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1 **Effects of dispersant-treated oil upon behavioural and metabolic parameters of the anti-**
2 **predator response in juvenile European sea bass (*Dicentrarchus labrax*).**

3

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5

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14

15 **ABSTRACT**

16 Acute exposure to oil and oil dispersants can cause a wide range of physiological
17 dysfunctions in marine fish species and evidences for consequences on behaviour are also
18 increasing. In response to the presence of predators or to food availability, the modulation of
19 locomotor activity and schools' behaviour enable fish to maximize their survival rates.
20 However, the degree to which this regulatory process is affected by exposure to oil and/or
21 dispersants is yet unknown. Here we investigated the effect of a 62-h experimental exposure
22 to dispersant-treated oil on the behavioural (shoal cohesion, spontaneous activity) and
23 metabolic (oxygen consumption) responses to simulated predation in juvenile European sea
24 bass, *Dicentrarchus labrax* L.. Our results suggest that exposure to petroleum hydrocarbons
25 may affect negatively individual fitness through impaired ability to respond to predation.

26 Shoal cohesion was not affected, but fish swimming activity was higher than control
27 individuals under predation pressure and the amplitude of their metabolic response was
28 significantly reduced. Fish recovered from alteration of their metabolic response 7 days post-
29 exposure. Additionally, a strong habituation component was observed in C fish and the
30 absence of such pattern in E fish suggest altered capacity to habituate over time to the
31 surrounding environment and possible impairments of the related cognitive performances.
32 Altogether, our data show that juvenile sea bass exposed to oil exhibit transient physiological
33 dysfunctions and impairments of complex behaviours that may have major population-level
34 consequences.

35

36 **KEYWORDS**

37 Behaviour, Metabolism, Oil spill, Teleost fish, Anti-predator response, European sea bass

38

39 **1. INTRODUCTION**

40 Among pollutants, crude oil remains a pervasive toxicant of global concern. Despite the
41 doubling of sea borne oil trade over the last 50 years, the number of oil spills has been
42 reduced by 92% (ITOPF Ltd, 2022). Yet, oil transport has resulted in the release of 153,000
43 tonnes of crude oil into the environment over the last decade (ITOPF Ltd, 2022). In response
44 to an oil spill, chemical dispersants are commonly used (Merlin et al., 2021) to breaking up
45 slicks into small droplets to enhance their natural dispersion and dilution at sea (*Dispersants:*
46 *surface application*, 2015). However, a major drawback of this technique is the increased
47 bioavailability of oil compounds (Brakstad et al., 2015; Ramachandran et al., 2004).

48

49 Over the past two decades, numerous studies have investigated the toxicity of crude oil and
50 dispersant-treated oil on different organisms (Beyer et al., 2016; Fingas, 2017; Pasparakis et

51 al., 2019). Most of these studies revealed that the toxicity of dispersed oil is higher than crude
52 oil alone due to the higher bioavailability of toxic components, especially polycyclic aromatic
53 hydrocarbons, PAHs (Esteban-Sánchez et al., 2021; Ramachandran et al., 2004). While
54 toxicity depends on species, life stage and the level of exposure, several studies revealed
55 detrimental effects, including mortality, of crude oil exposure on a wide variety of marine
56 organisms such as seabirds, sea turtles, marine mammals, and fish (Beyer et al., 2016; Mearns
57 et al., 2020; E. J. Ruberg et al., 2021; Elizabeth J. Ruberg et al., 2021). In the latter, a variety
58 of sublethal effects including stunted growth rate, deformities or physiological impairments
59 were observed (Cherr et al., 2017; Khursigara et al., 2019; Pasparakis et al., 2019).
60 Frequently reported common adverse physiological effects in fish include impaired sensory
61 capacities (Magnuson et al., 2020; Schlenker et al., 2019), altered metabolic and swimming
62 performances (Johansen and Esbaugh, 2017; Pan et al., 2018) and cardiac defects (Brette et
63 al., 2017; Nelson et al., 2017).

64
65 It has generally been presumed that cardiorespiratory impairment is the main driver of the
66 ecological impacts of oil exposure (Incardona et al., 2009, 2004). However, studies have
67 pointed out that oil exposure could disrupt other physiological functions linking physiology,
68 cognition and behaviour driving sublethal impairments in fish (Aimon et al., 2021; Jacquin et
69 al., 2020; Johansen et al., 2017; Khursigara et al., 2021). Recent transcriptomic studies have
70 added to this concept by highlighting disruptions of neurological and cognitive pathways
71 following oil exposure (Xu et al., 2019, 2017). Moreover, recent behavioural works have
72 demonstrated that exposure to crude or dispersed oil affect a wide range of behavioural
73 parameters such as activity level (Correia et al., 2007; Gonçalves et al., 2008; Khursigara et
74 al., 2021; Vignet et al., 2014), exploration (Aimon et al., 2021; Jacquin et al., 2017), social
75 dominance (Correia et al., 2007; Khursigara et al., 2018), prey-capture ability (M. Carvalho et

76 al., 2008; Rowsey et al., 2019; Woodward et al., 1987), risk taking (Aimon et al., 2021;
77 Johansen et al., 2017; Rowsey et al., 2019), anxiety (*Sciaenops ocellatus*; in Rowsey et al.,
78 2019), and alarm cue avoidance (*Stegastes partitus*; in Schlenker et al., 2019). This last study
79 even highlighted an increase in predator-induced mortality of coral reef fishes following oil
80 exposure. All these results suggest impairments of high-order cognitive processes associated
81 with risk perception and assessment (Johansen et al., 2017). While such behavioural
82 parameters can be very sensitive to contaminant exposure, there is a needed gap to address in
83 our understanding of the ecological consequences of sub-lethal oil exposure by investigating
84 its potential impact on complex behaviours, such as predator avoidance in a group of fish.

85
86 Any disruption of sociability may have consequences at population and biocenosis levels
87 (Maldonado-Chaparro et al., 2018) as collective behaviours such as shoaling for instance
88 (Pavlov and Kasumyan, 2000; Radakov and Williams, 1974) allows individuals to draw on a
89 full range of trade-offs to maximize feeding opportunities and lower predation risk (Clark and
90 Mangel, 1986; Godin, 1986; Krause et al., 2000; Krause and Ruxton, 2002; Pitcher and K.
91 Parrish, 1993; Pulliam and Caraco, 1984). Shoaling behaviour heavily relies on fish ability to
92 perceive external stimuli and to integrate this information centrally to adopt the most
93 appropriate behavioural response (Scott and Sloman, 2004; Weis, 2014). To our knowledge
94 only few studies have investigated the effect of acute crude or dispersed oil exposure upon the
95 shoal cohesion of gregarious fish, yielding to ambiguous results (Armstrong et al., 2019;
96 Jacquin et al., 2017). Indeed, Armstrong et al. (2019) revealed that shoal cohesion of Atlantic
97 croaker (*Micropogonias undulatus*) was significantly impaired following acute exposure to
98 2% oil, while Jacquin et al. (2017) did not find any effect of an acute short-term experimental
99 exposure to 50 % water-soluble fraction of oil upon shoaling behaviour in the Trinidadian
100 guppies (*Poecilia reticulata*). Moreover, even when effects of oil exposure upon fish shoaling

101 behaviour was observed, the capacity of these fish to recover from such effects was not
102 evaluated.

103

104 The objective of the present study was therefore to examine the potential effects of sub-lethal
105 exposure (62h) to an ecologically realistic dispersed oil mixture on physiological and
106 behavioural parameters of a gregarious fish, the European sea bass (*Dicentrarchus labrax*). In
107 juvenile fish, we investigated responsiveness and capacity to display appropriate shoaling
108 behavioural adjustments in response to simulated predation within the two weeks following
109 the oil exposure. We conducted two sets of experiments. The first set consisted of evaluating
110 fish physiological responsiveness to a threat with measures of metabolic rate, using
111 respirometry. The second set of experiments assessed behavioural adjustment of a free-
112 ranging group in an experimental arena. We hypothesized that exposure to dispersant-treated
113 oil (1) reduces resting metabolic rate by reducing anxiety level in respirometry chambers, (2)
114 decreases fish metabolic responsiveness to a stimulus (light), (3) reduces group cohesion and
115 activity, and (4) alters the shoal behavioural adjustment to a simulated aerial attack.

116

117 **2. MATERIALS AND METHODS**

118 **2.1 Animals**

119 Juvenile European Sea bass *Dicentrarchus labrax* (Linnaeus 1758) ($N=352$, age 1+;
120 mass= 57.65 ± 1.11 g, mean \pm s.e.m) were obtained from a fish farm (Les poissons du Soleil,
121 Balaruc les bains, France) and maintained in a 500 L indoor tank supplied with open-flow,
122 thermoregulated (15°C) and fully aerated sea water (salinity 32 ppt) at Ifremer (rearing
123 structure agreement B 29-212-05). Artificial lighting reproduced seasonal variation in local
124 photoperiod. Fish were fed 3 times a week *ad libitum* using commercial feed (Neo Start Coul
125 2, Le Gouessant, France). Experiments were non-invasive and were approved by the French

126 ethics committee in charge of animal experimentation n°74 (permit number:
127 APAFIS#13738-20 8022216252268 v4).

128

129 **2.2 Fish transport**

130 Exposures to dispersant-treated oil were conducted at the « Centre de documentation, de
131 recherche et d'expérimentation sur les pollutions accidentelles des eaux (Cedre, Brest,
132 France) » approximately 12km away from Ifremer laboratory. Fish were transported in a
133 sedated state to and from Cedre, in groups of 30 individuals placed in airtight plastic
134 containers (50 L) filled with 40 L of water containing a light dose of anaesthetic (MS-222; 20
135 mg L⁻¹). The volume above the water surface was filled with O₂ gaz. Upon arrival at Cedre,
136 the 60 fish were placed in a polyethylene tank (300 L) in which water temperature, salinity
137 and photoperiod were similar to those in their original rearing tank at Ifremer facilities.

