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

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ABSTRACT

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

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

KEYWORDS

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

1. INTRODUCTION

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

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

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

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

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

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

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

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

2. MATERIALS AND METHODS

2.1 Animals

Juvenile European Sea bass *Dicentrarchus labrax* (Linneaus 1758) (N=352, age 1^{+}; mass=57.65±1.11 g, mean±s.e.m) were obtained from a fish farm (Les poissons du Soleil, Balaruc les bains, France) and maintained in a 500 L indoor tank supplied with open-flow, thermoregulated (15°C) and fully aerated sea water (salinity 32 ppt) at Ifremer (rearing structure agreement B 29-212-05). Artificial lighting reproduced seasonal variation in local photoperiod. Fish were fed 3 times a week *ad libitum* using commercial feed (Neo Start Coul 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 O_2 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

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

Table 1. Experimental exposure to chemically dispersed oil, C: control; E: exposed, to 0.4 g L^{-1} of weathered crude Arabian light (CAL) added with 0.005 g L^{-1} of Finasol (© OSR 52, 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

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

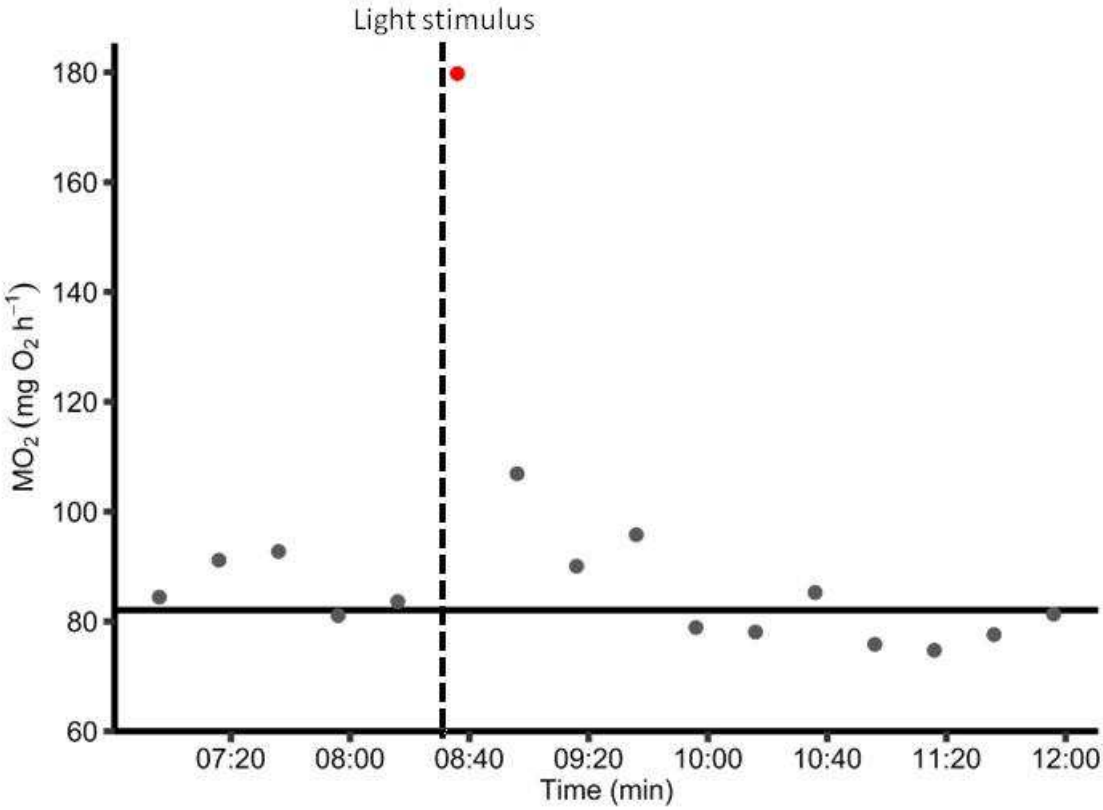
Tanks were continuously bubbled with air to maintain the oxygenation above 90% air saturation during the 62h exposure period. Following the exposure period, fish were bathed in clean seawater (1h) before their transfer back to Ifremer facilities (transportation procedure similar to the one described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

