Ecotoxicology and Environmental Safety 208 (2021) 111592

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Research paper

Effects of dispersant treated oil upon exploratory behaviour in juvenile European sea bass (*Dicentrarchus labrax*)

Cassandre Aimon [a,b,*], Christophe Lebigre [c], Nicolas Le Bayon [d], Stéphane Le Floch [b], Guy Claireaux [a]

[a] Université de Bretagne Occidentale, LEMAR (UMR 6539), Centre Ifremer de Bretagne, 29280 Plouzané, France
[b] CEDRE, Research Department, 715 rue Alain Colas, CS 41836, Brest 29218-Cedex 2, France
[c] Ifremer, Fisheries Science and Technology Unit (STH/LBH), Centre Ifremer de Bretagne, 29280 Plouzané, France
[d] Ifremer, LEMAR (UMR 6539), Cezon crude oil impacts the developing hearts of large predntre Ifremer de Bretagne, 29280 Plouzané, France

ARTICLE INFO

Edited by Professor Bing Yan

Keywords:
Behaviour
Oil spill
Teleost fish
Exploration
Open field test
European sea bass

ABSTRACT

Accidental spills are pervasive pollution in aquatic ecosystems. Resorting to chemical dispersant is one of the most implemented strategies in response to oil spills, but it results in an increase in the bio-availability of oil compounds known to disturb fish neurosensory capacities and hence fish habitat use. While it has become well established that acute oil exposure can cause a range of physiological defects, sub-lethal consequences on animal behaviour have only received recent attention. Here we investigated the effect of an exposure to a 62 h-dispersant treated oil on the exploration tendency (exploratory activity, and avoidance of unfamiliar open areas) of juvenile European sea bass. Three different concentrations of chemically dispersed oil were tested, low and medium conditions bracketing the range of likely situations that fish encounter following an oil spill, the high dose representing a more severe condition. Fish recovery capacities were also evaluated during 2 weeks post-exposure. Our results suggest a dose-response relationship; the low dose (0.048 ± 0.007 g L^{-1} of total petroleum hydrocarbons ([TPH])) had no effect on sea bass behavioural response to a novel environment while medium (0.243 ± 0.012 g L^{-1} [TPH]) and high (0.902 ± 0.031 g L^{-1} [TPH]) doses altered fish exploratory activity and their typical avoidance of unfamiliar open areas. Our experiment also suggest signs of recovery capacities in the first 10 days following oil exposure even if fish might need more time to fully recover from observed alterations. We discuss the possibility that observed alterations may result from a neurosensory or physiological known defects of oil exposure, causing anaesthetic-like sedative behaviours. Altogether, this study shows that juvenile sea bass exposed to oil spill exhibit transient behavioural impairments that may have major population-level consequences given the high mortality experienced by juveniles.

1. Introduction

Today's global economy heavily relies on the availability of oil reserves (Birol, 2015) and as production and refining sites are generally far apart, oil shipping is a continuously growing business with a doubling of oil transport activities over the last 40 years. Despite this rapidly expanding seaborne oil trade, improved security measures led to a decline of 92% in the annual number of oil spills > 7 tons over that period (International Tanker Owners Pollution Federation, 2017). Although less frequent, a total of 67,000 tons of oil have been released over the last decade with significant ecological and socio-economical consequences.

The application of chemical dispersants is a typical response to accidental oil spills as it contributes to the breaking oil slicks, facilitating the dissolution, evaporation and biodegradation of hydrocarbon compounds. However, a major drawback to such a treatment is the increased

Abbreviations: PAH, polycyclic aromatic hydrocarbon; OFT, open field test; CAL, crude Arabian light; H, high dose treatment; M, medium dose treatment; L, low dose treatment; E_H, exposed from the high dose treatment; E_M, exposed from the medium dose treatment; E_L, exposed from the low dose treatment; C_H, control from the highest dose treatment; C_M, control from the medium dose treatment; C_L, control from the low dose treatment; *Tswim*, total time spent swimming; *Dmoved*, total distance moved; *Velocity*, swimming speed; *TZoneC*, time spent in the central zone of the arena; [TPH], total petroleum hydrocarbon concentration; PCA, principal component analysis; PC, principal component; Liver ΣPAH, liver concentration in 21 polycyclic aromatic hydrocarbon compounds.

* Corresponding author at: Univ Brest, Ifremer, CNRS, IRD, LEMAR, F-29280 Plouzané, France.
E-mail address: cassandre.aimon@gmail.com (C. Aimon).

https://doi.org/10.1016/j.ecoenv.2020.111592
Received 8 September 2020; Received in revised form 27 October 2020; Accepted 30 October 2020
Available online 12 November 2020

bio-availability of oil compounds which can induce brand new functional impairments. For instance, dispersant alone or oil alone were shown to not affect hypoxia tolerance and swimming capacity in seabass while chemically dispersed oil impaired these functions (Mauduit et al., 2016). Fish are particularly exposed to oil compounds through their gills, skin and diet (Frapiccini et al., 2018; Tierney et al., 2013; van der Oost et al., 2003). Crude oil exposure has the potential to affect performance, structures and functions at organism, population, or community levels, with effects that may intensify or attenuate as they propagate across this hierarchy of biological levels (Fodrie et al., 2014). The study of the effects of oil exposure in fish has received considerable attention in the past two decades (Cherr et al., 2017; Khursigara et al., 2019; Pasparakis et al., 2019). Results have shown that in fish, oil exposure is related to increased mortality and to a range of sub-lethal effects including morphological and physiological disruptions at all developmental stages. These include cardiac impairments, that have been associated with impaired swimming performance and reduced maximum metabolic rate and aerobic metabolic scope (Claireaux et al., 2013; Johansen and Esbaugh, 2017; Mager et al., 2014). While the proximal detrimental effects of oil exposure have increasingly been recognised, their ultimate ecological significance is less clear. This gap in current understanding partly results from the persisting difficulties in linking fishes' physiology with their behavioural and ecological performance, in particular in an toxicological context (Ankley et al., 2010; Scott and Sloman, 2004).

Behavioural responses can be highly informative as they can provide insights onto fish strategy to mitigate the proximal, physiological and biochemical effects of their surrounding on their ecological performance and resulting fitness (Dell'Omo, 2002; Little et al., 1990). Changes in fish behavioural repertoire and capacity are indeed, likely to have potential impacts on intra- and inter-specific interactions with, in turn, possible consequences at population and biocoenosis levels. In fish, exploratory and risk-prone behaviours are linked to cognitive performances such as spatial learning, problem solving, memory, information transfer and processing (Archer and Birke, 1983; Griffin and Guez, 2014; Jacquin et al., 2017; Reader, 2015; Renner, 1990). Since these behavioural characteristics contribute to resource acquisition and predator escape, they are particularly associated with fish fitness.

Recent studies have highlighted that oil exposure alters fish sensory performance, including olfactory and visual performance, affecting their ability to collect information from their environment (Cave and Kajiura, 2018; Colavecchia et al., 2007; Magnuson et al., 2020, 2018; Martin et al., 2020; Schlenker et al., 2019; Xu et al., 2018). These experiments also indicated that sub-lethal oil exposure can modify detection and avoidance thresholds to essential cues. For instance, crucial response to conspecific alarm cues was reduced after oil exposure in bicolour damselfish (*Stegastes partitus*; Schlenker et al., 2019). Such capacity loss is likely to impair fish survival by hindering feeding and predator avoidance behaviours in oil-exposed fish (Colavecchia et al., 2007; M. Carvalho et al., 2008). Results from existing research suggest also alteration of spatial behaviours and cognitive abilities directly related with fitness and reproductive success. For instance, oil exposure decreased exploration in Trinidadian guppies (*Poecilia reticulata)* placed in an experimental maze (Jacquin et al., 2017). Additionally, acute crude oil exposure has been related to severe alteration of habitat selection in two species of damselfish (*Pomacentrus amboinensis* and *P. moluccensis*), exposed fish displaying riskier behaviour resulting in a nearly 3-fold increase in predation-related mortality (Johansen et al., 2017). Without drawing overly broad conclusion, available information illustrates the importance to have knowledge about oil effects upon critical behavioural characteristics in relation with fish ability to evaluate habitat quality and therefore to preserve ecological performance (Scott and Sloman, 2004).

Effects of oil exposure have been documented in a wide range of fish species (Anttila et al., 2017; Carls et al., 2008; Frapiccini et al., 2018; Hicken et al., 2011; Nelson et al., 2016; Schlenker et al., 2019) and at all life stages (Bautista et al., 2019; Hicken et al., 2011; Incardona et al., 2014; Mager et al., 2014) although, a broad intra- and inter-specific variability in the extent of these effects must be acknowledged (Beyer et al., 2016; Pasparakis et al., 2019). Furthermore, it has to be noted that there is a limited number of studies that considered these effects in a dose-response manner (Khursigara et al., 2019). Additionally and as already mentioned above, the long-term consequences of oil exposure upon fish ecological performance and fitness remains an open question. Available evidence suggests that physiological and behavioural impairments may persist well beyond the exposure period. For instance, in bicolour damselfish (*Stegastes partitus*) Schlenker et al. (2019) reported altered response to conspecific alarm cues that persisted 8 days following oil exposure. Similarly, zebra fish (*Danio rerio*) and mahi-mahi (*Coryphaena hippurus*) exposed to oil at embryonic stage displayed physiological impairments still perceptible at adult and juvenile stages respectively (Hicken et al., 2011; Mager et al., 2014). In juvenile red drum (*Sciaenops ocellatus*), persistent reduction of aerobic scope, burst- and critical swimming speeds were still observed six weeks after an acute, 24 h oil exposure (Johansen and Esbaugh, 2017). European sea bass also displayed reduced swim performance one month post-exposure even though, 10 months later, no residual effect were observable anymore (Mauduit et al., 2016).

The objective of this study was therefore to examine, in a dose-response manner, the effect of sub-lethal exposure (62 h) to a mixture of oil and dispersant on components of European sea bass (*Dicentrarchus labrax*) behavioural repertoire. This experiment was conducted using an open field test. We considered two types of behaviour: fish exploration tendency in a novel environment and their avoidance of an open (potentially dangerous) area. To investigate fish recovery capacities, the experiment was renewed six times over a period of 10 days post-exposure. More specifically, the research objective consisted in testing three hypotheses: (1) the exposure to dispersant-treated oil alters fish behaviour, reducing exploratory activity and increasing exposure to risk; (2) behavioural alterations increase when fish are exposed to larger doses; (3) recovery of fish behavioural performances is relatively rapid (within 2 weeks) post exposure. To test these hypotheses, we selected the European sea bass (*Dicentrarchus labrax*) because of its economical importance and role as a marine predator. Although acute exposure to dispersant treated oil has been shown to affect several physiological endpoints in this species (Claireaux et al., 2013; Mauduit et al., 2016; Milinkovitch et al., 2019; Nelson and Claireaux, 2005), to the best of our knowledge, effects on its behaviours had not been investigated so far.

