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Impact of dispersed fuel oil on cardiac mitochondrial function in polar cod *Boreogadus saida*

Matthieu Dussauze · Lionel Camus · Stéphane Le Floch
Karine Pichavant-Rafini · Perrine Geraudie · Nathalie Coquillé
Aline Amérand · Philippe Lemaire · Michael Theron

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Abstract In this study, impact of dispersed oil on cardiac mitochondrial function was assessed in a key species of Arctic marine ecosystem, the polar cod *Boreogadus saida*. Mature polar cod were exposed during 48 h to dispersed oil (mechanically and chemically) and dispersants alone. The increase observed in ethoxyresorufin-*O*-deethylase activity and polycyclic aromatic hydrocarbon metabolites in bile indicated no difference in contamination level between fish exposed to chemical or mechanical dispersion of oil. Oil induced alterations of O₂ consumption of permeabilised cardiac fibres showing inhibitions of complexes I and IV of the respiratory chain. Oil did not induce any modification of mitochondrial proton leak. Dispersants did not induce alteration of mitochondrial activity and did not increase oil toxicity. These data suggest that oil exposure may limit the fitness of polar cod and consequently could lead to major disruption in the energy flow of polar ecosystem.

Keywords Oil · Dispersant · *Boreogadus saida* · Cardiac mitochondria · Permeabilised fibre · Arctic

Introduction

For several decades, profound changes due, in part, to global warming have been occurring in ecosystems. Amongst them, polar areas appear particularly sensitive (Mackey et al. 2012). Indeed, in Arctic regions a major reduction in ice areas has been observed (Comiso et al. 2008; Perovich et al. 2008), opening up new shipping routes and making possible the exploration and exploitation of new promising oil reserves (USGS 2000; Pietri et al. 2008). The increase in traffic and human activities in these highly sensitive regions enhances the risk of their contamination with petroleum compounds. To respond to oil spills, chemical dispersants are commonly used, but these compounds may increase the effects of oil pollution and therefore their toxicity (Chapman et al. 2007). Thus, the question of the effects of both fuel oil and dispersed oil on Arctic species should be raised.

The polar cod, *Boreogadus saida*, a key species in Arctic ecosystems (Orlova et al. 2009; Hop and Gjøsæter 2013), is often used to assess oil pollution effects in cold waters (see for example Jonsson et al. 2010; Nahrgang et al. 2009; 2010; Gardiner et al. 2013). Moreover, this fish is considered as an indicator of the effects of environmental oil contamination in the Arctic environment (Stange and Klungsøyr 1997).

Numerous effects of petroleum compounds are reported in the literature leading to alterations of fish metabolism at several organisational levels: organism (Hose and Puffer 1984; Correa and Garcia 1990; Sharp et al. 1979; Serigstad and Adoff 1985; Davoodi and Claireaux 2007; Christiansen et al. 2010), organ (Hicken et al. 2011; Milinkovitch et al. 2012, 2013) or cell (Bains and Kennedy 2004). At a cellular level, it is interesting to note that the mitochondrion have been

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M. Dussauze (✉) · K. Pichavant-Rafini · A. Amérand · M. Theron
Laboratoire ORPHY EA4324, Université de Bretagne Occidentale, 6
Avenue le Gorgeu, CS 93 837, 29 238 Brest Cedex 3, France
e-mail: Matthieu.Dussauze@cedre.fr

M. Dussauze · S. Le Floch · N. Coquillé
Cedre, Centre de Documentation, de Recherche et
d'Expérimentations sur les Pollutions Accidentelles des Eaux, 715
rue Alain Colas, CS 41 836, Brest Cedex 2, France

L. Camus · P. Geraudie
Akvaplan-niva, High North Research Centre for Climate and the
Environment, 9296 Tromsø, Norway

L. Camus
Engineering and Safety Department, University of Tromsø, Tromsø,
Norway

P. Lemaire
Total Fluides Total Fluides, S.A. 92907 Paris la Défense, France

shown to be one of the target of the polycyclic aromatic hydrocarbons (PAHs) (Knecht et al. 2013). In particular, swollen mitochondria have been observed in Pacific herring larvae (Cameron and Smith 1980) and in fat head minnows (Norton et al. 1985). Petroleum compounds could also lead to alterations to mitochondrial oxidative phosphorylation, membrane potential or an increase in superoxide production (Salazar et al. 2004; Xia et al. 2004; Stabenau et al. 2008; Westman et al. 2013). However, to our knowledge, no direct assessment of petroleum compounds on mitochondrial function had been conducted on cold water fish.

