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 between late July and early August in the eastern Gulf of Finland to monitor biliary PAH metabolite concentrations. Sampling was carried out in the open sea off Haapasaari island from 2006 to 2009 and at two coastal locations east and west of the city of Hamina in 2008. Of the PAH metabolites, only 1-hydroxypyrene (1-OH pyrene) was detected at quantifiable levels in the bile of perch, and it was detected in nearly all perch. In addition, the total body weight and length and the liver and gonad weight were recorded. PAH metabolite concentrations were compared between the open sea and coastal samples and were examined in relation to body characteristics (body weight and length and proportional liver and gonad weight). There was 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. Some correlations between the body characteristics of perch and 1-OH pyrene were detected when analysed separately for annual observations, but none in the whole data set. 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.

Keywords: PAH metabolite, perch, bile, monitoring, 1-hydroxypyrene, Baltic Sea, Gulf of Finland

1. Introduction

The Baltic Sea is a highly polluted sea area due to its shallowness, long coastline and high human population 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 are toxic to biota, including fish (Billiard et al., 2008; Tuvikene, 1995). Fish are exposed to PAHs, for example, through respiration, ingestion of contaminated food and dermal absorption, and PAHs may interfere with growth and reproduction, damage 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 off-shore oil platforms, there have been indications of changes in tissue structure and altered disease frequencies in fish (Hylland et al., 2006).

Fish are generally able to metabolize PAHs relatively quickly, and these compounds do not therefore bioaccumulate in fish tissues (Jonsson et al., 2004; Maccubbin et al., 1988; Meador et al., 1995; Tuvikene, 1995). However, such activity causes an extra metabolic load and takes energy from other processes, such as growth. The half-lives of six PAHs (not including pyrene) were determined to be 1–4 days in rainbow trout (*Salmo gairdneri*), except for phenyl naphthalene, with a half-life of 25 days (Niimi and Dookhran, 1989). During a ten-

day recovery from a one-day exposure to 2 mg WSF of crude oil L^{-1}, 1-OH pyrene concentrations in the bile of perch females decreased on average by 39% from an initial value of 2 800 ng g^{-1} (Fahmy, 2013).

Recent 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; Vuontisjärvi et al., 2004; Vuorinen et al., 2006). The total concentration of PAH metabolites can be analysed by measuring fluorescence at certain wavelengths (FF method), or individual PAH metabolites can be determined by gas chromatography or high performance liquid chromatography (HPLC) (Beyer et al., 2010). The measurement of biliary PAH metabolites by the either of the chromatographic techniques is very specific and exposure to other environmental toxicants does not interfere with the measurement (Beyer et al., 2010). In addition, the concentrations of biliary PAH metabolites provide a very good dose–response relationship, and if not exposed to PAHs, no metabolites are detected (Collier and Varanasi, 1991). The FF method has been suggested to be adequate for monitoring purposes (Vuontisjärvi et al., 2004).

The most commonly detected PAH metabolite in fish bile has been 1-hydroxypyrene (Nagel et al., 2012; Ruczynska et al., 2011; Vuontisjärvi et al., 2004). Pyrene is a four-ring PAH compound, and crude oils contain approximately 3% PAHs, i.e., 3-6-ring PAHs (Neff, 1979). In the Baltic Sea environment, 1-hydroxyphenanthrene was also detected in a few flounders (*Platichthys flesus*) and in most of the eelpouts (*Zoarces viviparus*) out of seventy sampled specimens (Vuontisjärvi et al., 2004). Concentrations of PAH metabolites in fish bile are affected by gender, season and the feeding status (Brumley et al., 1998; Kammann, 2007; Richardson et al., 2004; Vuorinen et al., 2006).

In the Baltic Sea area, concentrations of PAH metabolites in fish bile had not been monitored over a period of years before the present study, and only sporadic investigations had been carried out (Table 1). In the EU project BEEP (Lehtonen et al., 2006), a three-year

sampling campaign was performed to investigate spatial, seasonal and gender effects on bile PAH metabolite concentrations in perch (*Perca fluviatilis*), flounder and eelpout (Vuorinen et al., 2006). These metabolites were also measured in the bile of perch caught in the Stockholm archipelago during a three-year survey campaign (Hansson et al., 2006b). Ruczynska et al. (2011) sampled flounder in 2008 from the Gulf of Gdansk and analysed bile for PAH metabolites. Vuorinen et al. (2003) and Pikkarainen (2006) measured PAH metabolites in the bile of perch caught in one-off sampling from the Gulf of Finland in 2001. In the North Sea, monitoring of bile PAH metabolites in dab (*Limanda limanda*) and flounder is included in the OSPAR convention (OSPAR, 2008), and the measurement of biliary PAH metabolites has been suggested to be adopted in monitoring in the Baltic Sea (Lehtonen et al., 2006).