138

139 **2.3 Experimental exposure**

140 Six hours following their arrival at Cedre, fish were moved per group of 10 to a new 300L
141 tank, randomly assigned to either control (C) or dispersed oil exposed (E) treatments. Fish
142 from the control treatment were maintained in clean water during the exposure phase while
143 fish allocated to the dispersant-treated oil treatment were exposed during 62h to 0.4 g L⁻¹ of
144 weathered crude Arabian light (CAL) added with 0.005 g L⁻¹ of chemical dispersant (Finasol
145 © OSR 52, Total Fluides, Paris France). This concentration of dispersant-treated oil was used
146 to mimic upper range of concentrations that fish are liable to encounter in the natural
147 environment (Kim et al., 2010; Sammarco et al., 2013; Spooner, 1970). CAL is an
148 international reference product previously used in several studies (Danion et al., 2011;
149 Dussauze et al., 2013; French-McCay et al., 2009). CAL is composed of 54% saturated
150 hydrocarbons, 10% polar compounds and 36% aromatic hydrocarbons. Four exposure trials

151 involving 30 fish per treatment condition, were successively conducted every eleven days
 152 (Table 1).

153

154 ***Table 1. Experimental exposure to chemically dispersed oil, C: control; E: exposed, to 0.4 g***
 155 ***L⁻¹ of weathered crude Arabian light (CAL) added with 0.005 g L⁻¹ of Finasol (© OSR 52,***
 156 ***Total Fluides, Paris France).***

	Date 2018-04-30 to 2018-05-03		Date 2018-05-14 to 2018-05-17		Date 2018-05-28 to 2018-05-31		Date 2018-06-11 to 2018-06-14	
Label	C	E	C	E	C	E	C	E
CAL (g L⁻¹)	0	0.4	0	0.4	0	0.4	0	0.4
Finasol (g L⁻¹)	0	0.005	0	0.005	0	0.005	0	0.005
Number of replicates	3	3	3	3	3	3	3	3
Number of fish per replicate	10	10	10	10	10	10	10	10

157

158 The mixture of CAL and dispersant was made in a glass bottle following the manufacturer's
 159 recommendation (dispersant/oil ratio of 4%), and was poured and weathered in the exposure
 160 tanks. The weathering process consisted of bubbling air for 5 hours to mimic ageing of an oil
 161 slick at sea (Nordvik, 1995). At that time, fish were introduced in the tanks. Both control and
 162 exposure tanks were equipped with a custom-made device. This device consisted of a funnel,
 163 placed on the water surface, connected to a 12V submersible bilge pump (L450-500GPH;
 164 Johnson) placed at the bottom of the tank. In the exposure tank, this device enabled us to
 165 mimic the mechanical dispersion of oil by waves at sea, while maintaining the exposure
 166 condition homogenous throughout the tank. Surface water and floating oil were sucked into
 167 the funnel, homogenized and delivered to the bottom of the tank (Milinkovitch et al., 2011).

168 Tanks were continuously bubbled with air to maintain the oxygenation above 90% air
169 saturation during the 62h exposure period. Following the exposure period, fish were bathed in
170 clean seawater (1h) before their transfer back to Ifremer facilities (transportation procedure
171 similar to the one described above).

172

173 **2.4 Respirometry**

174 Respirometry trials spread over 7-day periods, with 4 fish being tested simultaneously (2 fish
175 from the C and 2 fish from the E treatment). To this end, four intermittent-flow respirometers
176 (2 L) were submerged in a thermoregulated (15.0 ± 0.5 °C; Teco, Seachill TR20) and aerated
177 (>90% air saturation) water tank (200 cm × 60 cm × 40 cm). Flush pumps (Compact 600,
178 EHEIM, Germany) were used to create a water recirculation to each respirometry chamber.
179 These pumps were computer-controlled using AquaResp software (University of
180 Copenhagen, Helsingør, Denmark). Each respirometer had its own circulation loop to which
181 an optical oxygen probe was connected (Robust Oxygen Probe OXROB3, Pyroscience,
182 Germany or Dipping probe oxygen minisensor, PreSens, Germany). This probe was used to
183 continuously measure the dissolved oxygen (DO) concentration inside the chamber. Oxygen
184 probes were calibrated twice *i.e.*, prior to place the fish in the respirometers and then at day 4
185 of the trials.

186

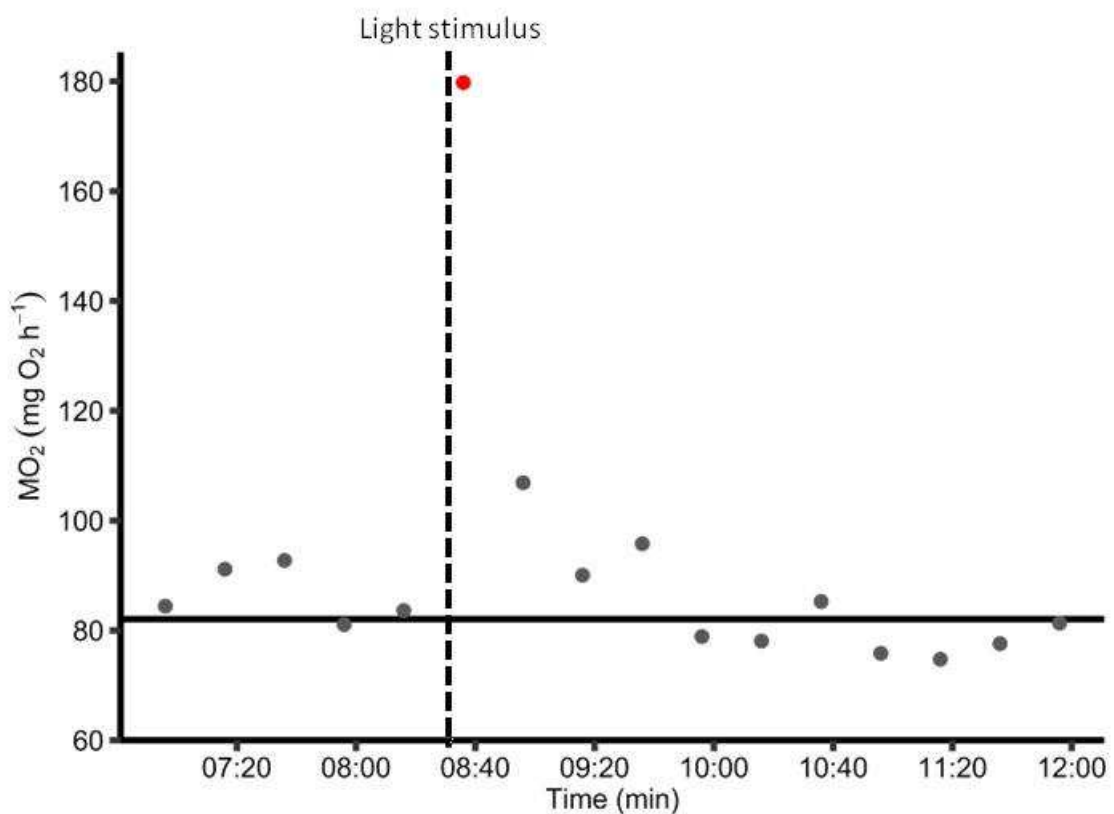
187 Fish were introduced in the respirometers 5 hours upon their return from Cedre (7 h post-
188 exposure). Oxygen consumption was measured over two period. The first period lasted 7 min
189 and corresponded to the flushing of the chamber with fully aerated water from the
190 surrounding tank. During the second period (13 min), the flushing pumps were turned off and
191 the decrease in DO was followed. The first minute of this sealed period was not taken into

192 account to calculate fish MO_2 , as it corresponded to the time needed to obtain reliable steady
193 state between the decrease in water DO and fish MO_2 . Water DO was always kept >85% sat.

194

195 Fish oxygen consumption was monitored over 7 days. To evaluate fish metabolic response to
196 a threat, a light stimulus was applied at 8:30am on days 1, 4, 5, 6 and 7. Each trial consisted of
197 turning on the room's lights (four neon tube lights 65W) for a few second while the rest of the
198 days these lights were turned off to maintain a relative darkness in the room (Fig. 1).

199



200

201 ***Fig. 1. MO_2 measured in one control fish over a morning and showing fish reaction to the***
202 ***light mediated stimulation occurring at 8:30am (red dot). Black line: Resting metabolic rate***
203 ***(RMR).***

204

205

206 **2.5 Shoaling behaviour**

207 To reveal the kinetic of post-exposure recovery, behavioural tests were conducted on days 1,
208 4, 6 and 8 post-exposure. On each of these days, five naïve groups of each treatment were
209 tested. The experimental arena consisted of a shallow rectangular tank (156 × 99 × 14 cm,
210 length, width, depth, respectively). Water characteristics in the arena were the same as those
211 of the rearing tanks. The experimental arena was screened from visual disturbance with a
212 curtain placed around and over it and it was homogenously lit with neon lamps placed on each
213 side. A retro-reflective adhesive foil (Loligosystem, Inc) was placed at the bottom of the
214 testing tank to enhance the contrast between the fish and the arena. A video camera (Logitech
215 webcam C930e, 15 frames s⁻¹) located 1m above the water surface was used to record fish
216 movement. The arena was emptied and refilled between each test.

217

218 For each trial, 4 naïve fish were randomly selected in the rearing tank and transferred without
219 emersion into the testing arena. Fish were then left undisturbed during 1h to allow them to
220 familiarize with this environment. The last minute of this 1h acclimation period was used as a
221 pre-stimulation control to evaluate the shoaling cohesion and swimming activity of the tested
222 group. Then an aerial predator attack was simulated with a slanting rope that ran diagonally
223 and downward over the experimental arena to simulate the glide path of a predator bird. For
224 the simulation, a life-sized polystyrene model of a generalized bird (73 cm × 30 cm) was
225 released and ran over this rope. This simulated predator was not visible to the fish before and
226 after the simulated attack. Fish behaviour was recorded over a 20-min period following the
227 simulated predator attack. Fish were then euthanised by overdose of anaesthetic.