2.4 Respirometry

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

Fish were introduced in the respirometers 5 hours upon their return from Cedre (7 h post-exposure). Oxygen consumption was measured over two period. The first period lasted 7 min and corresponded to the flushing of the chamber with fully aerated water from the surrounding tank. During the second period (13 min), the flushing pumps were turned off and 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

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

2.7 Data analysis and statistics

2.7.1 Respirometry

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

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

2.7.2. Shoaling behaviours

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

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

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

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

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

3. RESULTS

3.1 Exposure condition and bioaccumulation of contaminants

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

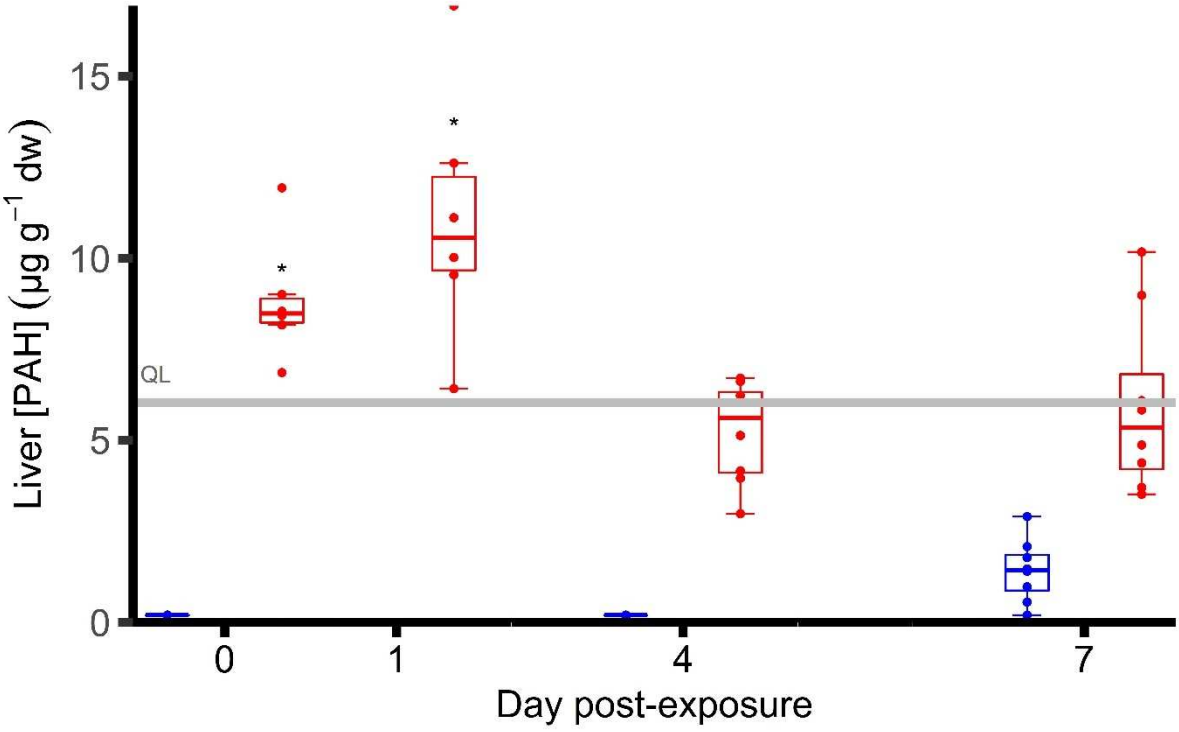
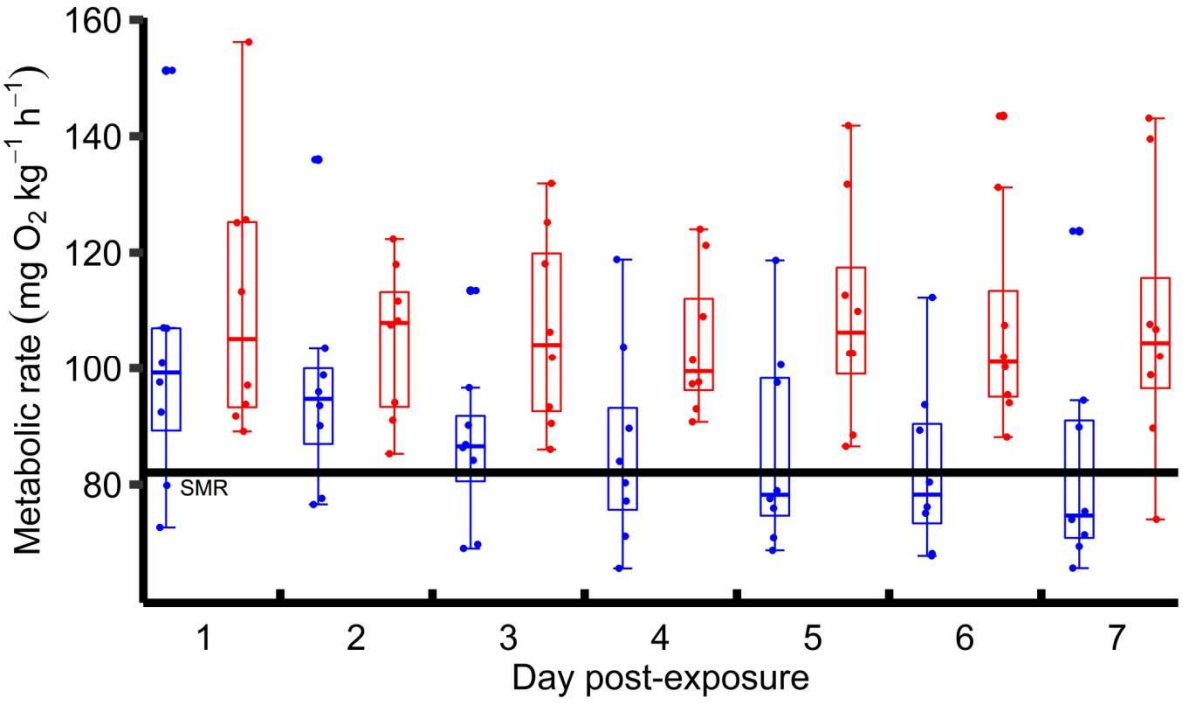