2. Materials and methods

2.1. Study animals

Juvenile (1^{+}) European sea bass *Dicentrarchus labrax* (Linneaus 1758), were obtained from a local fish farm (Aquastream, Lorient, France). Fish originated from a brood stock of wild fish initially caught off the coast of Morbihan, France. At each generation, newly caught breeders have been added to this stock in order to reduce inbreeding and domestication effects. Juveniles were conveyed to Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer) rearing facility in Brest. They were maintained in a 500 L indoor tank supplied with open-flow, thermoregulated (15 °C) and fully aerated sea water (salinity 32). Artificial lighting followed local photoperiod. Fish were fed 3 times a week ad libitum using commercial feed (Neo Start Coul 2, Le Gouessant, France). For this study, we used a total of 378 fish distributed equally among 3 experimental treatments labelled 'high' (n = 126), 'medium' (n = 126) and 'low' (n = 126). For logistic reasons, the treatments were experimented sequentially starting with high and finishing with low. Over the time span of the experiment fish mean mass increased slightly, being 9.05 ± 0.86 g, 12.35 ± 0.82 g and 13.73 ± 0.62 g for the high, medium and low treatment respectively. Experiments were non-invasive and were approved by the *French Ethics*

Committee in charge of Animal Experimentation n°74 (permit number: APAFIS#7783-2016112515512418 v3). They also followed institutional guidelines.

2.2. Fish transport

Fish exposure to the oil and dispersant mixtures were conducted at the Centre de documentation, de recherche et d'expérimentation sur les pollutions accidentelles des eaux (Cedre, Brest, France). Fish transportation to and from Cedre (travel time 30 min) was done using airtight plastic containers (50 L) filled with 40 L of water containing a light dose of anaesthetic (MS-222; 20 mg L^{-1}). The free volume above the water was filled with oxygen. Upon arrival at Cedre, fish were transferred to a polyethylene tank (300 L). Water temperature, salinity and photoperiod at Cedre were similar than in the original rearing tank.

2.3. Experimental exposure

As shown in Table 1, experiments spanned over 3 months. Three doses of dispersant-treated oil were successively tested in triplicate tanks. The highest dose (E_H) consisted in mixing 0.8 g L^{-1} of weathered crude Arabian light (CAL) with 0.01 g L^{-1} of chemical dispersant (Finasol OSR 52, Total Fluides, Paris France), the medium dose (E_M) consisted of 0.4 g L^{-1} of CAL added with 0.005 g L^{-1} of dispersant and the low dose (E_L) consisted of 0.2 g L^{-1} of CAL mixed with 0.0025 g L^{-1} of dispersant. Due to a lack of information regarding the possible lethal effect of oil, and in order to account for possible mortality, 63 fish were exposed to each experimental treatment group (E_H, E_M and E_L, in triplicate) although our objective was to analyze only 30 of them per group. These groups were compared to corresponding unexposed control groups labelled C_H, C_M and C_L (n = 63 in triplicate). Six hours following their arrival at Cedre facilities, fish were distributed among six polyethylene tanks (300 L), three of which housed the control fish (C) while the other three received the fish to be exposed to the experimental mixture (E).

Crude Arabian light and dispersant were mixed in a bottle in accordance with the manufacturer's recommendation (Dispersant/oil ratio: 4%). The mixture was then directly poured in the tanks and weathered by bubbling air during 5 h prior to the introduction of fish (Nordvik, 1995). Tanks were equipped with a custom-made device that mimicked oil mechanical dispersion by waves at sea while maintaining homogenous exposure conditions throughout the tanks. This device consisted in a funnel located at the surface and connected to a 12 V submersible bilge pump (L450-500GPH; Johnson) placed on the bottom of the tank. Oil floating at the surface was sucked through the device, homogenised with water and released at the bottom of the tank (Milinkovitch et al., 2011). Following the 62 h exposure period, control and exposed animals were bathed in clean sea water (1 h) and brought back to Ifremer facilities following the same transportation procedure as described above. At Ifremer, C and E fish were placed in two different tanks, meaning that treatment replicates were mixed after the exposure period.

Table 1
Experimental exposure. Experimental exposure to chemically dispersed oil, C: control; E: exposed, to different doses, H: high; M: medium; L: low. CAL: crude Arabian light.

	Date 2017-01-27 to 2017-01-30		Date 2017-03-17 to 2017-03-20		Date 2017-04-21 to 2017-04-24	
Label	C_H	E_H	C_M	E_M	C_L	E_L
CAL (g L^{-1})	0	0.8	0	0.4	0	0.2
Finasol (g L^{-1})	0	0.01	0	0.005	0	0.0025
Number of replicates	3	3	3	3	3	3
Number of fish per replicate	21	21	21	21	21	21

2.4. Behavioural assay to assess fish exploration tendency

Behavioural assays started the day following fish return to Ifremer facilities. Fish exploration tendency was examined using an open field test (OFT). To monitor the kinetics of post-exposure recovery, these tests were repeated over two weeks, on days 1, 2, 3, 8, 9 and 10 post-exposure (5 naïve fish per day). Given the long time span of the experiment (3 months; end of January to April 2017), each experimental treatment group (E_L, E_M, E_H) was compared against a corresponding control group (C_L, C_M, C_H), and between treatments comparisons are solely qualitative.

The day prior to the experimental test, fish were randomly selected in the rearing tanks and gently placed (without emersion) into individual confinement chambers. Confinement chambers consisted in an opaque PVC tube (13 cm × 5 cm length, diameter respectively) closed at both end with plastic meshing to allow water renewal inside the chamber (Aimon et al., 2019). Fish were left undisturbed 15 h in these chambers, placed side by side on the bottom of a tank that received the same water than the rearing tank. On the testing day, chambers were successively placed in an immersed plastic container (2 L) and gently moved to the experimental arena. We waited 3 min to enable fish to recover from potential disturbance before we opened, from a distance, one end of the chamber. Fish were allowed 1 min to exit the chamber or the opposite end from the exit was slowly lifted, also from a distance, to encourage the fish to swim out. This encouragement to leave the chamber had to be applied in 146 cases out of 156. The lifted chamber was then fully removed from the arena. A period of agitation was observed in all fish following their entrance in the arena. This 'flight response' typically lasted less than a minute. In order to standardise trials, the first minute following fish entrance in the arena was not subsequently used.

The experimental arena consisted in a white rectangular shallow tank (156 cm × 99 cm × 14 cm, length, width, depth, respectively) with a curtain placed around and over the experimental device to limit visual disturbances. Neon lamps were installed on each side of the arena to provide homogenous lighting (30 lux) and a video camera was placed 1 m above the water surface (Logitech webcam C930e, 15 frames s^{-1}). The bottom of the experimental tank was covered with a light retro-reflective adhesive foil to improve contrast between the fish and the background (Loligosystem, Inc). The open field test consisted in exposing a fish to a bare tank and to record its movements during the following 20 min. Three indices of exploratory activity were measured in 5-min increments: total time spent swimming (labelled *Tswim*), total distance moved (labelled *Dmoved*) and the swimming speed (labelled *Velocity*). Avoidance of open area was also evaluated by measuring the time spent in the central zone of the arena (labelled *TZoneC*). This central zone corresponded to the area situated at two body lengths from the walls (78 cm × 49.5 cm). Videos were analyzed using the video tracking software Lolitrack Version 4.2.0 (Loligosystem, Inc).

2.5. Chemical analyses

To characterise exposure conditions, total petroleum hydrocarbon concentration ([TPH]) was measured in triplicate in each exposure tank, with seawater samples being taken immediately before as well as 4, 24 and 48 h after fish introduction into the tanks. Seawater samples were extracted three times with 10 mL of dichloromethane Pestipur quality (SDS, Carlo Erba Reagent, France). The combined extracts were dried by filtering through anhydrous sodium sulphate and then analyzed using a spectrophotometer (Evolution 600 UV–VIS; Thermo Fisher Scientific) at 390 nm, as described by Fusey and Oudot (1976).

To assess fish contamination, two individuals per tank were euthanized at the end of the exposure phase and polycyclic aromatic hydrocarbons (PAH) concentrations in the bile and liver were measured. The presence of PAH metabolites in the bile was determined semi-quantitatively using a fluorospectrophotometer (Aas et al., 2000). A 5 nm slit width was used to measure the PAH metabolites on emission and excitation channels (Jasco FP-6200, Tokyo, Japan). Analyses were

conducted using three excitation-emission wavelengths i.e., 295–335 nm (naphthalene-type metabolites); 343–383 nm (four-ringed compounds including pyrene-type metabolites) and 380–430 nm (benzo[a] pyrene-type metabolites; Krahn et al., 1987; Lin et al., 1996; Aas et al., 2000). Liver concentrations of 21 polycyclic aromatic hydrocarbons (including the components listed by US-EPA) were measured by GC–MS as described by Lacroix et al. (2014). Briefly, liver PAH were extracted using an alkaline digestion combined with stir bar and were evaluated with sorptive extraction-thermal desorption-gas chromatography-mass spectrometry (SBSE-GC-MS). For the validation of this analytical method, quantification limit of each PAH was estimated. This measure allowed us to identify the lowest concentration of PAH in a liver sample that can be determined with acceptable precision and accuracy under the condition of the test described previously.

2.6. Statistical analysis

For every variable, while each treatment group was compared to the relevant control group, doses-response effect was evaluated qualitatively. Data were standardised and normalised before the subsequent analyses. As the three indices of exploratory activity (*Tswim*, *Dmoved* and *Velocity*) were highly correlated with one another (Appendices, Fig. A), principal component analysis (PCA) was used to reduce these variables to one principal component (PC) to represent fish exploratory activity. This PC was chosen according to Kaiser's criterion (Kaiser, 1961), retaining only factors with eigenvalues greater than 1. This PCA contained all fish in order to have a consistent measure and interpretation of fish exploratory activity across the different experimental treatments. The impact of dispersant-treated oil on juvenile sea bass behaviours was examined using linear mixed effects models in which the factors treatment, day post-exposure and the time steps of the open field test were used as fixed effects. To determine the best random effect, we used maximum likelihood estimations to compare models with fish ID as random effect or fish ID nested within each time step of the OFT. The performance of models with different random effects was compared using corrected Akaike information criterion (AIC). Because, the within fish changes in behaviour in the high treatment was limited, we only kept fish ID as random factor in the models for this treatment level. In contrast, fish ID nested within each time step of the OFT was set as a random effect to model intercepts and slopes in the models evaluating the effects on fish behaviours from the medium and low treatments. A stepwise backward reduction of the full model was applied to exclude non-significant interaction terms and fixed effects. The principal component analysis was conducted using FactoMineR package (Lê et al., 2008) and models were run using the nlme (Pinheiro et al., 2019) package in R version 3.5.1 (R Core Team, 2018). Model diagnostics were evaluated by visually inspecting the residuals. The normality of the data was calculated using Shapiro-Wilk test. An ANOVA was applied to determine whether there were differences in water concentration in total petroleum hydrocarbons and liver concentration in 21 polycyclic aromatic hydrocarbon compounds among treatment groups. A Tukey test was then carried out to assess significant differences between treatments. We classically set the statistical significance at $P < 0.05$.

