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Abbreviations: BAL: Brut Arabian Light; C: Control; CD: Chemically Dispersed oil using dispersant; D: Dispersant solution; MD: Mechanically Dispersed oil; WSF: Water Soluble Fraction

Abstract

This study evaluated the toxicity of dispersant application which is, in nearshore area, a controversial response technique to oil spill. Through an experimental approach with juveniles of *Liza aurata*, the toxicity of five exposure conditions was evaluated: (i) a chemically dispersed oil simulating dispersant application; (ii) a single dispersant as an internal control of chemically dispersed oil; (iii) a mechanically dispersed oil simulating natural dispersion of oil; (iv) a water soluble fraction of oil simulating an undispersed and untreated oil slick and (v) uncontaminated sea water as a control exposure condition. The relative concentration of PAHs (polycyclic aromatic hydrocarbons) biliary metabolites showed that the incorporation of these toxic compounds was increased if the oil was dispersed, whether mechanically or chemically. However, toxicity was not observed at the organism level since the aerobic metabolic scope and the critical swimming speed of exposed fish were not impaired.

Key words: Dispersant toxicity, Aerobic metabolic scope, Critical swimming speed, biliary metabolites, nearshore area, golden grey mullet.

I. Introduction

Over the past decades, oil spills have become a common occurrence; e.g. Amoco Cadiz in 1978, Erika in 1999, Prestige in 2002 and recently the Deepwater Horizon platform (2010). Nowadays, recovery and dispersion are the two mains techniques used to clean up an oil spill.

Recovery is based on an efficient containment of the oil slick and is therefore conducted when the oil is viscous, the water temperature low, and the sea surface flat (Merlin, 2005). On the other hand, dispersant application is operated if the oil is light, the water temperature high, and the sea rough enough to allow dispersion of the oil slick (Chapman et al., 2007). The dispersants used are surfactants (i.e. surface active agents) with a chemical affinity for both oil and water, enabling the petroleum to be mixed into the water column in small mixed oil-surfactant micelles (i.e. with a diameter lower than 100 µm) as described by Canevari (1978). By diluting the oil slick in the water column, dispersants prevent the arrival of the petroleum slick in ecologically sensitive nearshore habitats and reduce the risk of contamination in sea surface-dwelling organisms (e.g. seabirds, marine mammals). Moreover, by increasing the surface-to-volume ratio of the oil, dispersion of the slick accelerates bacterial degradation of hydrocarbons (Thiem, 1994; Churchill et al., 1995; Swannell and Daniel, 1999). In spite of these advantages, dispersant spraying may be considered as a controversial measure in nearshore areas. Indeed, because of the limited dilution potential of the oil in shallow waters, dispersant use may induce high concentrations of petroleum in the water column and thereby raises the toxicity for aquatic organisms. Thus, in order to provide a framework for dispersant use policies in nearshore areas, specific investigation are needed to evaluate the toxicity of its application. In past studies, the toxicity of the dispersant spraying technique was determined by evaluating the mortality of organisms exposed to a single dispersant solution (e.g. Perkins et al., 1973 in*Soleasolea*). More recent studies took into consideration the toxicity induced by the interaction between the dispersant and the petroleum, such as Lin et al. (2009) and Jung et al. (2009) in juvenile *Onchorhyncustshawytscha* and *Sebastesschlegeli*, respectively. These recent studies considered the toxicity of the chemically enhanced water accommodated fractions (CEWAF, described in Singer et al., 2000) : contamination solutions which did not contain the vast quantities of oil droplets formed *in situ* during the dispersion of an oil slick.

However, oil droplets (i) have been suggested as a determinant of dispersed oil toxicity by Brannon et al. (2006) and (ii) are particularly present in nearshore areas because of the mechanical agitation due to natural mixing processes (e.g. waves). Therefore, the present study is based on an experimental approach which takes into consideration the presence of these droplets in the water column in order to assess the actual toxicity of dispersant use in nearshore areas. Juvenile golden grey mullets (*Liza aurata*), a nearshore teleost species, were exposed to (i) Chemically Dispersed oil (CD) simulating dispersant application; (ii) Dispersant alone (D) as an internal control of CD; (iii) Mechanically Dispersed oil (MD) simulating natural dispersion of oil; (iv) Water Soluble Fraction of oil (WSF) simulating an undispersed and untreated oil slick and (v) uncontaminated sea water as a Control exposure condition (C).

For each condition, the level of exposure was evaluated through the concentration in seawater of total petroleum hydrocarbons (TPH) and through the concentration of the 16 priority pollutant Polycyclic Aromatic Hydrocarbons (PAH) listed by US EPA. In parallel, the concentration in the gallbladder of three biliary metabolites was estimated in order to provide information on PAH bioavailability. In addition, the contamination-related impairments were evaluated at the organism level by assessing the fish Aerobic Metabolic Scope (AMS, Fry, 1947). AMS is the difference between Active Metabolic Rate (AMR) and Standard Metabolic Rate (SMR), *i.e.* the maximal metabolic rate of an organism in a highly active state minus its metabolic rate when at rest (Fry, 1971). Thus, AMS estimates the instantaneous metabolism rate an organism may allocate to its energy-demanding activities (e.g. locomotion, digestion, feeding): AMS is, therefore, claimed to be a relevant proxy of fitness (e.g. Claireaux and Lefrançois, 2007). Environmental factors (e.g. temperature, dissolved oxygen, pollutants) are known to modulate AMS. For instance, in *Soleasolea,* hypoxia (e.g. 25% of air saturation) induced a decrease of Active Metabolic Rate (AMR) leading to a decrease of AMS

(Lefrançois and Claireaux, 2003). Specifically to petroleum hydrocarbon exposure, Davoodi and Claireaux (2007) highlighted a 30% decrease in AMS in *Soleasolea*. Such a reduction of AMS illustrates a diminished ability to cope with energy demanding activities, which is likely to result in a prioritization of internal energy flow towards short term survival activities to the detriment of somatic and/or gonadic growth (Claireaux and Lefrançois, 2007; Del Toro-Silva et al., 2008; Chabot and Claireaux, 2008). Thus, since (i) AMS is claimed to be a relevant proxy of fitness and since (ii) it is known to be modulated by pollutants, this study considered this biological parameter in order to estimate the impact of dispersant application upon a *Liza aurata* population.