Fig. 2. Liver concentration in 20 PAHs (µg g^{-1} dry weight) measured in fish exposed to chemically dispersed oil. *Blue: control fish; red: exposed fish. Sampling was performed directly at the end of the exposure phase: day 0 (C: N=6; E: N=8); one day later (C: N=0; E: N=7), four days post-exposure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

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; Kır 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

responded (showing a peak; Fig. 1), we evaluated the intensity of the response by calculating the ratio between the peak of MO_2 and the pre-stimulation MR. Over the 7 days post-exposure in the respirometry chambers, C fish displayed a reduction in the height of the post-stimulation peak in MO_2 while no change was observed in the E fish ($F_{1,32}$=5.274, P=0.028, Fig.4, Table S3). In addition, C fish displayed a more intense response to the light stimulus than E individuals, displaying larger ratio between the peak of MO_2 and the pre-stimulation MR, especially the first day of the week (Fig. 4, Table S3). At day 1 in the respirometry experiment, C fish responded to the light stimulus with a peak of MO_2 2.3 times higher than their pre-stimulation MR. In contrast, day 7 C fish showed a lower increase in MO_2 than day 1, with a peak 1.6 times higher than the pre-stimulus level (Fig. 4, Table S3). The 7th day post-exposure, ratio between the peak of MO_2 and the pre-stimulation MR was similar in E and C fish (Fig. 4). Furthermore, C individuals presented a reduction in intra-group variability over the week spent in the respirometer (Fig. 4). Over the experimental week, both treatments showed a reduction in the time taken to return to the pre-stimulation MR after the peak of MO_2 (Fig. 5). However, C fish displayed a steeper decreasing slope over the week than E fish ($F_{1,20}$=6.641, P=0.018, Table S4).