3. Results

3.1. Exposure conditions

At the three exposition doses tested, water concentration in total petroleum hydrocarbons ([TPH]) remained stable throughout the exposure periods (Fig. 1A). Experimental treatments corresponded to TPH of 0.902 ± 0.031 g L^{-1} for E_H, 0.243 ± 0.012 g L^{-1} for E_M and 0.048 ± 0.007 g L^{-1} for E_L. In the control groups (C_H, C_M, C_L), TPH concentrations were not statistically different from each other and were averaged (0.002 ± 0.0004 g L^{-1}, labelled C). Liver concentrations in 21 polycyclic aromatic hydrocarbon compounds (Liver ΣPAH) measured

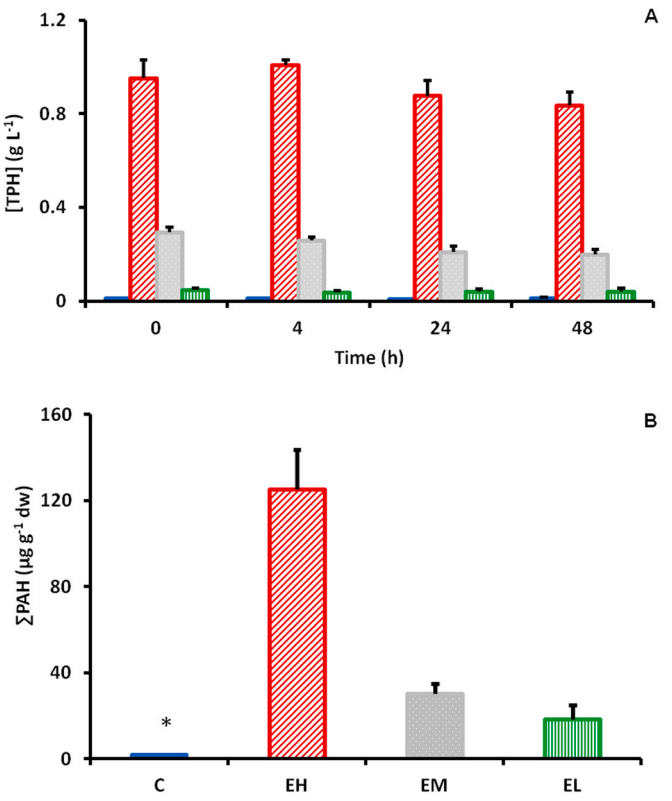

Fig. 1. Exposure to chemically dispersed oil in control (C; blue filled bar), high (E_H; red hatched bar), medium (E_M; grey pointed bar) and low (E_L; green vertical hatched bar) treatments. A) Water total petroleum concentration (g L^{-1}) measured throughout the 62 h-exposure period in tanks N = 3 (1 average concentration from three samples per tank × 3 replicate tanks). B) Liver concentration in 21 PAH (µg g^{-1} dry weight) measured in fish one day post-exposure. N = 3 (1 samples × 3 replicate tanks). Values for the C group were below the quantification limit but are shown for comparison with the other treatment groups. The error bars indicate the calculated SEM. * Represents significant difference ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

one day post-exposure in fish from the C_H, C_M and C_L conditions were below the quantification limit and have been averaged and labelled 'C' in Fig. 1B. In contrast, concentrations measured in fish from the E_H, E_M and E_L conditions were respectively 9.8, 2.4 and 1.4 times the quantification limit (Fig. 1B). Only ten (pyrene, fluorene, phenanthrene, naphthalene, benzothiophene, biphenyl, acenaphthene, dibenzothiophene, anthracene, chrysene) of the 21 PAH measured were at concentrations above the quantification limit (Appendices, Table A). Tricyclic PAH represented 80% of total liver PAH in the E_H and E_M exposed fish and nearly 70% of total liver PAH in E_L exposed fish (Table A).

3.2. Survival

No mortality was observed in the three controls groups and in the low exposure group (E_L). In the medium exposure (E_M) group, on the other hand, one fish (1.6%) died during the post-exposure 1 h cleaning period. In the high exposure treatment (E_H), there was a 71% mortality rate, death occurring both during and after the exposure period. Due to this high mortality rate, 18 fish instead of 30 were subsequently analyzed in this treatment group. For the other treatment groups, 30 fish in total ($N = 5$ fish per day post-exposure) were analyzed for behavioural response in the open field test (OFT).

3.3. Reduction and structuration of the variables

Qualitative observation allowed us to notice that exposed fish generally presented an anaesthetic-like sedative state i.e., slowness, with a head-up swimming posture. In such state, fish were often observed "drifting" across the experimental arena, a situation that was readily differentiable from a voluntary exploration of the novel environment. This was particularly noticeable in the E_H group during the first three days post-exposure.

Four variables were recorded during the OFT. Three of these variables assessed exploratory activity (total time spent swimming, labelled *Tswim*; distance moved, labelled *Dmoved*; swimming speed, labelled *Velocity*) while the fourth measured the time spent in the central zone (*TZoneC*). With the exception of *TZoneC*, variables were highly correlated to each other (coefficient of correlation >0.61; Fig. A). Thus, a principal component analysis (PCA) was conducted to combine *Tswim*, *Dmoved* and *Velocity* into one principal component (eigenvalue>1; Table 2). This principal component (PC1) was termed 'exploratory activity' and accounted for 86% of total variability. The two other principal components (PC2 and PC3) were ignored as their eigenvalue was < 1 and as they explained substantially less variance (Table 2).

In the following, we will inspect exploratory activity and *TZoneC* data by, firstly examining, in the high treatment group (H), the time-course of the above variables over the duration of the OFT. Secondly, by considering the post-exposure recovery period, we will investigate how decreasing exposure doses may have influenced fish response to the OFT and capacity to recover and finally by comparing qualitatively the three treatment conditions.

3.4. Exploratory activity

Over the 20-min test-time, exploratory activity progressively increased in individuals from the control treatment (C_H), and remained unchanged in high treatment exposed fish (E_H) ($F_{1,106}$ =19.97, $P < 0.01$; Table B; Fig. 2A). When examined over the 10-day post exposure period, the activity level displayed by C_H was stable. In the E_H group, on the other hand, fish showed lower overall exploratory activity in the experimental arena than C_H individuals during the first three days following exposure to oil + dispersant mixture, followed by a return to control level at day 8 ($F_{5,24}$ =2.64, $P = 0.04$; Table B; Fig. 3A). Over the 20-min test-time, control and exposed fish from the treatments M (C_M and E_M) displayed a similar pattern of exploratory activity than the C_H fish although fish from the E_M group showed higher overall exploratory activity than C_M ($F_{1,58}$ =5.34, $P = 0.02$; Table C; Fig. 2B). At longer-time scale fish from E_M and C_M groups displayed a constant level of exploration over the 10-day post exposure period (Fig. 3B). While, individuals from the E_H group returned to control level one week post-exposure, it was not the case for the E_M group ($F_{5,53} = 0.54$, $P = 0.75$; Table C, Fig. 3A & B). Additionally, in the medium treatment, differences between C and E fish were less prominent than in the high dose treatment. Finally, over the 20-min test-time, individuals from the low treatment (C_L and E_L) displayed similarly increasing exploratory activity (Fig. 2C) with no change in their overall level of exploratory activity over the 10-day, post-exposure monitoring period ($F_{5,174} = 0.48$, $P = 0.79$; Table D; Fig. 3C).

Table 2
Axes description of the principal component (PC) analysis.

	Variables	PC1 Exploratory activity	PC2	PC3
Eigenvalue		2.57	0.41	0.02
Percentage of variance		85.54	13.73	0.72
Cumulative percentage of variance		85.54	99.27	100.00
Loading	*Tswim*	**0.86**		
	Dmoved	**0.99**		
	Velocity	**0.92**		

3.5. Time spent in the central zone

In the treatment H, fish from the C_H group displayed a constant use of the central zone over the 20 min of the OFT whereas E_H fish showed a downward trend over the same period ($F_{1,106} = 10.36$, $P < 0.01$; Table E; Fig. 4A). At longer time scale (Fig. 5A) fish from E_H group spent more time in the central zone than fish from the corresponding control groups C_H during the first three days post-exposure ($F_{5,24} = 6.31$, $P < 0.01$; Table E; Fig. 5A). By the end of the test, however, *TZoneC* measured in E_H fish was back to control level (Fig. 5A). In the M treatment, we found that E_M fish spent more time in the central zone than C_M individuals ($F_{1,58} = 6.11$, $P = 0.02$; Table F; Fig. 4B & 5B). While, fish from E_M group spent significantly more time in the central zone than fish from the C_M group, they displayed a lower *TZoneC* than individuals from the E_H group (Fig. 4A & B). Additionally, for both C_M and E_M groups the use of the central zone remained constant over the 20-min test-time in the OFT ($F_{1,179} = 0.13$, $P = 0.72$; Table F; Fig. 4B). Furthermore, C_M and E_M fish displayed stable *TZoneC* over the 10 days, post-exposure monitoring period ($F_{5,53} = 0.33$, $P = 0.89$; Table F; Fig. 5B). In the L treatment, the use of the central zone did not differ between C_L and E_L groups ($F_{1,58} = 0.55$, $P = 0.46$; Table G) and remained constant over time for both groups ($F_{1,174} = 0.03$, $P = 0.87$; Fig. 4C). Moreover, no difference was observed over the different tested days post-exposure ($F_{5,175} = 1.22$, $P = 0.30$; Fig. 5C).

Observation of the complete dataset reveals that over the duration of the experiment (treatments H, M and L; February, March and April respectively), fish exploration was decreased (i.e., exploratory activity and *TZoneC*; Fig. 3 & 5). A progressive decrease in the behavioural response to the open field test was indeed, observable when comparing exploration from the different control groups. Individuals from all control groups displayed a similar increasing exploration patterns during the 20-min test-time in the OFT, however, exploration of fish from C_M and C_L groups was lower than in the H treatment. A parallel can be made between this difference in behavioural response between treatment conditions and the reduction in the extent of the behavioural changes due to the exposure to different oil + dispersant mixtures. In comparison to their corresponding control groups (C_M and C_H), exploration of E_M fish was less affected than E_H individuals.

