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Spatial distribution of polyfluoroalkyl compounds in dab (*Limanda limanda*) bile fluids from Iceland and the North Sea

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ABSTRACT

The spatial distribution of polyfluoroalkyl compounds (PFCs) was investigated in dab (*Limanda limanda*) bile fluids collected from Iceland and the North Sea. Concentrations of various PFCs, including perfluorinated sulfonates (C_4–C_6, C_8 PFSAs), perfluorinated carboxylic acids (C_9–C_{14} PFCAs) and *n*-methyl perfluorooctane sulfonamidoethanol (MeFOSE), were quantified. Perfluorooctane sulfonate (PFOS) was the predominant compound with highest concentrations along the Danish and German coast (mean 9.36 ng/g wet weight (ww)). Significantly lower PFOS concentrations were found at the other sampling stations in the North Sea and Iceland ($p < 0.01$, *t*-test). Conversely, the spatial distribution of the PFCAs in Iceland and the North Sea was more uniform. The most abundant PFCA was perfluorononanoic acid (PFNA), while the mean concentration decreased with increasing chain length from 4.7 ng/g ww for PFNA to 0.04 ng/g ww for perfluorotetradecanoic acid (PFTeDA). Overall, the different spatial distribution of PFCs indicates different origin of sources and different transportation mechanism.

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Polyfluoroalkyl compounds (PFCs) have received increasing public attention due to their persistence, bioaccumulation potential (Martin et al., 2003) and possible adverse effects on organisms (Joensen et al., 2009; Lau et al., 2007). PFCs are widely used as processing additives during fluoropolymer production and as surfactants in consumer applications, including surface coatings for carpets, furniture and paper products over the past 50 years (Kissa, 2001). PFCs in general bind to blood proteins (Jones et al., 2003) and the longer-chain PFCs are known to bioaccumulate (Martin et al., 2004). Scientific concern about PFCs increased due to their global distribution and ubiquitous detection in the environment, especially in marine mammals (Giesy and Kannan, 2001; Houde et al., 2006). As a result, the 3 M Company voluntarily phased-out the production of perfluorooctyl sulfonyl fluoride (POSF) in 2000 (Prevedouros et al., 2006). Furthermore, the European Union (EU) formed a directive which prohibits the general use of perfluorooctane sulfonate (PFOS) and its derivatives from June 2008 (European Community Directive, 2006). Since May 2009, PFOS has been included in Annex B of the Stockholm Convention on persistent organic pollutants (Stockholm Convention, 2009). However, the restrictions are focused only on the C_8 compounds, whereas related PFCs are still being produced. In addition, most studies concentrate only on selected PFCs (e.g. PFOS), while relatively little is known about the distribution of individual PFCs in marine wildlife, which is important for environmental risk assessments.

The aim of this study was to examine the levels and spatial distribution of PFCs in dab (*Limanda limanda*) bile fluids collected from the coast of Iceland and in the North Sea. Dabs were selected because they live in a large geographic range in shallow seas around Northern Europe, they are relatively sedentary in their habitat and they are sensitive to environmental pollutants. In this study, concentrations of 11 PFCs (i.e., C_4–C_6, C_8 perfluoroalkyl sulfonates (PFSAs), C_9–C_{14} perfluoroalkyl carboxylic acids (PFCAs) and *n*-methyl perfluorooctane sulfonamidoethanol (MeFOSE)) were quantified in 60 bile samples.

Dab bile samples were collected with trawl nets from the coast of Iceland (sampling station 1) and in the North Sea (sampling stations 2–6) in August and September 2008 ($n = 60$, see Fig. 1). For this study, 10 individuals per station of mature female and male dabs with a body length between 20 and 31 cm were used. All bile samples for PFC analyses were taken with Teflon-free syringes, placed into polyethylene cryogenic vials and stored in a −20 °C freezer until analysis. Details of the sampling parameters including sex, length and weight of the dabs are listed in Table S1 of the Supplementary material.