In this context, this work aims at assessing the effects of a dispersed fuel oil on mitochondrial activity from an active aerobic organ, i.e. the heart in *B. saida*. The fish were exposed for 48 h to mechanically dispersed oil, to two commercial formulations of dispersants and to the two corresponding chemically dispersed oils. Cardiac energy metabolism was evaluated by respirometry on permeabilised cardiac fibres. The exposure conditions were assessed by measuring the total petroleum hydrocarbon (TPH) concentration in the water, while the fish contamination status was evaluated through the presence of PAHs in the bile and measurement of ethoxresorufin-*O*-deethylase (EROD) activity.

Materials and methods

Animals

Polar cods (*B. saida*) were collected during summer 2011 in the waters of the Svalbard archipelago. They were acclimatised for 1 year in Troms Marin Yngel facility owned by Akvaplan-niva with a natural photoperiod. Forty-eight mature fish (mass, 94.4 ± 3.9 g; length, 23.5 ± 0.3 cm; mean \pm standard error of the mean) were used for this experimentation. Oxygen saturation was measured twice a day and was always higher than 90 %. The temperature (5.4 ± 0.2 °C) was measured daily.

Chemicals

Oil

The petroleum used in the study was a crude Arabian light (CAL). CAL is composed of 54 % saturated hydrocarbons, 10 % polar compounds and 36 % aromatic hydrocarbons and has been used in effect assessment studies (Rowe et al. 2009; Danion et al. 2011; Milinkovitch et al. 2011a; Claireaux et al. 2013). In this study, CAL was evaporated (with air bubbling) until a weight loss of 7 %. This process makes the lighter compounds to evaporate mimicking the weathering of an oil slick at sea (Milinkovitch et al. 2011a). The weathered CAL

contains 54 % saturated hydrocarbons, 12 % polar compounds and 34 % aromatic hydrocarbons.

Dispersants

Two dispersants were used in this study: Finasol OSR 52 a commercial formulation from TOTAL Fluides and Corexit 9500 from Nalco Company. They are both third-generation oil-based dispersant combining surfactants, amphiphilic molecules and solvents.

Experimental design

The fish were allocated to six experimental conditions: a control group (C), a group exposed to mechanically dispersed CAL (MD), two groups exposed to chemically dispersed CAL (Finasol OSR 52: CD-F and Corexit 9500: CD-C) and two groups exposed to the dispersants alone (Finasol OSR 52 alone: Da-F and Corexit 9500 alone: Da-C). In the case of the MD and CD conditions, 100 g of oil was poured in 105-L tanks; in the CD conditions, the dispersant oil ratio was 1/20. The details of the different treatments are given in Table 1.

The feeding was stopped 24 h before the experiment, and then the fish were randomly assigned to their experimental condition (eight fish per group) and placed in the 105-L exposure tanks for 48 h without water renewal. Each of these tanks was equipped with a pumping system allowing continuous water homogenisation (see Milinkovitch et al. 2011b for details).

Water samplings to measure TPH concentrations were performed at the beginning, after 24 h of exposure and at the end of the 48 h fish exposure period for the six experimental conditions. At the end of the exposure, the fish were sized, weighted and killed. The heart ventricle was sampled and placed in ice-cold medium isoosmotic solution (in mM: 152 NaCl, 3.4 KCl, 0.8 MgSO₄, 0.44 Na₂HPO₄, 0.44 KH₂PO₄, 5 NaHCO₃, 10 HEPES, 10 glucose, 2.5 CaCl₂, pH 7.8, 320 mOsmol). Liver and gall bladder were sampled and stored at -80 °C.

Table 1 Nominal concentrations of CAL and dispersants during the contamination

| Treatment | CAL (mg/L) | Finasol OSR 52 (mg/L) | Corexit 9500 (mg/L) |
|-----------|------------|-----------------------|---------------------|
| C | – | – | – |
| Da-F | – | 4.8 | – |
| Da-C | – | – | 4.8 |
| CD-F | 95 | 4.8 | – |
| CD-C | 95 | – | 4.8 |
| MD | 95 | – | – |

Analytical methods

Measurements of total petroleum hydrocarbon seawater concentrations

The TPH concentration (dissolved hydrocarbons and oil droplets) was measured in triplicate. One hundred millilitre samples of water were extracted twice 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 spectrophotometer, Unicam, France) as described by Fusey and Oudot (1976). The results are expressed in milligrams per litre, and the linearity of the response was checked between 5 and 100 mg/L.