4. Discussion

In the last decade there has been an increasing interest in understanding behavioural, ecological and evolutionary consequences of exposure to environmental toxicants (Bautista et al., 2019; Gerhardt, 2007; Khursigara et al., 2019; Scott and Sloman, 2004). Crude oil is a pervasive toxicant for marine organisms, known to cause mortality and a range of sub-lethal dysfunctions (e.g. of review, Pasparakis et al., 2019). However, the ensuing ecological significance of these effects remains uncertain, especially because the capacity of fish to recover from toxicant exposure is still poorly documented (Hicken et al., 2011; Johansen and Esbaugh, 2017; Mager et al., 2014; Mauduit et al., 2016). Present results suggested that dispersant treated oil affects juvenile sea bass behaviour in a dose-response manner. Although no difference with the control group was observed at the low dose (0.2 g L^{-1} oil + 0.0025 g L^{-1} dispersant), fish exposed to the medium (0.4 g L^{-1} oil + 0.005 g L^{-1} dispersant) and highest (0.8 g L^{-1} oil + 0.01 g L^{-1} dispersant) doses displayed altered exploratory activity and spent more time in the central, most exposed, zone of the open field. We also observed that fish displayed some recovery capacity over a period of 10 days post-exposure.

4.1. Exposure condition and dose-dependent effect

Fish exploratory activity and time spent in the central zone (*TZoneC)* in the control groups seemed to decrease over the span of the experiment. For instance, individuals from the C_H fish group were more active

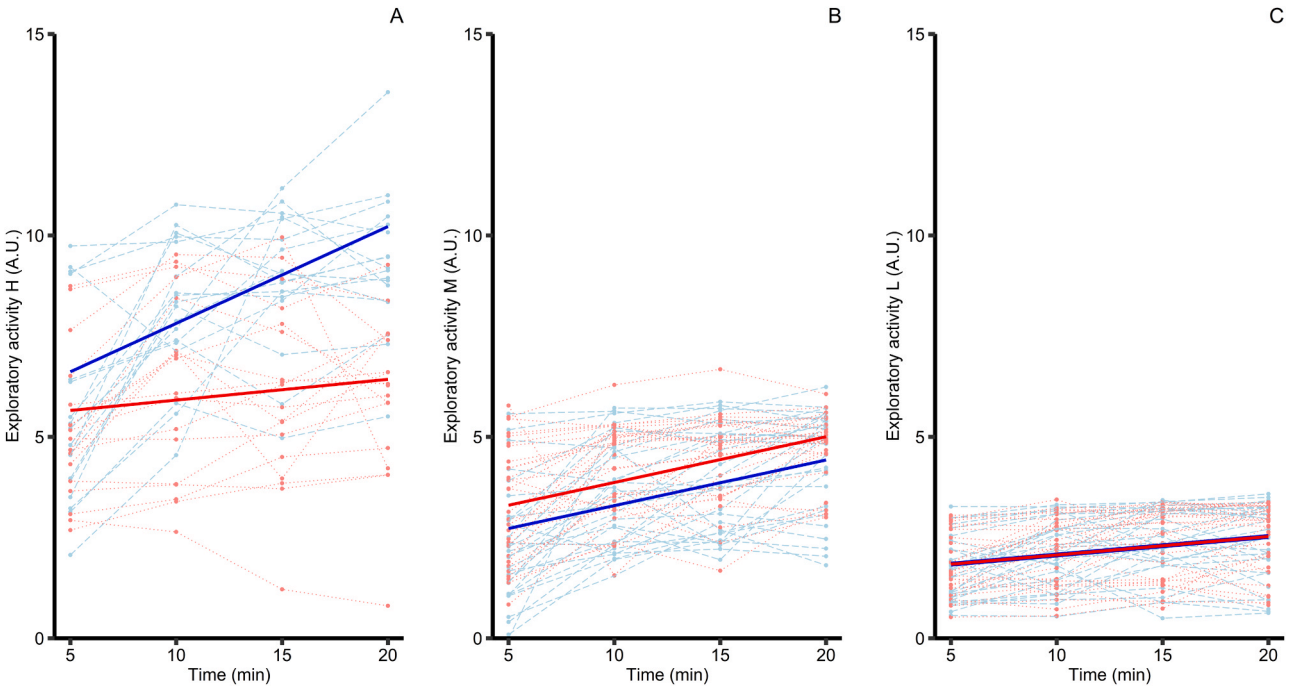

Fig. 2. Exploratory pattern over time in the open field test according to the dose treatment exposure. Blue: control fish; red: exposed fish; Solid lines: predicted models; dashed lines: individual changes in exploratory activity of control fish; dotted lines: individual changes in exploratory activity of exposed fish. A) H: high dose. B) M: medium dose. C) L: low dose. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

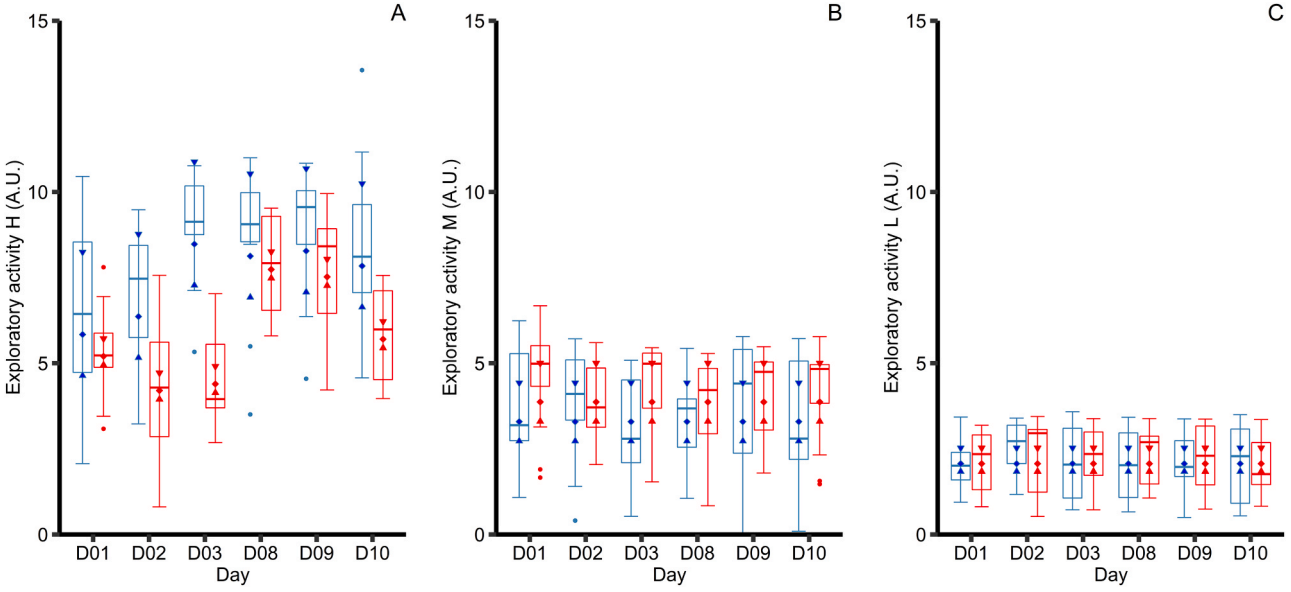

Fig. 3. Exploratory activity over post-exposure days 1, 2, 3, 8, 8, 9 and 10 according to the dose treatment exposure. Blue: control fish; red: exposed fish; Δ: predicted value after 5 min in the open field test (OFT)◇ predicted value after 10 min in the OFT; ▽: predicted value after 20 min in the OFT. The boundary of the box closest to zero indicates the 25th percentile, a black line within the box marks the median and the boundary of the box farthest from zero indicates the 75th percentile. 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. A) H: high dose. B) M: medium dose. C) L: low dose. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

than those from C_M, themselves showing higher exploration activity than fish from the C_L group. As the experiment lasted over a three months period, level of the behavioural response may have been influenced by different factors. Although the three sets of experiments were conducted at the same temperature, photoperiod-driven changes in fish physiological status or differences in age may have influenced fish behavioural responses (Lanteri et al., 2016; Oppedal et al., 2007; Veras et al., 2013). Therefore, the results suggest a dose-response relationship that could be a combined result of the exposure dose and time of the year. To investigate these potential effects, each treatment group (doses of crude oil exposure) has been compared to the corresponding control group to evaluate the effect of each dose.

To characterize exposure conditions, water concentration in total petroleum hydrocarbon ([TPH]) was monitored in all tanks and

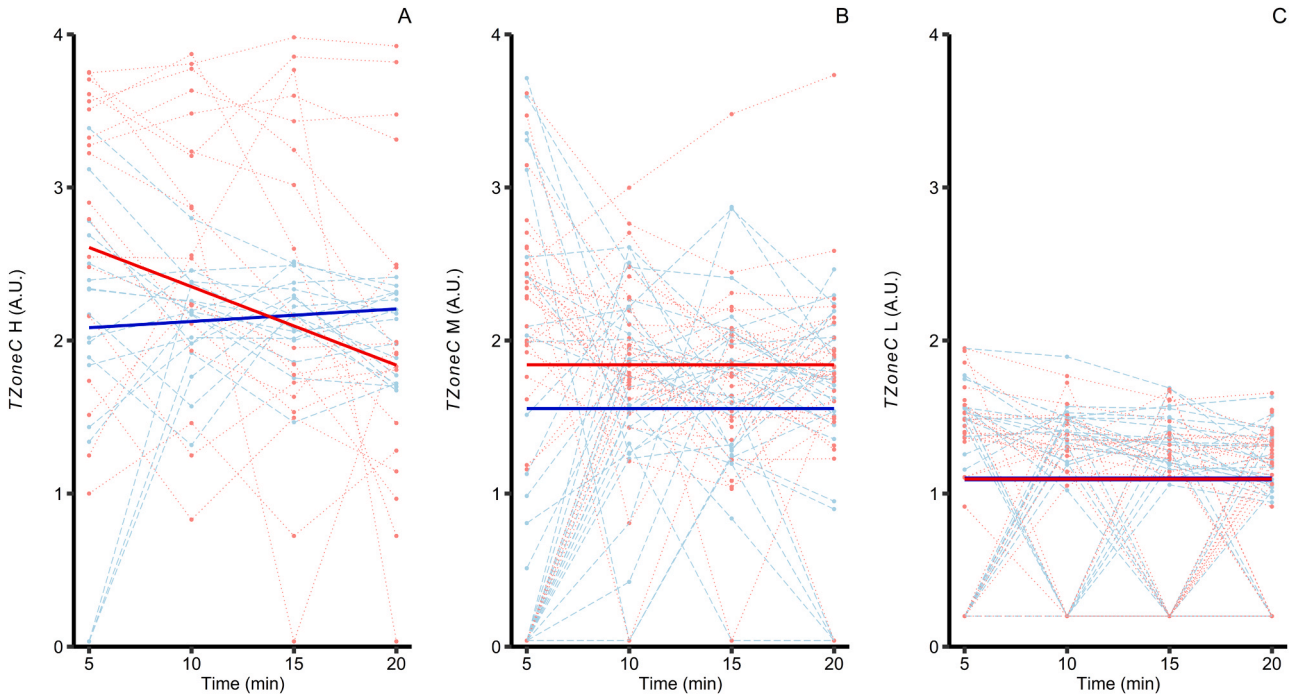

Fig. 4. Time spent in the central zone (*TZoneC*) over the 20 min of the open field test according to the dose treatment exposure. Blue: control fish; red: exposed fish. Solid lines: predicted models; dashed lines: trends of control individuals; dotted lines: trends of exposed individuals. A) H: high dose. B) M: medium dose. C) L: low dose. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