II. Material and Methods

2.1 Experimental organisms

Sixty juvenile golden grey mullets (*Liza aurata*), provided by CommercioPescaNovellame (Srl, Chioggia, Italy), were used in this experiment. The average length of the fish was 147.70 ± 0.49 mm and their average weight was 34.39 ± 0.50 g (mean ± standard error of the mean). Prior to the exposure studies, the fish were acclimatized for at least 3 weeks in 300-l flow-through tanks with the following physico-chemical parameters: dissolved oxygen: 91 ± 2% air saturation; salinity: 35 ± 1; temperature: 15 ± 0.1°C (mean ± standard error mean). During this period, fish were under natural photoperiod and fed daily with commercial food (Neosupra AL3 from Le Gouessant aquaculture).

2.2 Pollutants

2.2.1 Oil

An Arabian Crude Oil was selected for this study. Its composition was evaluated by CEDRE (CEntre of Documentation, Research and Experimentation on accidental water pollution, Brest, France), a laboratory certified according to ISO 9001 and ISO 14001. The oil was found to contain 54% saturated hydrocarbons, 36% aromatic hydrocarbons and 10% polar compounds. To simulate the natural behaviour of the oil after its release at sea (i.e. evaporation of light compounds), the oil was experimentally evaporated under atmospheric conditions. The resulting chemical composition of the oil was thereby 54% saturated hydrocarbons, 34% aromatic hydrocarbons and 12% polar compounds and its API (American Petroleum Institute) gravity was 33.

2.2.2 Dispersants

A formulation manufactured by Total Fluides was selected for its efficiency. The dispersant was composed of surfactants (blend of anionic and non ionic types) and solvents. It was a third generation dispersant (concentrated surfactant) deemed effective enough (preliminary determined by CEDRE, using the method NF.T.90-345),non-toxic at the concentration recommended by the manufacturer (preliminary determined by CEDRE assessing standard toxicity test: method NF.T.90-349) and biodegradable.

2.3 Contamination protocol

The experimental system employed is described in Milinkovitch et al. (2011). In short, it was made of five cylindrical tanks (diameter=1.1 m; height=0.4 m). Each tank comprised a funnel

connected to a Johnson L450 water pump which maintained a mixture of oil-dispersant droplets throughout the water column. The experimental system was set up in a temperature controlled room (15 ± 0.1°C). Five exposure conditions were tested. Prior to preparation of the exposure conditions, all the tanks were filled with 300-l of uncontaminated seawater provided by Oceanopolis (Brest, France). The control exposure condition was set up using seawater alone. The chemically dispersed (CD) oil exposure medium was made by pouring 20 g of petroleum and 1 g of dispersant into the funnel of the experimental system. The dispersant alone (D), as a positive control of CD, was made by pouring 1 g of dispersant into the funnel. The mechanically dispersed (MD) oil exposure medium was produced by pouring 20 g of petroleum into this funnel. For water-soluble fraction (WSF) a 20 g oil slick was contained using a plastic circle placed on the surface of the seawater. The funnel and the pump were only kept to maintain the same experimental conditions as for the other treatments. The oil slick remained at the surface without mixing and the fish were thereby only exposed to the soluble fraction of the oil.

For each exposure condition, 6 replicates of exposure were successively conducted. Two fish were exposed per replicate so that 12 fish were exposed to each of the five conditions. For each replicate, exposure lasted 48 h. Between consecutive exposures, the experimental tanks were cleaned using dichloromethane (Carlo ErbaReactifs, SDS, France), 12 hour evaporation phase was then conducted and tanks were finally thoroughly washed with freshwater. The absence of traces of dichloromethane was ensured by conducting gas chromatography-mass spectrometry. The fish were starved for 48 h prior to the bioassays and throughout the exposure period in order to avoid bile evacuation from the gallblader. Physicochemical parameters remained stable (**Table 1)**. No fish died during the all experiment.

2.4. Chemical analyses

2.4.1. Total petroleum hydrocarbon (TPH) seawater concentrations

TPH concentrations were measured for the 6 replicates of each exposure condition. For each replicate, three samples were made at the beginning (T = 0 h) and at the end (T = 48 h) of fish exposure. The mean of the three samples was considered representative of the TPH concentration at each time point. Extraction of samples was conducted with 10 ml of pestipur-quality dichloromethane (Carlo ErbaReactifs, SDS, France) which induced separation of the organic and aqueous phases. Then, water was extracted two additional times with the same volume of dichloromethane (2 x 10 ml). The extracts were dried using anhydrous sulphate and then treated using a UV spectrophotometer (UV-Vis spectrophotometer, Unicam, USA) at 390 nm as described by Fusey and Oudot (1976). According to CEDRE and taking into account the accuracy of the spectrophotometer (CEDRE property), results obtained with this method are not reliable below 1 mg/L.