Table 1. Reported concentrations of 1-hydroxypyrene (1-OH pyrene, analysed by HPLC) in the bile of fish from the Baltic Sea.

Species	Sampling location	Sampling time	1-OH pyrene, ng g^{-1}	Reference
Perch, *Perca fluviatilis*	Gulf of Finland	2001	55–160[a]	Vuorinen et al. (2003)
Salmon, *Salmo salar*	Gulf of Riga	1997	140[a]	Vuorinen et al. (2003)
Salmon, *Salmo salar*	Baltic Proper, SD28	1997	180[a]	Vuorinen et al. (2003)
Salmon, *Salmo salar*	Åland Sea, SD29	1997	13[a]	Vuorinen et al. (2003)
Perch, *Perca fluviatilis*	Western Gulf of Finland	2001	213–1149[b]	Pikkarainen (2006)
Perch, *Perca fluviatilis*	Stockholm archipelago	1999–2001	20–1300[a]	Hansson et al. (2006b)
Perch, *Perca fluviatilis*	Baltic Proper	2001, 2002	0–440[b]	Vuontisjärvi et al. (2004)
Flounder, *Platichthys flesus*	Baltic Proper	2001, 2002	0–1000[b]	Vuontisjärvi et al. (2004)
Eelpout, *Zoarces viviparus*	Baltic Proper	2001, 2002	0–1280[b]	Vuontisjärvi et al. (2004)
Cod, *Gadus morhua*	Baltic Proper	2001, 2002	0–310[b]	Vuontisjärvi et al. (2004)
Salmon, *Salmo salar*	Baltic Proper	1997	0–300[b]	Vuontisjärvi et al. (2004)
Flounder, *Platichthys flesus*	Wismar Bay	2001, 2002	110–590[a]	Vuorinen et al. (2006)
Flounder, *Platichthys flesus*	Lithuanian coast	2001	30[a]	Vuorinen et al. (2006)
Flounder, *Platichthys flesus*	Denmark Strait	2004	54–92[a]	Kammann (2007)
Dab, *Limanda limanda*	North Sea	2004	11–159[a]	Kammann (2007)
Flounder, *Platichthys flesus*	Gulf of Gdansk	2008	20–65[a]	Ruczynska et al. (2011)

[a]Range of mean concentrations; [b]Range of single concentration values

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 monitor the levels over a six-year period, because such monitoring has not previously been performed in the Gulf of Finland. The study was integrated with a long-term fish population status programme (Ådjers et al., 1996). In the case of a future large oil spill accident, background data on PAH metabolite concentrations would be valuable. Knowledge of the spatial variability in the PAH metabolite concentration was improved by sampling perch in two coastal bays in addition to the open sea.

2. Materials and methods

2.1. Sampling

As part of a long-term fish monitoring programme (Ådjers et al., 1996; Rahikainen and Vähänäkki, 2006; Saulamo, 2010; Saulamo et al., 2007), perch (*Perca fluviatilis* L.) were caught by gillnets on the southern coast of Haapasaari island in the Gulf of Finland (Fig. 1) annually between the 25 July and 15 August 2005–2009, i.e., clearly after the spawning period. The knot sizes of the gillnets used to catch perch of an appropriate size for the present study were 30 and 35 mm, and the nets were set overnight for 12 hours.

Only female perch were selected for the present study to eliminate the random effect of sex. A bile sample was drawn into a hypodermic needle and handled as described in Vuorinen et al. (2006). Fish were weighed, the total length was measured and the liver and gonads (except in 2007) were also weighted (Table 2). A piece of liver was dissected for analysis (not reported here), and the liver and bile samples were immediately frozen in liquid nitrogen. The operculum was removed for age determination. In 2008, perch were additionally caught and

similarly sampled from two coastal locations, which have been planned to be possible refuge harbour areas (Fig. 1). Bile and liver samples were transported to the laboratory and bile samples were stored at -80 °C until analysis.