Fixed wavelength fluorescence analysis of bile

Bile contained in gall bladder was used to perform semi-quantitative analysis of PAH biliary metabolite (Vuorinen et al. 2006). The bile was diluted in absolute ethanol (1:2,000). Fluorescence measurements were performed with a Kontron Instruments SFM25. The measurements were made at wavelengths of excitation and emission, 380:430 characteristics of benzo[*a*]pyrene type metabolites and 343:383 characteristics of 1-hydroxy-pyrene type metabolites (Lin et al. 1996; Aas et al. 2000). Values are expressed as arbitrary units (AU).

EROD activity in liver

EROD activity was measured on the microsomal fraction of liver according to Eggens and Galgani (1992). Activities were quantified by measuring the appearance of resorufin production in four replicates for 20 min at room temperature with a PerkinElmer Victor fluorimetric plate reader at 544:584 nm excitation/emission wavelengths, respectively. The total protein concentration in liver was assessed by spectrophotometry at 595 nm using the Bradford method. A resorufin standard curve (0–2 µM) was used to determine the reaction rates. EROD activity was calculated as picomoles per minute per milligram of total microsomal protein.

Tissue respiration

Measurement of O₂ consumption of permeabilised cardiac fibre was performed using four thermostated respiration chambers (volume, 1.4 mL) using an oxymeter Oxy-4 (Presens, Regensburg, Germany). Permeabilisation of cardiac fibres was performed following a method adapted for fish from Veksler et al. (1987), Toleikis et al. (1997) and Kuznetsov et al. (2008) at 4 °C using a saponine and collagenase solution. Briefly, cardiac fibres were cut in solution A (in mM: 5.5 EDTA, 2.5 MgCl₂, 10 imidazol, 20 HEPES, 70

KCl, 3.3 ATP, 2 PCr, 0.5 dithiothreitol; pH 7.4 at 4 °C) and were placed in incubation during 30 min in solution B (Solution A plus 0.05 mg/mL of saponine and 2 mg/mL of collagenase). Fibres were then washed 10 min in solution A and 10 min in respiration medium (in mM: 0.08 EDTA, 7.5 MgCl₂, 150 KCl, 20 Tris, 10 NaH₂PO₄, pH 7.4).

After permeabilisation, the fibres were placed in respiration chambers filled with respiration medium. All measurements were performed in excess of ADP. Successive addition of substrates (pyruvate/malate, succinate and ascorbate/TMPD), cytochrome C and oligomycin were performed into the respiration chamber. Details of these additions are reported in Table 2 and Fig. 1.

Cytochrome C was used to check the integrity of mitochondrial external membranes (Kuznetsov et al. 2008). Pyruvate/malate, succinate and ascorbate/TMPD allowed the analysis of different inputs in the mitochondrial respiratory chain. Oligomycin blocks the fifth complex of the respiratory chain and was used to measure the proton leak through the inner mitochondrial membrane. Proton leak was calculated as the O₂ consumption with oligomycin divided by the O₂ consumption with ascorbate/TMPD.

At the end of the experimentation, the fibres were dried and weighted to determine the O₂ consumption in micromoles O₂ per minute per milligram of dry tissue.

Statistical analysis

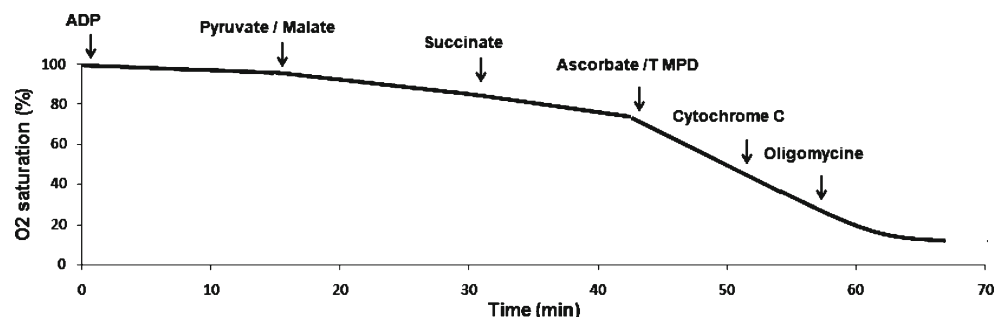
All analyses were performed using STATGRAPHICS software. First, all the data were tested with a Levene test to assess the variance homogeneity, and Kolmogorov–Smirnov and Shapiro–Wilk tests were used to ensure that data followed a normal distribution. The samples with a normal distribution were tested with a one-way ANOVA followed by a Fisher’s test. Samples that did not follow a normal distribution were tested with a Kruskal–Wallis analysis. All data are expressed as the mean±standard error of the mean.

