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Baseline concentrations of biliary PAH metabolites in perch (*Perca fluviatilis*) in the open Gulf of Finland and in two coastal areas

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ABSTRACT

Female perch (*Perca fluviatilis*) were sampled annually in late summer from 2006 to 2009 from the open sea of the eastern Gulf of Finland off Haapasaari island to monitor baseline biliary PAH metabolite concentrations. In addition, two coastal locations were sampled in 2008. PAH metabolite concentrations were compared between the open sea and coastal samples and between the sampling years and examined in relation to the body characteristics of perch. Of the PAH metabolites, only 1-hydroxypyrene (1-OH pyrene) was detected at quantifiable levels in the bile of nearly all perch individuals. There were some annual differences but no temporal trend in the concentration of biliary 1-OH pyrene in perch from Haapasaari. At the coastal locations, 1-OH pyrene concentrations in the bile of perch were significantly higher than in the open sea Haapasaari area, probably due to greater contamination of the coastal sites and differences in feeding behaviour. No correlations between the body characteristics of perch and 1-OH pyrene concentrations were detected. It is concluded that PAH metabolites in the bile of fish could be measured in the Gulf of Finland to detect oil spills in the open sea, and the cost-effective total fluorescence method could be used in such monitoring programmes.

1. Introduction

The Baltic Sea is a highly polluted sea area due to its shallowness, long coastline and high human population density with various industrial plants in the catchment area. The International Maritime Organization (IMO) has classified the Baltic Sea as a Particularly Sensitive Sea Area (PSSA) that needs special protection and into which no pollutants should be released.

However, vessel traffic, including oil transportation, is heavy and continually increasing (HELCOM, 2010). This is especially the case for the Gulf of Finland following the construction of large oil terminals on the Russian coast of the gulf. Both accidental and deliberate small (<1 m^3) oil spills occur at a rate of hundreds per year in the whole Baltic Sea area, although the number has been decreasing (HELCOM, 2015). Fortunately, no catastrophic oil accidents have occurred in the Gulf of Finland, but quite many accidents with oil spillages >100 t occurred in the area between 1970–1987 (Keinänen et al., 2012).

Oil contains polyaromatic hydrocarbons (PAHs), which comprise thousands of molecules, such as naphthalene, phenathrene, chrysene, pyrene and benzo(a)pyrene, formed from two or more fused aromatic (benzene) rings (Neff, 1985; Tuvikene, 1995). Oils spills are the main source of PAHs in marine and freshwater environments, while some PAHs are also pyrogenic, i.e., originate from various burning processes (Neff, 1985). Fish are exposed to dissolved or sediment-bound PAHs through the gills or dermal absorption, or via contaminated food (Logan, 2007). Generally, fish are able to metabolize PAHs relatively quickly, and these compounds do not therefore bioaccumulate in their tissues (Jonsson et al., 2004; Maccubbin et al., 1988; Meador et al., 1995; Neff, 1985; Tuvikene, 1995). PAH metabolites are excreted via the bile and kidneys (Kreitsberg et al., 2010). The half-lives of six PAHs were determined to be 1–4 days in rainbow trout (*Oncorhynchus mykiss*), except for phenyl naphthalene, with a half-life of 25 days (Niimi and Dookhran, 1989).

PAHs are toxic to biota, including fish (Billiard et al., 2008; Tuvikene, 1995), but not as parent compounds (Varanasi and Stein, 1991). The toxicity of PAHs is mediated through their activation in metabolism, for example to highly electrophilic arene oxides. These are intermediate metabolites in the oxidation of PAHs, are carcinogenic and can bind to cellular macromolecules, for example DNA, and affect their functions

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(Neff, 1985). The biotransformation of PAHs in fish causes an extra metabolic load and consumes energy that would be needed for normal metabolic and physiological processes. Consequently, PAHs may interfere with reproduction and growth, impair the immune system and cause lesions and tumours of the skin and liver (Logan, 2007). In the North Sea, which receives pollutants from atmospheric fallout and offshore oil platforms, there have been indications of changes in tissue structure and altered disease frequencies in fish (Hylland et al., 2006).

Recent or continuous exposure of fish to PAHs can be detected by analysing PAH metabolites in bile (Aas et al., 2000; Ariese et al., 1993; Beyer et al., 2010; Vethaak et al., 2016; Vuontisjärvi et al., 2004; Vuorinen et al., 2006). The most commonly detected PAH metabolite in fish bile has been 1-hydroxypyrene (1-OH pyrene) (Nagel et al., 2012; Ruczynska et al., 2011; Vuontisjärvi et al., 2004). Pyrene is a 4-ring PAH compound, and crude oils contain approximately 1% of 4-ring PAHs (Neff, 1979). In addition to 1-OH pyrene, 1-hydroxyphenanthrene was also detected in a few flounders (*Platichthys flesus*) and in most of the eelpouts (*Zoarces viviparus*) out of seventy fish specimens sampled from the Baltic Sea (Vuontisjärvi et al., 2004). In perch (*Perca fluviatilis*) and salmon (*Salmo salar*), only 1-OH pyrene was detected (Vuontisjärvi et al., 2004; Vuorinen et al., 2003).