Table 2. Percentage of individuals displaying an increased metabolic rate following the 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

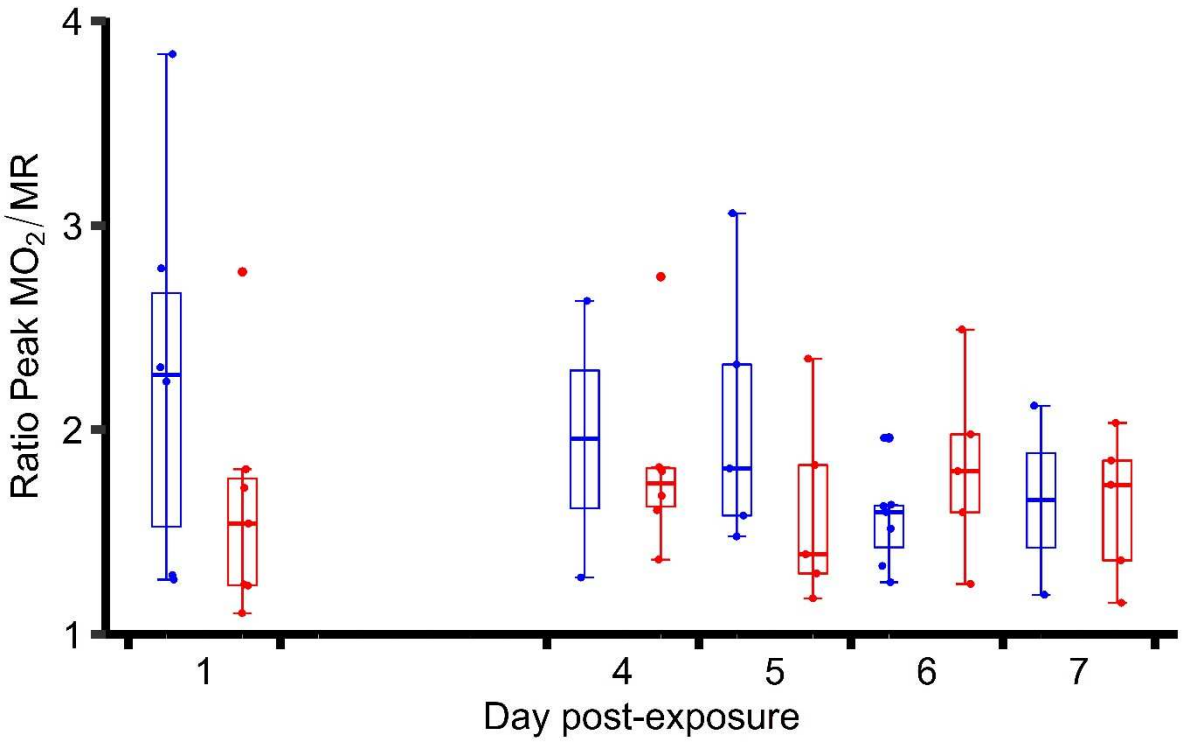


Fig. 4. Relationship between the treatment condition and the ratio (peak of MO_2/MR) over the 7 days post-exposure. *Only individuals showing a response to the light stimulus are represented. Sample sizes are therefore depending on the number of individuals that displayed an increase MO_2 following the light stimulus. Blue: control fish; red: exposed fish. The boundary of the box indicates 25th 50th and 75th percentiles. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate outliers outside the 10th and 90th percentiles. Linear mixed-effects model showed statistical significance of this relationship.*

