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Effects of crude oil exposure and elevated temperature on the liver transcriptome of polar cod (*Boreogadus saida*)

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ABSTRACT

Petroleum-related activities in the Arctic have raised concerns about the adverse effects of potential oil spill on the environment and living organisms. Polar cod plays a key role in the Arctic marine ecosystem and is an important species for monitoring oil pollution in this region. We examined potential interactions of oil pollution and global warming by analysing liver transcriptome changes in polar cod exposed to crude oil at elevated temperature. Adult males and females were kept at high (11 °C) or normal (4 °C) temperature for 5 days before exposure to mechanically dispersed crude oil for 2 days followed by recovery in clean sea water for 11 days at the two temperatures. Genome-wide microarray analysis of liver samples revealed numerous differentially expressed genes induced by uptake of oil as confirmed by increased levels of bile polycyclic aromatic hydrocarbon (PAH) metabolites. The hepatic response included genes playing important roles in xenobiotic detoxification and closely related biochemical processes, but also of importance for protein stress response, cell repair and immunity. Though magnitude of transcriptome responses was similar at both temperatures, the upregulated expression of *cyp1a1* and several chaperone genes was much stronger at 11 °C. Most gene expression changes returned to basal levels after recovery. The microarray results were validated by qPCR measurement of eleven selected genes representing both known and novel biomarkers to assess exposure to anthropogenic threats on polar cod. Strong upregulation of the gene encoding fibroblast growth factor 7 is proposed to protect the liver of polar fish with aglomerular kidneys from the toxic effect of accumulated biliary compounds. The highly altered liver transcriptome patterns after acute oil exposure and recovery suggests rapid responses in polar cod to oil pollutants and the ability to cope with toxicity in relatively short time.

1. Introduction

The Arctic contains one of the world's largest petroleum resources and the region is becoming increasingly accessible due to receding sea ice and technological advancements. Oil spills from operational or accidental discharges represent a major threat to Arctic marine organisms and therefore studies of the effects of oil pollution are given high priority. Polar cod (*Boreogadus saida*) is widely distributed in the Arctic seas of northern Greenland, Alaska, Canada and Russia, and plays a key role in the Arctic marine ecosystem by linking the food web between higher and lower trophic levels (Welch et al., 1992; Christiansen et al., 2012). The overlapping distribution with potential petroleum-related activities and its location in the ice edge, a natural oil spill sink, makes the polar cod an important species for monitoring oil pollution in the Arctic (Stange and Klungsøyr, 1997; Jonsson et al., 2010; Gardiner et al., 2013). Polar cod experiences temperatures from close to the freezing point of sea water to +7 °C in July, but the predicted warming of the Arctic may impair growth and reproduction (Doney et al., 2012; Nahrgang et al., 2014; Brown and Thatje, 2015). Further, petroleum exposure seemed to depress routine metabolism in polar cod (Christiansen et al., 2010). Melting ice increases risk of contact between fish and oil spills, while thermal stress may enhance vulnerability to toxic pollutants.

Polycyclic aromatic hydrocarbons (PAHs) are the primary toxic constituents in crude oil, and cytotoxic, immunotoxic, mutagenic

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and carcinogenic effects have been reported in diverse fish species (Reichert et al., 1998; Vogelbein, 2003; Reynaud and Deschaux, 2006). Exposure of embryos and fry to crude oil-derived PAH causes cardiac dysfunctions and abnormalities in neural system, spinal curvature and craniofacial structures (Carls et al., 2008; Hicken et al., 2011; Incardona et al., 2014). Biotransformation of lipophilic compounds forms water-soluble conjugates which are rapidly excreted via urine in fish with glomerular nephrons by a combination of glomerular filtration and tubular transport (James, 1987). The aglomerular kidney of polar fish protects against the loss of the vital antifreeze proteins, but hampers urinary secretion and causes the accumulation of toxic bile compounds (Dobbs et al., 1974; DeVries, 1988; Pritchard and Bend, 1984; Christiansen et al., 1996). Studies of biotransformation in oil-exposed fish, including polar cod, have commonly measured the gene expression and activities of enzymes involved in protection against xenobiotics and free radicals (van der Oost et al., 2003; Nahrgang et al., 2009, 2010a,b). Our knowledge about the toxic effects of PAH has been increased by expanding the repertoire of biomarkers, such as genes encoding peroxisomal enzymes of lipid metabolism (Bilbao et al., 2010), immune genes (Hur et al., 2013), alcohol dehydrogenase (Osorio-Yanez et al., 2012) and glycine N-methyltransferase, a mediator in the methionine and folate cycles (Fang et al., 2010). However, while the candidate gene approach is limited for elucidating the complex effects of PAH mixtures, transcriptome analyses of multiple genes may provide a comprehensive overview of the toxicogenomic responses.