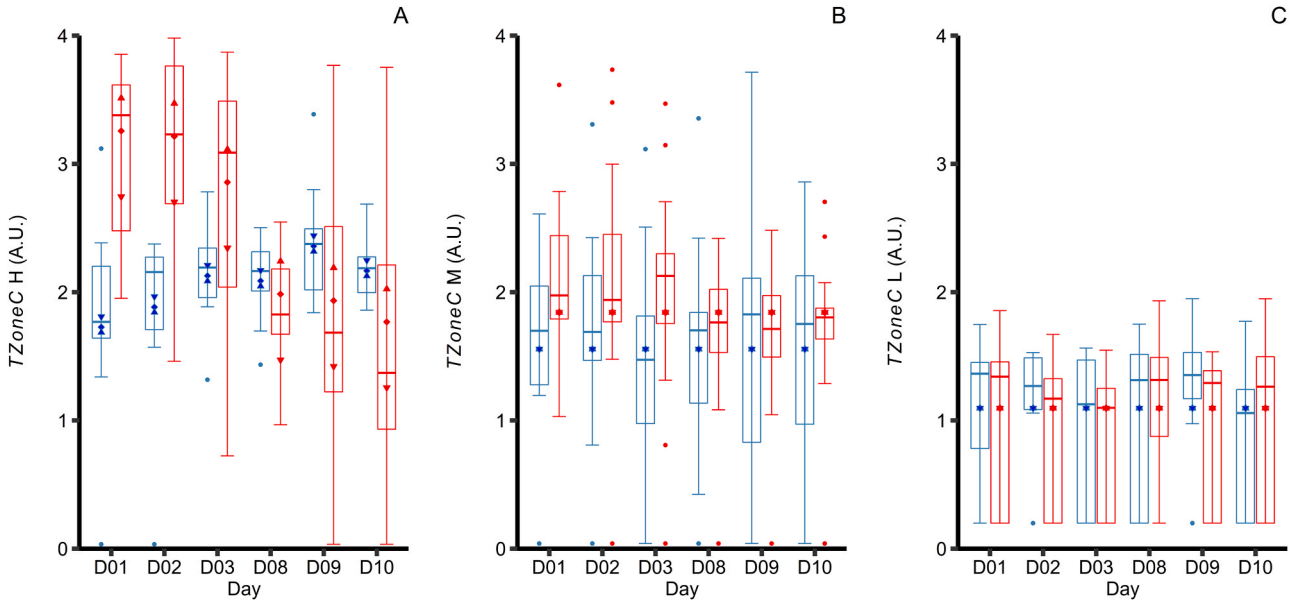

Fig. 5. Time spent in the central zone (*TZoneC*) over the 20 min of the open field test according to the dose treatment exposure. Blue: control fish; red: exposed fish; Δ: predicted value after 5 min in the open field test (OFT)◇ predicted value after 10 min in the OFT; Δ: predicted value after 20 min in the OFT. The boundary of the box closest to zero indicates the 25th percentile, a black line within the box marks the median and the boundary of the box farthest from zero indicates the 75th percentile. 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. A) H: high dose. B) M: medium dose. C) L: low dose. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

throughout fish exposure period. The high exposure condition (E_H) resulted in [TPH] (0.940 g L^{-1}) in the order of three times those classically considered as severe in such instances, although concentrations up to 11.4 g L^{-1} have been reported (Sammarco et al., 2013). In contrast, low (E_L: 0.048 g L^{-1}) and medium (E_M: 0.243 g L^{-1}) exposure conditions bracketed the range of situations that fish are liable to encounter following an oil spill and its treatment with dispersant (0.001–0.260 g L^{-1}; Kim et al., 2010; Sammarco et al., 2013; Spooner, 1970). Liver polycyclic aromatic hydrocarbon concentrations (PAH) measured in fish from E_L, E_M and E_H conditions were respectively 1.4, 2.4 and 9.8 times higher than in fish from their respective C groups (135.5 ng g^{-1} dw). In accordance with previous findings, behavioural alterations showed a clear dose-response relationship to oil exposure (Incardona et al., 2004; Rowsey et al., 2019). Although, no difference

with the control group was observed at the low dose, fish exposed to the medium and high doses displayed altered exploratory activity and spent more time in the central, most exposed, zone than fish from the corresponding control groups. However, and as suggested above, the experiment spanning over 3 months, this dose-response relationship may have resulted from the combined effects of the dose of exposure and the time of year. The highest dose tested in the present study (E_H) is clearly beyond those classically reported following and accidental spill (but see Sammarco et al. (2013)). The reason for testing such a condition was to draw a response pattern that would have been difficult to establish at less critical doses. It then allowed us ascertaining the progressive attenuation of the responses in the E_M and E_L treatments. This also enabled us to ascertain that an absence of effect in E_L actually represented a true negative.

After 62 h of exposure, we observed a 71% mortality rate in the E_H condition (0.8 g L^{-1} of oil mixed with 0.01 g L^{-1} of chemical dispersant) while no mortality was observed in the control groups and very few in the E_M (n = 1) and E_L (n = 0) groups. To our knowledge, only Dussauze et al., 2013 investigated the LC50 for similar pollutant mixture in European sea bass. This study reported that the LC50 in 1 g sea bass for a 96 h exposure was ca. 0.1 g L^{-1}. On the other hand, other studies did not observed any mortality in 17 g sea bass, exposed during 48 h to a mixture similar to ours (Anttila et al., 2017; Zhang et al., 2017). The mortality that we observed in the E_H group was therefore unexpected and may be related to the lower body mass of the fish used in our study (9.05 g) compared to that of Anttila et al. (2017) and Zhang et al. (2017). This result is also consistent with the exponential relationship between pollutants toxicity and fish body mass reported by Hedtke et al. (1982). Moreover, the duration of the exposure was increased by nearly 30% in the present case compared to Anttila et al. (2017) and Zhang et al. (2017). This may have contributed to increase even further the toxicity of the E_H condition. It is important emphasise that due to the high mortality rate observed in the E_H conditions, it is possible that the experimented subpopulation did not contain the full scope of phenotypic diversity initially present in this group. Unfortunately, we were unable to properly address this issue.

4.2. Exploration tendency

In the present study, the index of exploratory activity combines the time spent swimming, the distance moved and the swimming speed measured during the open field test (OFT). Following their transfer into the experimental arena, fish from the control groups (C_H, C_M, C_L) displayed a continuous increase in exploratory activity (+129%) over the duration of OFT. Similarly, fish exposed to low (E_L) and medium (E_M) doses of dispersant treated oil showed an increasing pattern over the 20-min test-time. In contrast, however, fish exposed to the high dose (E_H) exhibited a distinctive exploration pattern characterised by an overall lower activity and a less pronounced increase in exploratory activity (+40%) over the same time.

A progressively increasing locomotors activity following transfer into a novel environment has been suggested to reflect the gradual decrease in anxiety resulting from appropriation (Lister, 1990). It is believed that this increased exploration activity allows fish to gather information about the novel environment, its resources and risks (Archer and Birke, 1983; Renner, 1990; Laland and Reader, 1999; Champagne et al., 2010; Griffin and Guez, 2014; Reader, 2015; Jacquin et al., 2017). Thus, alteration of this behavioural process, even temporarily, is likely to affect individuals' capacity to rapidly tune the balance between risk taking and opportunity searching in the new environment, with potential consequences on fitness.

When exploring a new territory, fish typically display avoidance for open areas as they are associated with higher risk, in particular of encountering a predator with no solution to hide or escape (Brown and Nemes, 2008; Champagne et al., 2010; Dahlbom et al., 2011; Maximino et al., 2010; Perals et al., 2017; Sousa et al., 2006). Accordingly, although control sea bass increased their swimming activity over time, they remained close to the walls of the experimental arena, spending only ca. 2% of their time in the central zone of the arena. In many animals, including fish, thigmotaxis (i.e. wall-hugging) is considered as an anxiety indicator (Brown and Nemes, 2008; Champagne et al., 2010; Maximino et al., 2010; Sousa et al., 2006). At the beginning of the OFT, because of the size of the confinement chambers and the way they were positioned, fish always entered the arena in the central zone. Control individuals rapidly perceived the situation and swiftly swam to a corner or to a wall of the arena. As shown by the E_H group, oil-exposed fish tended to remain longer in the centre of the arena before moving towards corners or walls of the arena (Fig. 4A). However, over time risk perception was partially restored as indicated by the progressive drop in *TZoneC* observed from day 0 to day 8 post exposure (Fig. 5A). While, fish from the E_L group did not differ from the control pattern, fish exposed to E_M and E_H treatments spent respectively 10% and 32% more time in the central risky zone of the arena than corresponding control individuals (Fig. 4A & B). These results are consistent with those found in other species. For instance, Rowsey et al. (2019) reported an increase in the time spent in the exposed area of an experimental arena in oil-exposed larvae of *S. oscellatus*. However, this increase was also associated to a higher percentage of area explored and it was therefore interpreted as an increase in risk taking (Rowsey et al., 2019). In our study, exploratory activity of E_H fish was reduced compared to the corresponding control group, suggesting that the increase in *TZoneC* observed in E_H fish did not indicate increased inclination for risk-taking. It is noteworthy that altered risk perception, as well as reduced swimming ability and proficiency at manoeuvring, have been related to loss of sensory capacity (e.g. Stewart et al., 2017). In our study, oil-exposed fish were found to swim in an anaesthetic-like sedative behavioural state i.e., slowness, with a head-up swimming posture. This was particularly observed in the E_H group during the first three days post-exposure. Behavioural disruptions were described as fish "drifting" across the experimental arena rather than showing a voluntary exploration of the novel environment. This additional observation strengthens the idea that the higher *TZoneC* may be related to defects in cognitive or sensory systems rather than to an increase in risk taking.

Recent studies have highlighted that crude oil can disrupt visual and olfactory systems, hence reducing fish capacity to collect sensory information (Cave and Kajiura, 2018; Colavecchia et al., 2007; Magnuson et al., 2020, 2018; Martin et al., 2020; Schlenker et al., 2019; Xu et al., 2018). Observed modification in exposed fish exploratory behaviour could be associated to these impairments. However, Johansen et al. (2017) and Rowsey et al. (2019) suggest that similar increase in risk taking may results from impaired brain processing of the sensory information and decision making process.