Table 2 Added products in respiration chamber

| Products | Concentration (mM) | Injected quantity (µL) |
|--------------|----------------------|------------------------|
| ADP | 500 | 7 |
| Pyruvate | 1,000 | 12.5 |
| Malate | 500 | 12.5 |
| Succinate | 500 | 75 |
| Ascorbate | 2,000 | 20 |
| TMPD | 200 | 3 |
| Cytochrome C | 0.98 | 15 |
| Oligomycine | 3,16E ⁻⁰⁴ | 40 |

N,N,N,N',N'-tetramethyl-*p*-phenylenediamine dihydrochloride

Fig. 1 Profile of O₂ consumption of permeabilised heart fibres with addition of products into the respiration chamber



Results

Condition index

Fulton's indices of polar cod from the six experimental conditions are presented in Table 3. For the six experimental conditions, no significant difference was observed and the Fulton's indexes ranged between 0.68 ± 0.02 and 0.75 ± 0.10 .

Hydrocarbon exposure

Total petroleum hydrocarbon seawater concentrations

The mean TPH concentrations (in mg/L) for the 48 h of exposure are presented in Table 4. No hydrocarbons could be detected in the control condition and with the dispersants alone (Da-F and Da-C) during the 48 h of exposure. In the case of the three CAL containing conditions (CD-F, CD-C and MD), TPH concentrations were similar and situated between 40 and 49 mg/L.

Fixed wavelength fluorescence analysis of bile

The fluorescence intensities of bile samples at characteristic wavelengths of OH-BaP (343:383 nm) and OH-pyrene (380:430 nm) metabolites are presented in Table 5. After the

Table 3 Fulton's index for the six experimental conditions at the end of the contamination period

| | Fulton's index |
|------|-----------------|
| C | 0.69 ± 0.02 |
| Da-F | 0.73 ± 0.03 |
| Da-C | 0.75 ± 0.10 |
| CD-F | 0.75 ± 0.03 |
| CD-C | 0.68 ± 0.02 |
| MD | 0.70 ± 0.03 |

Mean \pm standard error of the mean ($n=8$)

C control, Da-F dispersant alone Finasol OSR 52, Da-C dispersant alone Corexit 9500, CD-F chemical dispersion of oil by Finasol OSR 52, CD-C chemical dispersion of oil by Corexit 9500, MD mechanical dispersion of oil

48 h of exposure, for the two couples of wavelengths, the two dispersant groups (Da-F and Da-C) were not statistically different from the control group (C). In CD-F, CD-C and MD conditions, bile fluorescence was significantly increased when compared to the control group. There was no statistical difference between the three CAL conditions (CD-F, CD-C and MD) at the two couples of wavelengths.

EROD activity in liver

After the 48 h of exposure, EROD activities in liver in the two dispersants groups (Da-F and Da-C) were not statistically different from the controls group (see Fig. 2). EROD activities for C, Da-F and Da-C were, respectively, 0.8 ± 0.3 , 0.8 ± 0.2 and 0.7 ± 0.2 pmol min⁻¹ mg⁻¹. In CD-F, DC-C and MD conditions, EROD activities were significantly increased to 2.6 ± 0.8 , 2.1 ± 0.7 and 3.9 ± 1.2 pmol min⁻¹ mg⁻¹, respectively. No difference was observed between the three oil conditions.

Tissue respiration

Mitochondrial integrity

Measurements of O₂ consumption after addition of ascorbate/TMPD and cytochrome C into the respiration chamber are reported in Table 6. For each condition, no significant difference was observed between O₂ consumption with ascorbate/TMPD and after addition of cytochrome C.

O₂ consumption of permeabilised cardiac muscle fibres

The results of O₂ consumption after addition of pyruvate/malate, succinate and ascorbate/TMPD are reported in Fig. 3. After pyruvate/malate addition, no difference was observed between the Da-F and C conditions. For these two conditions, O₂ consumptions were significantly higher compared to the MD condition. The two conditions with chemical dispersion of oil (CD-F and CD-C) were not statistically different from the two conditions with dispersant alone. After addition of succinate, no difference was observed between the six conditions. After addition of ascorbate/TMPD, O₂ consumption was similar for the four conditions with

Table 4 Total petroleum hydrocarbons concentrations over the contamination period (48 h) in milligrams per litre for the six experimental conditions

| | C | Da-F | Da-C | CD-F | CD-C | MD |
|---|-----|------|------|-------|------|-------|
| Total petroleum hydrocarbon concentrations (mg/L) | n.d | n.d | n.d | 46±10 | 49±8 | 40±12 |

Results in milligram per litre (mean±standard error of the mean; n=8)

n.d. not detected, C control), Da-F dispersant alone Finasol OSR 52, Da-C dispersant alone Corexit 9500), CD-F chemical dispersion of oil by Finasol OSR 52), CD-C chemical dispersion of oil by Corexit 9500), MD mechanical dispersion of oil

dispersants and MD conditions. While the four dispersant conditions were not different from the control condition, exposure to MD induced a significant decrease in O₂ consumption of permeabilised cardiac muscle fibres.

