 metabolites, the results of this study firstly demonstrate that WSF exposure may be less toxic  
475 than CD exposure. These results are in accordance with the TPH concentrations measured in

476 sea water, suggesting, in accordance with the literature (Perkins et al., 1973; Cohen and  
477 Nugegoda, 2000; Ramachandran et al., 2004; Lin et al., 2009), that the transfer of  
478 hydrocarbons (from the sea surface to the water column) due to dispersant application led to  
479 an increase of toxicity. Extrapolated to field operations, results of this study mean that  
480 containment and recovery, rather than chemical dispersion of the oil slick, must be conducted.  
481 However, depending on technical facilities and meteorological conditions, it is not always  
482 possible to contain the oil slick. In some oil spill situations (e.g. rough sea and low viscosity  
483 petroleum), dispersant is the only appropriated response technique.

484 Since a minimum sea energy is required before a dispersant functions effectively (Merlin,  
485 2005) and since nearshore areas are considered to be turbulent zones (due to waves, wind and  
486 swell) it seemed important, in this study, to evaluate the toxicity of dispersant application  
487 under a mixing process. Comparison of MD and CD showed a significant difference  
488 concerning low toxicity PAH-derived metabolites (naphthalene and pyrene) - these results are  
489 in accordance with the TPH concentrations measured in sea water -. However, no significant  
490 difference was found for benzo[*a*]pyrene-derived metabolites, which are considered to be  
491 carcinogenic and to induce reactive oxygen species. Moreover no significant difference was  
492 found between the glutathione content following the CD and MD treatments. Taken together,  
493 these results suggest (i) no increase of toxic compounds exposure due to dispersant  
494 application and (ii) no increase of organism detoxification response. These results suggest  
495 that, when an oil slick is naturally dispersed, the application of dispersant seems to not  
496 increase its environmental toxicity. These results are in accordance with a similar previous  
497 study (Milinkovitch et al., 2011).

498 However, several limits of this experimental approach compel us to be cautious in our  
499 conclusions. Indeed, the experimental approach is available only for a given turbulent mixing  
500 energy (the energy induced by the experimental system). Moreover, this experimental

501 approach only takes into account the toxicity to pelagic teleost fish while other components of  
502 the ecosystem are also likely to be impaired by dispersant application. An experimental  
503 approach considering the environmental conditions and other components of an ecosystem  
504 (benthic and demersal species) would provide supplementary information. In this context,  
505 further studies as part of the DISCOBIOL project will evaluate the impact of dispersed oil on  
506 burrowing organisms, demersal organisms (such as oysters) and pelagic species (such as  
507 golden grey mullet) within an enclosed ecosystem (mesocosm).

508

### 509 **Acknowledgements**

510

511 This study was supported by a PhD grant from the Conseil Général of the Charente-Maritime.  
512 The authors also wish to acknowledge CEDRE (CEntre de Documentation de Recherche et  
513 d'expérimentations sur les pollutions accidentelles des eaux), FREDD (Fédération de  
514 Recherche en Environnement et pour le Développement Durable), CPER (Contrat de Projet  
515 Etat-Région), Sophie Labrut and Jérôme Abadie from UMR 707 INRA-ONIRIS-University  
516 of Nantes for financial and technical support. Special thanks go to Marion Richard for her  
517 help and assistance during the study and to everybody who helped during Xynthia storm.

518

519

### 520 **References**

521

522 Aas, E., Baussant, T., Balk, L., Liewenborg, B., Andersen, O.K., 2000. PAH metabolites in  
523 bile, cytochrome P4501A and DNA adducts as environmental risk parameters for chronic oil  
524 exposure: a laboratory experiment with Atlantic cod. *Aquat. Toxicol.* 51, 241-258.

