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Effect of dispersed crude oil on cardiac function in seabass *Dicentrarchus labrax*

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HIGHLIGHTS

- *Dicentrarchus labrax* were used in this ecotoxicological study.
- Fish were exposed to dispersant, mechanical and chemical oil dispersion.
- Cardiac contraction parameters were impacted in the presence of oil.
- Cardiac energy metabolism was impacted by dispersant alone.

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ABSTRACT

In this study, the impact of dispersed oil was assessed in *Dicentrarchus labrax*, a fish frequently used as an oil contamination indicator species. Fish were exposed for 48 h to (mechanically and chemically) dispersed oil and dispersant alone. The impact of these exposure conditions was assessed on cardiac function by measuring (i) the contraction strength, the contraction and the relaxation speeds (ii) the cardiac energy metabolism using respirometry on permeabilized cardiac fibers. Compared to control, the increase of polycyclic aromatic metabolites observed in the bile indicated oil contamination in our fish. Following 48 h of oil exposure at realistic oil concentrations, alterations of cardiac performances were observed. A decrease in contraction strength, contraction and relaxation speeds was observed in the presence of oil without effect of dispersant on these three parameters. Looking at cardiac energy metabolism, dispersant alone decreases all the activity of the respiratory chain and increases the proton leak. From these results, it appears that the observed decrease in cardiac performance in fish exposed to oil was not linked to a decrease in energy availability.

1. Introduction

Global industrial development has resulted in a constant demand for oil, the most common world source of energy. As oil extraction areas are not distributed uniformly, large amounts must be transported across the seas leading to oil spills (Percebois, 2001). After the evaporation of volatile components, the oil is partially dissolved in sea water and broken up into microdroplets throughout the water column by physical mixing and degradation by microorganisms (Cerniglia, 1992). To limit oil spill damages, dispersants have been used, mainly in offshore areas, since the 1970s. However by increasing oil compounds' bioavailability, they may enhance pollution damages (Chapman et al., 2007). Thus, their use near the coast is still debated, due in part to the lack of knowledge of their effects on organisms, and the question of the effects of both fuel oil and dispersed oil on coastal organisms should be raised.

Petroleum compounds have been shown to affect numerous physiological functions such as respiration (Duarte et al., 2010), immunity (Fabiani et al., 1999; Reynaud et al., 2002), cell differentiation (Perez et al., 2003), development (Incardona et al., 2005), growth, reproduction and gene expression (Zhang et al., 2013). Alterations of fish metabolism are also reported (Davoodi and

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Claireaux, 2007). Amongst the functions studied, cardiac function seemed of great interest: (i) it could be altered by petroleum compounds (Hicken et al., 2011; Milinkovitch et al., 2012, 2013) and (ii) changes or alterations to the heart may have consequences on other physiological functions (Heideman et al., 2005). To allow heart contraction, energy production by cardiac mitochondria should be maintained. Petroleum compounds have been shown to alter mitochondrial oxidative phosphorylation and membrane potential, and to induce an increase in superoxide production leading to a potential decrease in ATP production (Salazar et al., 2004; Xia et al., 2004; Stabenau et al., 2008; Westman et al., 2013).

Thus, this work aims at assessing the effects of a dispersed fuel oil on the cardiac function of seabass, *Dicentrarchus labrax*. This fish, often used as a biological model, is highly represented in temperate coastal areas. Moreover, this species is exposed to xenobiotics *via* the ingestion of bioaccumulated molecules through the trophic network (Wolfe et al., 1998).

In this study, fish were exposed for 48 h to mechanically dispersed oil, to one commercial formulation of dispersant and to the corresponding chemically dispersed oil. Cardiac function was estimated by measuring contraction strength, contraction and relaxation speeds. Cardiac energy metabolism was evaluated by respirometry on permeabilized cardiac fibers. The exposure conditions were assessed by measuring the total petroleum hydrocarbon concentration and the oil droplet size in the water.

2. Material and methods

2.1. Animals

Experiments were conducted on seabass, *Dicentrarchus labrax* ($n = 32$; weight: 492 ± 41 g; length: 33 ± 1 cm; mean ± SD) purchased from Gravelines hatchery (Gravelines, France). Two weeks before the experiments, the fish were acclimatized to their 2500 L seawater tanks. The photoperiod was according to the season (February to March: 10 h light/14 h dark). The pH (8.0 ± 0.2), oxygen saturation (greater than 90%) and temperature (13.9 ± 0.4 °C) were measured daily. Fish were fed daily *ad libitum* (until they do not catch food anymore) with dried pellets (Neo Grower Extra Marin Col. 5® from Le Gouessant aquaculture). Around 400 g of food per kg of fish were distributed per day. The diet composition was 43% protein, 20% lipids, 3% cellulose, 5.6% ash, 10% moisture, 0.9% and 18.4% nitrogen free extract.