Target analytes included 36 ionic PFCs (i.e., PFCAs, PFSAs, perfluoroalkyl sulfinates (PFSiAs), perfluoroalkyl phosphonoic acids

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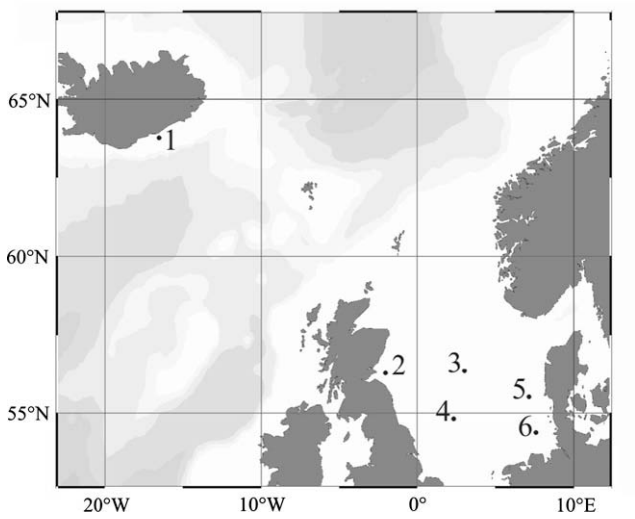

Fig. 1. Map showing the sampling stations for the dabs (*Limanda limanda*) at the coast of Iceland and in the North Sea.

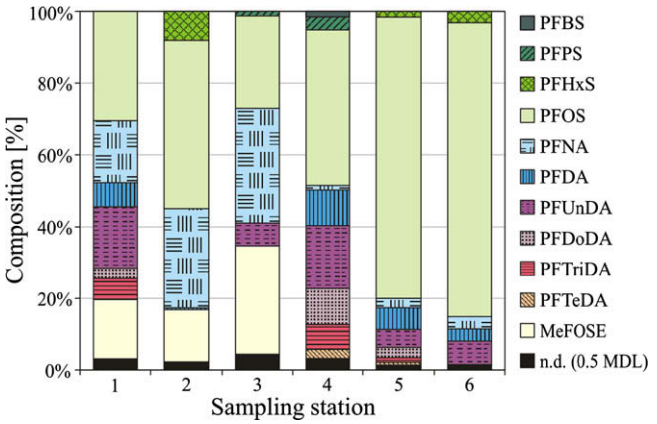

Fig. 2. Mean composition profile of individual PFCs in dab bile fluids from the coast of Iceland and the North Sea. *Note:* the PFC concentrations, which were not detected (n.d.), are shown as the sum of the half values of the method detection limit (MDL).

(PFPAs), fluorotelomer carboxylic acids (FTCAs), and unsaturated fluorotelomer carboxylic acids (FTUCAs)) as well as 7 neutral PFC precursor compounds (i.e., perfluoroalkanesulfonamides, perfluoroalkanesulfonamidoethanols) plus 20 mass-labelled internal standards (IS) (for details see Table S2 in the Supplementary material and (Ahrens et al., 2009c)).

Bile samples (~0.2 g) were extracted using solid–liquid extraction described elsewhere (Ahrens et al., 2009c). For the method blank, 1 mL of acetonitril was extracted in the same manner as the natural samples. The samples were analysed using high-performance liquid chromatography combined with negative ion electrospray tandem mass spectrometry (HPLC-(−)ESI-MS/MS) as previously described (Ahrens et al., 2009c). Quantification was performed using response factors calculated by a ten-point calibration curve (i.e., 1–3000 pg absolute injected). The co-elution of interferences (e.g. tauro-cholate bile salts) with the same transition ions of PFHxS and PFOS can lead to significant biases for their quantification (Chan et al., 2009; Lloyd et al., 2009). Over-reporting of the concentration levels of PFHxS and PFOS was avoided by (1) using a Synergi Hydro-RP 80A column for the HPLC system to separate the interferences from the perfluorohexane sulfonate (PFHxS) and PFOS peak (Chan et al., 2009) and (2) using the interference-free transition of *m/z* 499.0/98.8 for PFOS (see Fig. S1 in the Supplementary material). For the other PFCs no interferences were observed.

The analytical quality of the laboratory has been approved in interlaboratory studies (Van Leeuwen et al., 2009). As a standard procedure, blanks, instrument detection limits (IDLs), method quantification limits (MQLs) and recoveries of spiked samples were examined (for details see (Ahrens et al., 2009c)). All method blanks were under the MQL. The MQLs are in a range of a few tens of pg/g wet weight (ww). For the descriptive statistics, the Kaplan–Meier method was used (Helsel, 2006) and the statistical analyses were performed using SPSS for Windows (version 16) and Microsoft Excel.