525

526 Adams, G.G., Klerks, P.L., Belanger, S.E., Dantin, D., 1999. The effect of the oil dispersant  
527 omni-clean on the toxicity of fuel oil n° 2 in two bioassays with the sheepshead minnow  
528 *Cyprinodon variegatus*. Chemosphere 39, 2141-2157.  
529

530 Akerboom, T.P., Sies, H., 1981. Assay of glutathione, glutathione disulfide and glutathione  
531 mixed disulfides in biological samples. Methods Enzymol. 77, 373-382.  
532

533 Allen , J. I. & Moore, N. M., 2004. Environmental prognostics: Is the current use of  
534 biomarkers appropriate for environmental risk evaluation? Mar. Environ. Res. 58, 227-232.  
535

536

537 Almroth, B.C., Sturve, J., Stephensen, E., Holth, T.F., Förlin, L., 2008. Protein carbonyls and  
538 antioxidant defenses in corkwing wrasse (*Symphodus melops*) from a heavy metal polluted  
539 and a PAH polluted site. Mar. Environ. Res. 66, 271-277.  
540

541 Amiard-Triquet, C. and Amiard, J. C., 2008. Les biomarqueurs dans l'évaluation de l'état  
542 écologique des milieux aquatiques. Paris. pp. 372.  
543

544

545 Avci, A., Kaçmaz, M., Durak, I., 2005. Peroxidation in muscle and liver tissues from fish in a  
546 contaminated river due to a petroleum refinery industry. Ecotox. Environ. Saf. 60, 101-105.  
547

548

549 Baca, B., Ward, G.A., Lane, C.H., Schuler, P.A., 2005. Net environmental benefit analysis  
550 (NEBA) of dispersed oil on nearshore tropical ecosystems derived from International Oil Spill  
551 Conference, Miami, Florida, USA, pp. 1-4.

552

553 Bosveld, A.T.C., De Bie, P.A.F., Van den Brink, N.W., Jongepier, H., Klomp, A.V., 2002. In  
554 vitro EROD induction equivalency factors for the 10 PAH generally monitored in risk  
555 assessment studies in The Netherlands. *Chemosphere*, 49, 75-83.

556

557 Bruslé, J., 1981. Food and feeding in grey mullets, in: Oren O.H. (ed.), *Aquaculture of grey*  
558 *mulletts*. Cambridge University Press, Cambridge, pp. 185-217.

559

560 Camus, L., Aas, E., Børseth, J.F., 1998. Ethoxyresorufin-O-deethylase activity and fixed  
561 wavelength fluorescence detection of PAH metabolites in bile in turbot (*Scophthalmus*  
562 *maximus* L.) exposed to a dispersed topped crude oil in a continuous flow system. *Mar.*  
563 *Environ. Res.* 46, 29-32.

564

565 Canesi, L., Viarengo, A., Leonzio, C., Filippelli, M., Gallo, G., 1999. Heavy metals and  
566 glutathione metabolism in mussel tissues. *Aquat. Toxicol.* 46, 67-76.

567

568 Chapman, H., Purnell, K., Law, R.J., Kirby, M.F., 2007. The use of chemical dispersants to  
569 combat oil spills at sea: A review of practice and research needs in Europe. *Mar. Pollut. Bull.*  
570 54, 827-838.

571

572 Churchill, P.F., Dudley, R.J., Churchill, S.A., 1995. Surfactant-enhanced bioremediation.  
573 *Waste Manag.* 15, 371-377.

574

575 Cohen, A.M., Nugegoda, D., 2000. Toxicity of three oil spill remediation techniques to the  
576 Australian Bass (*Macquaria novemaculeata*). *Ecotoxicol. Environ. Saf.* 47, 178-185.



577

578 Cormack, D., 1977. Oil pollution. *Chem. Ind.* 14, 605-608.

579

580 Deisseroth, A., Dounce, A.L., 1970. Catalase: physical and chemical properties, mechanisms  
581 of catalysis, and physiological role. *Physiol. Rev.* 50, 319-375.

582

583 Eisler, R., 1987. Polycyclic aromatic hydrocarbon hazards to fish, wildlife, and invertebrates:  
584 a synoptic review U.S. Fish and Wildlife Service Biological Report, 85, 1.11.

585

586 Fusey, P., Oudot, J., 1976. Comparaison de deux méthodes d'évaluation de la biodégradation  
587 des hydrocarbures in vitro. *Mater. U. Organ.* 4, 241-251.