228

229 **2.6 Chemical analyses**

230 Exposure conditions were characterized by measuring total petroleum hydrocarbon
231 concentration ([TPH]) in triplicate in each exposure tank. Seawater samples were taken
232 immediately before fish introduction into the tank and after 4, 24 and 48h. These samples
233 were extracted three times with 10 mL of dichloromethane Pestipur quality (SDS, Carlo Erba
234 Reagent, France) before being dried by filtering through anhydrous sodium sulfate. The
235 combined extracts were then analysed using a spectrophotometer (Evolution 600 UV-VIS;
236 Thermo Fisher Scientific) at 390 nm, as described by Fusey and Oudot (1976).

237

238 To document fish contamination and the detoxification process, liver concentration of 20
239 polycyclic aromatic hydrocarbons (PAHs) were measured at days 0, 1, 4 and 7 post-exposure.
240 To measure the level of contamination, two fish per treatment were euthanized at the end of
241 the exposure phase, at day 0, after behavioural trials at days 1 and 4 post-exposure and at the
242 end of the respirometry experiment, at day 7 post-exposure. Liver [PAHs] (including the
243 components listed by US-EPA) were assessed using a gas chromatograph system Agilent
244 7890A coupled to an Agilent 7000 triple quadrupole mass spectrometer (Agilent
245 Technologies, Little Falls, USA), as described in Lacroix et al. (2014). Briefly, liver [PAHs]
246 were extracted using alkaline digestion combined with stir bar sorptive extraction and
247 thermal-desorption–gas chromatography mass spectrometry (SBSE–TD–GC–MS). The GC–
248 MS device equipped with a Thermal Desorption Unit (TDU) and a Multipurpose Sampler
249 (Gerstel, Mülheim an der Ruhr, Germany) enabled automatic introduction of bars into the
250 TDU. Two ions were monitored for each PAH; one for quantification (quantifier ion) and the
251 second to confirm the analyte (qualifier ion). The Mass Hunter software (Agilent
252 Technologies, Little Falls, USA) was used to perform data analysis. Calculation of the target
253 PAH/deuterated PAH ratio enabled quantification of the analytes. Analytical method was
254 validated by determining the quantification limit of each PAH. This measure estimated the

255 lowest [PAH] in a liver sample that can be measured with acceptable precision and accuracy
256 under the stated conditions of the test.

257

258 **2.7 Data analysis and statistics**

259 **2.7.1 Respirometry**

260 $\dot{M}O_2$ was determined for each measurement cycle by calculating the slope of declining DO in
261 the respirometry chamber using a linear regression. MO_2 values were corrected for
262 background bacterial MO_2 (typically <5% of fish MO_2). Night and day metabolic rates were
263 determined using a quantile method ($q = 0.2$) by applying an R script (Chabot et al., 2016) to
264 the continuous $\dot{M}O_2$ measurements obtained during nights (11pm to 5am) or days (7am to
265 1pm). The reaction to the light stimulus was evaluated by noting if the fish showed an
266 increase in MO_2 compared to previous metabolic rate (MR) after light stimulation. For fish
267 that showed an increase in MO_2 in response to light stimulus, the ratio between the peak of
268 MO_2 and the MR (measured between 7am and 1pm) was calculated to estimate the intensity
269 of this reaction. Furthermore, fish recovery capacity was also assessed by noting the time to
270 return to previous MR after the MO_2 peak occurred. At the end of the last trial, day 7, we
271 removed fish from the chamber and measured background MO_2 (30min). The entire system
272 was then disinfected using household bleach.

273

274 The effects of treatment and day's post-exposure on metabolic rate and behavioural
275 measurements were examined using linear mixed effects models. Linear mixed-effects model
276 was used to test for the effects of treatment, day post-exposure and their interaction on the
277 presence of a response to the light stimulus, with fish identification number as random effect.
278 A stepwise backward reduction of the full models was applied by excluding sequentially non-
279 significant effects to identify the most parsimonious model.

280

281 **2.7.2. Shoaling behaviours**

282 Principal component analyses (PCA) were used to combine three indices of fish activity, the
283 total time spent swimming (labelled *Tswim*), the total distance moved (labelled *Dmoved*) and
284 the swimming speed (labelled *Velocity*), into principal components (PCs). PCA's were
285 applied to two datasets: (i) over the behavioural data recorded during the minute before the
286 simulated predation (t-1; individuals' baseline behavioural characteristics); (ii) over the
287 behavioural data collected during the entire post-stimulation period (from t-1 to t+20). For the
288 measurements conducted over the 20 minutes following the stimulus, measures were made in
289 1-min increments, during the first five minutes following the stimulus (t+1, t+2, t+3, t+4, t+5)
290 and at 10 (t+10) and 20 (t+20) minutes post-stimulus. We used Kaiser's criterion to select the
291 number of PCs (Kaiser, 1961). Linear mixed effect models were then used to quantify the
292 main effects of treatment and day post-exposure on fish activity and inter-individual distance
293 within the group. Fish identification number was used as a random effect. Again, a stepwise
294 backward reduction of the full models was applied to identify the most parsimonious model.

295

296 Group cohesion/disintegration was estimated using the mean inter-individual distance within
297 the group. The swimming activity was assessed through the analysis of the principal
298 component combining *Tswim*, *Dmoved* and *Velocity*. Fish response to the model predator was
299 measured using the parameters of inter-individual distance and swimming activity described
300 previously. The intensity of this response and the time to return to previous levels of shoaling
301 and activity were monitored by measuring differences between these variables one minute
302 before the stimulus (t-1) and during the first five minutes following the stimulus and at 10-
303 and 20-minutes post-stimulus (at t+1, t+2, t+3, t+4, t+5, t+10 and t+20). Negative values
304 therefore indicate a reduction in activity or inter-individual distance compared to the

305 behavioural level expressed at t-1. The video tracking software Lolitrack Version 4.2.0
306 (Loligosystem, Inc) was used to analyse the videos and to calculate the following behavioural
307 parameters: Activity (*Tswim*), Distance moved, Velocity and inter-individual distance
308 between each individual and its neighbours.

309

310 In the exposed treatment, Student's tests were carried out to determine whether water
311 concentration in total petroleum hydrocarbons was different from zero and liver concentration
312 in 20 polycyclic aromatic hydrocarbon compounds was above the quantification limit.

313

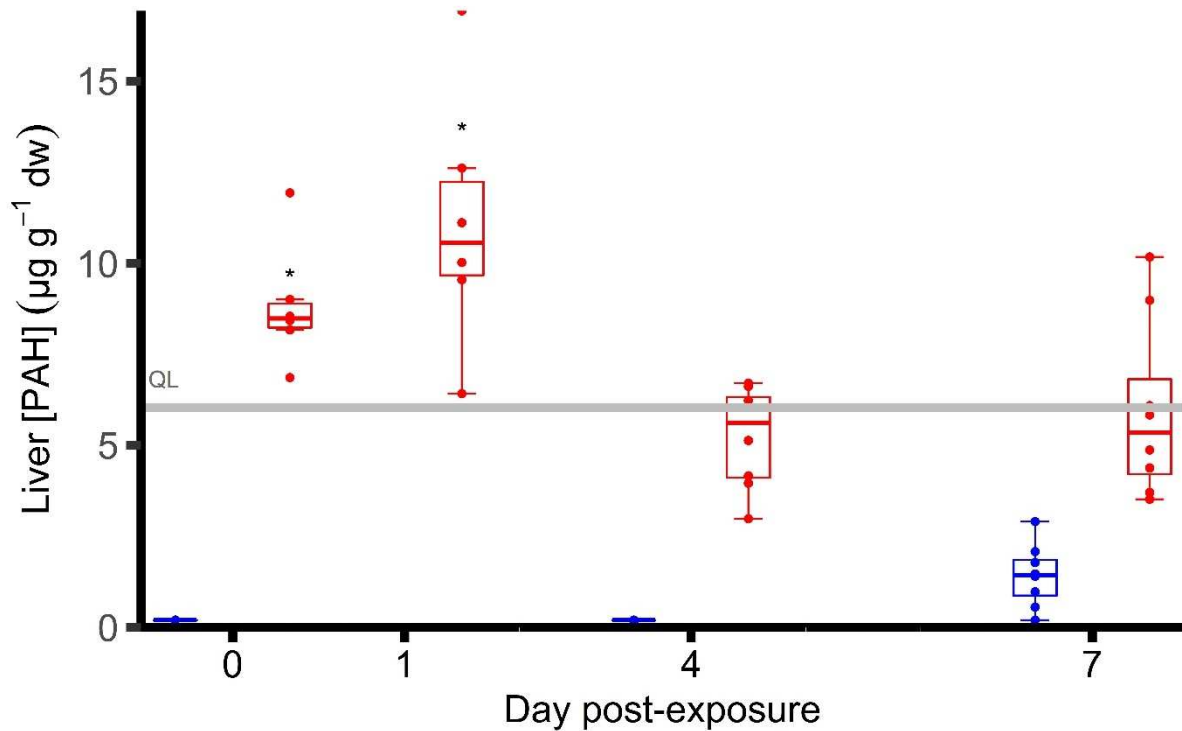
314 All statistical analyses were conducted on R version 3.5.1 (R Core Team, 2018). The principal
315 component analyses were carried out using FactoMineR package, ANOVA analyses were
316 carried out on the 'stat' package and mixed models were implemented using the 'nlme'
317 package (Le and Husson, n.d.; Pinheiro et al., 2019; R Core Team, 2013). Model diagnostics
318 were evaluated by visually inspecting the residuals. Statistical significance was set to $P <$
319 0.05.

320

321 **3. RESULTS**

322 **3.1 Exposure condition and bioaccumulation of contaminants**

323 Water TPH concentration was not significantly different from 0 in the control treatment (C)
324 while it reached $0.131 \pm 0.023 \text{ g L}^{-1}$ in the exposed tank (E). The liver concentrations of 20
325 PAHs compounds measured in fish from the C treatment were below the quantification limit.
326 In the E treatment, [PAHs] peaked at day 1 post exposure ($10 \mu\text{g g}^{-1}$ dry weight) followed by
327 a decrease to below the quantification limit at days 4 and 7 (QL; t-test QL vs Day 4: $t_7=-1.62$,
328 $P=0.15$; t-test QL vs Day 7: $t_7= -0.10$, $P=0.92$; Fig. 2).



