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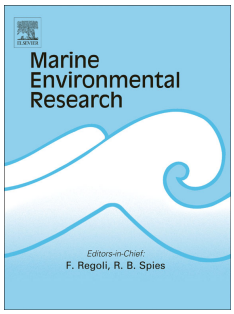

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EROD activity in liver and gills of polar cod (*Boreogadus saida*) exposed to waterborne and dietary crude oil

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Abstract

Polar cod *Boreogadus saida* an indicator species for biomonitoring in the Arctic was exposed to crude oil in waterborne and dietary experiments. Ethoxyresorufin O-deethylase (EROD) activity was measured in liver and gills of polar cod at week 0, 2 and 4 of exposure and following 2 weeks of depuration. EROD increased significantly and dose-dependently in both tissues through both exposure routes. Levels were very low in gills compared to liver reflecting the tissue-specific metabolism capacities and tissue-specific response kinetics were also observed. Furthermore, a significant increase of gill EROD was shown in dietary exposed fish, demonstrating a substantial transport of PAHs via the systemic circulation. To conclude, this study gave some preliminary information on the EROD response in terms of levels, dose dependency and timing, in gills of PAH exposed polar cod.

Key words: crude oil, EROD activity, polar cod, Arctic, gills, liver

1. Introduction

Biomarkers are developed for routine environmental monitoring to assess adverse effects of pollutants on the biological systems and to provide earlier and more sensitive signals than conventional endpoints such as growth, survival and reproduction (Ryan and Hightower, 1996). These measurements have gained broad acceptance and are used in several monitoring programmes (JAMP, 1998; Hylland et al., 2008).

With expansion of oil and gas activities in the Arctic, biomarkers are being developed for Arctic species. Recent studies have shown the relevance of polar cod *Boreogadus saida* as an indicator species for the implementation of biomarker based environmental monitoring in the Arctic (Nahrgang et al., 2010; accepted). Indeed, biomarkers of PAH exposure, i.e. hepatic CYP1A, GST activities and their mRNA expressions measured in the liver and PAH metabolites in the bile, showed significant dose-dependent inductions in polar cod exposed to crude oil via the water (Nahrgang et al., accepted) and the diet (Nahrgang et al., 2010). Nevertheless, gills, which are the principal site of xenobiotic uptake in fishes exposed via the water, have been considered a tissue of choice in several fish species for measuring biomarker responses such as the ethoxyresorufin O-deethylase (EROD) activity (Jönsson et al., 2002; Andersson et al., 2007; Jönsson et al., 2009). To date, EROD activity has never been measured in polar cod gills. Hence, the present study aims to investigate the response of gill EROD activity in polar cod exposed to crude oil through waterborne and dietary exposures and compare EROD levels between gills and liver and exposure routes. This study is a complementary work to larger studies on biomarker responses by Nahrgang et al. (2010; accepted).

2. Materials and Methods

Polar cod were caught with a bottom trawl in Rijpfjorden (Svalbard) and kept on deck in a tank with running seawater during 24 h while being transferred to the Kings Bay Marine Laboratory in Ny-Ålesund (latitude ~ 79° N). Polar cod were acclimated to the laboratory conditions for 1 month in 700 L holding tanks with running and filtered seawater and were fed weekly till satiation with the pelagic amphipod *Themisto libellula*. The photoperiod was constant dimmed light during periods of acclimation and experiments (October-November 2007). Polar cod were transferred into experimental tanks (160 L) at a density of 24 fish per tank, 2 weeks prior experimental start.

In the waterborne crude oil exposure polar cod (Total N=96) were exposed to the water soluble fraction of North Sea crude oil, using oiled rock columns (Carls et al., 1999). Briefly, gravels were mixed with crude oil at concentrations of 3, 6 and 12 g crude oil kg^{-1} gravel corresponding to low, medium and high oil treatments respectively. The oiled gravels were inserted in 1 m high PVC columns. Particle filtered and UV treated seawater percolated upwards through the columns (960 ml min^{-1}) to extract the water soluble fraction into the experimental tanks. The control tank received water percolated through a column containing clean gravel. Fish were exposed continuously for 4 weeks. The columns were then removed, and tanks were provided with clean water for 2 weeks for studying the effect of a depuration period on EROD activity. Total PAH concentration in the water is presented in Table 1.