588

589 Gautier, D., Hussenot, J. (Eds.), 2005. Les mulets des mers d'Europe. Synthèse des  
590 connaissances sur les bases biologiques et les techniques d'aquaculture. Ifremer, Paris.

591

592 George-Ares, A., Clark, J.R., 2000. Aquatic toxicity of two Corexit dispersants. *Chemosphere*  
593 40, 897-906.

594

595 Gérard-Monnier, D., Erdelmeier, I., Régnard, K., Moze-Henry, N., Yadan, J., Chaudière, J.,  
596 1998. Reactions of 1-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals.  
597 Analytical applications to a colorimetric assay of lipid peroxidation. *Chem. Res. Toxicol.* 11,  
598 1176-1183.

599

600 Halliwell, B. & Gutteridge, J. M. C., 1999. Free radicals in biology and medicine. Oxford  
601 University Press, Oxford. pp. 192.

602

603 Hannam, M.L., Bamber, S.D., Galloway, T.S., John Moody, A., Jones, M.B., 2010. Effects of  
604 the model PAH phenanthrene on immune function and oxidative stress in the haemolymph of  
605 the temperate scallop *Pecten maximus*. *Chemosphere* 78, 779-784.

606

607 Jung, J.H., Yim, U.H., Han, G.M., Shim, W.J., 2009. Biochemical changes in rockfish,  
608 *Sebastes schlegeli*, exposed to dispersed crude oil. *Comparative Biochemistry and Physiology*  
609 *Part C: Toxicology & Pharmacology* 150, 218-223.

610

611 Kopecka-Pilarczyk, J., Correia, A.D., 2009. Biochemical response in gilthead seabream  
612 (*Sparus aurata*) to in vivo exposure to a mix of selected PAH. *Ecotoxicol. Environ. Saf.* 72,  
613 1296-1302.

614

615 Lemaire-Gony, S., Lemaire, P., 1993. Interactive effects of cadmium and benzo(a)pyrene on  
616 cellular structure and biotransformation enzymes of the liver of the European eel *Anguilla*  
617 *anguilla*. *Aquat. Toxicol.* 22, 145-160.

618

619 Lessard, R.R., DeMarco, G., 2000. The significance of oil spill dispersants. *Spill Sci.*  
620 *Technol. Bull.* 6, 59-68.

621

622 Lin, C.Y., Anderson, B.S., Phillips, B.M., Peng, A.C., Clark, S., Voorhees, J., Wu, H.-D.I.,  
623 Martin, M.J., McCall, J., Todd, C.R., Hsieh, F., Crane, D., Viant, M.R., Sowby, M.L.,  
624 Tjeerdema, R.S., 2009. Characterization of the metabolic actions of crude versus dispersed oil  
625 in salmon smolts via NMR-based metabolomics. *Aquat. Toxicol.* 95, 230-238.

626

627 Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and  
628 oxidative damage in aquatic organisms. *Mar. Pollut. Bull.* 42, 656-666.  
629

630 Lunel, T., 1995. The Braer oil spill: oil fate governed by dispersion. International Oil Spill  
631 Conference, Long Beach, California, USA.  
632

633 Maracine, M., Segner, H., 1998. Cytotoxicity of metals in isolated fish cells: Importance of  
634 the cellular glutathione status. *Comparative Biochemistry and Physiology Part A* 120, 83-88.  
635

636 Maria, V. L., Correia, A. C. & Santos, M. A., 2002. *Anguilla anguilla* L. biochemical and  
637 genotoxic responses to Benzo[a]pyrene. *Ecotoxicol. Environ. Saf.*, 53, 86-92.  
638

639

640 Martinho, F., Leitao, R., Neto, J.M., Cabral, H.N., Marques, J.C., Pardal, M.A., 2007. The use  
641 of nursery areas by juvenile fish in a temperate estuary, Portugal. *Hydrobiologia* 587, 281-  
642 290.  
643  
644

645 McKeown, B.A., 1981. Long-term sublethal and short-term high dose effects of physically  
646 and chemically dispersed oil on accumulation and clearance from various tissues of juvenile  
647 coho salmon, *Onchorhynchus kitsutch*. *Mar. Environ. Res.* 1, 295-300.  
648  
649