Concentrations of individual PFCs in bile fluids of dabs collected at the coast of Iceland and in the North Sea are shown in Table 1. In this study, 11 of 43 PFCs were quantified in bile samples (i.e., perfluorobutane sulfonate (PFBS), perfluoropentane sulfonate (PFPS), PFHxS, PFOS, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTriDA), perfluorotetradecanoic acid (PFTeDA) and MeFOSE). PFOS was the dominant compound with a maximum concentration of 19.7 ng/g ww (sampling station 5). The shorter-chain PFSAs (i.e., PFBS, PFPS, and PFHxS) made only a mean contribution of ~5% to the ∑PFSAs. In contrast to the PFSAs, only the longer-chain PFCAs (C_9–C_{14}) could be detected in dab biles, which suggests a higher bioaccumulation potential of PFSAs compared to PFCAs (Martin et al., 2004). The ∑PFCA concentrations ranged between 0.02 and 10.5 ng/g ww with PFNA, PFDA and PFUnDA as the dominating compounds, whereas perfluorooctanoic acid (PFOA) was not detected. The

Table 1
Average concentrations (ranges) in dab (*Limanda limanda*) bile fluids from the coast of Iceland and the North Sea in ng/g ww ($n = 6 \times 10$).[a]

Analyte	Sampling station					
	1	2	3	4	5	6
PFBS	<0.10	<0.10	<0.10	0.09 (<0.10–0.28)	<0.10	<0.10
PFPS[b]	<0.06	<0.06	0.06 (<0.06–0.51)	0.21 (<0.06–2.06)	<0.06	<0.06
PFHxS	<0.03	0.58 (<0.03–3.78)	<0.03	<0.03	0.20 (<0.03–1.85)	0.37 (<0.03–1.96)
PFOS	1.99 (<0.30–4.47)	3.40 (<0.30–7.65)	1.21 (<0.30–4.85)	2.52 (<0.30–7.29)	9.34 (1.10–19.7)	9.39 (<0.30–18.0)
PFNA	1.13 (<0.02–2.85)	2.01 (<0.02–7.35)	1.49 (<0.02–3.66)	0.08 (<0.02–0.30)	0.33 (0.06–0.69)	0.37 (<0.02–1.55)
PFDA	0.44 (<0.02–1.16)	0.02 (<0.02–0.10)	<0.02	0.57 (<0.02–2.33)	0.71 (0.03–2.41)	0.41 (0.03–1.26)
PFUnDA	1.11 (<0.01–2.67)	<0.01	0.31 (<0.01–1.41)	1.01 (<0.01–3.36)	0.62 (0.01–2.48)	0.75 (0.01–1.73)
PFDoDA	0.19 (<0.01–0.52)	<0.01	<0.01	0.57 (0.08–1.99)	0.32 (<0.01–1.40)	<0.01
PFTriDA	0.39 (<0.02–0.66)	<0.02	<0.02	0.41 (<0.02–2.67)	0.18 (<0.02–0.45)	<0.02
PFTeDA	<0.03	<0.03	<0.03	0.15 (<0.03–1.51)	0.08 (<0.03–0.59)	<0.03
NMeFOSE	1.07 (<0.08–2.41)	1.06 (<0.08–2.90)	1.42 (<0.08–2.51)	<0.08	<0.08	<0.08
∑PFCAs	3.26 (0.36–7.09)	2.03 (0.02–7.35)	1.80 (0.45–3.66)	2.79 (0.73–10.5)	2.25 (0.14–6.69)	1.53 (0.05–3.62)
∑PFCs	6.32 (1.04–12.8)	7.07 (1.67–13.8)	4.50 (1.13–9.76)	5.61 (0.73–14.4)	11.79 (1.42–28.3)	11.29 (1.78–21.6)

[a] <*x* = lower than the respective method detection limit (MDL).
[b] To be considered as estimated, because no standard was available.