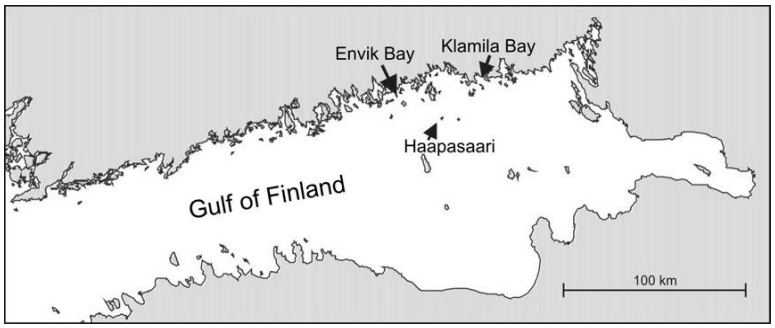

Fig. 1. Sampling locations for perch. Sampling was performed at Haapasaari in 2005–2009 and in the coastal Envik Bay and Klamila Bay in 2008.

Table 2. Mean (± SE) weight, length, condition factor (CF), liver somatic (LSI) and gonadosomatic (GSI) index, and the number (N) of female perch caught in different years from the open sea at Haapasaari and two coastal locations (Envik Bay and Klamila Bay). A different letter as a superscript denotes a significant difference ($p < 0.05$) between the annual means.

Year	Location	Weight, g	Length, mm	CF	LSI	GSI	N
2005	Haapasaari	185.9 ± 7.8^{b}	248 ± 4^{b}	1.21 ± 0.04^{a}	1.05 ± 0.04^{a}	0.89 ± 0.05^{a}	31
2006	Haapasaari	163.4 ± 7.0^{b}	241 ± 3^{b}	1.12 ± 0.02^{a}	1.14 ± 0.10^{a}	0.91 ± 0.08^{a}	57
2007	Haapasaari	183.5 ± 11.6^{b}	245 ± 6^{b}	1.17 ± 0.02^{a}			34
2008	Haapasaari	164.3 ± 18.5^{b}	235 ± 9^{b}	1.19 ± 0.03^{a}	0.99 ± 0.09^{a}	0.95 ± 0.11^{a}	12
2008	Envik Bay	101.3 ± 12.7^{a}	203 ± 8^{a}	1.15 ± 0.04^{a}	0.94 ± 0.10^{a}	0.92 ± 0.10^{a}	12
2008	Klamila Bay	97.3 ± 12.5^{a}	204 ± 8^{a}	1.07 ± 0.03^{a}	0.91 ± 0.05^{a}	0.74 ± 0.05^{a}	12
2009	Haapasaari	174.3 ± 11.5^{b}	243 ± 4^{b}	1.18 ± 0.04^{a}	1.13 ± 0.05^{a}	0.95 ± 0.08^{a}	26

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148 *2.2. Chemical analysis*

149 Biliary PAH metabolites were analysed by HPLC as described in Vuontisjärvi et al.
150 (2004), with changes in volumes as described in the following. Bile samples (10 µL) were
151 hydrolyzed with β-glucuronidase / aryl sulfatase (10 µL, Merck) at 37 °C for 2 hours in 150
152 µL (total volume topped with Millipore purified H_2O). Proteins were precipitated with HPLC-
153 grade acetonitrile (150 µL) and the samples were centrifuged (Heraeus Fresco 21) at 10 000
154 rpm for 5 minutes. The supernatant was filtered through a 0.2-µm syringe filter and a 5-µL
155 aliquot was injected into a UHPLC system with a fluorescence detector (excitation and
156 emission wavelengths 346 nm and 384 nm, respectively). An external standard curve was
157 prepared from 2 to 100 ng 1-hydroxypyrene (1-OH pyrene) mL^{-1} and it was always run with
158 bile sample sets. The uncertainty of analysis was 20% and the quantification limit 5.0 ng g^{-1}.
159 The recovery was 93% and results were corrected for this recovery.

160 The chromatographic system consisted of a Waters Acquity UPLC binary pump,
161 autosampler and fluorescence detector. The separation column comprised a C-18 type BEH
162 UPLC column (1.7 µm particles, 1 x 100 mm) and the pump was operated at a flow rate of
163 150 µL min^{-1}. Separation was performed with a gradient program from 10% to 80%
164 acetonitrile in the B pump and with 0.1% trifluoroacetic acid in water in the A pump. The
165 retention time of 1-OH pyrene was 6.5 minutes and the total run time was 15 minutes.