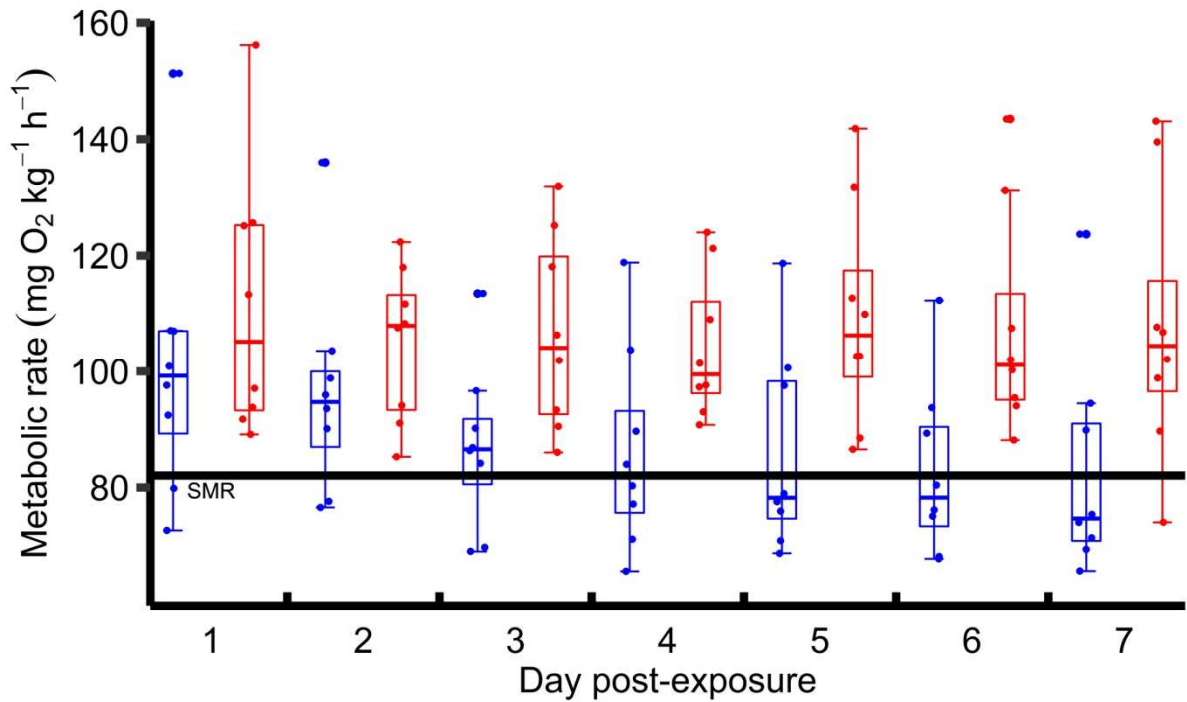
329

330 *Fig. 2. Liver concentration in 20 PAHs ($\mu\text{g g}^{-1}$ dry weight) measured in fish exposed to*
 331 *chemically dispersed oil. Blue: control fish; red: exposed fish. Sampling was performed*
 332 *directly at the end of the exposure phase: day 0 (C: N=6; E: N=8); one day later (C: N=0; E:*
 333 *N=7), four days post-exposure (C: N=8; E: N=8) and seven days post-exposure at the end of*
 334 *the respirometry experiment (C: N=8; E: N=8). The grey solid line indicates the*
 335 *quantification limit (QL) that is the lowest [PAH] in a liver sample that is measured with*
 336 *acceptable precision and accuracy under the stated conditions of the test. Below QL, PAHs*
 337 *concentration can be considered as zero. T-test was used to determine whether liver*
 338 *concentration in 20 PAHs was above QL. The boundary of the box indicates 25th 50th and*
 339 *75th percentiles. Whiskers above and below the box indicate the 10th and 90th percentiles.*
 340 *Points above and below the whiskers indicate outliers outside the 10th and 90th percentiles. **
 341 *Represents significant difference ($p < 0.05$).*

342

343 **3.2 Respirometry**

344 MO_2 from the control treatment (C) decreased and reached standard metabolic rate (SMR)
345 level found in the literature (Claireaux and Lagardère, 1999; Kir and Demirci, 2018) within 3-
346 4 days post-exposure in the respirometry chambers. In contrast, exposed fish (E) displayed a
347 higher MO_2 than C individuals, that remained above SMR over the 7 days post exposure
348 ($F_{1,94}=13.162$, $P<0.001$; Table S1; Fig. 3).



349
350 **Fig. 3. Evolution of the relationship between night metabolic rate and treatment condition**
351 **over the 7 days post-exposure.** Blue: control fish (N=8); red: exposed fish (N=8); black solid
352 line: Standard metabolic rate (SMR) reported in literature. The boundary of the box indicates
353 25th 50th and 75th percentiles. Whiskers above and below the box indicate the 10th and 90th
354 percentiles. Points above and below the whiskers indicate outliers outside the 10th and 90th
355 percentiles. Linear mixed-effects model showed statistical significance of this relationship.

356
357 There was no significant difference among treatment groups (GLMM, $Z=1.135$, $P=0.257$) or
358 days post-exposure ($Z=-1.374$, $P=0.169$) with regards to the number of fish that showed a
359 respiratory response to the light stimulus (Table S2, Table 2). Among the individuals that

360 responded (showing a peak; Fig. 1), we evaluated the intensity of the response by calculating
 361 the ratio between the peak of MO₂ and the pre-stimulation MR. Over the 7 days post-exposure
 362 in the respirometry chambers, C fish displayed a reduction in the height of the post-
 363 stimulation peak in MO₂ while no change was observed in the E fish ($F_{1,32}=5.274$, $P=0.028$,
 364 Fig.4, Table S3). In addition, C fish displayed a more intense response to the light stimulus
 365 than E individuals, displaying larger ratio between the peak of MO₂ and the pre-stimulation
 366 MR, especially the first day of the week (Fig. 4, Table S3). At day 1 in the respirometry
 367 experiment, C fish responded to the light stimulus with a peak of MO₂ 2.3 times higher than
 368 their pre-stimulation MR. In contrast, day 7 C fish showed a lower increase in MO₂ than day
 369 1, with a peak 1.6 times higher than the pre-stimulus level (Fig. 4, Table S3). The 7th day
 370 post-exposure, ratio between the peak of MO₂ and the pre-stimulation MR was similar in E
 371 and C fish (Fig. 4). Furthermore, C individuals presented a reduction in intra-group variability
 372 over the week spent in the respirometer (Fig. 4). Over the experimental week, both treatments
 373 showed a reduction in the time taken to return to the pre-stimulation MR after the peak of
 374 MO₂ (Fig. 5). However, C fish displayed a steeper decreasing slope over the week than E fish
 375 ($F_{1,20}=6.641$, $P=0.018$, Table S4).

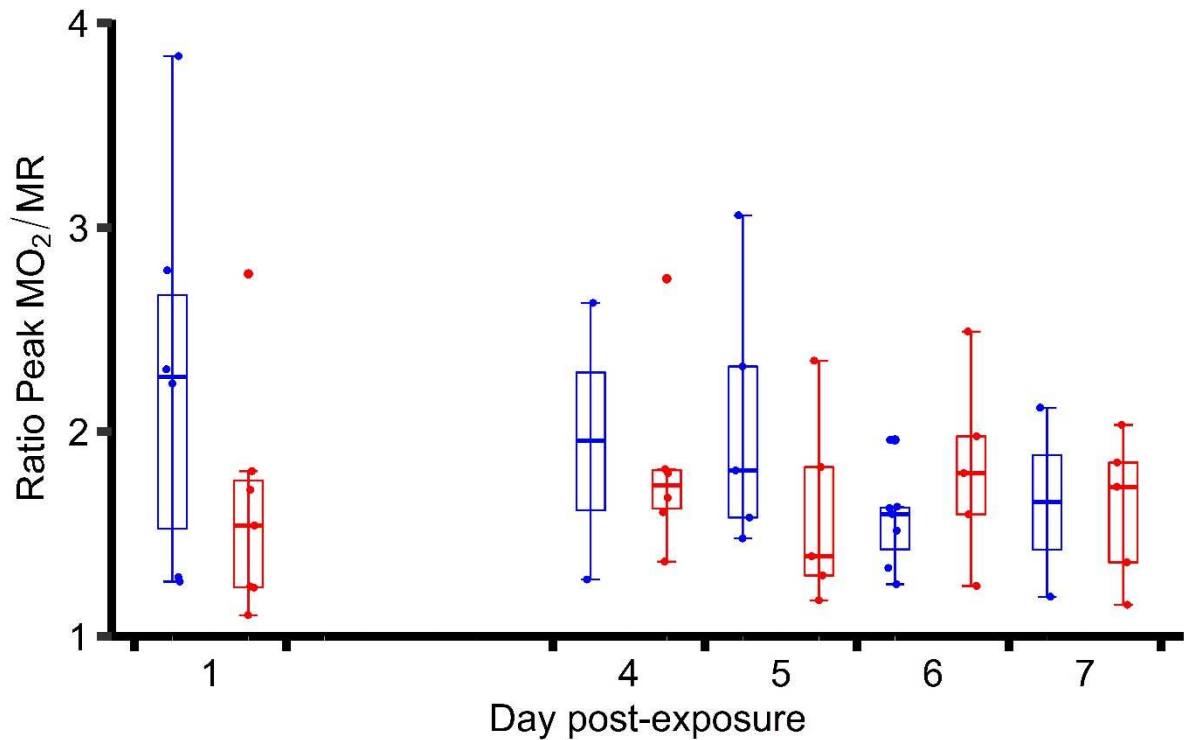
376

377 ***Table 2. Percentage of individuals displaying an increased metabolic rate following the***
 378 ***light stimulus over the 7 days post-exposure.***

Percentage of individuals showing a reaction to the light stimulus					
Days	1	4	5	6	7
C	87.5	66.7	62.5	87.5	50
E	100	100	71.4	62.5	100