650 Mendonça Duarte, R., Tomio Honda, R., Luis Val, A., 2010. Acute effects of chemically  
651 dispersed crude oil on gill ion regulation, plasma ion levels and haematological parameters in  
652 tambaqui (*Colossoma macropomum*). *Aquat. Toxicol.* 97, 134-141.  
653

654 Merlin, F.X., 2005. Traitement aux dispersants des nappes de pétrole en mer. CEDRE  
655 (CEntre de Documentation de Recherche et d'expérimentations sur les pollutions accidentelles  
656 des eaux), Brest, France.

657

658 Milinkovitch, T., Ndiaye, A., Sanchez, W., Le Floch, S., Thomas-Guyon, H., 2011. Liver  
659 antioxidant and plasma immune responses in juvenile Golden grey mullet (*Liza aurata*)  
660 exposed to dispersed crude oil. *Aquat. Toxicol.* 101:155-154.

661

662 Milinkovitch, T., Kanan, R., Thomas-Guyon, H., Le Floch, S., Effects of dispersed oil  
663 exposure on bioaccumulation of polycyclic aromatic hydrocarbons and mortality of juvenile  
664 *Liza ramada*. Accepted for publication in *Science of the total environment*.

665

666 Mitchell, F.M., Holdway, D.A., 2000. The acute and chronic toxicity of the dispersants  
667 Corexit 9527 and 9500, water accommodated fraction (WAF) of crude oil, and dispersant  
668 enhanced WAF (DEWAF) to *Hydra viridissima* (green hydra). *Water Res.* 34, 343-348.

669

670 Nahrgang, J., Camus, L., Gonzalez, P., Goksøyr, A., Christiansen, J.S., Hop, H., 2009. PAH  
671 biomarker responses in polar cod (*Boreogadus saida*) exposed to benzo(a)pyrene. *Aquat.*  
672 *Toxicol.* 94, 309-319.

673

674 Oliveira, M., Pacheco, M., Santos, M. A., 2007. Cytochrome P4501A, genotoxic and stress  
675 responses in Golden grey mullet (*Liza aurata*) following short-term exposure to  
676 phenanthrene. *Chemosphere*, 66, 1284-1291.

677

678 Oliveira, M., Pacheco, M., Santos, M.A., 2008. Organ specific antioxidant responses in  
679 golden grey mullet (*Liza aurata*) following a short-term exposure to phenanthrene. *Sci. Total*  
680 *Environ.* 396, 70-78.

681

682 Ortiz-Delgado, J. B., Segner, H., Arellano, M. & Sarasquete, C., 2007. Histopathological  
683 alterations, EROD activity, CYP1A protein and biliary metabolites in Gilthead seabream  
684 *Sparus aurata* exposed to Benzo(a)pyrene. *Histology and Histopathology*, 22, 417-432.

685

686 Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative  
687 characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 70, 158-169.

688

689 Perkins, R.A., Rhoton, S., Behr-Andres, C., 2005. Comparative marine toxicity testing: A  
690 cold-water species and standard warm-water test species exposed to crude oil and dispersant.  
691 *Cold Regions Sc.Technol.* 42, 226-236.

692

693 Petry, T., Schmidz, P., Slatter, C., 1996. The use of toxic equivalency factors in assessing  
694 occupational and environmental health risk associated with exposure to airborne mixtures of  
695 polycyclic aromatic hydrocarbons (PAH). *Chemosphere*, 4, 639-648.

696

697 Ramachandran, S.D., Hodson, P.V., Khan, C.W., Lee, K., 2004. Oil dispersant increases PAH  
698 uptake by fish exposed to crude oil. *Ecotoxicol. Environ. Saf.* 59, 300-308.

699

700 Salar Amoli, H., Porgamb, A., Bashiri Sadr, Z. & Mohanazadeh, F., 2006. Analysis of metal  
701 ions in crude oil by reversed-phase high performance liquid chromatography using short  
702 column. *J. Chrom. A*, 1118, 82-84.