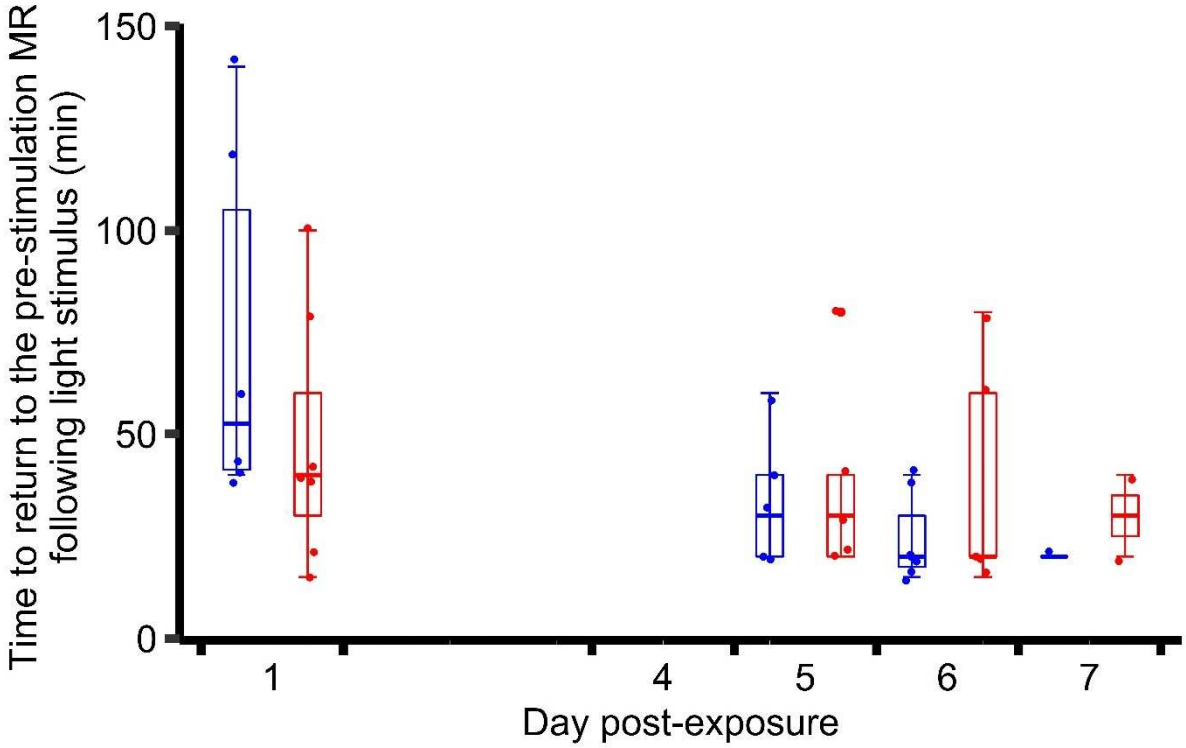


Fig. 5. Relationship between the treatment condition and the time to return to the pre-stimulation MR following the peak of MO_2 in response to the light stimulus over the 7 days post-exposure. *Only individuals showing a response to the light stimulus are represented. Effectives are therefore depending on the number of individuals that displayed an increase in MO_2 following the light stimulus. Blue: control fish; red: exposed fish. The boundary of the box indicates 25th 50th and 75th percentiles. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate outliers outside the 10th and 90th percentiles. Linear mixed-effects model showed statistical significance of this relationship.*

3.4 Shoaling behaviour

3.4.1 Reduction and structuration of behavioural variables

Four variables were recorded during behavioural tests. Three of these variables allowed evaluating fish swimming activity (total time spent swimming, labelled *Tswim*; distance

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

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

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

3.4.2 Comparing treatments

3.4.2.1 Pre-stimulus shoaling behaviour

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

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

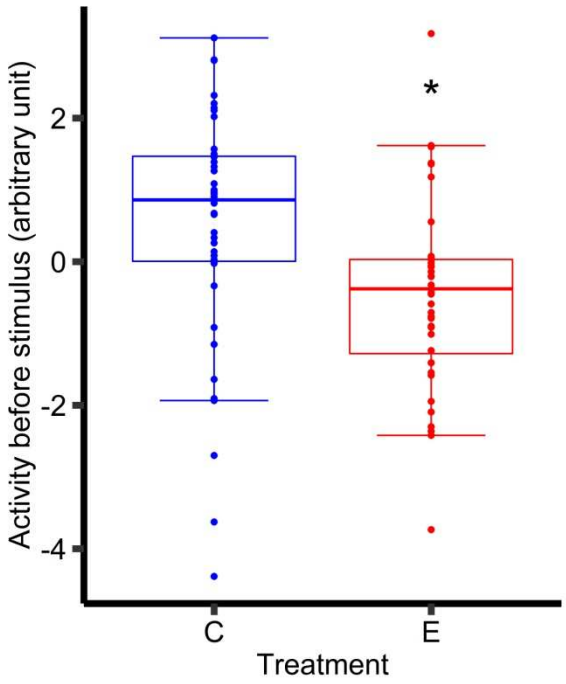


Fig. 6. Effect of treatment condition on the Activity level of the group before stimulus (t-1). *Scores of all fish tested within the week post-exposure: blue: control fish (N=40); red: exposed fish (N=40). The boundary of the box indicates 25th 50th and 75th percentiles. Whiskers above and below the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