379

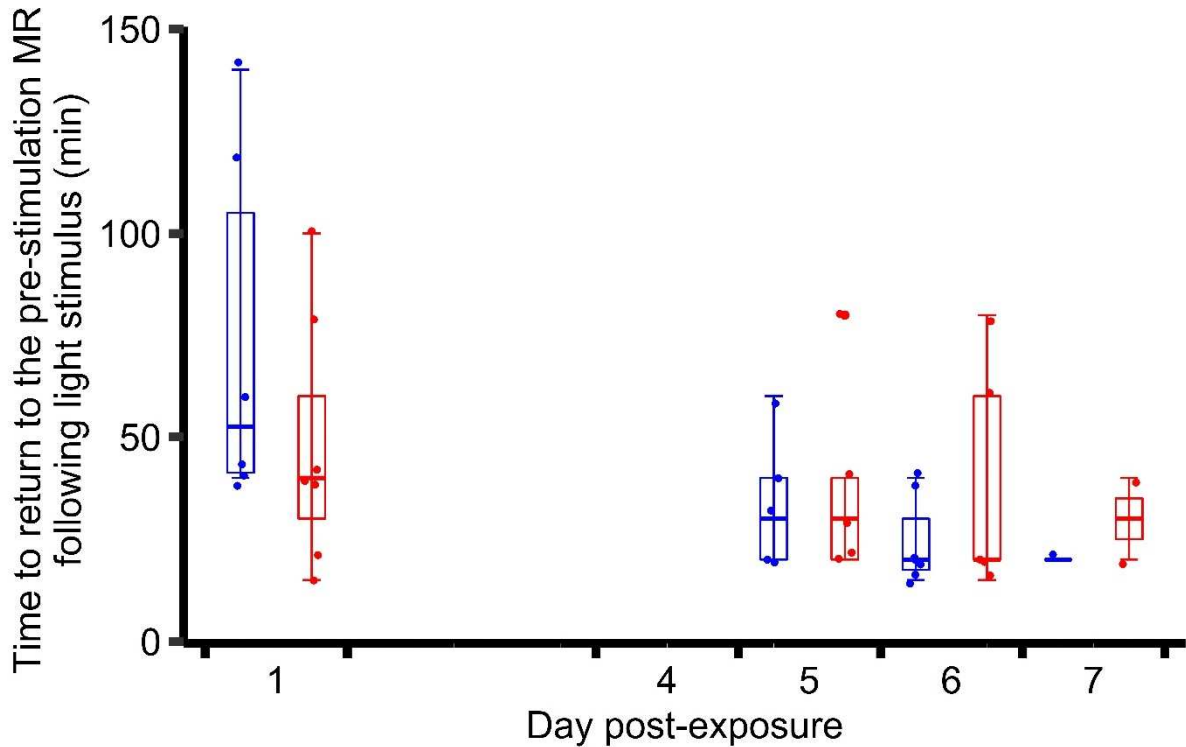
380



381

382 *Fig. 4. Relationship between the treatment condition and the ratio (peak of MO₂/MR) over*
 383 *the 7 days post-exposure. Only individuals showing a response to the light stimulus are*
 384 *represented. Sample sizes are therefore depending on the number of individuals that*
 385 *displayed an increase MO₂ following the light stimulus. Blue: control fish; red: exposed fish.*
 386 *The boundary of the box indicates 25th 50th and 75th percentiles. Whiskers above and below*
 387 *the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate*
 388 *outliers outside the 10th and 90th percentiles. Linear mixed-effects model showed statistical*
 389 *significance of this relationship.*

390



391

392 *Fig. 5. Relationship between the treatment condition and the time to return to the pre-*

393 *stimulation MR following the peak of MO_2 in response to the light stimulus over the 7 days*

394 *post-exposure. Only individuals showing a response to the light stimulus are represented.*

395 *Effectives are therefore depending on the number of individuals that displayed an increase in*

396 *MO_2 following the light stimulus. Blue: control fish; red: exposed fish. The boundary of the*

397 *box indicates 25th 50th and 75th percentiles. Whiskers above and below the box indicate the*

398 *10th and 90th percentiles. Points above and below the whiskers indicate outliers outside the*

399 *10th and 90th percentiles. Linear mixed-effects model showed statistical significance of this*

400 *relationship.*

401

402 **3.4 Shoaling behaviour**

403 **3.4.1 Reduction and structuration of behavioural variables**

404 Four variables were recorded during behavioural tests. Three of these variables allowed

405 evaluating fish swimming activity (total time spent swimming, labelled *Tswim*; distance

406 moved, labelled *Dmoved*; swimming speed, labelled *Velocity*) and one variable measured
 407 group cohesion (mean inter-individual distance within the group, labelled *Inter-individual*
 408 *distance*). The principal component analysis (PCA) loaded with the variables *Tswim*, *Dmoved*
 409 and *Velocity* showed that only one principal component had an eigenvalue greater than 1
 410 (Table 3). This PC termed ‘Activity’ explained 82% of the total variance in behaviour
 411 measured in the minute before the stimulus, and 79% of the whole dataset (Table 3).

412

413 **Table 3. Description of the principal components (PC) analyses.**

		All data	
	Variables	Minute Pre-stimulus	1 min pre-stimulus + 20 minutes post- stimulus
		PC1	PC1
		Activity	Activity
Eigenvalue		2.449	2.382
Percentage of variance		81.643	79.394
Loading	<i>Tswim</i>	0.785	0.776
	<i>Dmoved</i>	0.982	0.985
	<i>Velocity</i>	0.932	0.899

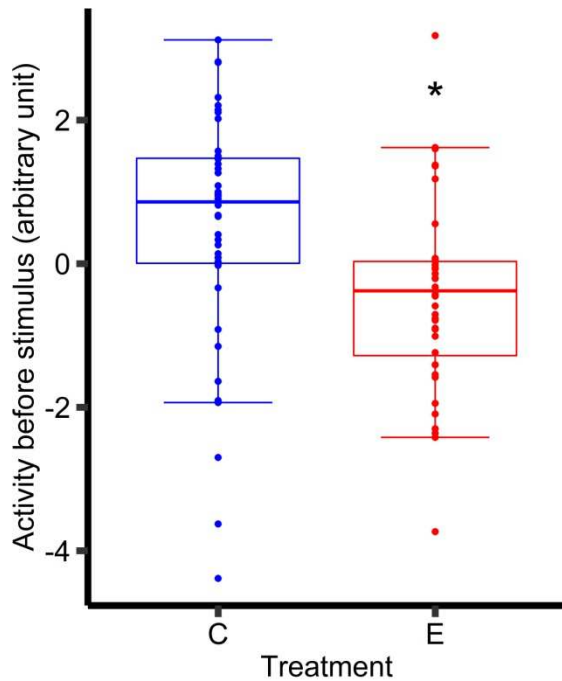
414

415 **3.4.2 Comparing treatments**

416 **3.4.2.1 Pre-stimulus shoaling behaviour**

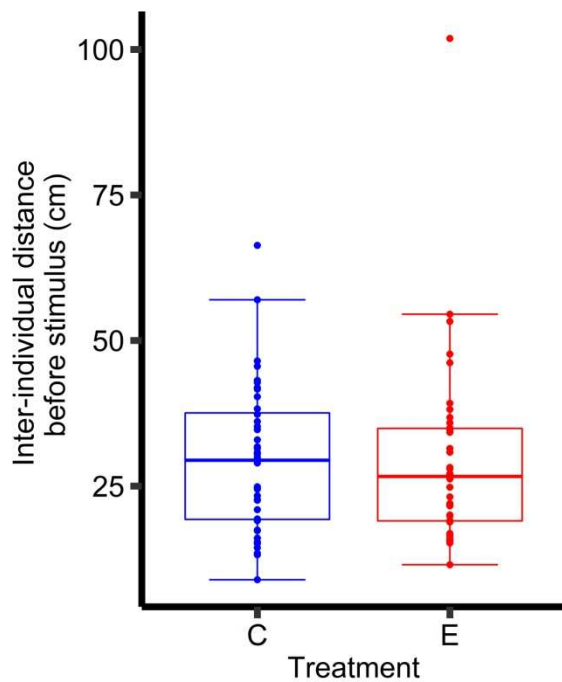
417 Before being exposed to the model predator (t-1) fish from the C treatment displayed
 418 significantly higher activity level than those of the E treatment ($F_{1,78}=7.622$, $P=0.007$; Table
 419 S1; Fig. 6). There was, however, no difference between days post-exposure ($F_{3,75}=0.860$,

420 $P=0.466$; Fig. S5), suggesting that oil exposed fish did not enter into a recovery process over
421 the 8 days of the experiment. Moreover, there was no statistically significant difference in the
422 inter-individual distance between treatment groups ($F_{1,75}=0.002$, $P=0.961$; Fig. 7, Table S6).
423 There was no detectable effect of the days post-exposure on inter-individual distances within
424 groups ($F_{3,76}=0.849$, $P=0.472$; Fig. S6).



425
426 **Fig. 6. Effect of treatment condition on the Activity level of the group before stimulus (t-1).**
427 Scores of all fish tested within the week post-exposure: blue: control fish (N=40); red:
428 exposed fish (N=40). The boundary of the box indicates 25th 50th and 75th percentiles.
429 Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and
430 below the whiskers indicate outliers outside the 10th and 90th percentiles. * Represents
431 significant difference ($p < 0.05$).

432



433

434 **Fig. 7. Effect of treatment on mean inter-individual distance within the group before**
 435 **stimulus (t-1).** Scores of all groups tested within the week post-exposure, blue: control fish
 436 (N=40); red: exposed fish (N=40). The boundary of the box indicates 25th 50th and 75th
 437 percentiles. Whiskers above and below the box indicate the 10th and 90th percentiles. Points
 438 above and below the whiskers indicate outliers outside the 10th and 90th percentiles.