2.4.2. Polycyclic aromatic hydrocarbon (PAH) seawater concentrations

Two replicates were analyzed at the beginning (T=0 h) and end (T=48 h) of fish exposure for each replicate. Sixteen PAHs (alkylated and parents), listed by US EPA as priority pollutants, were quantified according to the method described by Roy et al. (2005). After sampling, a 24-hour settling phase to separate oil droplets and particulate matter from the seawater was conducted. Then, 150 µL of a solution of five perdeuterated internal standards in acetonitrile (Naphthalene d_8, Biphenyl d_{10}, Phenanthrene d_{10}, Chrysene d_{12}, and Benzo[a]pyrene d_{12} at respective concentrations of 210, 110, 210, 40 and 40 µg/mL, Sigma-Aldrich, France) were diluted in 10 ml of absolute methanol (Sigma-Aldrich, France) and the resulting solution was added to the liquid phase of samples. Using the stir bar sorptive extraction technique (SBSE –

Stir bar coated with PDMS, Gerstel, USA) and thermal desorption coupled to capillary gas chromatography-mass spectrometry (GC–MS), the PAHs were extracted from the seawater and treated. The GC was a HP7890 series II (Hewlett Packard, Palo Alto, CA, USA) coupled with a HP5979 mass selective detector (MSD) (Electronic Impact: 70eV, voltage: 2,000 V). According to the procedure of Roy et al. (2005), the quantification limit for each PAH was 1 ng/L.

2.5 Evaluation of aerobic metabolic scope and critical swimming speed

2.5.1 Experimental set-up

Two identical swimming tunnels (LoligoApS., Denmark) were employed to challenge simultaneously two fish of each exposure replicates. Swimming tunnel allowed controlling fish activity and measuring the associated oxygen consumption. The swimming tunnels were adapted from those used in Vagner et al. (2008), although were smaller in size (75 l instead of 150 l). Each swimming tunnel was composed of a swimming respirometer (11 l) and a buffer tank. The swimming respirometer was made of a chamber (40 x l0 x 10 cm) where the fish was placed to be tested. The water flow was generated by a motor fitted with a three bladed-propeller. Some deflectors and a plastic honeycomb promoted rectilinear flow with a uniform velocity profile (vertical and horizontal). A peristaltic pump created a continuous water flow from the respirometer to a flow-through cell oxygen microsensor (Presens, Germany). This oxygen probe was connected to an oxymeter (Microx, Presens, Germany) which was interfaced to a computer via a RS232 port. A data acquisition program (Oxyview) was used to record oxygen saturation every ten seconds. The buffer tank, where temperature and oxygen were controlled using a thermoregulator and an air pump respectively, was connected to the

swimming respirometer via a flush pump which allowed exchange of water between the two compartments. This water flow renewed oxygen between consecutive measurements and also maintained a constant temperature in the swimming respirometer.

2.5.2 Experimental protocol

At the end of the 48 h contamination period, the fish were gently caught and their fork length (L) was measured before introducing them into the swimming respirometer where they recovered for one night prior to the swim challenge. During the recovery period, the water flow was maintained at a low swimming speed of 0.5 $L.s^{-1}$. When the experiment started, water flow was increased by steps of 1.5 $L.s^{-1}$ from 0.5 to 3.5 $L.s^{-1}$, and steps of 0.75 $L.s^{-1}$ for further increase. The increment duration was 20 min. The fish was considered fatigued if it did not manage to swim against the current and fell against the grid at the rear of the swimming chamber. Then the experiment was stopped and the speed was decreased to 0.5 $L.s^{-1}$. The fish were allowed to recover for a couple of hours before being removed from the swimming respirometer and euthanized using 2 ml of eugenol (99 %, Sigma Aldricht chemicals, France) in 5 ml of seawater. The gallbladder was removed for PAH biliary metabolite analysis. The fish length, mass, width and height were measured. The net oxygen consumption (i.e. the microbial oxygen consumption) was measured to be further subtracted from the measured oxygen consumption.

2.5.3 Calculations

2.5.3.1 Oxygen consumption (MO_2)

Oxygen consumption $MO_{2(meas)}$ is expressed in $mgO_2.kg^{-1}.h^{-1}$ and calculated using the following formula:

$$MO_{2(meas)}=\Delta[O_2].\ \Delta t^{-1}.V.M_{meas}^{-1}$$

where $\Delta[O_2]$ in $mgO_2.l^{-1}$ is the variation in oxygen concentration during the measurement period (Δt in hours), V (L) is the volume of the respirometer minus the volume of the fish, M_{meas}(kg) is the fish mass.

Since an allometric relation exists between oxygen consumption and body mass, $MO_{2(meas)}$ was corrected using the following formula:

$$MO_{2cor=}\ MO_{2meas}.(M_{meas}.M_{cor}^{-1})^{1-A}$$

where MO_{2cor} ($mgO_2.kg^{-1}.h^{-1}$) is the oxygen consumption related to a standard fish of 0.1 kg (M_{cor}) fish, MO_{2meas} ($mgO_2.kg^{-1}.h^{-1}$) is the oxygen consumption calculated for experimented fish whose mass was M_{meas}(kg) and A is the allometric constant describing the relation between oxygen consumption and body mass. In the case of this study, we used A=0.8 as employed by Vagner et al. (2008) in the flathead grey mullet (*Mugilcephalus*).

2.5.3.2 Critical swimming speed (U_{crit})

The critical swimming speedis expressed in $L.s^{-1}$ and calculated using the Brett formula (1964):

$$U_{crit}=U_t+t_1.t^{-1}.U_1$$

whereU_t ($L.s^{-1}$) is the highest velocity maintained for an entire swimming step, t_1 (min) is the amount of time spent at the fatigue velocity, t(min) is the prescribed swimming period (i.e. 20 min in the present study) and U_1 is the increment velocity (0.75 or 1.5 $L.s^{-1}$).