Another known effect of crude oil compounds is non-polar narcosis which affects neuronal cells and disrupts central nervous system function and signalling pathways (Barron et al., 2004; Gonçalves et al., 2008; Hsieh et al., 2006; van Brummelen et al., 1998; van Wezel and Opperhuizen, 1995). In fish, a narcosis state has been associated to altered capacities to acquire and process information from the surrounding environment, resulting in reduced consciousness, general slowness, sleepiness, disorientation and impaired coordination and swimming (Barron et al., 2004; Gerlai et al., 2000; Vignet et al., 2014). While, this mode of toxicity has been related to low-molecular-weight, 2- ring, polycyclic aromatic hydrocarbon (PAH) (Stieglitz et al., 2016; van Brummelen et al., 1998), compounds found in the sea bass liver consisted primarily in higher molecular weight molecules. Narcosis may therefore not be the main mode of toxicity for the observed behavioural impairments. The tricyclic PAH (flurorene, phenantrene, dibenzothiophene) represented 70–80% of the total PAH in our sea bass liver. This larger fraction of tricyclic PAH molecules is most likely resulting from the weathering process which enhanced evaporation of lower-molecular-weight compounds (Carls et al., 1999; Heintz et al., 1999; Short and Heintz, 1997). Toxicity of higher molecular weight

constituents such as tricyclic PAH, has been found to drive mortality and sub-lethal cardiac impairment (Esbaugh et al., 2016; Incardona et al., 2011, 2004). An extensive amount of work has reported alterations of aerobic scope and swimming performance suggested to be associated to cardiac impairments (Brette et al., 2017; Davoodi and Claireaux, 2007; Johansen and Esbaugh, 2017; Mager et al., 2014; Mauduit et al., 2016; Nelson et al., 2016, 2017). Yet, other impairments than cardiorespiratory injuries might also influence physiological and behavioural alterations induced by crude oil exposure. Indeed, other studies showed uncoupling effect of crude oil exposure upon swimming performance and aerobic scope (Johansen and Esbaugh, 2017; Mager et al., 2014).

Regardless of the underlying mechanism, it is crucial to better understand the ecological consequences of crude oil exposure. Our results are consistent with those found in other fish species following exposure to oil compounds (Vignet et al., 2014; Jacquin et al., 2017; Rowsey et al., 2019; Johansen et al., 2017). For instance, an exposure to pyrene, fluorene and phenanthrene, which are among the PAH present in our mixture (Table A), has been shown to decrease exploration tendency i.e., altered and riskier habitat settlement, reduced number of zone explored, less success in reaching the farthest area of a maze, less time spent in the upper water layer of a tank and less time spent swimming (Jacquin et al., 2017; Johansen et al., 2017; Vignet et al., 2014). Such behavioural impairments may have important fitness consequences such as increased predator-induced mortality and reduced prey-capture ability (Johansen et al., 2017; Rowsey et al., 2019). The relevance of sub-lethal toxicity endpoints stems from the principle that sub-lethal injuries lead to ecological consequences (Ankley et al., 2010). In the present study, we have demonstrated that dispersant treated oil exposure has the capacity to disrupt exploratory activity and to induce risk-taking behaviours, which can carry a suite of fitness consequences such as increased predation-induced mortality. Furthermore, our results brings new insight in the interpretation of these behavioural disruptions. Present study suggests alterations to perception or cognition rather than a simple increase in risk-taking. In accordance with Jacquin et al. (2020) these results strengthen the hypothesis that behaviour and cognition can be linked in syndromes including as well physiology and fitness which could be disrupted by pollutants.

4.3. Recovery capacities

Our results suggest partial recovery of exploration and risk-taking within the first 10 days following the exposure. As illustrated on Figs. 3 and 5, exploratory activity and *TZoneC* of E_H fish are primarily altered during the first three days post-exposure and became closer to those of control fish at day 8 post-exposure. This result shows that fish do have recovery capacities (although not complete by day 10 post-exposure). As discussed previously E_H fish displayed the greatest behavioural alterations with high presence in the central zone and reduced exploratory activity. Moreover, the inter-individual variance in *TZoneC* of the E_H group was particularly high (Fig. 4A) and we found no sign of recovery for this high inter-individual variance over the 10 testing-days (Fig. 5A). This supports the idea that E_H fish were still suffering from impaired behaviour after 10 days post-exposure. This idea of incomplete recovery of E_H fish behaviours is strengthen by the absence of recovery in E_M fish, exposed to a lower dose of dispersant treated oil than E_H individuals. Thus, while the level of exploratory activity and *TZoneC* displayed by E_H fish is becoming closer to C_H individuals over the 10 days post-exposure, exposed individuals may need more time to fully recover under such harsh condition.

In accordance with previous research on crude oil exposure, our findings suggest that the effects of crude oil on fish performances and their consequences on individual's fitness can last well past the time of initial damage (Hicken et al., 2011; Johansen and Esbaugh, 2017; Mager et al., 2014; Mauduit et al., 2016). Johansen and Esbaugh (2017) notably reported alteration of the aerobic scope that persisted at least 6 weeks after a 24 h exposure to crude oil. Furthermore, oil spills can produce scenarios where impaired individuals must interact with unaffected fish, placing exposed fish at a competitive disadvantage. For instance, a recent study from Khursigara et al. (2019) has shown that crude oil exposure induced a competitive disadvantage in red drum, exposed individuals being more likely to be subordinate relative than non- or less-exposed individuals. While, the duration of the effects of acute oil exposure remains unclear, its effect on individuals' competitive abilities and resilience to environmental stressors, is still a crucial concern as it can lead to a decline in fitness.

It is possible that fish recovery is linked to tissues detoxification. Unfortunately we could not test this hypothesis as liver concentration in toxicants was only measured one day post-exposure and we therefore lack information about the time course of the detoxification process. Other studies are therefore needed to investigate simultaneously individuals' behavioural recovery and their tissues' detoxification.

5. Conclusion

Our results reinforce current literature showing that individuals' behaviour is altered by the presence of oil in the water, and we further show that fish can recover, at least partially, following exposure. Exploration is an important aspect of animal behaviour, allowing the acquisition of information about the environment and the mapping of potential habitats. Knowledge outcome from exploratory behaviour can be used in the search of resources, such as food, or to avoid risky situations. In addition, information gathering has been linked to problem solving capacity, learning and behavioural innovation, all predictive of animal cognitive performance (Archer and Birke, 1983; Griffin and Guez, 2014; Jacquin et al., 2017; Laland and Reader, 1999; Reader, 2015; Renner, 1990). Reduced exploratory activity and increased time exposure in the open area of the novel environment suggest impairments of fish cognitive performances that can particularly be linked to foraging and predator avoidance. Despite signs of recovery, the harshest and medium conditions altered juvenile sea bass exploration tendency for more than 10 days post-exposure. Such altered exploratory behaviour following oil exposure could therefore affect fish environmental use and have major fitness consequences.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. C. Aimon was supported by a joint CEDRE-University of Bretagne Occidentale doctoral grant.

CRediT authorship contribution statements

C.A., G.C. and S.L.F. conceived the research; C.A., G.C. and N.L.B. designed the experiments; C.A. conducted the experiments; C.A. and C.L. performed statistical analyses; C.A. prepared the initial manuscript and all authors contributed to later revisions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors would like to thanks M. Hervy (Aquativ) for providing the fish as well as the members of the PFOM-ARN laboratory (Ifremer) for their technical assistance. We also thank Luana Albert for her assistance with fish maintenance and laboratory work. This article is dedicated to Nicolas Le Bayon who passed away while this article was in preparation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111592.

References

Aas, E., Beyer, J., Goksoyr, A., 2000. Fixed wavelength fluorescence (FF) of bile as a monitoring tool for polyaromatic hydrocarbon exposure in fish: an evaluation of compound specificity, inner filter effect and signal interpretation. Biomarkers 5, 9–23. https://doi.org/10.1080/135475000230505.

Aimon, C., Le Bayon, N., Le Floch, S., Claireaux, G., 2019. Food deprivation reduces social interest in the European sea bass Dicentrarchus labrax. J. Exp. Biol. 222, 190553 https://doi.org/10.1242/jeb.190553.

Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J. E., Villeneuve, D.L., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29, 730–741. https://doi.org/10.1002/etc.34.

Anttila, K., Mauduit, F., Le Floch, S., Claireaux, G., Nikinmaa, M., 2017. Influence of crude oil exposure on cardiac function and thermal tolerance of juvenile rainbow trout and European sea bass. Environ. Sci. Pollut. Res. 24, 19624–19634. https://doi.org/10.1007/s11356-017-9609-x.

Archer, J., Birke, L.I.A., 1983. Exploration in Animals and Humans. Van Nostrand Reinhold, UK.

Barron, M.G., Carls, M.G., Heintz, R., Rice, S.D., 2004. Evaluation of fish early life-stage toxicity models of chronic embryonic exposures to complex polycyclic aromatic hydrocarbon mixtures. Toxicol. Sci. 78, 60–67. https://doi.org/10.1093/toxsci/kfh051.

Bautista, N.M., Pothini, T., Meng, K., Burggren, W.W., 2019. Behavioral consequences of dietary exposure to crude oil extracts in the Siamese fighting fish (Betta splendens). Aquat. Toxicol. 207, 34–42. https://doi.org/10.1016/j.aquatox.2018.11.025.

Beyer, J., Trannum, H.C., Bakke, T., Hodson, P.V., Collier, T.K., 2016. Environmental effects of the Deepwater Horizon oil spill: a review. Mar. Pollut. Bull. 110, 28–51. https://doi.org/10.1016/j.marpolbul.2016.06.027.

Birol, F., 2015. Changes in global energy: will they be led by policies or driven by events? Annales des Mines - Responsabilite et Environnement, pp. 21–24. N° 77.

Brette, F., Shiels, H.A., Galli, G.L.J., Cros, C., Incardona, J.P., Scholz, N.L., Block, B.A., 2017. A novel cardiotoxic mechanism for a pervasive global pollutant. Sci. Rep. 7, 41476. https://doi.org/10.1038/srep41476.

Brown, G.R., Nemes, C., 2008. The exploratory behaviour of rats in the hole-board apparatus: is head-dipping a valid measure of neophilia? Behav. Process. 78, 442–448. https://doi.org/10.1016/j.beproc.2008.02.019.

Carls, M.G., Holland, L., Larsen, M., Collier, T.K., Scholz, N.L., Incardona, J.P., 2008. Fish embryos are damaged by dissolved PAHs, not oil particles. Aquat. Toxicol. 88, 121–127. https://doi.org/10.1016/j.aquatox.2008.03.014.

Carls, M.G., Rice, S.D., Hose, J.E., 1999. Sensitivity of fish embryos to weathered crude oil: Part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval pacific herring (Clupea pallasi). Environ. Toxicol. Chem. 18, 481–493. https://doi.org/10.1002/etc.5620180317.

Cave, E.J., Kajiura, S.M., 2018. Effect of deepwater horizon crude oil water accommodated fraction on olfactory function in the Atlantic Stingray, Hypanus sabinus. Sci. Rep. 8, 15786. https://doi.org/10.1038/s41598-018-34140-0.

Champagne, D.L., Hoefnagels, C.C., de Kloet, R.E., Richardson, M.K., 2010. Translating rodent behavioral repertoire to zebrafish (Danio rerio): relevance for stress research. Behav. Brain Res. 214, 332–342. https://doi.org/10.1016/j.bbr.2010.06.001.