In the dietary exposure, polar cod (Total N=72) were fed with *T. libellula* which was blended with gelatine powder and North Sea crude oil at a final concentration of 0.5 and 2 mg crude oil g^{-1} wwt food for low and high oil treatments respectively and gelatine powder only for the control diet. Total PAH concentration was 0.1, 18 and 43 µg g^{-1} wwt diet in the control, low and high oiled diet respectively. During 4 weeks, polar cod were fed once a week, as a group, assuming an administered dose of 4 g diet per fish (~ 20%

body weight). Fish were then fed 2 additional weeks with the control diet for studying the effect of a depuration period on EROD activity.

Polycyclic aromatic hydrocarbons (16 Environmental Protection Agency [EPA] priority PAHs and naphthalenes, phenanthrenes and dibenzothiophenes [NPD]) were analyzed in the water samples of the waterborne experiment and in the food of the dietary experiment by Gas chromatograph/Mass Spectrometry (GC/MS). Further details of the experimental setups and the analysis of polycyclic aromatic hydrocarbons are given in Nahrgang et al. (accepted) and Nahrgang et al. (2010) for the waterborne and dietary exposures respectively.

In both experiments, polar cod (n=6/treatment) were sampled at week 0, week 2 and 4 of exposure and following 2 weeks of depuration (week 6). In the waterborne experiment, the water was percolated through the columns 48 h before the transfer of polar cod into the experimental tanks to ensure the volatilization of BTEX compounds (benzene, toluene, ethylbenzene, and xylene) and an absence of particulate oil. Therefore, the first sampling is noted “week 0+”, due to a delay of 7 h between the first and the last fish sampled at this time. In the dietary experiment, polar cod were first sampled before the exposure started and sampling is noted “week 0”. Polar cod were sacrificed by sharp blow to the head. Fork length (± 0.1 cm), weight (± 0.1 g) and gender were recorded. Liver slices and gill filaments were placed into separate cryovials, snap frozen in liquid nitrogen and stored at -80 °C prior to analysis.

Ethoxyresorufin O-deethylase (EROD) activity was measured on the microsomal fraction of liver and gill tissue. Briefly, liver and gill samples were homogenized at 4 °C in a phosphate buffer pH 7.4 containing 50 mM Tris, 150 mM KCl, 1 mM EDTA, 1 mM dithiothreitol and 20% glycerol. Microsomal fractions was extracted via successive centrifugations of the supernatant (10,000×g for 30 min and 50,000×g for 2 h at 4◦C) and

dissolution of the microsomal pellets in Tris buffer (pH 7.4). Ethoxyresorufin O-deethylase activity (pmol resorufin min^{-1} mg^{-1} protein) was determined according to Eggens and Galgani (1992). The reaction mix consisted of 10 µl microsomal fraction in 100 mM of Tris-phosphate buffer (pH 8), ethoxyresorufin 2 µM as substrate in a final volume of 230 µl. Reaction started by adding 0.25 mM NADPH in the microwells. The resorufin production was measured in four replicates during 20 min at room temperature with a fluorimetric plate reader PerkinElmer Victor at 544/584 nm excitation/emission wavelengths, respectively. A resorufin standard curve (0–2 µM) was used for determination of the reaction rates in pmol of resorufin produced min^{-1} mg^{-1} of total microsomal protein. Total concentration of microsomal protein was determined according to Bradford (1976).

Statistical analyses were performed on Statistica® (Ver. 6.1). No gender differences were found for the parameters examined and data were pooled in analyses. Requirements for homogeneity and normality (Levene test, $p \leq 0.05$) were not met, thus the non-parametric Mann–Whitney *U*-test was used for comparison of the exposed groups with the control group at each sampling time. The significance level for all analyses was $p \leq 0.05$.

3. Results and Discussion

EROD activity was the same in control fish from both experiments i.e. 3.1 ± 0.5 pmol min^{-1} mg^{-1} protein in the liver and < 0.02 pmol min^{-1} mg^{-1} protein in the gills of polar cod. The maximum EROD activity was ~ 60 pmol min^{-1} mg^{-1} protein in the liver and < 3 pmol min^{-1} mg^{-1} protein in the gills of fishes exposed through the water (Fig. 1). In the dietary exposed fish, the maximum activities were lower, i.e. ~ 50 pmol min^{-1} mg^{-1} protein in the liver and < 0.6 pmol min^{-1} mg^{-1} protein in the gills (Fig. 2). The

significantly lower levels of EROD activity in gills compared to liver support similar findings observed in other fish species (e.g. Andersson et al., 2007; Ortiz Delgado et al., 2009). The difference in absolute levels between liver and gills are related to the amount of CYP1A proteins in these tissues. Indeed, the liver is the main metabolizing organ in the fish, containing larger amounts of enzymes (Hinton et al., 2005).