2.5.3.3 Standard metabolic rate (SMR), active metabolic rate (AMR) and aerobic metabolic scope (AMS)

As expected, oxygen consumption increased exponentially with swimming speed (Brett, 1964) so that SMR can be assessed from the following equation:

$$MO_2 = SMR\ exp^{bU}$$

Where SMR is the intercept (i.e. the MO_2 when U=0 $L.s^{-1}$), b is a constant, U is the swimming speed and MO_2 is the oxygen consumption ($mgO_2.h^{-1}.kg^{-1}$).

AMR is evaluated as the maximum oxygen consumption measured during the swimming test. Finally, AMS is the difference between AMR and SMR. U_{crit}, SMR, AMR and AMS were assessed for each fish.

2.6. Fixed wavelength fluorescence analysis

Four µL of bile extracted from the gallbladder of fish were diluted in 996 µL of absolute ethanol (VWR International) in quartz cuvettes. Fixed wavelength fluorescence (FF) was then measured on a spectrofluorimeter (SAFAS Flx-Xenius, Monaco). Excitation-emission wavelength pairs 290:335, 341:383, 380:430 were employed to detect naphthalene-derived metabolites, pyrene-derived metabolites and benzo[*a*]pyrene-derived metabolites, respectively

(Aas et al., 2000). The FF values are expressed as arbitrary units of fluorescence and provide an estimation of the relative concentration of metabolites between the five different exposure conditions (C, CD, MD, WSF, D).

2.7 Statistical analysis

The statistical analysis was carried out using Statistica software. The homoscedasticity (using the Barlett test) and normality (using the Kolmogorov-Smirnoff test) of data were demonstrated for SMR, AMR, AMS and the fixed wavelength fluorescence. Therefore, for each of these variables, a one-way analysis of variance (one-way ANOVA) was performed in order to test for significant differences due to exposure conditions. For critical swimming speed, homoscedasticity and normality were not respected, therefore a Kruskal-Wallis test was conducted. When necessary, a Tukey post-hoc test was conducted to detect significant differences between exposure conditions. Patterns of TPH and PAH concentrations were analysed using a one-way repeated measure ANOVA with exposure condition as a between factor and time (i.e. at the beginning and at the end of the experiment) as a within factor. When a significant difference was detected, HSD Tukey post hoc test was applied. The results were considered significantly different if $P_{value}<0.05$. The results were expressed as means ± standard error means.

III. Results

3.1. Total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) concentrations (**Table 2**).

Neither TPHs nor PAHs were detected in C and D exposure conditions. TPHs were not detected in WSF exposure conditions while PAHs were detected.

At T=0 h, even if TPH concentrations tended to be higher in CD than in MD exposure, the results did not differ significantly. At T=48 h, the TPH concentration remained stable in CD while it significantly decreased in MD exposure which resulted in a TPH concentration, at T=48 h, significantly lower in the mechanically dispersed oil solution than in the chemically dispersed oil.

At T=0 h, the concentrations of PAHs in CD and MD exposure conditions were significantly higher than in WSF. At T=48 h, the PAH concentration significantly decreased in CD and MD exposure, while no significant decrease was observed in WSF. Although not statistically different, the concentration of PAH (at T=48 h) was higher in MD and CD than in WSF.

Concerning the 16 US EPA PAHs **(Table 3)**, the pattern was similar, whatever the exposure conditions. Naphthalene (alkylated and parents) concentration represented the major proportion of dissolved PAHs. While the concentration of two and three ring compounds (from naphthalene to fluoranthene, **Table 3**) tended to represent the higher proportion of PAHs dissolved in seawater, heavier PAHs (four rings and more) showed low concentrations. Regarding variation over time, concentrations of two and three ring compounds tended to decrease during exposure contrary to the concentration of heavier PAHs. However, readers must take into account that these results were not statistically tested, due to the high variability between replicates.

3.2 Fixed wavelength fluorescence analysis (**Figure 1**)

Fixed wavelength fluorescence analysis did not reveal any significant differences in the relative concentration of naphthalene derived metabolites between exposure conditions. On the contrary, a significant increase in the relative concentration of pyrene derived metabolites was observed following both MD and CD exposure, when compared to C, D and WSF. The same pattern was obtained for benzo[*a*]pyrene derived metabolites.

3.3 Aerobic metabolism (**Figure 2**) and critical swimming speed (U_{crit}, **Figure 3**)

For all conditions of exposure, the increasing swimming speed of the fish induced an exponential increase in oxygen demand (results not shown). This exponential increase in oxygen consumption was followed by a plateau as the fish fatigued. Concerning SMR and AMR **(Figure 2)**, no significant difference was found between exposure conditions. Due to high AMR values, AMS tended to be higher in the Control exposure condition than in contaminant exposure conditions even if no significant difference was found between conditions. U_{crit}**(Figure 3)** showed the same pattern as AMS.

4. Discussion

The aim of this study was to assess the toxicity of dispersant use in nearshore areas. Our experimental approach aimed at evaluating the toxicity of dispersant application on an oil slick under mixing processes to simulate turbulent energy that is a characteristic of nearshore areas (e.g. waves) and also an abiotic condition required for dispersant use (Merlin, 2005).