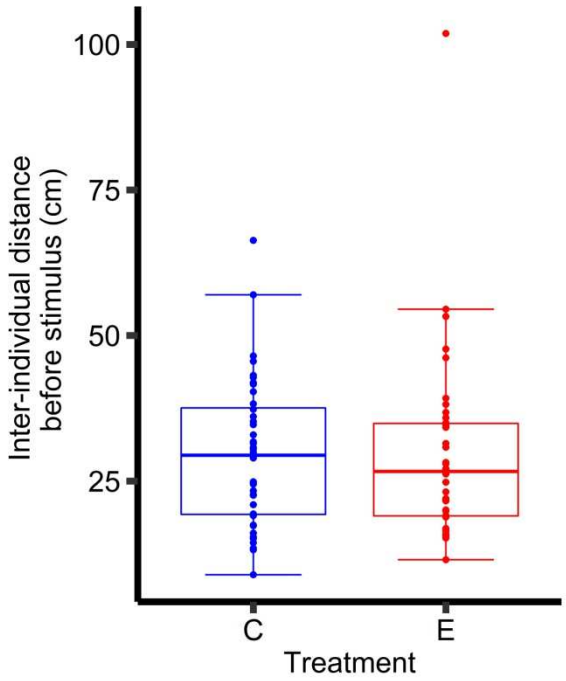
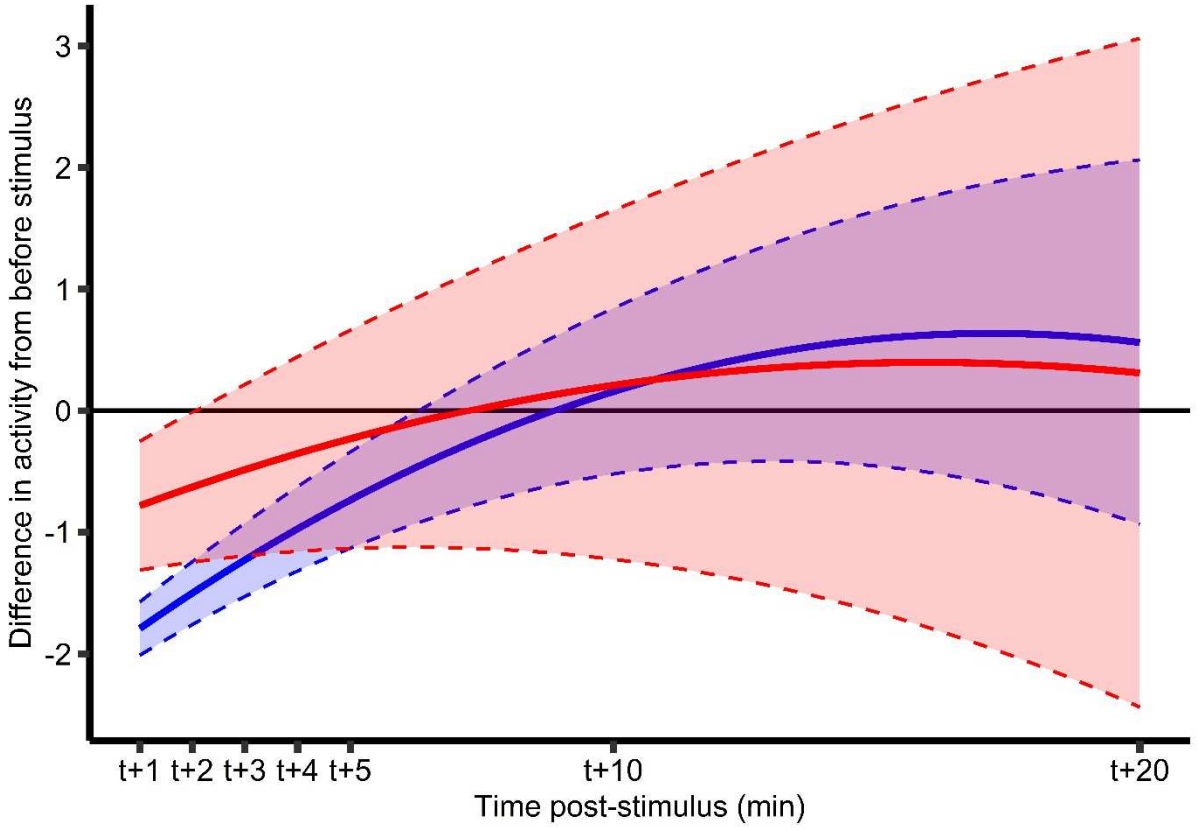