EROD activity in liver and gills and for both exposure routes was increased after 2 weeks of exposure compared to controls. The 2 week sampling window was too large to determine when EROD activity was first increased. Nahrgang et al. (accepted) showed however, that *cyp1a1* mRNA expression in the liver was induced in the first hours of the waterborne exposure, suggesting that hepatic EROD activity may have been increased early in the experiment.

In the waterborne experiment, the increase in EROD activity seemed faster in the liver than in the gills, with maximum levels reached at week 2 and a declining trend at week 4 in the liver, except in the highest treatment (Fig. 1). By contrast, EROD activity in the gills increased linearly over the entire exposure. Considering the continuous decrease in PAH concentration in the water over the experiment (Table 1), these results suggest that gill EROD activity was not directly driven by the PAH uptake from the water but rather by the continuous synthesis of CYP1A proteins and a potential accumulation of PAHs in this tissue, which has lower metabolism capacity than the liver. Furthermore, above an induction threshold, the amount of PAH required to maintain the EROD activity at a high level may be lower.

In the dietary experiment, EROD activity in the liver was high compared to controls and at similar levels at week 2 and 4 for the oil treatments (Fig. 2). The exposure pressure was maintained continuously through the weekly feeding, contrasting the decreasing PAH concentration of the waterborne experiment. These differences between

exposure routes may explain partly the differences in the observed EROD levels over time. The significant increase of EROD activity in the gills of dietary exposed polar cod reflected a substantial transport of PAHs to the gills via the systemic circulation. In this experiment, EROD activity in the gills and liver was probably driven by the tissue distribution of PAHs through the systemic circulation and the tissue-specific metabolism capacity.

Following 2 weeks of depuration, EROD activity in exposed polar cods from both exposures decreased in liver and gills. In the waterborne exposure, EROD activity remained significantly higher in the liver of the medium and high oil exposed fishes and in the gills of fishes from all oil treatments compared to controls. In the dietary exposure, EROD activity was significantly higher in liver and gills of fish from the highest oil treatment compared to the controls while EROD activity returned to basal levels in polar cod from the low oil treatment. After 2 weeks depuration period, PAHs were undetected in the water of the waterborne experiment and PAH metabolites were undetected in the bile of polar cod from both exposures (Nahrgang et al., 2010; accepted), suggesting that the PAHs were unlikely to cause these persisting activities. It is however difficult to draw a conclusion based on EROD activity alone and additional data such as the accumulation of PAHs in the tissues would be highly relevant to include in further experiments. An alternate hypothesis to the persistence of EROD activity following 2 weeks of depuration period may be the specific properties of enzymes in cold-adapted species such as protein stability or low protein degradation rate (Pörtner et al., 2005).

This study shows that EROD activity in gills of polar cod was significantly and dose-dependently increased and discriminated the exposure time points even better than in liver. Nevertheless, in field-based studies, where PAH contamination levels may be even lower than in the present experiments and where environmental confounding factors

may occur, the detection limit of the EROD assay, which may be close to the levels reported in the gills (< 3 pmol min^{-1} mg^{-1} protein), may become an issue. Furthermore, the design of the present experiments did not allow studying thoroughly the response kinetics of EROD activity in the liver and gills of polar cod exposed to PAHs. In future experiments, it would thus, be interesting to further investigate these kinetics by enlarging the sampling window and including further parameters such as the bioaccumulation of PAHs in the liver and gills.

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Table 1 Total concentration (µg L^{-1}) in polycyclic aromatic hydrocarbons (26 PAHs) in the water of the control, low, medium and high crude oil treatments. Asterisk indicates that the total PAH was underestimated. Na: not available.

Treatment/Time (weeks)	0	2	4	2-week depuration
Control	0.06	na	0.004	0.002
Low oil	14.6	1.6	0.4	0.004
Medium oil	18.1	4.0	na	0.003
High oil	40.2*	10.8	4.3	0.002

FIGURE CAPTIONS

Figure 1. Ethoxyresorufin O-deethylase (EROD) activity (pmol min^{-1} mg^{-1} proteins) (A) in the liver (from Nahrgang et al., accepted) and (B) gills of polar cod *Boreogadus saida* (n=6) exposed to low, medium and high levels of waterborne crude oil and controls. Fish were sampled at 0, 2 and 4 weeks of exposure and after 2 weeks of depuration (week 6). Values are shown as means ± SE. Asterisks indicate significant difference ($p \leq 0.05$) between exposed groups and the control group.