Proton leak

Results of proton leak for the six experimental conditions are reported in Fig. 4. No statistical variation was observed between the six experimental conditions after measurement of the proton leak.

Discussion

Fish

In this study, 48 polar cod were exposed to fuel oil in order to evaluate the effects of petroleum compounds on the mitochondrial activity of an organ of interest the heart. The Fulton indices of these fish ranged from 0.68 to 0.75. These values are higher than one that reported by Nahrgang et al. (2010). This difference could be explained by the conditions of stabulation. In this study, the fish were fed daily with commercial food, and no energetic cost was linked to prey catching or predator escape.

Table 5 Biliary fluorescence

| | 343:383 | 380:430 |
|------|--------------|----------------|
| C | 6.8±0.3(a) | 19.5±0.8 (a) |
| Da-F | 7.2±0.3(a) | 18.7±0.6 (a) |
| Da-C | 7.4±0.6 (a) | 17.4±0.5 (a) |
| CD-F | 21.4±1.9 (b) | 179.7±19.2 (b) |
| CD-C | 23.9±3.6 (b) | 209.5±40.7 (b) |
| MD | 22.0±1.9 (b) | 197.8±23.2 (b) |

Results expressed as arbitrary fluorescence units (mean±standard error of the mean; n=8). Values not sharing common letters indicate a significant difference (P<0.05)

C control, Da-F dispersant alone Finasol OSR 52, Da-C dispersant alone Corexit 9500, CD-F chemical dispersion of oil by Finasol OSR 52, CD-C chemical dispersion of oil by Corexit 9500), MD mechanical dispersion of oil

Contamination

In this experiment, polar cod (*B. saida*) were exposed for 48 h to mechanically dispersed weathered CAL, to two dispersants and to the corresponding chemically dispersed oil. TPH measurement was used to monitor exposure conditions, while fish contamination was assessed by measuring EROD activity and bile fluorescence.

The weathering of the oil simulates 12 h ageing of a slick at sea, 12 h being a minimum response time, and the use of CAL allowed comparison with previous studies on temperate species (Luna-Acosta et al. 2011; Milinkovitch et al. 2011a).

The results of TPH measurements show no detectable levels of hydrocarbons in the control and dispersant alone conditions (C, Da-C and Da-F). In the case of the mechanical and chemical dispersion, the measured concentrations were nearly half of the nominal concentration (40–49 mg/L vs 95 mg/L). Similar experiments with the same weathered CAL and similar contamination setups have shown that chemical dispersions lead to higher TPH concentrations than mechanical dispersions at 14–15 °C (Milinkovitch et al. 2011b, 2012). In the case of this study, the low temperature (4 °C) could be responsible for the lower efficiency of the chemical dispersion.

These concentrations can be considered as representative of environmental oil contamination after oil spills at sea where TPH in the top 10 m of the water column can reach 20–60 mg/L (Spooner 1970; Lunel et al. 1997; Lewis et al. 1998; Lessard and Demarco 2000).

In this work, individual contamination levels were estimated by measuring biliary fluorescence and EROD activity, two efficient biomarkers of exposure to petroleum compounds in fish. The analysis of biliary PAH fluorescence is recognised as an efficient way to assess recent exposure to PAH (Aas et al. 2000; van der Oost et al. 2003). EROD activity is a catalytic measurement of cytochrome P4501A induction, responding to a multitude of chemicals in fish (Whyte et al. 2000); it is an efficient biomarker of exposure to petroleum compounds (Goksøyr and Förlin 1992; Lin et al. 1996; Ramachandran et al. 2004). These two parameters were used to assess the effectiveness of the contamination. The results of OH-pyrene and OH-BaP metabolites are equivalent: Once the background fluorescence of control and dispersant alone groups (due to auto-fluorescence, Vuontisjärvie et al. 2004) has been set