703

704 Sies, H., 1999. Glutathione and its role in cellular functions. *Free Rad. Biol. Med.* 27, 916-  
705 921.

706

707 Spooner, M.F., 1970. Oil spill in Tarut Bay, Saudi Arabia. *Mar. Pollut. Bull.* 1, 166-167.

708

709 Stegeman, J.J., 1987. Cytochrome P450 isozymes and monooxygenase activity in aquatic  
710 animals. *Environ. Health Perspect.* 71, 87-95.

711

712 Stentiford, G. D., Longshaw, M., Lyons, B. P., Jones, G., Green, M. & Feist, S. W., 2003.  
713 Histopathological biomarkers in estuarine fish species for the assessment of biological effects  
714 of contaminants. *Mar. Environ. Res.* 55, 137-159.

715

716 Sun, Y., Yu, H., Zhang, J., Yin, Y., Shi H., Wang, X., 2006. Bioaccumulation, depuration and  
717 oxidative stress in fish (*Carassius auratus*) under phenanthrene exposure. *Chemosphere* 63,  
718 1319-1327.

719

720 Tiehm, A., 1994. Degradation of polycyclic aromatic hydrocarbons in the presence of  
721 synthetic surfactants. *Appl. Environ. Microbiol.* 60, 258-263.

722

723 van der Oost, R., Beyer, J. & Vermeulen, N. P. E., 2003. Fish bioaccumulation and  
724 biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol.*, 13, 57-  
725 149.

726

727 Wooliams, J.A., Wiener, G., Anderson., P.H., McMurray, C.H., 1983. Variation in the  
728 activities of glutathione peroxidase and superoxide dismutase and in the concentration of  
729 copper in the blood in various breed crosses of sheep. *Research in Veterinary Science* 34,  
730 253-256

731

732 Yin, Y., Jia, H., Sun, Y., Yu, H., Wang, X., Wu, J., Xue, Y., 2007. Bioaccumulation and ROS  
733 generation in liver of *Carassius auratus*, exposed to phenanthrene. *Comparative Biochemistry*  
734 *and Physiology Part C: Toxicology & Pharmacology* 145, 288-293.

735

736 **Table 1:** TPH and dispersant nominal concentration in the five exposure media at the  
737 beginning (T=0 h) and at the end of the exposure (T=48 h) to C (Control), CD (Chemically  
738 Dispersed oil), MD (Mechanically Dispersed oil), WSF (Water Soluble Fraction of oil) and D  
739 (Dispersant).

740 Values are expressed as mean  $\pm$  standard error mean of both experimental replicates. n.d. =  
741 not detected.

742

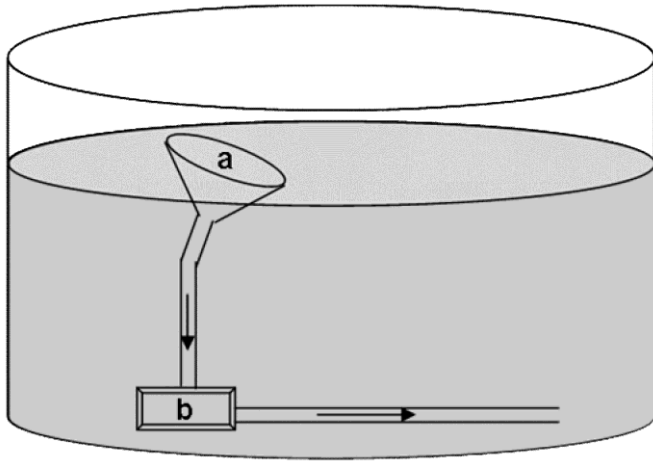
	[TPH] <sub>T=0h</sub> (mg/L)	[TPH] <sub>T=48h</sub> (mg/L)	[Dispersant] <sub>nom.</sub> (mg/L)
C	n.d.	n.d.	n.d.
CD	46.4	14.9	3.33
MD	39.2	10.7	n.d.
WSF	n.d.	n.d.	n.d.
D	n.d.	n.d.	3.33