4.1. TPH concentration, PAH concentration and relative concentration of biliary metabolites

Concentrations of TPHs (**Table 2**) in both CD and MD exposure present the same order of magnitude as the concentrations measured on oil spill sites or field studies. For instance, Cormack (1977) measured concentrations of 18 mg/L in the top 30 cm of the water column after chemical dispersion and Spooner (1970) measured 50 mg/L of naturally dispersed oil after an oil spill in TarutBay (Saudi Arabia). In the present study, the TPH concentration was found to be significantly lower in MD than in CD at the end of the 48 h exposure period. This phenomenon was probably due to the petroleum adherence to the experimental system (in particular to the funnel) which was observed for MD exposure but not CD exposure. This reduction of petroleum adherence due to dispersant has even been discussed in Milinkovitch et al. (2011) and is in accordance with a field study of Baca et al. (2006) in which dispersant permitted to reduce the adherence of petroleum to natural substrate such as sea grasses and corals.

With regards to the kinetics of hydrocarbon concentration and exposure period, our experimental approach was used to expose fish to a possible scenario. Indeed, in most oil spills in offshore areas, TPH concentrations have decreased drastically after 2-5 hours (Lessard and Demarco, 2000) during which a localized oil slick is dispersed and the hydrocarbons are disseminated. On the contrary, in the shallow waters of nearshore areas, the lower dilution potential (due to the size of the water column) is likely to reduce the dissemination speed. Moreover in case of a tanker grounding in coastal areas, the continuous release of oil together with the turbulent mixing process may cause the TPH concentration to be maintained in the water column. For instance, in the case of the Braer oil spill, which

occurred in rough sea conditions (up to 10 Beaufort), dispersion was maintained for over a week (Lunel, 1995). Our experimental approach exposed fish to a halfway scenario (between a drastic decrease and one week of dispersion) in which the concentration was maintained (for CD exposure) or decreased slowly (for MD exposure) over a 48 h period.

With regards to the sum of PAH concentrations, a higher concentration was observed for MD and CD exposure than for WSF exposure. This was probably due to the fact that oil droplets (observed for CD and MD exposure) have a larger surface/volume ratio than an oil slick, inducing enhanced solubilisation of PAHs. Our results showed that, during the 48 h exposure, PAH concentration decreased in MD as well as CD treatments. Regarding to the concentrations of the 16 PAHs, this decrease seems to be due to a loss of the lighter compounds (from naphthalene to phenanthrene) because of their volatilization and/or photolysis as suggested by Huang et al. (2004).

Concerning PAH biliary metabolites, concentrations of pyrene and benzo[*a*]pyrene type were relatively higher in MD and CD than for other exposure conditions (WSF, D, C) which suggests a higher incorporation of these compounds when the oil was dispersed (mechanically and/or chemically). The similar patterns observed for MD and CD exposure suggest that dispersant application did not increase PAH bioavailability. On the other hand, the invariability of naphthalene type metabolites between all the conditions was probably due to the high turnover of light PAH which were rapidly bioaccumulated (as described in *Mytilusedulis* in Baussant et al. 2001) and metabolized in the tissues so that metabolites may not have been accumulated in the gallbladder of contaminated fish.

4.2. Aerobic metabolic scope and critical swimming speed

Results in this study conform to those observed in the literature (Brett, 1964; Webb, 1975; Vagner et al., 2008). Indeed, in accordance with the literature, the increasing swimming speed induced an exponential elevation in oxygen demand (MO_2) followed by a plateau when *Liza aurata* approached U_{crit} (data not shown). Moreover, SMR and AMR values obtained in this study for control fish (109 $mgO_2.kg^{-1}.h^{-1}$ and 958 $mgO_2.kg^{-1}.h^{-1}$, respectively) are of the same order of magnitude as the results obtained in *Mugilcephalus*, a close species tested at 20°C (76 and approximately 700 $mgO_2.kg^{-1}.h^{-1}$, respectively, Vagner et al., 2008). The comparison of U_{crit} values obtained with values observed in literature is limited due to the difference of abiotic and/or biotic experimental parameters (such as temperature, species or size of the fish) which influence the metabolic activity and by the way the swimming performance of the fish.

Although precocious biomarkers, such as biliary metabolites, revealed an increase of hydrocarbons exposure due to contamination, measurements of SMR and AMR in *Liza aurata* did not reveal any differences between exposure conditions leading, as expected, to an unchanged AMS. AMS illustrates the energy an organism is able to mobilize for sustaining activities (Fry, 1971; Priede, 1985; Claireaux and Lefrançois, 2007). With a constant AMS, energy demanding activities such as locomotion, digestion or growth are therefore expected to remain unimpaired in contaminated golden grey mullets. As a consequence, in our study, swimming performance, measured by critical swimming speed, was also not altered by contaminant exposure.

These results are in accordance with results obtained by McKenzie et al. (2007) which suggested that organic pollutants did not affect swimming performance whereas inorganic pollutants such as heavy metals (Wilson et al. 1994 ; Pane et al., 2004) or ammonia (McKenzie et al., 2003) did.

However, in contrast to the results of this study, a study by Davoodi and Claireaux (2007) showed that exposure to petroleum decreased the active metabolic rate of the common sole

(*Soleasolea*) while the standard metabolic rate remained unchanged. The same authors (Claireaux et al., 2004 ;Claireaux and Davoodi, 2010) suggested that reduction in AMR could be explained by the impairment of heart rate and stroke volume which led to diminished cardio-respiratory performances, as well as by the alteration of gill functional integrity which reduce oxygen diffusion across the respiratory epithelium and into the blood. Although these studies exposed effects of petroleum upon aerobic metabolism, readers must take into account that the experimental protocol showed a fuel to water ratio which was 76 times higher than in our study. Moreover, unlikely our study, exposure period (5 days), contamination protocol and petroleum composition in these studies did not simulate actual organism exposure during dispersant application.