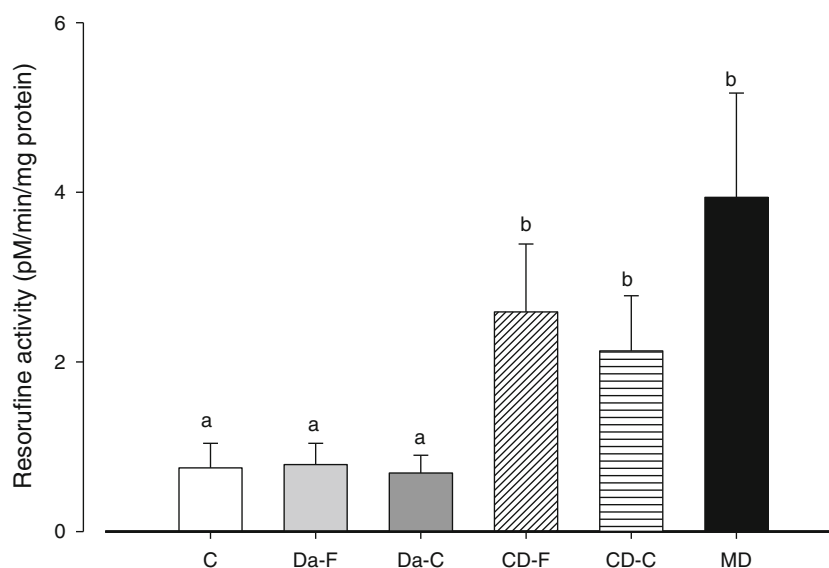


Fig. 2 Measurement of EROD activity in the liver of polar cod. Results are expressed as Resorufine activity in picomolar per minute per milligram protein (mean±standard error of the mean; $n=8$). *C* control (open bar), *Da-F* dispersant alone Finasol OSR 52 (light grey bar), *Da-C* dispersant alone Corexit 9500 (dark grey bar), *CD-F* chemical dispersion

of oil by Finasol OSR 52 (diagonally striped bar), *CD-C* chemical dispersion of oil by Corexit 9500 (horizontally striped bar) and *MD* mechanical dispersion of oil (closed bar). Values not sharing common letters in the column indicate a significant difference ($P<0.05$)

apart, they clearly show that results from the three oil exposure conditions (*MD*, *CD-C* and *CD-F*) are not different. This conclusion is consistent with TPH results and further supported by the EROD measurements: There is no significant difference between the *CD-F*, *CD-C* and *MD* group activities, and these groups exhibit results significantly higher than *C*, *Da-C* and *Da-F* groups.

Contamination effect of on tissue respiration

Studies on mammalian cells have shown that after contamination PAH are preferentially localised in the mitochondria

Table 6 Measurements of O_2 consumption after addition of ascorbate/TMPD and cytochrome C for the six experimental conditions

| | O_2 consumption ($\mu\text{mol } O_2 \text{ min}^{-1} \text{ mg}^{-1}$ of dry tissues) | |
|------|---|-----------------|
| | Ascorbate/TMPD | Cytochrome C |
| C | 0.35 ± 0.05 | 0.31 ± 0.05 |
| Da-F | 0.30 ± 0.05 | 0.24 ± 0.03 |
| Da-C | 0.25 ± 0.06 | 0.23 ± 0.04 |
| CD-F | 0.23 ± 0.06 | 0.18 ± 0.03 |
| CD-C | 0.23 ± 0.03 | 0.24 ± 0.04 |
| MD | 0.20 ± 0.03 | 0.18 ± 0.01 |

Results expressed as micromoles O_2 per minute per milligram of dry tissues (mean±standard error of the mean; $n=8$)

C control), *Da-F* dispersant alone Finasol OSR 52, *Da-C* dispersant alone Corexit 9500, *CD-F* chemical dispersion of oil by Finasol OSR 52, *CD-C* chemical dispersion of oil by Corexit 9500, *MD* mechanical dispersion of oil

(Zhu et al. 1995). Once in these organelles, they can be metabolised by mitochondrial cytochrome P450 (Jung and Di Giulio 2010), leading to more reactive and more toxic compounds. Consequently, the mitochondrion appears to be a particularly interesting target for studies of PAH effects. The literature on PAH effects on mitochondria is scarce, but they have been shown to adversely impact mitochondrial function. In *Rana pipiens* exposed to pyrene, Stabenau et al. (2008) evidenced alterations of muscle mitochondria oxygen consumption and membrane potential. In zebra fish embryos, a reduction of mitochondrial oxygen consumption rate (Knecht et al. 2013) has been demonstrated after exposure to a cocktail of PAH.