Cherr, G.N., Fairbairn, E., Whitehead, A., 2017. Impacts of petroleum-derived pollutants on fish development. Annu. Rev. Anim. Biosci. 5, 185–203. https://doi.org/10.1146/annurev-animal-022516-022928.

Claireaux, G., Théron, M., Prineau, M., Dussauze, M., Merlin, F.-X., Le Floch, S., 2013. Effects of oil exposure and dispersant use upon environmental adaptation performance and fitness in the European sea bass, Dicentrarchus labrax. Aquat. Toxicol. 130–131, 160–170. https://doi.org/10.1016/j.aquatox.2013.01.004.

Colavecchia, M.V., Hodson, P.V., Parrott, J.L., 2007. The relationships among CYP1A induction, toxicity, and eye pathology in early life stages of fish exposed to oil sands. J. Toxicol. Environ. Health Part A 70, 1542–1555. https://doi.org/10.1080/15287390701384726.

Dahlbom, S.J., Lagman, D., Lundstedt-Enkel, K., Sundström, L.F., Winberg, S., 2011. Boldness predicts social status in zebrafish (Danio rerio). PLoS One 6, e23565. https://doi.org/10.1371/journal.pone.0023565.

Davoodi, F., Claireaux, G., 2007. Effects of exposure to petroleum hydrocarbons upon the metabolism of the common sole Solea solea. Mar. Pollut. Bull. 54, 928–934. https://doi.org/10.1016/j.marpolbul.2007.03.004.

Dell'Omo, G., 2002. Behavioural Ecotoxicology. John Wiley & Sons.

Dussauze, M., Le Floch, N., Le Floch, S., Merlin, F., Théron, M., Pichavant-Rafini, K., 2013. Toxicity of oil-dispersant mixtures on juveniles sea bass (Dicentrarchus labrax). Proceedings of the 36th AMOP Technical Seminar on Environmental Contamination and Response 422–432.

Esbaugh, A.J., Mager, E.M., Stieglitz, J.D., Hoenig, R., Brown, T.L., French, B.L., Linbo, T. L., Lay, C., Forth, H., Scholz, N.L., Incardona, J.P., Morris, J.M., Benetti, D.D., Grosell, M., 2016. The effects of weathering and chemical dispersion on Deepwater Horizon crude oil toxicity to mahi-mahi (Coryphaena hippurus) early life stages. Sci. Total Environ. 543, 644–651. https://doi.org/10.1016/j.scitotenv.2015.11.068.

Fodrie, F.J., Able, K.W., Galvez, F., Heck, K.L., Jensen, O.P., López-Duarte, P.C., Martin, C.W., Turner, R.E., Whitehead, A., 2014. Integrating organismal and population responses of estuarine fishes in Macondo spill research. BioScience 64, 778–788. https://doi.org/10.1093/biosci/biu123.

Frapiccini, E., Annibaldi, A., Betti, M., Polidori, P., Truzzi, C., Marini, M., 2018. Polycyclic aromatic hydrocarbon (PAH) accumulation in different common sole (Solea solea) tissues from the North Adriatic Sea peculiar impacted area. Mar. Pollut. Bull. 137, 61–68. https://doi.org/10.1016/j.marpolbul.2018.10.002.

Fusey, P., Oudot, J., 1976. Comparaison de deux méthodes d'évaluation de labiodégradation des hydrocarbures in vitro. Mater. Org. 241–251.

Gerhardt, A., 2007. Aquatic behavioral ecotoxicology—prospects and limitations. Hum. Ecol. Risk Assess. Int. J. 13, 481–491. https://doi.org/10.1080/10807030701340839.

Gerlai, R., Lahav, M., Guo, S., Rosenthal, A., 2000. Drinks like a fish: zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects. Pharmacol. Biochem. Behav. 67, 773–782. https://doi.org/10.1016/S0091-3057(00)00422-6.

Gonçalves, R., Scholze, M., Ferreira, A.M., Martins, M., Correia, A.D., 2008. The joint effect of polycyclic aromatic hydrocarbons on fish behavior. Environ. Res. 108, 205–213. https://doi.org/10.1016/j.envres.2008.07.008.

Griffin, A.S., Guez, D., 2014. Innovation and problem solving: a review of common mechanisms. Behav. Processes 109, 121–134. https://doi.org/10.1016/j.beproc.2014.08.027.

Hedtke, J.L., Robinson-Wilson, E., Weber, L.J., 1982. Influence of body size and developmental stage of coho salmon (Oncorhynchus kisutch) on lethality of several toxicants. Fundam. Appl. Toxicol. 2, 67–72.

Heintz, R.A., Short, J.W., Rice, S.D., 1999. Sensitivity of fish embryos to weathered crude oil: Part II. Increased mortality of pink salmon (Oncorhynchus gorbuscha) embryos incubating downstream from weathered Exxon valdez crude oil. Environ. Toxicol. Chem. 18, 494–503. https://doi.org/10.1002/etc.5620180318.

Hicken, C.E., Linbo, T.L., Baldwin, D.H., Willis, M.L., Myers, M.S., Holland, L., Larsen, M., Stekoll, M.S., Rice, S.D., Collier, T.K., Scholz, N.L., Incardona, J.P., 2011. Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. Proc. Natl. Acad. Sci. USA 108, 7086–7090. https://doi.org/10.1073/pnas.1019031108.

Hsieh, S.-H., Tsai, K.-P., Chen, C.-Y., 2006. The combined toxic effects of nonpolar narcotic chemicals to Pseudokirchneriella subcapitata. Water Res. 40, 1957–1964. https://doi.org/10.1016/j.watres.2006.03.026.

Incardona, J.P., Collier, T.K., Scholz, N.L., 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. Toxicol. Appl. Pharmacol. 196, 191–205. https://doi.org/10.1016/j.taap.2003.11.026.

Incardona, J.P., Linbo, T.L., Scholz, N.L., 2011. Cardiac toxicity of 5-ring polycyclic aromatic hydrocarbons is differentially dependent on the aryl hydrocarbon receptor 2 isoform during zebrafish development. Toxicol. Appl. Pharmacol. 257, 242–249. https://doi.org/10.1016/j.taap.2011.09.010.

Incardona, J.P., Gardner, L.D., Linbo, T.L., Brown, T.L., Esbaugh, A.J., Mager, E.M., Stieglitz, J.D., French, B.L., Labenia, J.S., Laetz, C.A., Tagal, M., Sloan, C.A., Elizur, A., Benetti, D.D., Grosell, M., Block, B.A., Scholz, N.L., 2014. Deepwater Horizon crude oil impacts the developing hearts of large predatory pelagic fish. Proc. Natl. Acad. Sci. USA 111, E1510–E1518. https://doi.org/10.1073/pnas.1320950111.

International Tanker Owners Pollution Federation, 2017. The International Tanker Owners Pollution Federation Limited Oil Tanker Spill Statistics 2017.

Jacquin, L., Dybwad, C., Rolshausen, G., Hendry, A.P., Reader, S.M., 2017. Evolutionary and immediate effects of crude-oil pollution: depression of exploratory behaviour across populations of Trinidadian guppies. Anim. Cogn. 20, 97–108. https://doi.org/10.1007/s10071-016-1027-9.

Jacquin, L., Petitjean, Q., Cote, J., Laffaille, P., Jean, S., 2020. Effects of pollution on fish behavior, personality, and cognition : some research perspectives. Front. Ecol. Evol. 8. https://doi.org/10.3389/fevo.2020.00086.

Johansen, J.L., Allan, B.J.M., Rummer, J.L., Esbaugh, A.J., 2017. Oil exposure disrupts early life-history stages of coral reef fishes via behavioural impairments. Nat. Ecol. Evol. 1, 1146. https://doi.org/10.1038/s41559-017-0232-5.

Johansen, J.L., Esbaugh, A.J., 2017. Sustained impairment of respiratory function and swim performance following acute oil exposure in a coastal marine fish. Aquat. Toxicol. 187, 82–89. https://doi.org/10.1016/j.aquatox.2017.04.002.

Kaiser, H.F., 1961. A note on Guttman's lower bound for the number of common factors. Br. J. Stat. Psychol. 14, 1–2. https://doi.org/10.1111/j.2044-8317.1961.tb00061.x.

Khursigara, A.J., Ackerly, K.L., Esbaugh, A.J., 2019. Oil toxicity and implications for environmental tolerance in fish. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 220, 52–61. https://doi.org/10.1016/j.cbpc.2019.03.003.

Kim, M., Yim, U.H., Hong, S.H., Jung, J.-H., Choi, H.-W., An, J., Won, J., Shim, W.J., 2010. Hebei Spirit oil spill monitored on site by fluorometric detection of residual oil in coastal waters off Taean, Korea. Mar. Pollut. Bull. 60, 383–389. https://doi.org/10.1016/j.marpolbul.2009.10.015.

Krahn, M.M., Burrows, D.G., MacLeod, W.D., Malins, D.C., 1987. Determination of individual metabolites of aromatic compounds in hydrolyzed bile of English sole (Parophrys vetulus) from polluted sites in Puget Sound, Washington. Arch. Environ. Contam. Toxicol. 16, 511–522. https://doi.org/10.1007/BF01055807.

Lacroix, C., Le Cuff, N., Receveur, J., Moraga, D., Auffret, M., Guyomarch, J., 2014. Development of an innovative and "green" stir bar sorptive extraction–thermal desorption–gas chromatography–tandem mass spectrometry method for quantification of polycyclic aromatic hydrocarbons in marine biota. J. Chromatogr. A 1349, 1–10. https://doi.org/10.1016/j.chroma.2014.04.094.

Laland, K.N., Reader, S.M., 1999. Foraging innovation in the guppy. Anim. Behav. 57, 331–340. https://doi.org/10.1006/anbe.1998.0967.

Lanteri, G., Giardina, A., Arfuso, F., Rizzo, M., Giannetto, C., Piccione, G., 2016. Photic entrainment of daily rhythm pattern of locomotor activity in sea bass (Dicentrarcus labrax). Biol. Rhythm Res. 47, 69–76. https://doi.org/10.1080/09291016.2015.1084154.

Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Softw. 25, 1–18. https://doi.org/10.18637/jss.v025.i01.

Lin, E.L.C., Cormier, S.M., Torsella, J.A., 1996. Fish biliary polycyclic aromatic hydrocarbon metabolites estimated by fixed-wavelength fluorescence: comparison with HPLC-fluorescent detection. Ecotoxicol. Environ. Saf. 35, 16–23. https://doi.org/10.1006/eesa.1996.0077.

Lister, R.G., 1990. Ethologically-based animal models of anxiety disorders. Pharmacol. Ther. 46, 321–340. https://doi.org/10.1016/0163-7258(90)90021-S.

Little, E.E., Archeski, R.D., Flerov, B.A., Kozlovskaya, V.I., 1990. Behavioral indicators of sublethal toxicity in rainbow trout. Arch. Environ. Contam. Toxicol. 19, 380–385.