166 As a quality control, a laboratory control sample prepared from fish bile was run with
167 each sample set. The laboratory has participated in intercalibration exercises for the
168 determination of PAH metabolites in fish bile samples both by HPLC and FF (Kammann et
169 al., 2013).

170

171 *2.3. Statistical calculations*

The condition factor (CF) of perch was calculated as CF = 10^5 * weight (g) / length (mm)^3 and the relative liver (LSI) and gonad (GSI) weight as a percentage of the body weight.

Variance homogeneity was tested using Levene's test. The statistical significance of differences between the years and locations was tested by one-way ANOVA with the *post hoc* Student-Neuman-Keuls test for significant differences ($p < 0.05$) between the sample means.

Statistical tests were performed using the Statistical Analysis System, SAS ver 9.4 (SAS Institute Inc., 2008).

3. Results and discussion

3.1. Coastal versus open sea locality

Of the 16 analysed PAH metabolites, only 1-OH pyrene was detected at quantifiable levels in the bile of perch. The concentrations of 1-OH pyrene in perch bile were several times higher at the two coastal locations, Envik Bay and Klamila Bay, than in the open sea archipelago of Haapasaari in the same year, and significantly ($p < 0.05$) higher in Envik Bay (Fig. 2). The mean concentrations at the two coastal locations did not differ significantly from each other. Anthropogenic sources are probably reflected in the bile of the coastal perch, because in addition to oil spills, 1-OH pyrene may originate from various burning processes and also be transported via the atmosphere (Anderson and Lee, 2006), and apparently also with run-off waters. This is supported by the fact that the mean 1-OH pyrene concentration and its variation were numerically higher at Envik Bay, which is located closer to an industrial and highly populated area than the Klamila Bay. As reviewed by Tuvikene (1995), PAHs entering the aquatic environment are mostly localised in rivers, estuaries and coastal waters. The coastal mean values of the present study were up to two to three times higher than have earlier been measured in the bile of perch caught near an oil refinery at Sköldvik, Gulf of

Finland, in 2001 (Vuorinen et al., 2003). In the bile of perch caught in 2001 from the western coast of the Gulf of Finland (Table 1), 1-OH pyrene concentrations analysed by HPLC were even higher (Pikkarainen, 2006), being nearly twice as high as in the present study in perch from Envik Bay. At highly contaminated sites, the 1-OH pyrene concentrations in fish bile may be much higher than in the perch of the present study. For example, 2000–300 000 ng 1-OH pyrene g^{-1} bile was detected in English sole (*Parophrys vetulus*) caught from the side of the Puget Sound polluted by aromatic hydrocarbons (Krahn et al., 1987).

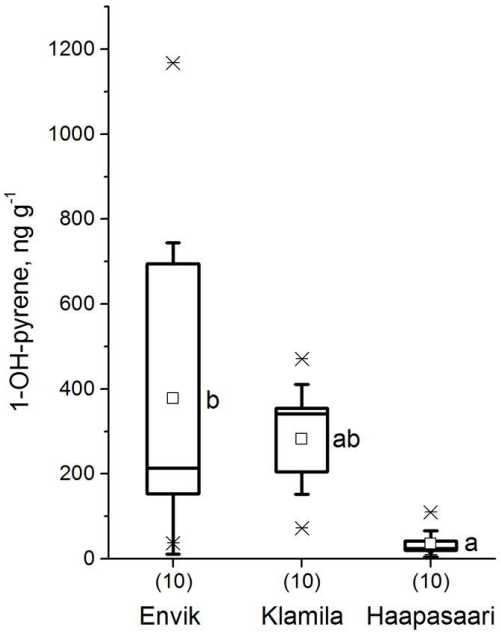

Fig. 2. Box plots of 1-OH pyrene concentrations in the bile of female perch caught in two coastal bays (Envik Bay and Klamila Bay) and in the open sea at Haapasaari in 2008. Whiskers depict 1 x SD, asterisks the minimum and maximum observations, upper and lower parts of the boxes 25 and 75% of observations, horizontal lines the median value and small squares the mean concentrations. A different letter close to the means denotes a significant difference ($p < 0.05$) between the means. The sample size is indicated in parentheses.