The total concentration of PAH metabolites in fish bile can be analysed by measuring fluorescence at certain wavelengths (FF method), whereas individual PAH metabolites can be determined by gas chromatography or high performance liquid chromatography (HPLC) (Beyer et al., 2010). The concentrations of biliary PAH metabolites provide a good dose–response relationship, and if not exposed to PAHs, no metabolites are detected (Collier and Varanasi, 1991). However, concentrations of PAH metabolites in fish bile are affected by the sex, season and feeding status (Brumley et al., 1998; Kammann, 2007; Richardson et al., 2004; Vethaak et al., 2016; Vuorinen et al., 2006). The measurement of biliary PAH metabolites by either of the chromatographic techniques is very specific, and the presence of other environmental toxicants does not interfere with the measurement (Beyer et al., 2010). The measurement of biliary PAH metabolites in fish has been suggested to be adopted in monitoring in the Baltic Sea areas (Lehtonen et al., 2006). The FF method has been concluded to be adequate for monitoring purposes (Vuontisjärvi et al., 2004).

The aim of the present study was to investigate, by analysing PAH metabolites in bile samples, whether perch in the open sea archipelago at Haapasaari are exposed to PAHs, and to monitor the baseline metabolite levels over a five-year period. Such monitoring has not previously been performed in the Gulf of Finland, and this is why the analytical method for individual PAH metabolites was chosen. The perch was selected as the target species because it is common and because sampling for the present study could be integrated with sampling for a separate long-term fish population status programme (Ådjers et al., 1996). In the event of future large oil spills or accidents, background data on PAH metabolite concentrations would be valuable.

2. Materials and methods

2.1. Sampling

Perch (*Perca fluviatilis* L.) were caught by gillnets on the southern coast of Haapasaari island (60°17; 27°10) in the Gulf of Finland (Fig. 1) as part of a long-term fish monitoring programme (Ådjers et al., 1996; Rahikainen and Vähänäkki, 2006; Saulamo et al., 2007; Saulamo, 2010). Sampling was performed annually between 25 July and 15 August 2005–2009, i.e., four to six weeks after the spawning period. In addition, perch were sampled during the same time period in 2008 from two coastal stations planned as refuge harbours: Klamila Bay (60°28; 27°29) to the east and Envik Bay (60°25; 27°41) to the west of the city Kotka (Fig. 1) (Saulamo, 2010). The mesh sizes of the gillnets used to catch perch of an appropriate size for the present study were 30 and 35 mm (knot to knot), and the nets were set overnight for 12 h.

Only female perch were analysed in the present study to eliminate the random effect of sex. The perch were stunned by a blow on the head and opened, and a bile sample was drawn into a hypodermic needle and handled as described in Vuorinen et al. (2006). The bile samples were immediately frozen in liquid nitrogen. The total body weight and the total length of the fish were measured and the liver and gonads (except in 2007) were also weighed (Table 1). Bile samples were transported in liquid nitrogen to the laboratory and stored at −80 °C until analysis.

2.2. Chemical analysis

The biliary PAH metabolites to be analysed were selected on the basis of a literature survey associated with a laboratory experiment (Vuorinen et al., 2003), in which perch were exposed to the water-soluble fraction (WSF) of Russian crude oil and various PAH metabolites were examined in the bile. Four PAH metabolites (2-OH naphthalene, 1,2-OH chrysene, 1-OH phenanthrene, 1-OH pyrene) were selected for analysis, in addition to benzo(a)pyrene, which is on the priority substance list of the EU Water Framework Directive (EC, 2013). However, screening of a random set of samples revealed no other metabolites except 1-OH pyrene, and thus only this was further analysed. 1-OH pyrene has often been used as a marker metabolite of exposure of fish to PAH compounds (Beyer et al., 2010).

The biliary PAH metabolite 1-OH pyrene was analysed by HPLC as described in Vuontisjärvi et al. (2004), with modifications to the sample volumes as described in the following. Bile samples (10 μL) were hydrolysed with β-glucuronidase/aryl sulfatase (10 μL, Merck) at 37 °C for 2 h in a total volume of 150 μL (made up to volume with Millipore purified H_2O). Proteins were precipitated with HPLC-grade acetonitrile (150 μL) and the samples were centrifuged (Heraeus Fresco 21) at 10 000 rpm for 5 min. The supernatant was filtered through a 0.2-μm syringe filter and

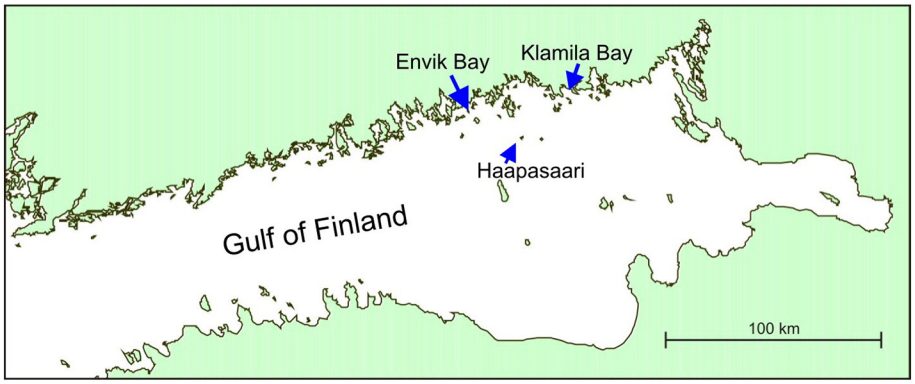

Fig. 1. Sampling locations for perch. Sampling was performed at Haapasaari in 2005–2009 and in the coastal Envik Bay and Klamila Bay in 2008.

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