Studies on joint effects of PAH and elevated water temperature on fish are scarce. Exposure of juvenile Atlantic cod to oil dispersants caused greater induction of hepatic EROD activity at higher temperature (Lyons et al., 2011), while gill CYP1A levels and EROD activity in North Sea dab (*Limanda limanda*) exposed to polyaromatic contaminants were inversely related to water temperature (Sleiderink et al., 1995). Rainbow trout exposed to PAH at 12 °C and 24 °C showed quantitative and qualitative differences in temporal dynamics of biomarkers (Brinkmann et al., 2013). Here, we examined transcriptome changes in the liver of polar cod induced by acute crude oil exposure combined with elevated temperature to assess the adverse effects of oil spills in the Arctic combined with the predicted climatic changes. Gene expression (in this aticle gene expression is taken as synonymous to gene transcription, although it is acknowledged that gene expression is additionally affected, e.g., by translational efficiency and mRNA and protein stability) was analysed with a genome-wide oligonucleotide microarray based on Atlantic cod genome sequences (Krasnov et al., 2013), and selected genes representing novel biomarker candidates were validated by quantitative qPCR. For exposure concentration verifications water samples were analyzed for total hydrocarbon content (THC) and PAH concentrations, and the bioavailability of the dispersed oil was measured by semi-quantification of biliary PAH metabolites.

2. Material and methods

2.1. Fish collection and maintenance

Polar cod were caught outside Svalbard in January, 2013 by a benthic trawl (Campelen Super 1800) equipped with a fish-lift to prevent the fish from being injured during trawling. The fish were kept on board the vessel in a circular transport tanks supplied with running seawater during transport to Akvaplan–Niva's marine facility in Tromsø, Norway. The fish were placed in a 1000 L holding tank for acclimation and maintenance at water temperature of 3–4 °C and simulated ambient Svalbard photoperiod (January: 0 L:24D, February–April: gradual change from 9 L:15D to 24 L:0D, May: 24 L:0D) until the start of the experiment in May 2013. The fish were hand fed twice a week with a commercial marine fish feed (Skretting, 3–4 mm dry pellets).

2.2. Experimental set-up

In total 56 adult fish (three months post-spawning) of mixed gender with body weight of 28.9 ± 18.0 g and total length of 16.4 ± 4.5 cm were divided randomly into four 120-L tanks (n = 14 per tank) with circulating seawater at the ambient temperature of 4 °C for two weeks. The water temperature in two tanks was gradually increased to 11 °C during 4 days, and the fish were maintained at either 4 or 11 °C for 5 days. All fish were then transferred to 4 equivalent tanks for 2-day exposure to mechanically dispersed crude oil or clean seawater (control) at 4 or 11 °C (see Section 2.3). Seven fish from each tank were then sampled randomly, while the remaining fish were transferred back to the clean water tanks at 4 or 11 °C and sampled after 11 days of recovery. The fish were hand-fed every day, except for 2 days before and during oil exposure.

2.3. Oil exposure

The work was carried out in accordance with the laws and regulations controlling experiments/procedures in live animals in Norway and has been approved by the Norwegian Animal Research Authority (NARA; ID 5561). A static, spiked oil exposure treatment was performed according to the protocol developed by CEDRE, France, for the DISCOBIOL project (Milinkovitch et al., 2013). Naphthenic crude oil (Troll) with an initial concentration of 67 mg oil/L sea water was added through a funnel fixed at the surface 24 h before the fish were transferred to the experiment tanks and exposed to the dispersed oil for 48 h. The funnel was connected to a pump at the bottom of the tank ensuring a homogenous mixture of oil and water throughout the water column. Oxygen was percolated into the water of the exposure tanks through air stones to ensure >90% O_2-saturation throughout the exposure period. The same experimental system was used for the control tanks without addition of oil. Water samples for chemical analysis were taken

Table 1
Selected genes and primer sequences (5'–>3') for RT-qPCR verification.

Gene	Symbol	Forward primer	Reverse primer
Aryl-hydrocarbon receptor repressor b	*ahrr*	CAAGCGAATCCAGAGAAACC	GCGTAGAACACCATCCCATC
TCDD-inducible poly [ADP-ribose] polymerase	*tiparp*	TCAACATCAAGGAGGGCTTC	AAGAGGAGGGGTGAGGAGAA
ATP-binding_cassette,_sub-family G (WHITE), member 2	*abcg2*	TGGCGTACCAGGGAGTAGAT	TTTGGTGTAAGCGATGGACA
UDP glucuronosyltransferase	*ugt2a2*	AATGGTTGCCTCAGAACGAC	GCACTTCCAGCCTCAAGATG
Cytochrome P450, family1, member C2	*cyp1c2*	TGTCTGGAAGCCTGTCTGTG	CGTTGCCGTATTTCTTAGCC
Cytochrome P450, family 1, member A1	*cyp1a1*	CCACCCCGAGATGCAGG	CGAAGGTGTCTTTGGGGA
Phosphoserine aminotransferase	*psat*	TGAGTGTCCTGTGGTCTTCG	ACTTCTGCTTGTTGAGCGTCT
3-phosphoadenosine 5-phosphosulfate synthase 2	*papss2*	GTGATGGAGGGAGGTGATTG	CAGTGGAGTGAGGCGGTATT
Fibroblast growth factor 7	*fgf7*	CGGCAAGGAGATGTTTATCG	GAAGTGGGACGCTATGTGCT
Gamma-crystallin B	*crygb*	CATGTCCAACTGCATGTCCT	CATCATCCTGCCCCTGTACT
Mitochondrial uncoupling protein 2	*ucp2*	GCCATCCTCAAACACAACCT	ATGTAGCGGGTCTTCACCAC
Ubiquitin	*ubi*	GGCCGCAAAGATGCAGAT	CTGGGCTCGACCTAAGAGT