3.2. Temporal variation in biliary PAH metabolites of perch

Biliary 1-OH pyrene concentrations of perch from the open sea archipelago off Haapasaari differed significantly ($p < 0.05$) between years, the concentrations having been clearly and significantly lowest in the first monitoring year, 2005 (Fig. 3). However, no clear temporal trend was observed in perch during the five-year monitoring period. Indeed, the mean 1-OH pyrene concentrations in perch bile in 2006, 2007 and 2009 were nearly equal, with a significant decrease in 2008 (Fig. 3). In all these years, the 1-OH pyrene concentrations were on average clearly lower than in the bile of perch in the two coastal bays. The variation among fish at all sampling times was large (Fig. 3), although less than in the coastal locations. However, 1-OH pyrene was detected in nearly all samples. This indicates that perch are continuously exposed to pyrene, but individual differences in behaviour or feeding status may cause the variation in pyrene concentrations. Oil is sparingly dissolved in water, and there may have been large differences in exposure of individual fish. In laboratory experiments, 1-OH pyrene concentrations were also quite variable in the bile of perch exposed to the water soluble fraction (WSF) of crude oil (Fahmy, 2013).

On average, the 1-OH pyrene concentrations in the bile of perch were approximately one half of those detected in the bile of salmon caught from the open sea of the Baltic Proper or the Gulf of Bothnia in 1997 (Vuorinen et al., 2003). In flounder caught in the Baltic Sea near the Denmark Strait in 2004, the biliary concentrations of 1-OH pyrene were on average similar to those in perch of the present study, but those in dab from the North Sea were on average lower than in perch (Kammann, 2007). Ruczynska et al. (2011) detected similar concentrations of 1-OH pyrene in the bile of flounders from the Gulf of Gdansk (Table 1) to those in perch of the present study. The low concentrations of biliary 1-OH pyrene in perch also reported in other studies (Table 1) suggest that pollution by PAH compounds is continuous. No major oil accidents have occurred in the Baltic Sea during the 2000s, but

100–140 ship accidents, mostly in harbours, have been reported annually (Keinänen et al., 2012), in addition to open sea spills (HELCOM, 2015). As 1-OH pyrene also originates from various burning processes, it has apparently been transported via the atmosphere (Anderson and Lee, 2006).

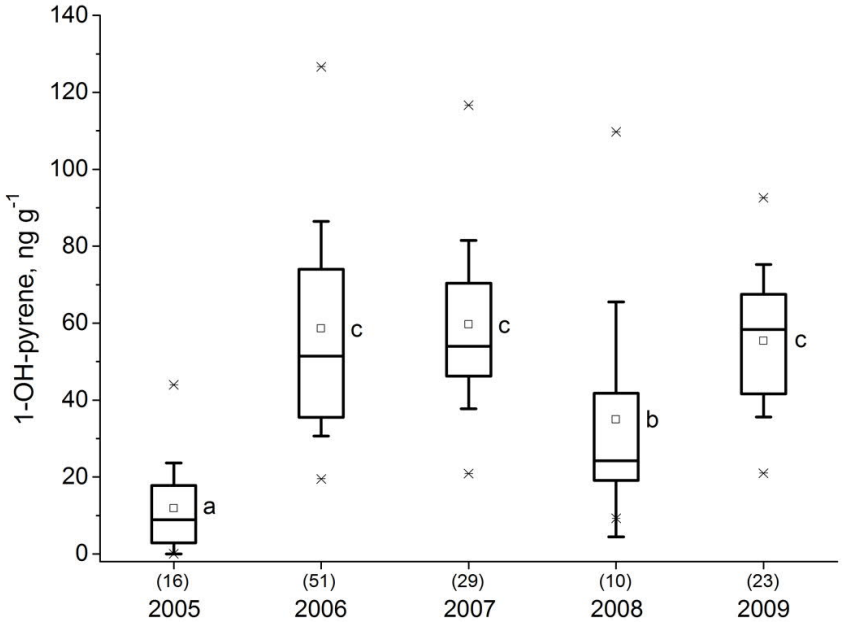

Fig. 3. Box plots of 1-OH pyrene concentrations in the bile of perch caught in the open sea at Haapasaari in 2005–2009. Whiskers depict 1 x SD, asterisks the minimum and maximum observations, upper and lower parts of the boxes 25 and 75% of observations, horizontal lines the median value and small squares the mean concentrations. A different letter close to the means denotes a significant difference ($p < 0.05$) between the means. The sample size is indicated in parentheses.