5. Conclusion

Through an experimental approach, this study aimed to assess the toxicity of dispersant application. Although biliary metabolites revealed increased exposure to PAHs following dispersed oil exposure, no effect was highlighted regarding aerobic metabolic scope nor critical swimming speed. This lack of effect may indicate that the fitness of the fish was not impaired (Fry, 1971; Priede, 1985). However, the lack of significance could also be due to the short exposure time employed in order to simulate the toxicity of oil spill: this study focused on the acute toxicity of oil spill but, in nearshore areas, the contamination of the sediment and the consequent retention of heavy PAHs also induce chronic exposure. Thus, an approach considering the long term toxicity of dispersant use would be relevant in order to give supplementary information concerning the environmental risk of this response technique.

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Table 1: Physicochemical Parameters Monitored over the Experimental Period. Values are the mean of six tank replicates (± standard error mean). AS = air saturation

	Temperature (ºC)	Oxygen (% AS)	pH
C	14.39 ± 0.15	99.64 ± 1.4	8.05 ± 0.01
CD	14.49 ± 0.13	99.47 ± 1.6	7.98 ± 0.02
MD	14.68 ± 0.11	100.71 ± 2.0	8.03 ± 0.03
WSF	14.57 ± 0.13	98.13 ± 3.2	8.02 ± 0.03
D	14.46 ± 0.12	99.55 ± 1.5	8.03 ± 0.02

Table 2: TPH concentration and concentration of the sum of 16 parents and alkylated US-EPA PAH (ΣPAH) in the five treatments at the beginning (T=0 h) and at the end of the exposure (T=48 h) for C (Control), CD (Chemically Dispersed oil), MD (Mechanically Dispersed oil), WSF (Water Soluble Fraction of oil) and D (Dispersant). For each contaminant measurements (TPH and PAH),different letters in the same row indicate significant difference of concentration between T = 0h and T = 48 h ($p<0.05$); different symbols in the same column indicate significant difference of concentration between exposure conditions ($p<0.05$). Values are the mean of six tank replicates (± standard error mean). n.d. means non detected PAH or TPH compounds

	$[TPH]_{T=0h}$ (mg/L)	$[TPH]_{T=48h}$ (mg/L)	$[\Sigma PAH]_{T=0h}$ (µg/L)	$[\Sigma PAH]_{T=48h}$ (µg/L)
C	n.d.	n.d.	n.d.	n.d.
CD	$44.0 \pm 3.0^{a,*}$	$38.2 \pm 2.8^{a,*}$	$60.1 \pm 9.3^{a,*}$	$15.6 \pm 3.3^{b,\dagger}$
MD	$29.2 \pm 5.6^{a,*}$	$14.2 \pm 3.1^{b,\dagger}$	$36.9 \pm 6.3^{a,*}$	$1.8 \pm 0.4^{b,\dagger}$
WSF	n.d.	n.d.	$3.3 \pm 0.6^{a,\dagger}$	$0.5 \pm 0.1^{a,\dagger}$
D	n.d.	n.d.	n.d.	n.d.

Table 3: Concentration of 16 parents and alkylated (C1-, C2-,C3-,C4-) US-EPA PAH in sea water during CD (Chemically Dispersed oil), MD (Mechanically Dispersed oil) and WSF

(Water Soluble Fraction of oil) exposures. Values are the mean of six tank replicates (± standard error mean). n.d. means non detected PAH compounds