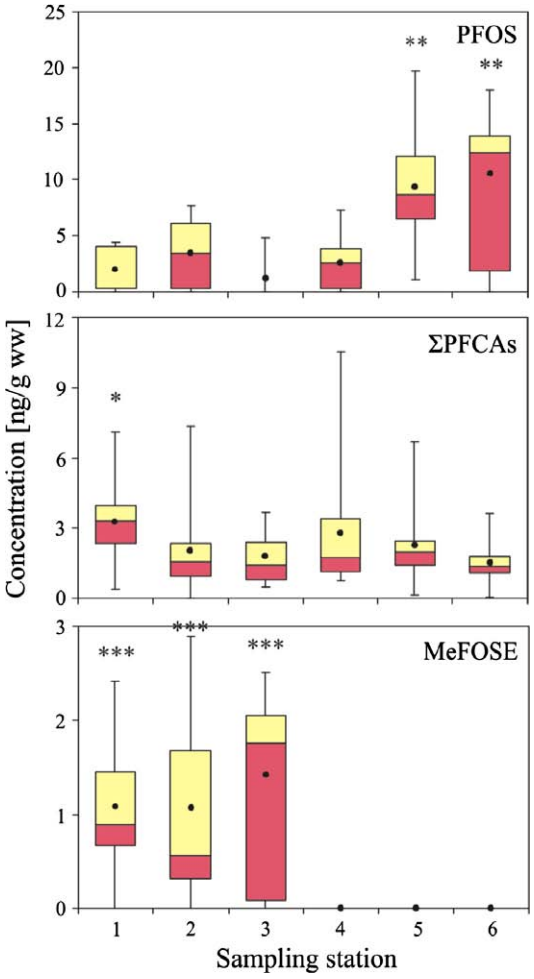

Fig. 3. PFOS, ∑PFCAs and MeFOSE concentration in dab bile fluids from the coast of Iceland and the North Sea. PFOS concentrations at sampling stations 5 and 6 are significantly higher than at sampling stations 1–4 ($p < 0.01$ (**), *t*-test), ∑PFCAs concentrations at sampling station 1 are significantly higher than at sampling station 6 ($p < 0.05$ (*), *t*-test) and MeFOSE concentrations at sampling stations 1–3 are significantly higher than at sampling stations 4–6 ($p < 0.001$ (***), *t*-test). Mean concentrations are indicated as a black circle, while the yellow and red boxes show 25% and 75% percentiles and the error bars represent the minimum and maximum concentrations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mean PFCA concentration decreased with increasing chain length from 4.7 ng/g ww for PFNA to 0.04 ng/g ww for PFTeDA. The presence of MeFOSE at sampling stations 1–3 may indicate incomplete biotransformation of MeFOSE to PFOS in the liver which can be excreted into the bile (Tomy et al., 2004). This is consistent with the positive correlation of MeFOSE and PFOS with each other ($p < 0.001$, Pearson correlation, see Table S3 in the Supplementary material). Interestingly, the concentrations of PFNA and MeFOSE were negatively correlated with the fish length ($p < 0.01$, Pearson correlation) and fish weight ($p < 0.05$, Pearson correlation) (see Table S3 in the Supplementary material), which indicates that with increasing age of the dabs the contamination of these compounds decreases.

The contribution of individual PFCs in dab bile from the coast of Iceland and the North Sea is shown in Fig. 2. Overall, PFOS, PFNA and MeFOSE had the highest mean contribution with ~45%, ~14% and ~14%, respectively, to the ∑PFCs. PFOS was the predominant compound at sampling stations 5 and 6 with a mean contribution of ~80%, while its mean contribution at sampling stations 1–4 ranged between 27% and 48%. A high contribution was found for PFNA and MeFOSE at sampling stations 1–3 ranging from 18–33% to 15–32%, respectively. PFUnDA had a high contribution at sampling stations 1 and 4 with both ~18%. Similar PFC distributions were observed previously in liver samples from the Canadian Arctic with PFOS and the longer-chain PFCAs as the predominant compounds (Martin et al., 2004).