2.2. Chemicals

The petroleum used in this study was a Crude Arabian Light (CAL) composed of 54% saturated hydrocarbons, 10% polar compounds and 36% aromatic hydrocarbons. CAL was evaporated (with air bubbling) until a weight loss of 7%. This process caused the lighter compounds to evaporate mimicking the weathering of an oil slick at sea (Milinkovitch et al., 2011). This weathered CAL was used in other ecotoxicological studies (Milinkovitch et al., 2012; Claireaux et al., 2013; Theron et al., 2014).

Finasol OSR 52, a commercial formulation from TOTAL Fluides (Puteaux, France), was used as dispersant in this study. It is a third generation oil-based dispersant combining surfactants (amphiphilic molecules) and solvents. Components of Finasol OSR52 were: docusate sodium (20–25%); Hydrocarbons, C1–C14, n-alkanes, ioalkanes and cylics (<2%); aromatics (15–20%); 2-methoxymethylethoxy propanol (15–20%); Carboxylic acids, diC6-12 compounds, with ethanolamine and boric acid compound with ethanolamine (0–2%); Ethanolamine (0–1%). According to the safety data sheet, physicochemical parameters of Finasol OSR 52 were: viscosity 30.1–36.7 $m^2\ s^{-1}$ at 40 °C; pH 9–10.5; density 990–1015 $kg\ m^{-3}$ at 20 °C; boiling point higher than 150 °C; flash point higher or equal than 65 °C.

2.3. Experimental design

The fish were allocated to four experimental conditions: a control group (C), a group exposed to mechanically dispersed CAL (MD), a group exposed to chemically dispersed CAL (Finasol OSR 52: CD) and a group exposed to the dispersant alone (D). In the case of the MD and CD conditions, 25 g of oil was poured into 300 L seawater tanks (concentration around 80 $mg\ L^{-1}$), in the CD condition 1.25 g of dispersant was also added (dispersant oil ratio: 1/20) in accordance with the manufacturers terms of use. For the D condition, 1.25 g of dispersant was added to the 300 L seawater tank.

Feeding was stopped 24 h before the experiment; the fish were then randomly assigned to their experimental condition (8 fish per group) and placed in the 300 L exposure tanks for 48 h without water renewal. The total petroleum hydrocarbon (TPH) concentration was measured at the beginning, after 24 h of exposure and at the end of the 48 h fish exposure period for the four experimental conditions. The obtained results allowed us to adjust the TPH concentration to around 80 $mg\ L^{-1}$ by adding the appropriate CAL quantity when necessary. Each of these tanks was equipped with a pumping system allowing continuous water homogenization (see Milinkovitch et al., 2011 for details).

At the end of the exposure, the fish were sized, weighed and killed with a cerebral dislocation. The heart was sampled and placed in ice cold medium isoosmotic solution (in mM: NaCl 152, KCl 3.4, $MgSO_4$ 0.8, Na_2HPO_4 0.44, KH_2PO_4 0.44, $NaHCO_3$ 5, Hepes 10, Glucose 10, $CaCl_2$ 2.5, pH 7.8, 320 $mosmol^{-1}$). Gall-bladders were also sampled and stored at −80 °C.

2.4. Measurements of Total Petroleum Hydrocarbon (TPH) seawater concentrations

The total petroleum hydrocarbon concentration was measured in triplicate. One hundred mL samples of water were extracted three times with 10 mL of dichloromethane (Carlo Erba Reactifs, SDS). The combined organic phases were dried on anhydrous sulphate and the absorbance was measured at 390 nm (UVeVis spectrophometer, Unicam, France) as described by Fusey and Oudot (1976). The results are expressed in $mg\ L^{-1}$, and the linearity of the response was checked between 5 and 100 $mg\ L^{-1}$.

2.5. Measurements of droplet size

Droplet size (diameter in microns) in the CD and MD conditions were measured 6 h after the beginning of fish exposure. The measurements were performed by laser granulometry (Malvern Mastersizer 2000, Malvern Instruments Ltd, Worcestershire, United Kingdom) based on the principle of Fraunhofer according to the intensity of diffracted radiation, where the diffraction angle depends on the particle size. It is completed in our case with the Mie theory taking into account the refractive indices of the sample and the carrier medium (ISO 13320-1, 1999). A flow rate of 1200 $mL\ min^{-1}$ and an obscuration of 10 ± 0.01% were the conditions used during the measurements.

2.6. Fixed wavelength fluorescence analysis of bile

Bile contained in gall-bladder was used to perform semi-quantitative analysis of PAH biliary metabolites (Vuorinen et al., 2006). The bile was diluted in absolute ethanol (1/2000). Fluorescence measurements were performed with a Jasco FP-6200 (Tokyo, Japan). The measurements were made at wavelengths of excitation