The lack of a temporal trend may be due to effective control measures by authorities. All HELCOM member states regularly conduct aerial surveillance flights to monitor illegal

and accidental oil spills in the Baltic Sea (HELCOM, 2010), and the number and size of these has decreased from about 600 oil spills in the early 1990s to close 100 in 2014 (HELCOM, 2015). This development has occurred despite the fact that oil transportation in the Baltic Sea more than doubled between 1997–2008 (HELCOM, 2010) and was predicted to increase in the Gulf of Finland by more than ten times from 1995 to 2015 (Jolma and Hietala, 2009); in fact, oil transportation increased by a factor of seven (web reference). It is possible that exposure of perch to PAHs has actually decreased along with the diminished number of oil spills reported (HELCOM, 2015). This could be an explanation for the lower levels of 1-OH pyrene in the bile of perch of the present study compared to those in samples of feeding salmon from the open sea in autumn ten years earlier (Vuorinen et al., 2003).

3.3. Biliary PAH metabolites and body characteristics of perch

Perch caught from the Haapasaari waters in different years did not differ from each other in terms of mean body length or weight, but those caught at the two coastal locations were significantly ($p < 0.05$) smaller than the perch from Haapasaari (Table 2). When analysing the whole data set, the average CF, LSI and GSI (Table 2) were similar ($p > 0.05$) in all perch populations. However, taking weight as a covariate, the average CF of perch from Haapasaari was significantly ($p < 0.05$) higher than that of perch from Klamila Bay, potentially reflecting better feeding resources in the open sea area. The CF of perch from Envik Bay did not differ significantly from the CF of perch of the two other areas in 2008. In that year, the GSI of perch from Klamila Bay was significantly ($p < 0.05$) lower than that of perch from Envik Bay, but the GSI of perch from Haapasaari did not differ ($p > 0.05$) from those of the coastal locations. Long-term monitoring data on perch from Swedish reference areas in the Baltic Proper revealed a decrease in the GSI that along with changes in some other variables was interpreted to result from environmental pollution (Hansson et al., 2006a).

The concentrations of 1-OH pyrene in bile did not correlate with length, weight or CF, or with the proportional liver (LSI) or gonad (GSI) weight of female perch from Haapasaari in all data combined (Table 3). However, calculating Pearson correlations separately for each monitoring year revealed significant negative correlations between 1-OH pyrene and weight ($r = -0.310$, $p = 0.027$) and CF (-0.290, $p = 0.039$) in 2006, when the highest individual 1-OH pyrene concentrations were detected. Moreover, there were nearly significant negative correlations between the bile 1-OH pyrene concentration and the LSI in 2005 ($r = -0.492$, $p = 0.053$) and in 2009 ($r = -0.389$, $p = 0.055$). In a laboratory experiment, exposure of fasting perch for 7 and 21 days to the water-soluble fraction of crude oil resulted in a decrease in the LSI, and significant negative correlations of the LSI with 1-OH pyrene, 2-OH naphthalene and 1,2-OH chrysene in females, and with the two last mentioned metabolites in males (Vuorinen et al., 2003). The proportional weight of gonads (GSI) correlated positively and highly significantly ($p < 0.0001$) with the proportional liver weight (LSI). This apparently results from the liver synthesising vitellogenin to be transported via blood to the developing ovaries. Consequently, the liver weight increases during vitellogenesis (Mommsen and Walsh, 1988). This development thus appears rather normal in the early stage of the reproductive cycle, despite perch being exposed, evidently slightly, to the PAH compound pyrene. On the other hand, higher PAH metabolite levels in bile of eelpout from the Gulf of Finland compared to the Gulf of Riga were associated with a lower LSI and condition factor and with higher geno- and cytotoxicity and parasite infections (Kreitsberg et al., 2012). Moreover, pollution from offshore oil platforms in the North Sea has been suspected to associate with structural changes in tissues and both decreases and increases in some disease prevalences in fish (Hylland et al., 2006).

Table 3. Pearson correlation coefficients with the p-value and number of observations (in parentheses) in female perch caught from Haapasaari waters in 2005–2009.