In this study, the permeabilisation of cells using saponin and collagenase made possible the analysis of functional mitochondria in situ, in their normal intracellular position, and the use of selected substrates and inhibitors allowed the analysis of different elements of the respiratory chain.

A preliminary step for this type of approach is the verification of the mitochondrial integrity after the permeabilisation process. This can be done by the addition of cytochrome C under maximal oxygen consumption conditions with ascorbate and TMPD as substrates (Kuznetsov et al. 2008). In our case, the addition of the exogenous cytochrome C had no effects on O_2 consumption, showing that the integrity of mitochondria external membranes was preserved.

Pyruvate/malate, succinate and ascorbate/TMPD are respectively substrates of the first second and fourth complexes of the respiratory chain. The measurement of maximal ADP-stimulated (state 3) respiration with these substrates made

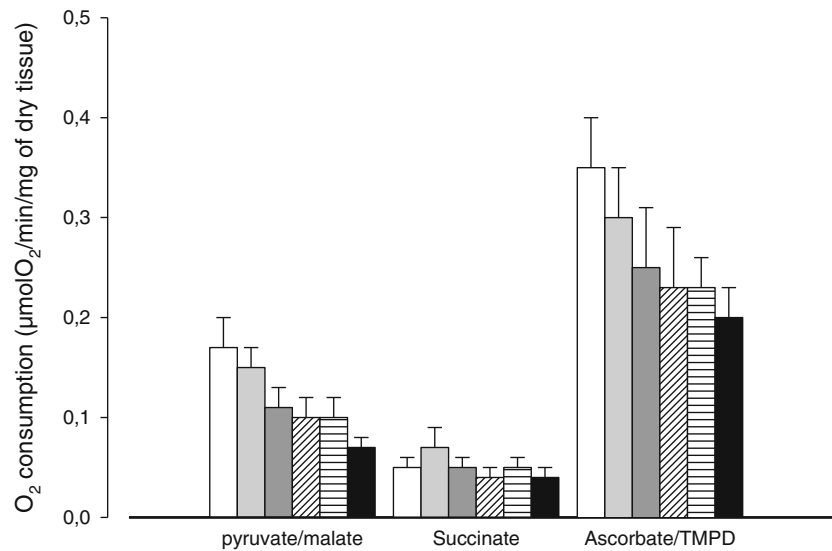


Fig. 3 O₂ consumption of permeabilised cardiac muscle fibres for the six experimental conditions after addition of substrates. Results expressed as micromoles O₂ per minute per milligram of dry tissues (mean±standard error of the mean; n=8). C control (open bar), Da-F dispersant alone Finasol OSR 52 (light grey bar), Da-C dispersant alone Corexit 9500 (dark grey bar), CD-F chemical dispersion of oil by Finasol OSR 52 (diagonally striped bar), CD-C chemical dispersion of oil by Corexit 9500 (horizontally striped bar) and MD mechanical dispersion of oil (closed bar). Values not sharing common letters in the column indicate a significant difference (P<0.05)

reduced when ascorbate/TMPD is used as a substrate. These results are consistent with the works of Stabenau et al. (2008) and Knecht et al. (2013) cited previously and showing a reduction in respiratory chain activity after contamination. When F₀-F₁ ATPase (the fifth complex of the respiratory chain) is inhibited by oligomycin, the remaining oxygen consumption is mainly due to proton leak through the inner mitochondrial membrane (Kuznetsov et al. 2008). In the

possible the analysis of different parts of the respiratory chain. The results presented here show that exposure to fuel oil (either mechanically or chemically dispersed) induces a reduction in the maximal O₂ consumption when the respiratory chain is fed with pyruvate/malate, but no effect was observed when succinate is used. The cytochrome oxidase (complex IV) activity is also altered after exposure to mechanically dispersed oil since the state 3 respiration rate is significantly

reduced when ascorbate/TMPD is used as a substrate. These results are consistent with the works of Stabenau et al. (2008) and Knecht et al. (2013) cited previously and showing a reduction in respiratory chain activity after contamination. When F₀-F₁ ATPase (the fifth complex of the respiratory chain) is inhibited by oligomycin, the remaining oxygen consumption is mainly due to proton leak through the inner mitochondrial membrane (Kuznetsov et al. 2008). In the