743

744

745

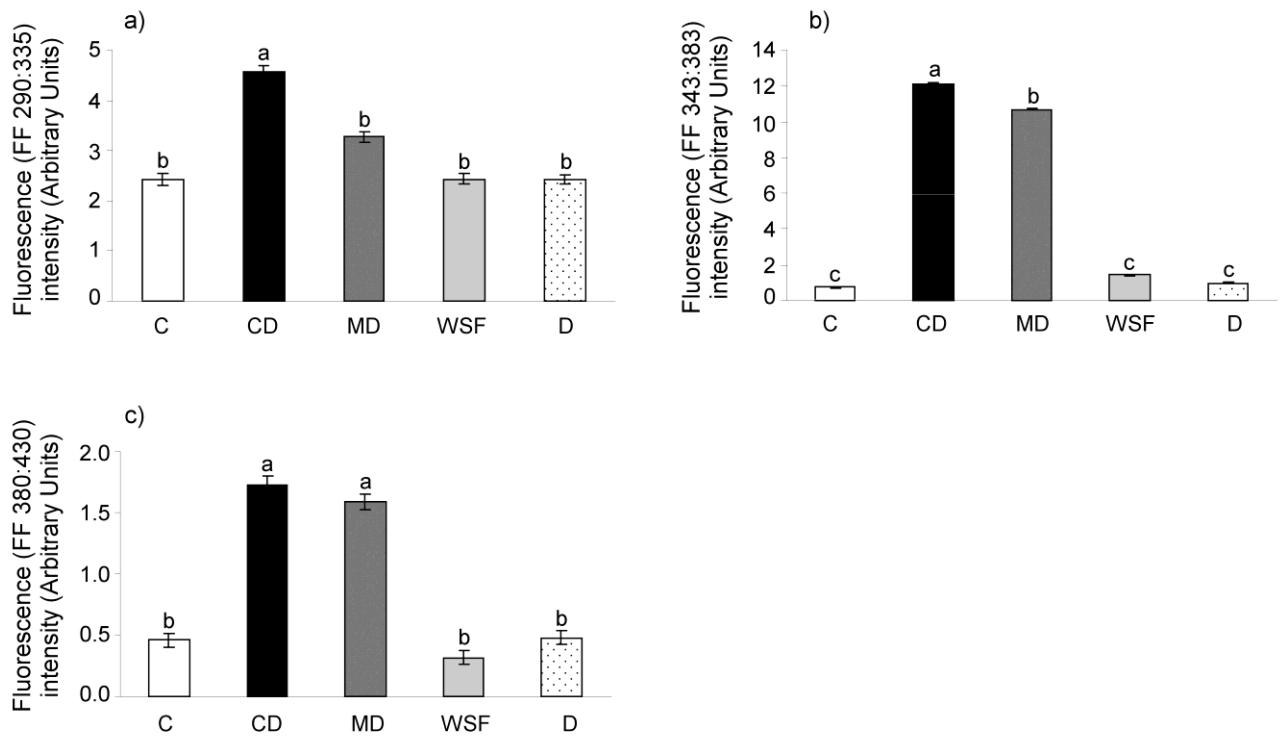
746



747

748 **Figure 1:** The experimental system constituted of a funnel (a) linked to a water pump (b) in a  
749 300-l sea tank. → indicates the direction of seawater and/or contaminants through the  
750 experimental system