Fig. 7. Effect of treatment on mean inter-individual distance within the group before stimulus (t-1). *Scores of all groups tested within the week post-exposure, blue: control fish (N=40); red: exposed fish (N=40). The boundary of the box indicates 25th 50th and 75th percentiles. Whiskers above and below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

3.4.2.2 Response to the model predator

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

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

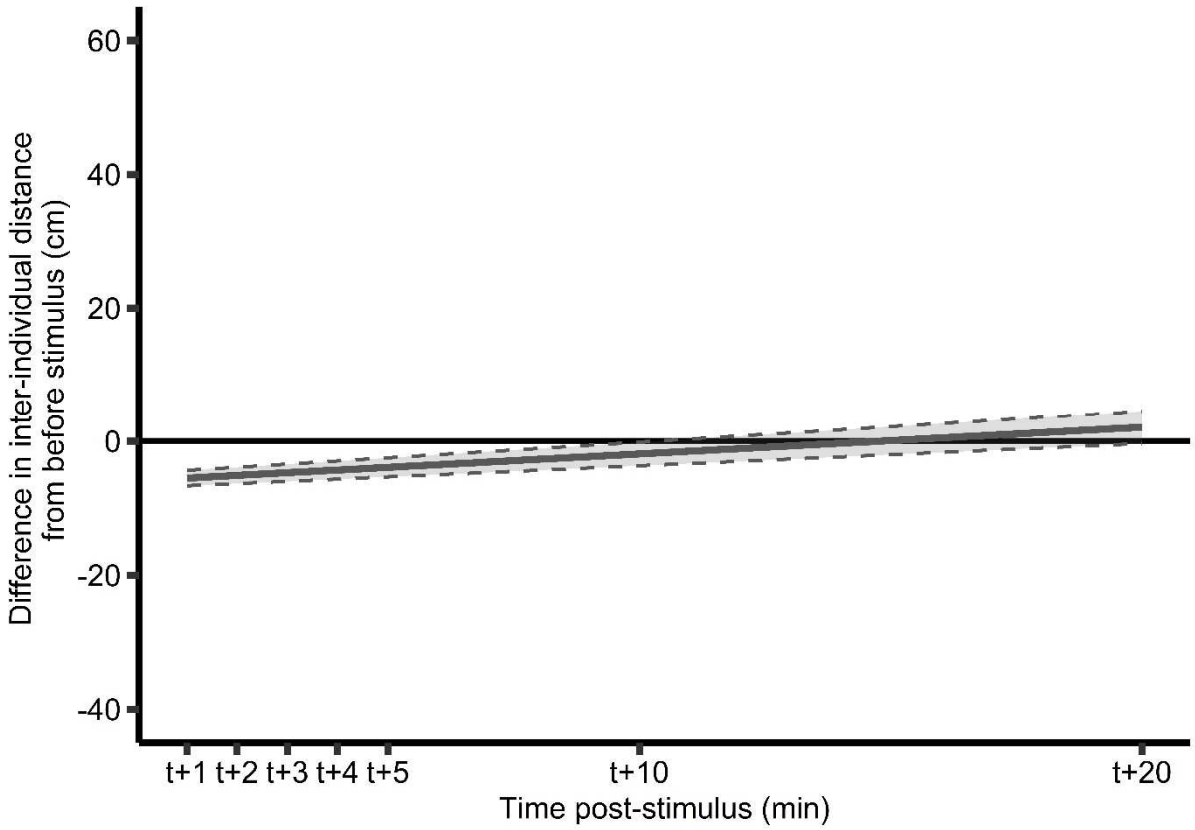


Fig. 9. Evolution of inter-individual distance within group over the 20 minutes following the simulated aerial predator attack. *Points represent for each individual, the difference between the measures taken at time post-stimulus and t-1. Black horizontal line: relative inter-individual distance before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