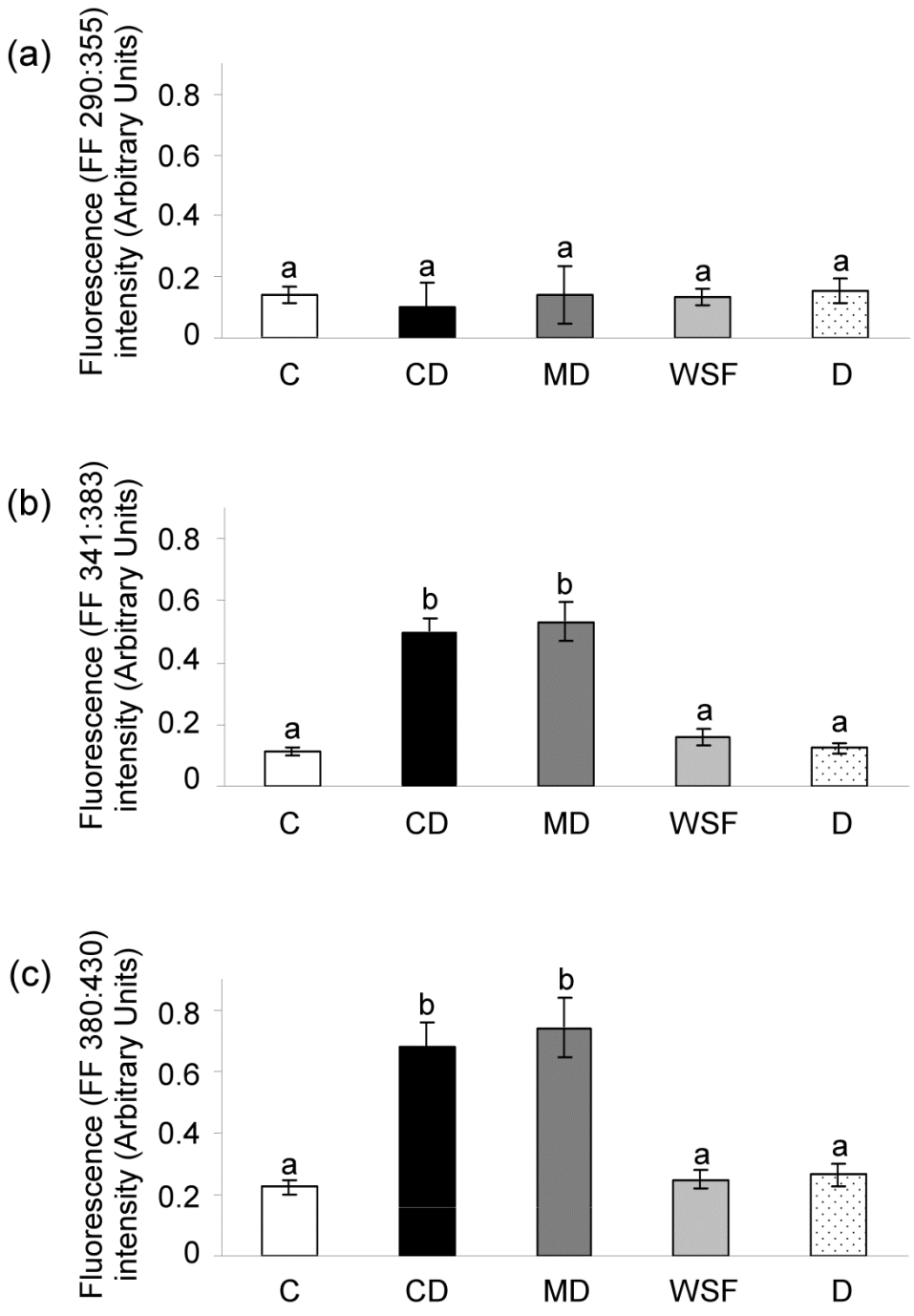
16 US-EPA PAH (parents and alkylated)	**Concentration (ng/L) at T=0h and T=48h**					
	T= 0 h			**T=48 h**		
	CD	**MD**	**WSF**	**CD**	**MD**	**WSF**
Naphtalene	526 ± 149	676 ± 191	301 ± 72	469 ± 43	71 ± 16	48 ± 6
C1-Naphtalene	5798 ± 1064	4451 ± 1363	1040 ± 255	1444 ± 698	150 ± 37	111 ± 17
C2-Naphtalene	21948 ± 4808	12553 ± 3181	833 ± 167	3641 ± 1725	167 ± 35	105 ± 15
C3-Naphtalene	23828 ± 4195	12398 ± 2436	497 ± 181	4276 ± 1204	156 ± 26	55 ± 7
C4-Naphtalene	4294 ± 602	4654 ± 2441	171 ± 55	1513 ± 180	312 ± 48	23 ± 4
Acénaphtylene	8 ± 8	9 ± 7	1 ± 0	27 ± 2	1 ± 0	n.d.
Acénaphtene	33 ± 3	32 ± 3	3 ± 0	46 ± 3	1 ± 0	n.d.
Fluorene	283 ± 22	202 ± 12	6 ± 2	121 ± 21	2 ± 1	n.d.
C1-Fluorene	370 ± 39	206 ± 31	9 ± 3	169 ± 22	10 ± 3	2 ± 0
C2-Fluorene	224 ± 27	128 ± 19	7 ± 2	168 ± 13	29 ± 7	2 ± 1
C3-Fluorene	62 ± 9	27 ± 10	2 ± 1	60 ± 8	29 ± 4	1 ± 0
Phenanthrene	600 ± 29	343 ± 47	18 ± 4	477 ± 66	5 ± 1	5 ± 2
Anthracene	153 ± 82	47 ± 42	2 ± 1	141 ± 56	2 ± 0	2 ± 1
C1-Phenanthrenes/Anthracene	868 ± 45	456 ± 85	16 ± 5	591 ± 122	12 ± 6	5 ± 1
C2-Phenanthrenes/Anthracene	472 ± 41	274 ± 52	22 ± 9	530 ± 37	108 ± 22	4 ± 1
C3-Phenanthrenes/Anthracene	183 ± 25	104 ± 24	12 ± 8	241 ± 33	107 ± 15	3 ± 1
C4-Phenanthrenes/Anthracene	4 ± 3	n.d.	3 ± 3	3 ± 4	n.d.	1 ± 1
Fluoranthene	4 ± 1	10 ± 8	4 ± 2	28 ± 1	2 ± 1	1 ± 0
Pyrene	10 ± 1	15 ± 9	6 ± 3	31 ± 1	6 ± 1	1 ± 0
C1-Fluoranthenes/Pyrenes	17 ± 5	9 ± 2	n.d.	26 ± 4	13 ± 2	n.d.
C2-Fluoranthenes/Pyrenes	29 ± 8	15 ± 4	12 ± 10	48 ± 7	26 ± 3	n.d.
C3-Fluoranthenes/Pyrenes	27 ± 8	22 ± 15	22 ± 17	42 ± 8	19 ± 3	1 ± 1
Benzo[*a*]anthracene	3 ± 0	37 ± 28	3 ± 2	130 ± 38	10 ± 7	2 ± 2
Chrysene	64 ± 7	72 ± 31	11 ± 5	225 ± 44	40 ± 7	7 ± 4
Benzo[*b+k*]fluoranthene	33 ± 15	48 ± 37	15 ± 6	474 ± 177	97 ± 75	51 ± 47
Benzo[*a*]pyrene	32 ± 13	17 ± 11	20 ± 10	225 ± 80	87 ± 61	12 ± 11
Benzo(*g,h,i*)perylene	91 ± 25	46 ± 24	37 ± 11	231 ± 36	123 ± 65	18 ± 10
Indeno(*1,2,3-cd*)pyrene	71 ± 16	29 ± 8	41 ± 12	86 ± 32	105 ± 71	22 ± 11
Dibenzo(*a,h*)anthracene	105 ± 24	46 ± 16	56 ± 17	132 ± 43	149 ± 91	33 ± 17