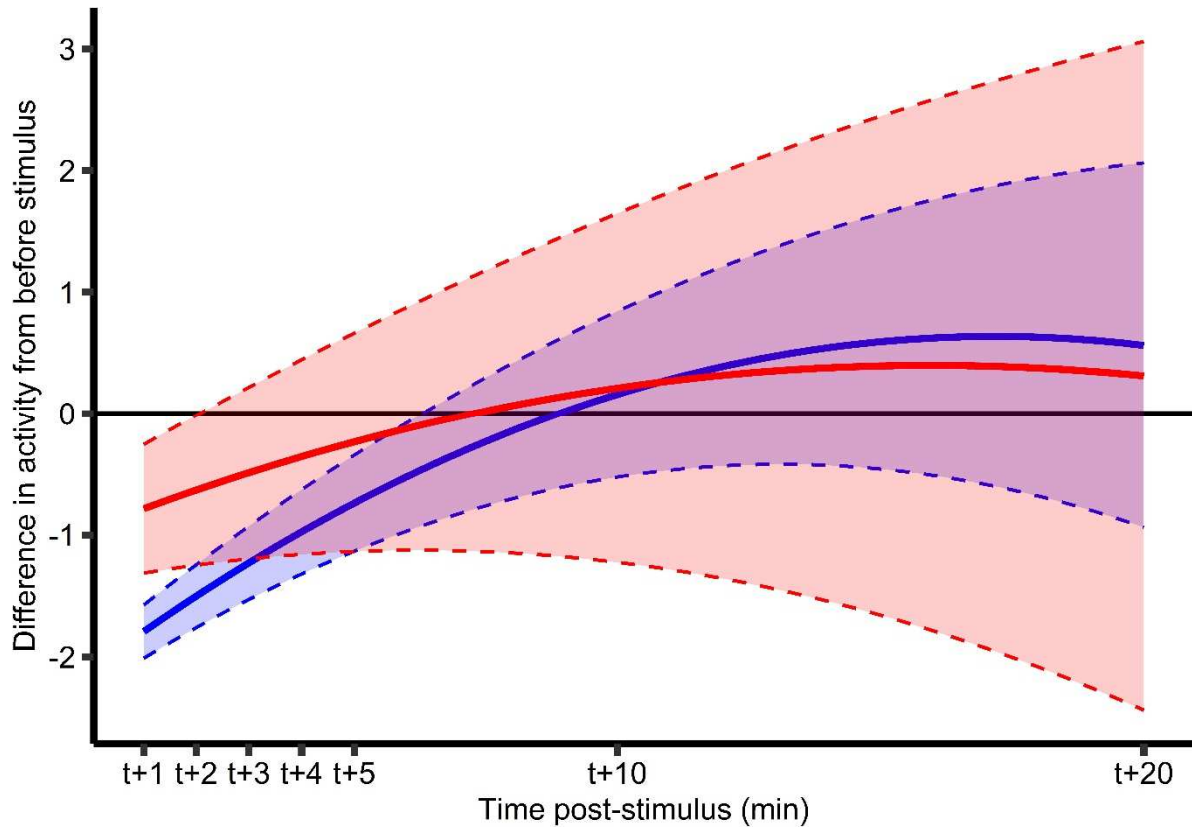
439

440 3.4.2.2 Response to the model predator

441 Following the simulated predation, fish from both treatments displayed a reduction in activity
 442 below the pre-stimulus level. Immediately after the simulated predation activity level of C
 443 fish was substantially lower than E fish activity level (Fig. 8). Both C and E fish displayed a
 444 recovery trend, activity level returning to its initial level between 5 to 10 minutes post-
 445 stimulus (Fig. 8). However, E individuals showed a slower rate of recovery of their activity
 446 over the 20 minutes post-stimulus in comparison to C fish (Linear mixed model:
 447 $F_{1,470}=34.305$, $P<0.001$; Fig. 8 and Table S7). Consistent with our analyses at t-1, C fish
 448 displayed higher activity level than E individuals at the end of the 20 minutes. Furthermore,

449 no effect of the day post-exposure upon fish activity was observed ($F_{3,74}=1.584$, $P=0.200$;
450 Table S7), suggesting no recovery over the week post-exposure.

451



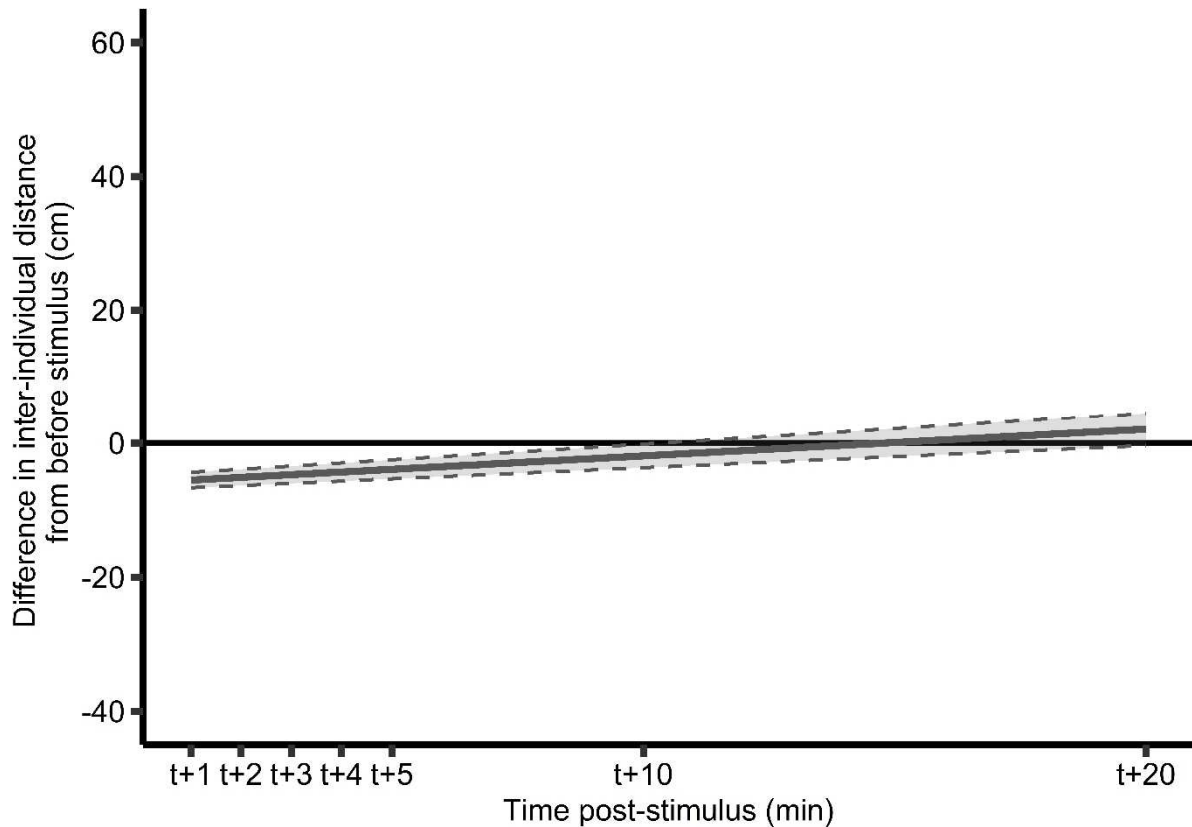
452

453 **Fig. 8. Evolution of the relationship between Activity and treatment condition over the 20**
454 **minutes following the simulated aerial predator attack that occurred at t+0. Blue: control**
455 **fish (N=40); red: exposed fish (N=40), solid lines: predicted values from linear mixed-effects**
456 **model showing significant statistical difference in the relationship between treatment, dotted**
457 **lines: standard errors, black horizontal line: represent the relative activity level before**
458 **stimulus at t-1**

459

460 Within the first minute following the stimulus, fish from both treatments showed a rapid
461 decrease in the inter-individual distance within groups (negative values in Fig. 9). Then, over
462 the next 20 minutes post-stimulus inter-individual distance progressively returned to pre-

463 stimulation level (Linear mixed model: $F_{1,470}=34.669$, $P<0.001$; slope 'Time'=0.401; Fig. 9
 464 and Table S8); full recovery being reached at *ca.* 10 minutes post-stimulus. There was no
 465 effect of the treatment or the day post-exposure upon fish inter-individual distance
 466 (Treatment: $F_{1,74}=1.551$, $P=0.217$; Day: $F_{3,75}=1.831$, $P=0.149$; Table S8).
 467



468
 469 **Fig. 9. Evolution of inter-individual distance within group over the 20 minutes following**
 470 **the simulated aerial predator attack.** Points represent for each individual, the difference
 471 between the measures taken at time post-stimulus and $t-1$. Black horizontal line: relative
 472 inter-individual distance before stimulus ($t-1$); solid grey line: predicted values from linear
 473 mixed-effect model of the evolution in inter-individual distance over the 20 minutes post-
 474 stimulus irrespective of the treatment group; dotted lines: standard errors of the model's
 475 predicted values; blue points: control fish ($N=40$); red points: exposed fish ($N=40$).
 476

477 **DISCUSSION**

478 Collective behaviours such as shoaling are meaningful complex behaviours of animals
479 involving perception, attention, and cognition (Scott and Sloman, 2004; Weis, 2014).
480 Shoaling behaviour notably allows individuals to draw on a full range of trade-offs to
481 maximize feeding opportunities and lower predation risk (Ward and Webster, 2016). Thus,
482 any disruption of such collective response may have consequences at population and
483 biocenosis levels (Maldonado-Chaparro et al., 2018). Characteristics of collective predator
484 avoidance in fish is well documented (Pavlov and Kasumyan, 2000; Ward and Webster,
485 2016), but little is known about the potential effects of sub-lethal exposure to pervasive
486 pollutants such as dispersant-treated oil on this complex behaviour. In this study, we
487 demonstrated that juvenile European sea bass, a gregarious fish species, displayed altered
488 anti-predator response following exposure to sub-lethal dose of dispersant-treated oil. While
489 our experiment indicated that dispersant-treated oil exposure did not affect the number of
490 individuals responding to the light stimulus (responsiveness), it showed that the metabolic
491 response was significantly lower in oil exposed fish, which supports our initial hypothesis.
492 Furthermore, in comparison to C individuals, E fish displayed higher activity level following
493 the simulated predation increasing, therefore, their exposure at risk under potential predation
494 pressure. Contrary to our initial hypotheses, our data showed that oil exposed fish displayed
495 higher metabolic rate than control individuals. Additionally, we could not find any effect of
496 oil exposure on the cohesion of the group before or after the simulated predation.