	Length	CF	LSI	GSI	1-OH pyrene
Weight	0.912	0.499	-0.105	-0.145	-0.134
	<.0001	<.0001	0.240	0.106	0.124
	(160)	(160)	(126)	(125)	(132)
Length		0.204	-0.234	-0.231	-0.126
		0.010	0.008	0.010	0.150
		(160)	(126)	(125)	(132)
CF			-0.121	-0.137	-0.123
			0.179	0.129	0.160
			(126)	(125)	(132)
LSI				0.705	-0.098
				<.0001	0.325
				(125)	(102)
GSI					-0.077
					0.442
					(102)

3.4. Biliary PAH metabolites as a biomarker

PAH metabolites in the bile of fish provide a specific and dose-responsive indication of exposure to PAHs (Collier and Varanasi, 1991) or a biomarker of ongoing or recent exposure of fish to PAHs (Beyer et al., 2010; Lehtonen et al., 2006). An extensive oil spill at the Butinge oil terminal on the Lithuanian coast was still detectable by the FF method as bile PAH metabolites in flounders after seven months (Barsiene et al., 2006). This biomarker has been used in many studies to demonstrate accidental exposure of fish to PAHs (Kammann, 2007; Kreitsberg et al., 2010; Lee and Anderson, 2005; Nagel et al., 2012; Ruddock et al.,

2002; Ruddock et al., 2003), and included as one of the biomarkers in the monitoring programme of OSPAR in the North Sea (OSPAR, 2008). Thus far, unequivocal evidence linking this specific biomarker with higher order effects such as reproductive failures is lacking (Lee and Anderson, 2005), as is the case for biomarkers in general (Forbes et al., 2006). However, the combination of biomarkers with advanced statistical analysis, such as principal component analysis, might reveal associations between the physiological status of fish and environmental contaminants (Gagnon and Rawson, 2016). Biomarkers might also be related to phenotyping responses in fish (Houde et al., 2014), as was the approach in the present study.

Variability was detected in the biliary 1-OH pyrene concentrations of individual perch in the present study. While no clear pattern was detected, this might, along with variation in exposure, partly result from differences in the feeding status of perch on various sampling occasions. Samples of the present study were collected annually at the same time and only from female perch, and seasonal and sex effects were thus excluded (Kammann, 2007; Ruddock et al., 2002; Vuorinen et al., 2006). Bile is a part of the normal digestion, and the bile volume as well as the concentration of metabolites in bile are therefore affected to some degree by the feeding status of fish (Brumley et al., 1998; Collier and Varanasi, 1991; Richardson et al., 2004).

Attempts have been made to minimize random error in biliary PAH metabolite concentrations when sampling populations by standardisation procedures, such as normalising the results against the bile biliverdin or protein concentration (Collier and Varanasi, 1991; Kammann, 2007; Ruddock et al., 2002; Ruddock et al., 2003; Vuorinen et al., 2006). In the present study, such procedures were not tested, although biliverdin normalisation has been reported to reduce variation (Collier and Varanasi, 1991; Kammann, 2007; Ruddock et al., 2003). However, biliverdin or protein normalisation, although it affected metabolite results

348 measured by the FF method, did not affect results for 1-OH pyrene in the HPLC method
349 (Vuorinen et al., 2006) that was used in the present study. Moreover, biliverdin normalisation
350 did not consistently reduce variation in various PAH metabolite concentrations measured in
351 the bile of plaice (*Pleuronectes platessa*), and thus it was suggested that PAH metabolite
352 concentrations should be preferably reported both as raw data and normalised by biliverdin
353 (Richardson et al., 2004). Measurement of total fluorescence is simple and cost effective, as
354 bile samples only need to be diluted before measuring fluorescence. Because no other PAH
355 metabolites except 1-OH pyrene were detected in perch bile in the present study, and total
356 fluorescence correlates well with the bile 1-OH pyrene concentration, FF would be the
357 method of choice for monitoring purposes (Vuontisjärvi et al., 2004; Vuorinen et al., 2006).
358
359 4. Conclusions
360
361 On the basis of PAH metabolite concentrations in the bile of perch caught in the open
362 sea archipelago, pollution by PAH compounds is continuous in the Gulf of Finland. However,
363 the biliary PAH metabolite concentrations in perch are rather low in the open sea and several
364 times lower than at the coastal locations. Therefore, larger changes in PAH exposure at open
365 sea locations should be readily detectable. Thus, the present monitoring data on PAH
366 metabolites in fish bile in the Gulf of Finland provide a background reference in case of a
367 larger oil accident. Because no other PAH metabolites except 1-OH pyrene were detected in
368 bile, the cost-effective FF method would be appropriate for monitoring purposes.
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