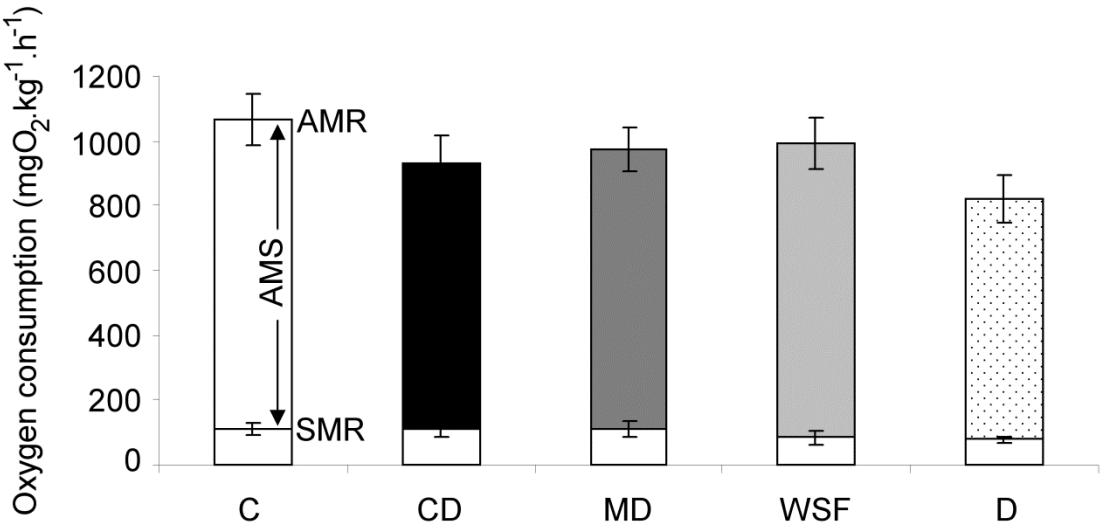
Figure captions

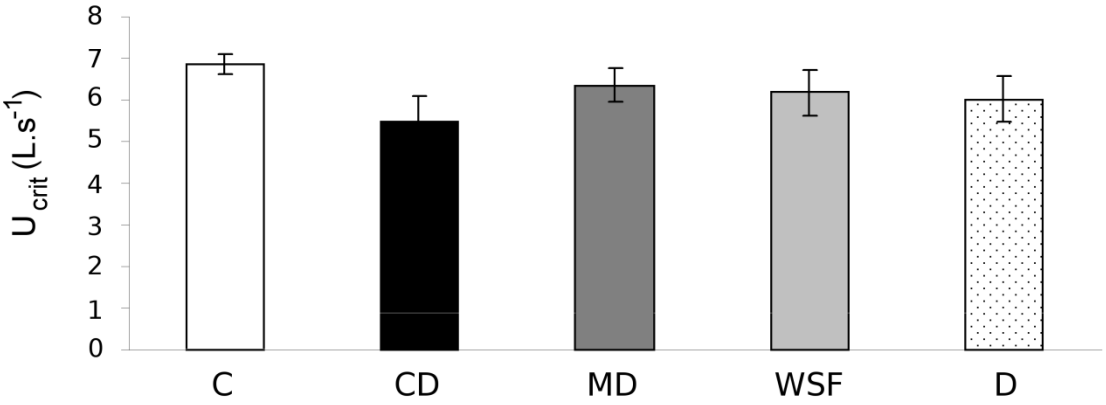
Figure 1: Fixed wavelength fluorescence (FF) of bile reflecting biliary PAHs metabolites levels after 48 h exposure to Control (C), Chemically Dispersed oil (CD), Mechanically Dispersed oil solution (MD), Water Soluble Fraction (WSF) and Dispersant (D) solution: (a) FF 290:335 (naphthalene derived type of metabolites); (b) FF 341:383 (benzo[*a*]pyrene type of metabolites); (c) FF 380:430 (pyrene derived type of metabolites). Levels expressed as fluorescence intensity. Values represent mean ± standard error (n=10 per treatment). Bars not sharing common letters indicate asignificant difference**,** where $P < 0.05$.

Figure 2: Standard metabolic rate (**SMR**), Active metabolic rate (**AMR**) and Aerobic metabolic scope (**AMS**) of golden grey mullets exposed to Control (C), Chemically Dispersed oil (CD), Mechanically Dispersed oil (MD), Water Soluble Fraction (WSF) and Dispersant (D) solution. Results are expressed as mean values (± standard error mean, n=12). No significant difference was found.

Figure 3: Critical swimming speed (U_{crit}) of golden grey mullets exposed to Control (C), Chemically Dispersed oil solution (CD), Mechanically Dispersed oil solution (MD), Water Soluble Fraction (WSF) and Dispersant (D) solution. Results are expressed as mean values (± standard error mean, n=12). No significant difference was found.


(a)
Fluorescence (FF 290:355) intensity (Arbitrary Units)
0.8
0.6
0.4
0.2
0
a
a
a
a
a
C
CD
MD
WSF
D
(b)
Fluorescence (FF 341:383) intensity (Arbitrary Units)
0.8
0.6
0.4
0.2
0
a
b
b
a
a
C
CD
MD
WSF
D
(c)
Fluorescence (FF 380:430) intensity (Arbitrary Units)
0.8
0.6
0.4
0.2
0
a
b
b
a
a
C
CD
MD
WSF
D


Oxygen consumption (mgO2.kg-1.h-1)
1200
1000
800
600
400
200
0
AMR
AMS
SMR
C
CD
MD
WSF
D


Ucrit (L.s-1)
8
7
6
5
4
3
2
1
0
C
CD
MD
WSF
D