497

498 **4.1 Exposure condition and bioaccumulation of contaminants**

499 To characterize exposure conditions, water concentration in total petroleum hydrocarbon
500 ([TPH]) was monitored throughout fish exposure period. As expected, E treatment (TPH=
501 0.131 g L⁻¹) was in the range of situations that fish are liable to encounter in the wild

502 following an oil spill and its treatment with dispersant (0.001 to 0.260 g L⁻¹; Kim et al., 2010;
503 Sammarco et al., 2013; Spooner, 1970). We confirmed oil contamination of fish by analysing
504 the hepatic concentration of 20 polycyclic aromatic hydrocarbons (PAHs) concentrations. As
505 expected, no significant trace of oil contamination was detected in the control treatment group
506 while [PAHs] measured in the exposed individuals at the end of the exposure period and at
507 day 1 post-exposure were respectively 1.3 and 1.7 times higher than the QL. In teleost fish as
508 in other organisms, the liver plays a central role in the metabolism of PAHs and in the
509 detoxification process (Stein et al., 2010). Fish are known to rapidly metabolize PAHs in the
510 liver and secrete it into the bile (Maccubbin et al., 1988; Snyder et al., 2019; Varanasi et al.,
511 1989). Thus, the increase in liver [PAHs] that we observed in oil exposed fish group from day
512 0 to day 1 post-exposure was unexpected. This delayed increase in liver [PAHs] may indicate
513 metabolization of residues of PAHs accumulated in other tissues. At 4- and 7-days post-
514 exposure, mean liver [PAHs] was back below the QL confirming the efficiency of fish
515 detoxification mechanisms.

516

517 **4.2 Respirometry**

518 **4.2.1 Standard metabolic rate**

519 As expected, over the days post-introduction into the respirometers C individuals showed a
520 decrease in MR, reaching level of reported SMR from the literature, resulting from lower
521 spontaneous activity and reduced stress due to acclimation to their new environment.

522

523 Because stress such as unfamiliarity or handling may elevate MO₂ substantially (Chabot et al.,
524 2016; Fry, 1971; Smit, 1965), we used the measures of MR as indicators of anxiety in fish.
525 We initially hypothesized that oil exposure would disturb the perception or cognition abilities
526 of the exposed fish, leading to decreased anxiety while in the respirometry chamber. We

527 expected such alteration to be reflected through a lower MR in E fish compared to C fish.
528 Contrary to our expectation, we found that E individuals displayed higher MR than C fish all
529 week, with no recovery observed within 7 days post-exposure. These results are in accordance
530 with previous study showing higher oxygen consumption in Australian Bass (*Macquaria*
531 *novemaculeata*) 4 days following exposure to dispersant-treated oil (Cohen et al., 2001).
532 Davoodi & Claireaux (2007b) also reported that following a 48h period of severe oil
533 exposure, the resting metabolic rate of the European common sole (*Solea solea*) tended to
534 increase. Despite this increase in resting oxygen demand of E versus C (107.45 ± 5.19 vs
535 82.89 ± 4.17 $\text{mgO}_2 \text{ kg}^{-1} \text{ h}^{-1}$), MR of E fish largely remained within the range of seabass
536 aerobic capacities at 15°C (Claireaux and Lagardère, 1999). This higher resting MR observed
537 in E fish could result from an increase metabolic requirement in relation with the
538 detoxification process (Correia et al., 2007; Reddy and Bhagyalakshmi, 1994; Sørensen et al.,
539 2009).

540

541 **4.2.2 Responsiveness to light stimulus**

542 Fish from both treatments displayed similar responsiveness when stimulated with light
543 stimulus *i.e.*, displayed a transient increase in O_2 demand. This result confirmed those
544 obtained in other studies indicating the absence of effects of oil exposure on the percentage of
545 fish responding to a threatening stimulus (Johansen et al., 2017; Khursigara et al., 2021;
546 Milinkovitch et al., 2019). However, these three studies also revealed that when present, fish
547 locomotor response was altered both in terms of velocity and directionality. These results
548 seem to be in agreement with the changes we observed in fish physiological and behavioural
549 responses to a threat *i.e.*, displayed transients increase in O_2 demand and reduction in activity.

550

551 Following light stimulation, E individuals exhibited lower amplitude in the peak of MO_2
552 compared to C fish. This difference can have two origins which are not mutually exclusives
553 *i.e.*, a limitation of metabolic pathways and a difference in the perception/assessment of the
554 simulated predation. The light stimulation used in the present study has been shown to induce
555 a rapid increase in MO_2 to level corresponding to the maximum metabolic rate (MMR)
556 measured after individual chasing (Claireaux, pers com.). The lower amplitude in the peak of
557 MO_2 that we found in E fish might therefore suggest a reduction in their MMR. This
558 suggested limitation of the metabolic pathways is in agreement with previous publications
559 reporting reduced aerobic scope or MMR after oil exposure (Ackerly and Esbaugh, 2020;
560 Davoodi and Claireaux, 2007; Johansen and Esbaugh, 2017; Mager et al., 2014; Pan et al.,
561 2018; Stieglitz et al., 2016). This impairment could result, for instance, from reduced oxygen
562 supply/transport capacity along the oxygen cascade from the gill to the mitochondria. Future
563 works could further explore this hypothesis. The second hypothesis to explain this lowered
564 metabolic response to the stimulus can be altered perception and/or assessment of the
565 nature/severity of the threat. Recent works have highlighted the potential of oil exposure to
566 disrupt sensory systems as well as neuronal and cognitive processing (Jacquin et al., 2020;
567 Johansen et al., 2017; Xu et al., 2019, 2017). It is therefore possible that oil exposure could
568 alter a fish's capacity to acquire and process information from the surrounding environment
569 possibly resulting in inappropriate physiological and behavioural responses. Future studies
570 comparing the activities of the visual system between C and E fish would allow to directly
571 test this hypothesis.

572

573 **4.2.3 Post-stimulation recovery**

574 At day 1 post-exposure, E fish returned faster to their MR (48 ± 12 min) than C fish
575 (74 ± 18 min). This faster return to pre-stimulation MR may indicate lower sensitivity to the

576 light stressor or may just result from the fact that pre-stimulation MR in E fish was lower than
577 C fish. Time to return to pre-stimulation level displayed a strong habituation component in the
578 control fish. The habituation component is illustrated by the decrease over time of the peak
579 amplitude and of the time to return to the pre-stimulus MR. Such habituation pattern as well
580 as the acclimation capacity displayed by C fish to the respirometry chamber are indicators of
581 animal cognitive performances. Indeed, such habituation pattern is associated with learning
582 abilities, memory, information transfer and processing (Archer and Birke, 1983; Griffin and
583 Guez, 2014; Jacquin et al., 2017; Reader, 2015; Renner, 1990). The absence of such pattern in
584 E fish suggest, therefore, altered capacity to habituate over time to the surrounding
585 environment and possible impairments of the related cognitive performances. This is
586 consistent with previous studies reporting a reduced capacity to habituate to environmental
587 stimuli and a reduced learning ability following exposure to PAH (Geier et al., 2018; Knecht
588 et al., 2017).

589

590 **4.3 Shoaling behaviour**

591 Fish swimming activity in the experimental arena was measured by the combined analysis of
592 the time spent swimming, the distance moved and the swimming speed (Baker et al., 2018;
593 Little and Finger, 1990). Measures of group activity level conducted one hour after their
594 introduction into the experimental arena were considered to reflect fish standard activity level
595 in undisturbed and familiar conditions. As expected in such conditions, E fish displayed an
596 activity level 2 times lower than C fish. This reduction in activity level after oil exposure is in
597 agreement with previous studies (Aimon et al., 2021; Gonçalves et al., 2008; Little and
598 Finger, 1990; Woodward et al., 1987). For instance, Gonçalves et al. (2008) reported an
599 increase in the percentage of juvenile gilthead seabream (*Sparus aurata*) showing non-
600 locomotor activity following exposure to PAHs for 4 days. Hypoactivity can have major

601 ecological consequences through the disruption of crucial behaviours such as foraging,
602 predation avoidance or reproduction (Krause and Ruxton, 2002; Lima and Dill, 1990; Sih et
603 al., 2004; Weis et al., 2001). For instance, lower swimming activity can interfere with prey
604 capturing ability, lessening the searching area and reducing the chance to encounter potential
605 preys (Smith and Weis, 1997; Weis and Khan, 1991) with consequences on the amount of
606 energy available for growth (Little et al., 1990; Weis et al., 2001). Furthermore, we found that
607 the reduced activity level displayed by E fish was associated with altered behavioural
608 response to a simulated aerial attack stimulus.

609

610 The primary cause of mortality in juveniles seabass is predation (Almany and Webster, 2006).
611 Antipredator behaviours are therefore of critical importance. Usually, once under attack from
612 a predator, fish tend to minimize movements and to shoal in order to benefit from numerical
613 dilution and confusion effect for the predator, in addition of additive vigilance (Clark and
614 Mangel, 1986; Godin, 1986; Krause et al., 2000; Krause and Ruxton, 2002; Pitcher and K.
615 Parrish, 1993; Pulliam and Caraco, 1984). The visual stimulation used in the present work *i.e.*,
616 model predator passing over the arena, was thought to mimic an aerial predatory attack. It was
617 designed to evaluate fish behavioural response to the presence of a potential danger.
618 Following the stimulus, fish from both experimental treatments displayed the typical
619 reduction in activity and increased group cohesion, as illustrated by a reduction in the inter-
620 individual distance. However, E fish showed a less marked reduction in activity after the
621 stimulus than C individuals leading them to display higher and certainly inappropriate activity
622 level in a potentially dangerous situation.