Figure 2. Ethoxyresorufin O-deethylase (EROD) activity (pmol min^{-1} mg^{-1} proteins) (A) in the liver (from Nahrgang et al., 2010) and (B) gills of polar cod *Boreogadus saida* (n=6) exposed to low and high levels of dietary crude oil and controls. Fish were sampled at 0, 2 and 4 weeks of exposure and after 2 weeks of depuration (week 6). Values are shown as means ± SE. Asterisks indicate significant difference ($p \leq 0.05$) between exposed groups and the control group.

Figure 1

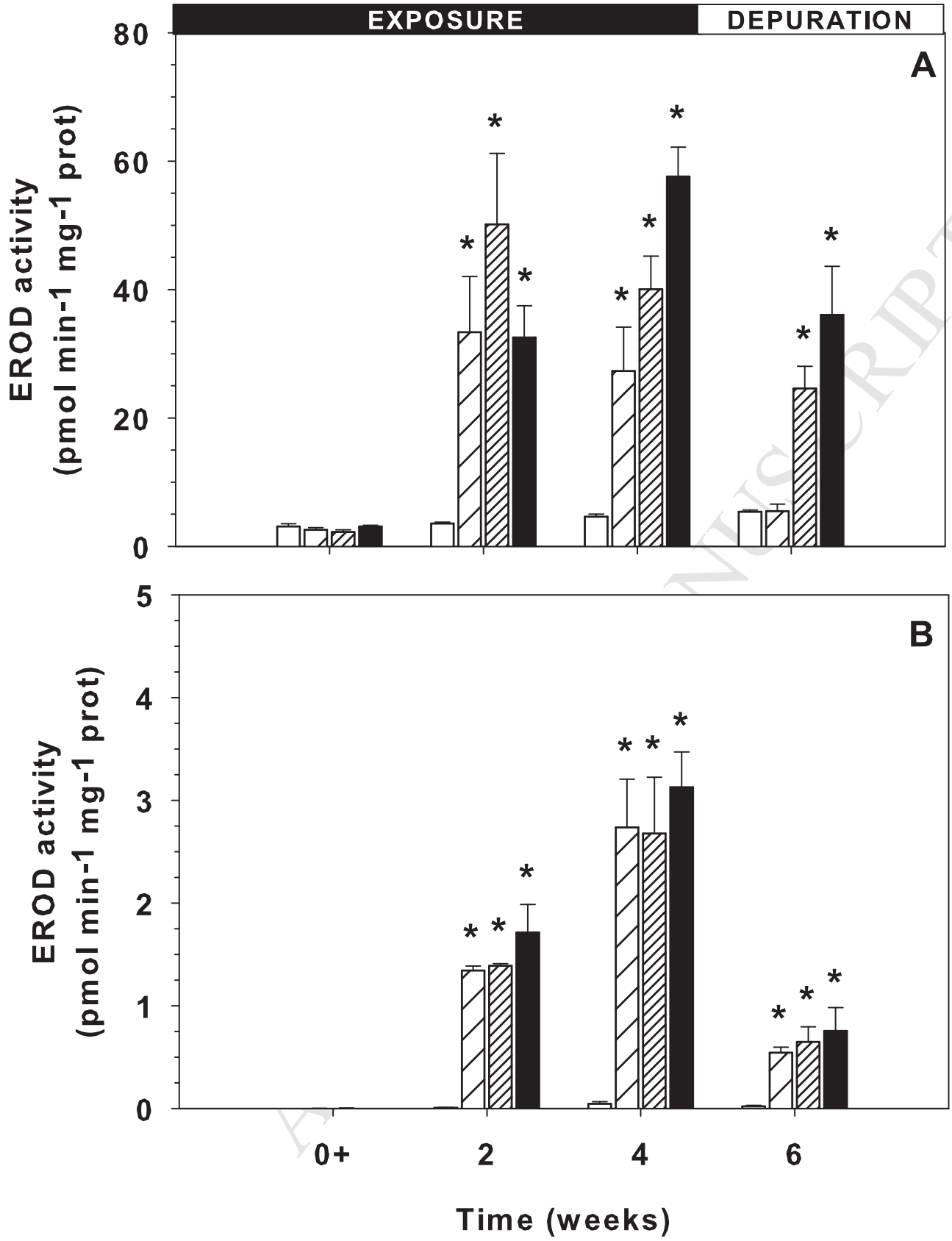

Figure 2