The spatial distributions of PFOS, ∑PFCA and MeFOSE in dabs from the coast of Iceland and the North Sea are shown in Fig. 3. The mean concentrations of PFOS at sampling stations 5 and 6 close to the Danish and German coast were 9.34 ng/g ww and 9.39 ng/g ww, respectively. Significantly lower mean PFOS concentrations were found at sampling stations 1–4, ranging from 1.21 ng/g ww to 3.40 ng/g ww ($p < 0.01$, *t*-test). The reason could be the influence of the nearby industrial area of the Danish and German coast, where the rivers have been identified as important input sources for PFCs resulting in a PFOS concentration level of 0.69–3.95 ng/L in surface water of the German Bight (Ahrens et al., 2009a). Conversely, the mean concentration of ∑PFCA was significantly higher at sampling station 1 (i.e., 3.26 ng/g ww) in comparison to sampling station 6 (i.e., 1.53 ng/g ww) ($p < 0.05$, *t*-test). However, no significant differences for ∑PFCAs were observed for the other sampling stations, which indicates a relatively homogeneous distribution of PFCAs. The mean concentration of MeFOSE was significantly higher at sampling stations 1–3 with mean concentrations between 1.06 ng/g ww and 1.42 ng/g ww in comparison to sampling stations 4–6, where MeFOSE was not detected. It is possible that MeFOSE had its origin from atmospheric deposition; however, the exact contamination source is not known.

The different spatial distributions of PFOS and PFCAs in dabs correspond with the spatial distribution of PFCs in marine mammals, for which fish is the main food source. The PFOS concentrations in harbour seals (*Phoca vitulina*) from the German Bight were a factor of 10–50 higher than in Arctic ringed seals (*Pusa hispida*), while the PFCA concentrations were only a factor of 2 higher in harbour seals (Ahrens et al., 2009b; Butt et al., 2007). These results indicate that PFOS is mainly found near source areas such as the Danish and German coasts, whereas the distribution of PFCAs is more uniform due to their potential for long-range transportation via volatile precursors (Young et al., 2007) and/or directly by ocean currents (Armitage et al., 2006). A different spatial distribution of PFOS and PFCAs can also be found in harbour porpoise (*Phocoena phocoena*) livers (Van de Vijver et al., 2004) and guillemot (*Uria aalge*) eggs (Löfstrand et al., 2008). Similar to this study, decreasing PFOS concentrations were observed from north to south and a more uniform distribution of PFCAs in Northern Europe.

Overall, the presence of high levels of PFCs in the bile of dabs is an interesting observation, because the bile fluid is secreted by hepatocytes from the liver and is important for the excretion of several compounds including organic pollutants. Furthermore, dabs are good bioindicator of sediment pollution because they live in the benthic zone and their diet consists of zoobenthos. This could be problematic, because PFCs have the potential to bioaccumulate in the marine food web (Martin et al., 2003) and are potentially harmful to marine mammals (Ishibashi et al., 2008). In addition, consumption of the contaminated dabs could possibly be a source of PFCs to humans (Falandysz et al., 2006). Further investigations concerning the accumulation potential and whole body burdens in marine wildlife are necessary to assess potential adverse effects of PFCs. This study provides data on the baseline concentrations and spatial distribution for individual PFCs in dabs. The investigation of the spatial and temporal changes of PFC contamination in marine wildlife is an important challenge for the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.marpolbul.2009.10.007.

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Mercury in wahoo, *Acanthocybium solandri*, from offshore waters of the southeastern United States and the Bahamas

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ABSTRACT

Wahoo, *Acanthocybium solandri*, are predatory oceanic fish that occur and are harvested in all tropical and subtropical oceans. Total mercury concentrations analyzed in dorsal muscle tissue of 208 wahoo from offshore waters of the southeastern United States and the Bahamas ranged from 0.021 to 3.4 mg/kg (wet weight), with a mean of 0.50 mg/kg (± 0.595 SD). Analyses indicated significant positive linear relationships between mercury and length, as well as, age of wahoo. The piscivorous nature, generally high trophic position, fast growth rate, and associated high metabolism of wahoo within tropical offshore pelagic environments may lead to comparatively higher concentrations of mercury over relatively short time periods. Mercury in wahoo, a highly mobile species consisting of one world-wide population, is regionally influenced by large-scale spatial differences in available mercury in selected prey fish species - many of which have been found to contain relatively high concentrations of mercury.

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