DISCUSSION

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

4.1 Exposure condition and bioaccumulation of contaminants

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

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

4.2 Respirometry

4.2.1 Standard metabolic rate

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

Because stress such as unfamiliarity or handling may elevate MO_2 substantially (Chabot et al., 2016; Fry, 1971; Smit, 1965), we used the measures of MR as indicators of anxiety in fish. We initially hypothesized that oil exposure would disturb the perception or cognition abilities of the exposed fish, leading to decreased anxiety while in the respirometry chamber. We

expected such alteration to be reflected through a lower MR in E fish compared to C fish. Contrary to our expectation, we found that E individuals displayed higher MR than C fish all week, with no recovery observed within 7 days post-exposure. These results are in accordance with previous study showing higher oxygen consumption in Australian Bass (*Macquaria novemaculeata*) 4 days following exposure to dispersant-treated oil (Cohen et al., 2001). Davoodi & Claireaux (2007b) also reported that following a 48h period of severe oil exposure, the resting metabolic rate of the European common sole (*Solea solea*) tended to increase. Despite this increase in resting oxygen demand of E versus C (107.45±5.19 vs 82.89±4.17 $mgO_2\ kg^{-1}\ h^{-1}$), MR of E fish largely remained within the range of seabass aerobic capacities at 15°C (Claireaux and Lagardère, 1999). This higher resting MR observed in E fish could result from an increase metabolic requirement in relation with the detoxification process (Correia et al., 2007; Reddy and Bhagyalakshmi, 1994; Sørensen et al., 2009).

4.2.2 Responsiveness to light stimulus

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

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

4.2.3 Post-stimulation recovery

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

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

4.3 Shoaling behaviour

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

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

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

4.4 Recovery over the week post-exposure

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

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

4. Conclusion

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

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

Acknowledgments

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Appendices

Table A.1 Linear mixed effect model evaluating the relationship between the treatment 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*

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

Table A.2 Backward stepwise reduction of the full model evaluating the relationship between the treatment condition and the time post-exposure upon the percentage of fish 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

Table A.3 Linear mixed effect model evaluating the relationship between the treatment 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*

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

Table A.4 Linear mixed effect model evaluating the relationship between the treatment condition and the day post-exposure upon the time to return to the pre-stimulation MO_2 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*

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

Table A.5 Backward stepwise reduction of the full model evaluating the relationship 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 *

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

Table A.6 Backward stepwise reduction of the full model evaluating the relationship between, the treatment condition and the day post-exposure upon inter-individual distance 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

Table A.7 Backward stepwise reduction of the full model evaluating the relationship between, the behavioural trend over time (20min post-stimulus), the treatment condition 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) +Day:I(Time^2)	Treatment:Day:Time		1.17	0.32
Treatment:Time+Treatment:Day +Day:Time+Treatment:I(Time ^2)	Day:I(Time^2)		0.44	0.72
Treatment:Time+Treatment:Day +Day:Time+Treatment:I(Time ^2)	Treatment:Day		1.06	0.37
Treatment:Time+Day:Time +Treatment:I(Time ^2)	Day:Time		1.65	0.18
Day+Treatment:Time +Treatment:I(Time ^2)	Day		1.58	0.20
Treatment:Time +Treatment:I(Time ^2)		Treatment	5.56	0.02*
		Time	257.52	<0.01*
		Treatment:Time	34.31	<0.01*
		Treatment:I(Time ^2)	22.96	<0.01*

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

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).*

735

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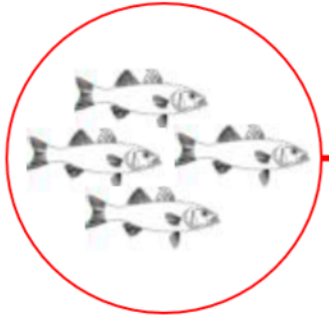
Juvenile European sea bass
D. labrax (mass = 57g, 1+)



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