M. Carvalho, P.S., Costa B. Kalil, D.D., A. Novelli, G.A., D. Bainy, A.C., M. Fraga, A.P., 2008. Effects of naphthalene and phenanthrene on visual and prey capture endpoints during early stages of the dourado. Mar. Environ. Res. 66, 205–207. https://doi.org/10.1016/j.marenvres.2008.02.059.

Mager, E.M., Esbaugh, A.J., Stieglitz, J.D., Hoenig, R., Bodinier, C., Incardona, J.P., Scholz, N.L., Benetti, D.D., Grosell, M., 2014. Acute embryonic or juvenile exposure to deepwater horizon crude oil impairs the swimming performance of Mahi-Mahi (Coryphaena hippurus). Environ. Sci. Technol. 48, 7053–7061. https://doi.org/10.1021/es501628k.

Magnuson, J.T., Bautista, N.M., Lucero, J., Lund, A.K., Xu, E.G., Schlenk, D., Burggren, W.W., Roberts, A.P., 2020. Exposure to crude oil induces retinal apoptosis and impairs visual function in fish. Environ. Sci. Technol. 54, 2843–2850. https://doi.org/10.1021/acs.est.9b07658.

Magnuson, J.T., Khursigara, A.J., Allmon, E.B., Esbaugh, A.J., Roberts, A.P., 2018. Effects of Deepwater Horizon crude oil on ocular development in two estuarine fish species, red drum (Sciaenops ocellatus) and sheepshead minnow (Cyprinodon variegatus). Ecotoxicol. Environ. Saf. 166, 186–191. https://doi.org/10.1016/j.ecoenv.2018.09.087.

Martin, C.W., McDonald, A.M., Rieucau, G., Roberts, B.J., 2020. Previous oil exposure alters oil avoidance behavior in a common marsh fish, the Gulf Killifish Fundulus grandis. bioRxiv 2020.06.15.152413. https://doi.org/10.1101/2020.06.15.152413.

Mauduit, F., Domenici, P., Farrell, A.P., Lacroix, C., Le Floch, S., Lemaire, P., Nicolas-Kopec, A., Whittington, M., Zambonino-Infante, J.L., Claireaux, G., 2016. Assessing chronic fish health: an application to a case of an acute exposure to chemically treated crude oil. Aquat. Toxicol. 178, 197–208. https://doi.org/10.1016/j.aquatox.2016.07.019.

Maximino, C., de Brito, T.M., da Silva Batista, A.W., Herculano, A.M., Morato, S., Gouveia, A., 2010. Measuring anxiety in zebrafish: a critical review. Behav. Brain Res. 214, 157–171. https://doi.org/10.1016/j.bbr.2010.05.031.

Milinkovitch, T., Antognarelli, F., Lacroix, C., Marras, S., Satta, A., Le Floch, S., Domenici, P., 2019. The effect of hypoxia and hydrocarbons on the anti-predator performance of European sea bass (Dicentrarchus labrax). Environ. Pollut. 251, 581–590. https://doi.org/10.1016/j.envpol.2019.05.017.

Nelson, D., Heuer, R.M., Cox, G.K., Stieglitz, J.D., Hoenig, R., Mager, E.M., Benetti, D.D., Grosell, M., Crossley, D.A., 2016. Effects of crude oil on in situ cardiac function in young adult mahi-mahi (Coryphaena hippurus). Aquat. Toxicol. 180, 274–281. https://doi.org/10.1016/j.aquatox.2016.10.012.

Nelson, D., Stieglitz, J.D., Cox, G.K., Heuer, R.M., Benetti, D.D., Grosell, M., Crossley, D.A., 2017. Cardio-respiratory function during exercise in the cobia, Rachycentron canadum: the impact of crude oil exposure. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 201, 58–65. https://doi.org/10.1016/j.cbpc.2017.08.006.

Milinkovitch, T., Ndiaye, A., Sanchez, W., Le Floch, S., Thomas-Guyon, H., 2011. Liver antioxidant and plasma immune responses in juvenile golden grey mullet (Liza aurata) exposed to dispersed crude oil. Aquat. Toxicol. 101, 155–164. https://doi.org/10.1016/j.aquatox.2010.09.013, 1.

Nelson, J.A., Claireaux, G., 2005. Sprint swimming performance of juvenile European sea bass. Trans. Am. Fish. Soc. 134, 1274–1284. https://doi.org/10.1577/T04-087.1.

Nordvik, A.B., 1995. The technology windows-of-opportunity for marine oil spill response as related to oil weathering and operations. Spill Science & Technology Bulletin 2, 1. https://doi.org/10.1016/1353-2561(95)00013-T.

Oppedal, F., Juell, J.-E., Johansson, D., 2007. Thermo- and photoregulatory swimming behaviour of caged Atlantic salmon: implications for photoperiod management and fish welfare. Aquaculture 265, 70–81. https://doi.org/10.1016/j.aquaculture.2007.01.050.

Pasparakis, C., Esbaugh, A.J., Burggren, W., Grosell, M., 2019. Physiological impacts of Deepwater Horizon oil on fish. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 224, 108558 https://doi.org/10.1016/j.cbpc.2019.06.002.

Perals, D., Griffin, A.S., Bartomeus, I., Sol, D., 2017. Revisiting the open-field test: what does it really tell us about animal personality? Anim. Behav. 123, 69–79. https://doi.org/10.1016/j.anbehav.2016.10.006.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2019. nlme : Linear and Nonlinear Mixed Effects Models.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Reader, S.M., 2015. Causes of individual differences in animal exploration and search. Top. Cogn. Sci. 7, 451–468. https://doi.org/10.1111/tops.12148.

Renner, M.J., 1990. Neglected aspects of exploratory and investigatory behavior. Psychobiology 18, 16–22. https://doi.org/10.3758/BF03327209.

Rowsey, L.E., Johansen, J.L., Khursigara, A.J., Esbaugh, A.J., 2019. Oil exposure impairs predator–prey dynamics in larval red drum (Sciaenops ocellatus). Mar. Freshw. Res. https://doi.org/10.1071/MF18263.

Sammarco, P.W., Kolian, S.R., Warby, R.A.F., Bouldin, J.L., Subra, W.A., Porter, S.A., 2013. Distribution and concentrations of petroleum hydrocarbons associated with the BP/Deepwater Horizon Oil Spill, Gulf of Mexico. Mar. Pollut. Bull. 73, 129–143. https://doi.org/10.1016/j.marpolbul.2013.05.029.

Schlenker, L.S., Welch, M.J., Meredith, T.L., Mager, E.M., Lari, E., Babcock, E.A., Pyle, G.G., Munday, P.L., Grosell, M., 2019. Damsels in distress: oil exposure modifies behavior and olfaction in bicolor damselfish (Stegastes partitus). Environ. Sci. Technol. 53, 10993–11001. https://doi.org/10.1021/acs.est.9b03915.

Scott, G.R., Sloman, K.A., 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat. Toxicol. 68, 369–392. https://doi.org/10.1016/j.aquatox.2004.03.016.

Short, J.W., Heintz, R.A., 1997. Identification of *Exxon Valdez* oil in sediments and tissues from Prince William sound and the Northwestern Gulf of Alaska based on a PAH weathering model. Environ. Sci. Technol. 31, 2375–2384. https://doi.org/10.1021/es960985d.

Sousa, N., Almeida, O.F.X., Wotjak, C.T., 2006. A hitchhiker's guide to behavioral analysis in laboratory rodents. Genes Brain Behav. 5 (Suppl 2), 5–24. https://doi.org/10.1111/j.1601-183X.2006.00228.x.

Spooner, M., 1970. Oil spill in Tarut Bay, Saudi Arabia. Mar. Pollut. Bull. 1, 166–167. https://doi.org/10.1016/0025-326X(70)90296-1.

Stewart, W.J., Johansen, J.L., Liao, J.C., 2017. A non-toxic dose of cobalt chloride blocks hair cells of the zebrafish lateral line. Hear. Res. 350, 17–21. https://doi.org/10.1016/j.heares.2017.04.001.

Stieglitz, J.D., Mager, E.M., Hoenig, R.H., Benetti, D.D., Grosell, M., 2016. Impacts of Deepwater Horizon crude oil exposure on adult mahi-mahi (Coryphaena hippurus) swim performance. Environ. Toxicol. Chem. 35, 2613–2622. https://doi.org/10.1002/etc.3436.

Tierney, K.B., Kennedy, C.J., Gobas, F., Gledhill, M., Sekela, M., 2013. 1 - Organic Contaminants and Fish. In: Tierney, K.B., Farrell, A.P., Brauner, C.J. (Eds.), Fish Physiology, Organic Chemical Toxicology of Fishes. Academic Press, pp. 1–52 https://doi.org/10.1016/B978-0-12-398254-4.00001-7.

van Brummelen, T.C., van Hattum, B., Crommentuijn, T., Kalf, D.F., 1998. Bioavailability and ecotoxicity of PAHs. In: The Handbook of Environmental Chemistry. PAHs and Related Compounds: Biology. Springer-Verlag Berlin Heidelberg, pp. 203–263.

van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6.

van Wezel, A.P., Opperhuizen, A., 1995. Narcosis due to environmental pollutants in aquatic organisms: residue-based toxicity, mechanisms, and membrane burdens. Crit. Rev. Toxicol. 25, 255–279. https://doi.org/10.3109/10408449509089890.

Veras, G.C., Murgas, L.D.S., Rosa, P.V., Zangeronimo, M.G., Ferreira, M.S., da, S., Leon, J.A.S.-D., 2013. Effect of photoperiod on locomotor activity, growth, feed efficiency and gonadal development of Nile tilapia. Rev. Bras. Zootec. 42, 844–849. https://doi.org/10.1590/S1516-35982013001200002.

Vignet, C., Menach, Le, Lyphout, K., Guionnet, L., Frère, T., Leguay, L., Budzinski, D., Cousin, H., Bégout, M.-L, X., 2014. Chronic dietary exposure to pyrolytic and petrogenic mixtures of PAHs causes physiological disruption in zebrafish—part II: behavior. Environ. Sci. Pollut. Res. 21, 13818–13832. https://doi.org/10.1007/s11356-014-2762-6.

Xu, E.G., Magnuson, J.T., Diamante, G., Mager, E., Pasparakis, C., Grosell, M., Roberts, A.P., Schlenk, D., 2018. Changes in microRNA–mRNA signatures agree with morphological, physiological, and behavioral changes in larval Mahi-Mahi Treated with deepwater horizon oil. Environ. Sci. Technol. 52, 13501–13510. https://doi.org/10.1021/acs.est.8b04169.

Zhang, Y., Mauduit, F., Farrell, A.P., Chabot, D., Ollivier, H., Rio-Cabello, A., Le Floch, S., Claireaux, G., 2017. Exposure of European sea bass (Dicentrarchus labrax) to chemically dispersed oil has a chronic residual effect on hypoxia tolerance but not aerobic scope. Aquat. Toxicol. 191, 95–104. https://doi.org/10.1016/j.aquatox.2017.07.020.