751

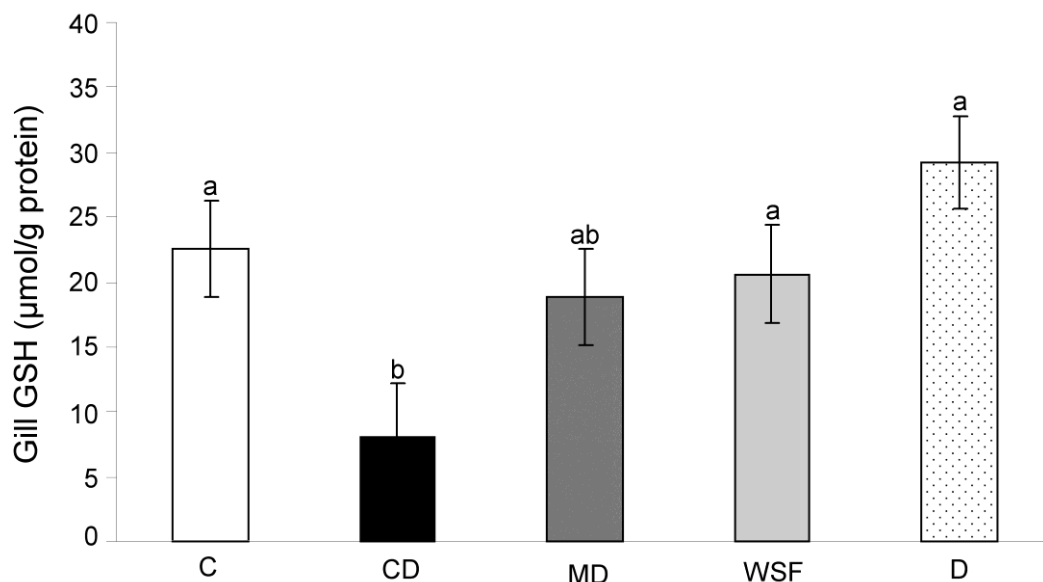


752



753 **Figure 2:** Concentration of biliary PAH metabolites measured by fixed wavelength  
754 fluorescence (FF) levels after 48 h exposure to Control solution (C), Chemically Dispersed oil  
755 solution (CD), Mechanically Dispersed oil solution (MD), Water Soluble Fraction (WSF)  
756 solution and Dispersant solution (D) : (a) FF 290:335 (naphthalene type derived metabolites);  
757 (b) FF 343:383 (pyrene derived type of metabolites); (c) FF 380:430 (benzo[*a*]pyrene type of  
758 metabolites). Levels are expressed as fluorescence intensity. Values represent mean  $\pm$   
759 standard error (n=10 per treatment). Different letters above bars indicate a significant  
760 difference, where  $P < 0.05$ .