623

624 Similarly to C individuals, activity level increased over time post-stimulus in E fish to return
625 to background level at t-1. Compared to C fish, however, E individuals showed a higher

626 activity level and returned slightly faster to background activity level, between 5 to 10
627 minutes post-stimulus. These changes in the behavioural response, with inappropriate level of
628 activity, can be critical for individual survival by increasing the risk of predator-induced
629 mortality. For instance, when E fish return to standard activity level while the environment is
630 still unsecure, they are more prone to predation risk than usual. Accordingly, previous studies
631 looking at the effects of petroleum hydrocarbons compounds on fish behaviour showed
632 reduced sheltering and shoaling behaviours, increased risk taking and altered antipredator
633 behaviours, such as escape response (Gonçalves et al., 2008; Johansen et al., 2017;
634 Khursigara et al., 2021; Milinkovitch et al., 2019). Moreover, exposure to PAHs has been
635 shown to increase predator-induced mortality in six species of Pomacentridae and Lethrinidae
636 families presenting such behavioural alterations (Johansen et al., 2017). Altogether our data
637 suggest that exposure to petroleum hydrocarbons may affect negatively individual fitness
638 through impaired ability to respond to predation.

639

640 In the present study, exposure to dispersant-treated oil affected shoal activity but not its
641 cohesion. This results is not surprising as it has already been shown that swimming activity is
642 the most sensitive behavioural indicator of animal disturbance (Little et al., 1990). Our results
643 agree with the current literature, showing impairment in activity before mortality occurs.

644

645 **4.4 Recovery over the week post-exposure**

646 Reported behavioural impairments might be detrimental for sea bass and it is therefore
647 critically important that recovery takes place as early as possible to preserve individuals from
648 additional jeopardy. To assess the recovery capacities of exposed individuals, we monitored
649 the previously discussed behavioural parameters over one-week post-exposure. Concerning
650 the altered spontaneous activity level pre- and post- stimulus, no recovery was shown over the

651 8 days of the experiment. However, our results showed that at day 7 post-exposure, E and C
652 fish expressed similar respiratory response to the light stimulus (1.6-fold the pre-stimulation
653 MR). Few studies have examined fish recovery capacities of physiological or behavioural
654 performances after oil exposure (Hicken et al., 2011; Johansen and Esbaugh, 2017; Mager et
655 al., 2014b; Mauduit et al., 2016; Zhang et al., 2017) but, to our knowledge, the present work is
656 the first to address the recovery of the response to a simulated predation. Our results suggest
657 that recovery of metabolic response to a threat could occur within 1 to 2 weeks post-exposure
658 to dispersant-treated oil, a result consistent with previous data indicating that recovery of
659 exploratory behaviour occurs in European seabass juveniles within two-weeks following an
660 exposure to similar concentration of dispersant-treated oil (Aimon et al., 2021). This rapid
661 recovery contrasts with data related to recovery of hypoxia tolerance that was suggested to
662 occur between 5 to 10 months post-exposure to oil (Mauduit et al., 2016; Zhang et al., 2017).
663 Thus, fish may recover behavioural and some of their physiological performances faster than
664 other.

665

666 **4. Conclusion**

667 In conclusion, this study provides additional experimental support that oil exposure can
668 jeopardize survival through altered antipredator response. Our data show that exposure to
669 dispersant-treated oil does not inhibit the classical antipredator response. Oil exposed fish
670 responded to the threat by increasing MO_2 and group cohesion and by displaying reduced
671 activity. However, this response was altered compared to the control fish. We observed lower
672 amplitude of the metabolic response to light mediated stimulation as well as higher activity
673 level following a simulated aerial attack. Moreover, absence of the typical habituation pattern
674 displayed by C fish, to lower their anxiety level over time in respirometry chamber, indicates
675 alteration of related cognitive performances. Overall, these results suggest that dispersant-

676 treated oil may disturb fish capacity to acquire and process information from external stimuli.
677 While previous works extensively addressed the mechanisms related to cardiotoxicity of oil
678 compounds, our study highlights the need for further investigations of the effects of these
679 chemicals on neurological and cognitive performances. Recovery was observed for the
680 physiological response to the light stimulus within 7 days post-exposure showing the transient
681 nature of these impairments. Future studies should investigate recovery capacities of fish
682 behavioural and physiological performances over a longer period post-exposure. Such altered
683 physiological and behavioural responses to simulated predation clearly suggest that oil
684 exposure can have major consequences for individuals' survival and hence for population
685 dynamics.

686

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691 assistance. We also thank the four anonymous reviewers for constructive comments. This
692 article is dedicated to Nicolas Le Bayon who passed away while this article was in
693 preparation.

694

695 **Appendices**

696

697 *Table A.1 Linear mixed effect model evaluating the relationship between the treatment*
698 *condition and the time post-exposure upon night resting metabolic rate.*

Model	Dropped term	Retained term	F-value	p-value
-------	--------------	---------------	---------	---------

Treatment:Day	(Intercept)	2.07	<0.01*
	Treatment	4.84	0.04*
	Day	14.82	<0.01*
	Treatment:Day	13.16	<0.01*

699 * Represents significant effects ($p < 0.05$).

700

701 *Table A.2 Backward stepwise reduction of the full model evaluating the relationship*
702 *between the treatment condition and the time post-exposure upon the percentage of fish*
703 *showing a metabolic response to the light mediated stimulation.*

Model	Dropped term	Retained term	z-value	p-value
Treatment:Day	Treatment:Day		-0.60	0.55
Treatment+Day	Treatment		1.14	0.26
Day	Day		-1.37	0.17

704

705 *Table A.3 Linear mixed effect model evaluating the relationship between the treatment*
706 *condition and the day post-exposure upon the ratio (Peak of MO_2 /pre-stimulation MR).*

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day		(Intercept)	225.35	<0.01*
		Treatment	0.93	0.35
		Day	4.85	0.04*
		Treatment:Day	5.27	0.03*

707 * Represents significant effects ($p < 0.05$).

708

709 *Table A.4 Linear mixed effect model evaluating the relationship between the treatment*
 710 *condition and the day post-exposure upon the time to return to the pre-stimulation MO₂*
 711 *after the light mediated stimulation.*

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day		(Intercept)	50.12	<0.01*
		Treatment	0.09	0.77
		Day	22.56	<0.01*
		Treatment:Day	6.64	0.02*

712 * Represents significant effects ($p < 0.05$).

713

714 *Table A.5 Backward stepwise reduction of the full model evaluating the relationship*
 715 *between the treatment condition and the day post-exposure upon Activity at t-1.*

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day	Treatment:Day		0.60	0.62
Treatment+Day	Day		0.86	0.47
Treatment		Treatment	7.62	0.01 *

716 * Represents significant effects ($p < 0.05$).

717

718

719 *Table A.6 Backward stepwise reduction of the full model evaluating the relationship*
 720 *between, the treatment condition and the day post-exposure upon inter-individual distance*
 721 *within the group, at t-1.*

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day	Treatment:Day		1.46	0.23
Treatment+Day	Treatment		<0.01	0.96

Day

Day

0.85

0.47

722 * *Represents significant effects ($p < 0.05$).*

723

724

725 *Table A.7 Backward stepwise reduction of the full model evaluating the relationship*
 726 *between, the behavioural trend over time (20min post-stimulus), the treatment condition*
 727 *and the day post-exposure upon Activity.*

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day:(Time+I(Time ²))	Treatment:Day:I(Time ²)		0.89	0.45
Treatment:Time+Treatment:Day				
+Day:Time+Treatment:I(Time ²)	Treatment:Day:Time		1.17	0.32
+Day:I(Time ²)				
Treatment:Time+Treatment:Day	Day:I(Time ²)		0.44	0.72
+Day:Time+Treatment:I(Time ²)				
Treatment:Time+Treatment:Day	Treatment:Day		1.06	0.37
+Day:Time+Treatment:I(Time ²)				
Treatment:Time+Day:Time	Day:Time		1.65	0.18
+Treatment:I(Time ²)				
Day+Treatment:Time	Day		1.58	0.20
+Treatment:I(Time ²)				
Treatment:Time		Treatment	5.56	0.02*
+Treatment:I(Time ²)		Time	257.52	<0.01*
		Treatment:Time	34.31	<0.01*
		Treatment:I(Time ²)	22.96	<0.01*

728 * Represents significant effects ($p < 0.05$).

729

730

731 *Table A.8 Backward stepwise reduction of the full model evaluating the relationship*
732 *between, the behavioural trend over time (20min post-stimulus), the treatment condition*
733 *and the day post-exposure upon inter-individual distance within the group.*

Model	Dropped term	Retained term	F-value	p-value
Treatment:Day:(Time+I(Time ²))	Treatment:Day:Time		0.02	1.00
Treatment:Time+Treatment:Day +Day:Time+Treatment:I(Time ²)	Treatment:Day:I(Time ²)		0.14	0.94
Treatment:Time+Treatment:Day +Day:Time+Treatment:I(Time ²)	Treatment:I(Time ²)		1.01	0.37
Treatment:Time+Treatment:Day +Day:Time+Day:I(Time ²)	Treatment:Day		1.29	0.28
Treatment:Time+Day:Time + Day :I(Time ²)	Treatment:Time		1.67	0.20
Treatment+ Day:Time +Day:I(Time ²)	Day:Time		1.70	0.17
Treatment+ Time+Day:I(Time ²)	Day: I(Time ²)		1.08	0.40
Treatment+ Time+Day	Treatment		1.55	0.22
Time+Day	Day		1.83	0.15
Time		Time	34.67	<0.01*

734 * Represents significant effects ($p < 0.05$).

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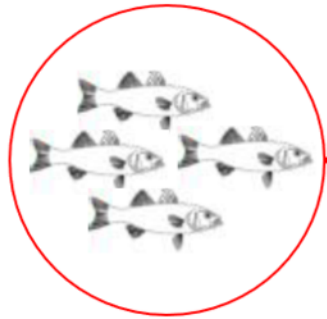
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Juvenile European sea bass
D. labrax (mass = 57g, 1+)



Exposure

0.4 g L⁻¹ Crude Arabian Light
+ 0.005 g L⁻¹ Dispersant OSR 52



Return to clean
water



Predation pressure

Recovery over 8 days post-exposure

Metabolic response

↓

Day 7 recovered

Activity

↑

No recovery

Shoal cohesion

=

No change