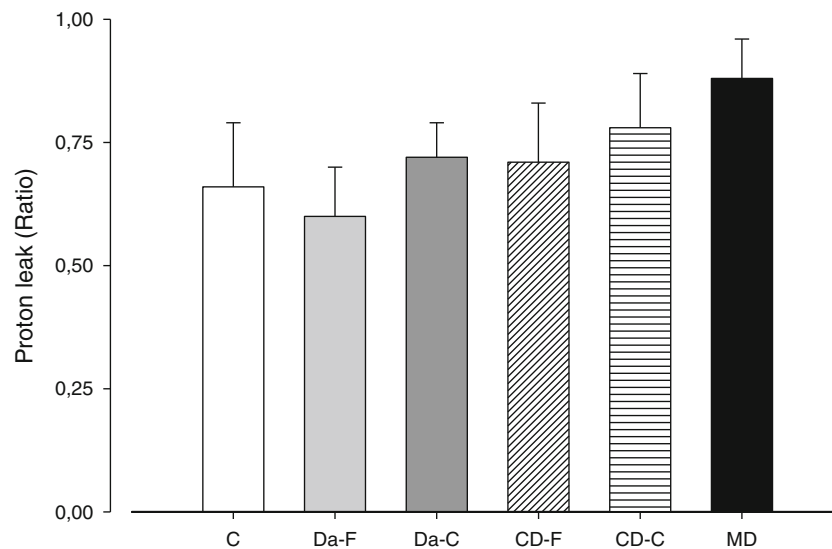


Fig. 4 Measurement of proton leak of permeabilised cardiac muscle fibres for the six experimental conditions. Results expressed as (mean±standard error of the mean; n=8). C control (open bar), Da-F dispersant alone Finasol OSR 52 (light grey bar), Da-C dispersant alone Corexit 9500 (dark grey bar), CD-F chemical dispersion of oil by Finasol OSR 52 (diagonally striped bar), CD-C chemical dispersion of oil by Corexit 9500 (horizontally striped bar) and MD mechanical dispersion of oil (closed bar). Values not sharing common letters in the column indicate a significant difference (P<0.05)

reduced when ascorbate/TMPD is used as a substrate. These results are consistent with the works of Stabenau et al. (2008) and Knecht et al. (2013) cited previously and showing a reduction in respiratory chain activity after contamination. When F₀-F₁ ATPase (the fifth complex of the respiratory chain) is inhibited by oligomycin, the remaining oxygen consumption is mainly due to proton leak through the inner mitochondrial membrane (Kuznetsov et al. 2008). In the

present work, no modification of this proton leak could be observed in any groups. This result, taken together with the fact that oxygen consumption with succinate was not modified, makes unlikely the hypothesis of an internal membrane modification that could in turn induce a modification of the microenvironment of the respiratory chain complexes or the permeability of the inner membrane to ions. The hypothesis of an effect on the complexes of the respiratory chain seems more promising. In fact, PAH exposure has been shown to induce superoxide generation in cell cultures (Xia et al. 2004) and up-regulation of oxidative stress-related genes in zebrafish (Knecht et al. 2013). Furthermore, oxidative stress is known to induce alterations of complexes I, III and IV of the respiratory chain (Musatov and Robinson 2012). These elements and the results of our study are compatible with the hypothesis of a reactive oxygen species mediated inhibition of the respiratory chain by PAH.

As far as we know, this work is the first study into the effects of dispersants on mitochondrial activity. Oxygen consumption results show that the dispersant groups (Da-F, Da-C, CD-F and CD-C) exhibited intermediate results between the control and the MD condition. It appears that, in most cases, the dispersant did not induce a decrease in energy production by mitochondria and that the dispersants did not increase the oil toxicity.

Conclusion

This study was performed to assess the potential toxicity of fuel oil and dispersed oil on the mitochondrial metabolism of a key species in Arctic ecosystem. It clearly demonstrates that fuel oil impairs mitochondrial respiratory chain activity, confirming on a polar species results observed on temperate and tropical animals. The results obtained provide new elements on the mechanism of oil toxicity showing an inhibition of the first and the third complexes of the respiratory chain. Finally, this study is the first analysis of dispersant effects on mitochondrial metabolism; it makes it possible to conclude that the dispersants tested alone have low effects and do not worsen the effects of oil contamination. In order to enhance our comprehension of the effects of the contamination on the mitochondrial respiratory chain, further experiments are still needed; they will make possible the determination of the affected complexes.

Since mitochondria activity is fundamental, these data suggest that oil exposure may limit the fitness of polar cod in their environment by limiting prey catching, mating, and predator avoidance behaviours. Because of their important function in the Arctic marine food web (Bradstreet et al. 1986; Welch et al. 1992), the effects of oil on polar cod metabolism could lead to major disruption in the energy flow of the polar ecosystem. There is still a clear need for

experiments filling the gap between mechanistic approaches of oil effects and their potential environmental consequences.

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