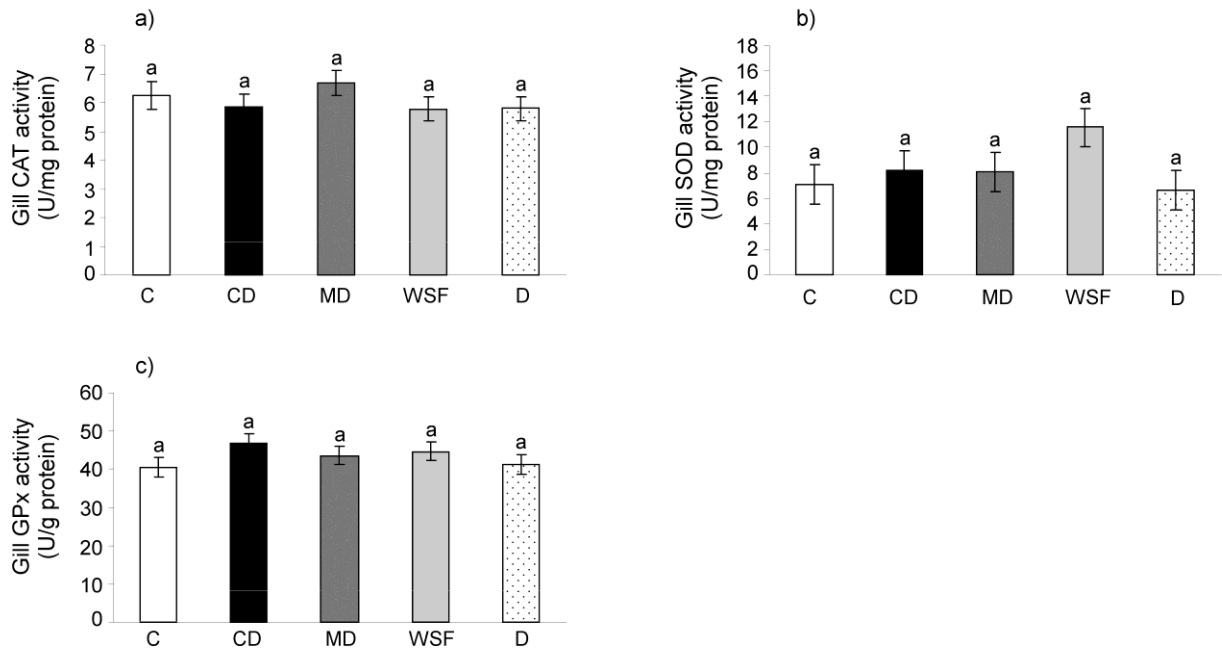
761



762

763 **Figure 3:** Total glutathione (GSH) content in gills of *Liza aurata* after 48 h exposure to  
764 Control solution (C), Chemically Dispersed oil solution (CD), Mechanically Dispersed oil  
765 solution (MD), Water Soluble Fraction (WSF) solution and Dispersant solution (D). Values  
766 represent mean  $\pm$  standard error (n=10 per treatment). Different letters above bars indicate a  
767 significant difference, where  $P < 0.05$ .

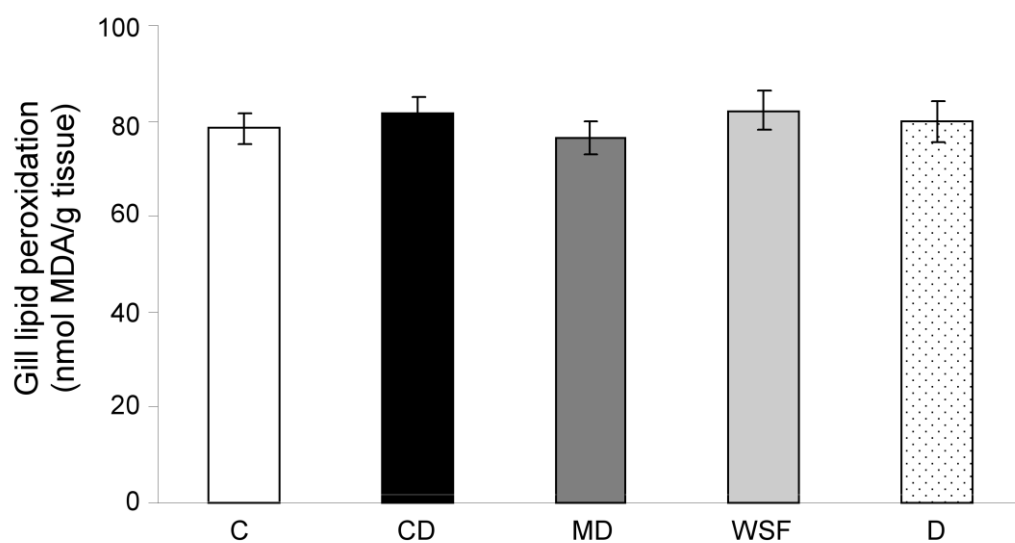
768



769

770 **Figure 4:** a) Catalase (CAT) activity, b) Superoxide Dismutase (SOD) activity and c)  
 771 Glutathione Peroxidase (GPx) activity in gills of *Liza aurata* after 48 h exposure to Control  
 772 solution (C), Chemically Dispersed oil solution (CD), Mechanically Dispersed oil solution  
 773 (MD), Water Soluble Fraction (WSF) solution and Dispersant solution (D). Values represent  
 774 mean  $\pm$  standard error (n=10 per treatment). Different letters above bars indicate a significant  
 775 difference, where  $P < 0.05$ .

776



777

778 **Figure 5:** Lipid peroxidation in gills of *Liza aurata* after 48 h exposure to Control solution  
 779 (C), Chemically Dispersed oil solution (CD), Mechanically Dispersed oil solution (MD),  
 780 Water Soluble Fraction (WSF) solution and Dispersant solution (D). Values represent mean  $\pm$   
 781 standard error (n=10 per treatment). Different letters above bars indicate a significant  
 782 difference, where  $P < 0.05$ .

783

784

785

786