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Temporal Trends (1981–2013) of Per- and Polyfluoroalkyl Substances and Total Fluorine in Baltic cod (*Gadus morhua*)

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Abstract: Temporal trends from 1981 to 2013 of 28 per- and polyfluoroalkyl substances (PFASs) were investigated in liver tissue of cod (*Gadus morhua*) sampled near southeast Gotland, in the Baltic Sea. A total of 10 PFASs were detected, with Σ_{28}PFAS geometric mean concentrations ranging from 6.03 to 23.9 ng/g ww. Perfluorooctane sulfonate (PFOS) was the predominant PFAS, which increased at a rate of 3.4% per year. Most long-chain perfluoroalkyl carboxylic acids increased at rates of 3.9 to 7.3% per year except for perfluorooctanoate (PFOA), which did not change significantly over time. The perfluoroalkyl acid precursors perfluorooctane sulfonamide (FOSA) and 6:2 fluorotelomer sulfonic acid were detected, of which the former (FOSA) declined at a rate of –4.4% per year, possibly reflecting its phase-out starting in 2000. An alternate time trend analysis from 2000 to 2013 produced slightly different results, with most compounds increasing at slower rates compared to the entire study period. An exception was perfluorohexane sulfonate (PFHxS), increasing at a faster rate of 3.7% measured from 2000 on, compared to the 3.0% per year measured starting from 1981. Analysis of the total fluorine content of the samples revealed large amounts of unidentified fluorine; however, its composition (organic or inorganic) remains unclear. Significant negative correlations were found between concentrations of individual PFASs (with the exception of PFOS) and liver somatic index. In addition, body length was negatively correlated with PFOA and perfluorononanoate, but positively correlated with perfluorododecanoate (PFDoDA) and FOSA. Additional studies on endocrine, immunological, and metabolic effects of PFAS in marine fish are essential to assess the environmental risk of these substances. *Environ Toxicol Chem* 2020;39:300–309. © 2019 SETAC

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INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are a class of anthropogenic chemicals, some of which occur ubiquitously in biotic and abiotic media (Giesy and Kannan, 2001; Buck et al. 2011; Houde et al. 2011). Due to the unique combination of hydro- and lipophobicity imparted by perfluoroalkyl chains, PFASs are used in many industrial and commercial applications, including fire-fighting foams, fluoropolymer production aids, pesticides, cosmetics, coatings for textiles, and food-contact materials (Kissa, 2001). However, concerns surrounding their environmental persistence, long-range transport potential, bioaccumulation in the food chain, and adverse effects in humans have led to a number of voluntary and regulatory actions aimed at controlling and reducing the production and use of certain PFASs. In 2000, the 3M company announced the production phase-out of perfluorooctanesulfonyl fluoride (POSF)–based products such as perfluorooctane sulfonate (PFOS; 3M Company, 2000). Furthermore, PFOS, its salts, and POSF were listed under Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009 (Stockholm Convention, 2009), whereas perfluorooctanoate (PFOA) production and use in Europe will be prohibited under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as of 2020 (Commission Regulation [EU], 2017). Long-chain perfluoroalkyl carboxylic acids (PFCAs, C9-C14) and perfluorohexane sulfonate (PFHxS) have been included in the Candidate List of Substances of Very High Concern by the European Chemicals Agency (ECHA) and are under consideration for inclusion in the Stockholm Convention (European Chemicals Agency 2017). Despite these steps, manufacture of many PFASs is ongoing.

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For example, PFOS production in China has continued, and many fluorochemical manufacturers have introduced alternative formulations which are claimed to be more environmentally friendly (Xie et al. 2012). However, some of these alternatives display similar physico-chemical properties to legacy PFAS (Gomis et al. 2015). A recent report by the Organization for Economic Co-operation and Development (OECD) lists 4730 PFAS-related CAS registry numbers, an immense quantity to monitor and regulate if handled on a substance-by-substance basis (Ritscher et al. 2018). In response, several analytical techniques have emerged over recent years for quantifying total fluorine and/or extractable organic fluorine (EOF) in environmental samples and consumer products, which complement compound-specific PFAS analysis (Miyake et al. 2007; Yeung et al. 2009; Robel et al. 2017; Schultes et al. 2018, 2019). By combining these data using fluorine mass balance calculations, the fraction of unidentified fluorine in a sample can be estimated (Schultes et al. 2018).

PFAS can be released into the environment via different pathways. Although direct emissions to air and water from production sites play a major role (Shi et al. 2015; Gebbink et al. 2017; Chen et al. 2018), other indirect point sources such as sewage and wastewater treatment plants, industries, and aqueous fire fighting foam (AFFF)-usage sites are important contributors to environmental emissions as well (Schultz et al. 2004; Becker et al. 2008; Clara et al. 2008; Murakami et al. 2009; Müller et al. 2011; McGuire et al. 2014). The Baltic Sea is a highly contaminated brackish ecosystem, which is under additional stress from other environmental pressures such as acidification, eutrophication, and climate change (Backer et al. 2010; Omstedt et al. 2012; Andersson et al. 2015). The unusually long residence time of water in the Baltic Sea (~30 yr) and its large catchment area of 85 million people favors accumulation of contaminants (Snoeijs-Leijonmalm and Andren, 2017; The Baltic Sea Experiment, 2018). Riverine discharge and atmospheric deposition are regarded as the major input pathways for perfluoroalkyl acids (PFAAs; Filipovic et al. 2013). Perfluoroalkyl acids are mainly stored in the water column as opposed to sediment (Filipovic et al. 2013), and are therefore available for uptake by fish through the gills. Perfluorooctane sulfonate and other PFASs can accumulate in blood and liver tissues of higher-trophic-level species (Houde et al. 2006; Haukås et al. 2007). Bioconcentration factors and bioaccumulation factors have been shown to increase with perfluorocarbon chain length (Martin et al. 2003; Kannan et al. 2005; Arnot and Gobas, 2006).

Baltic cod (*Gadus morhua*) are bottom-dwelling predatory fish that feed primarily on herring and sprat, but also on small cod (Pachur and Horbowy, 2013). Due to the relatively smaller number of marine mammals in the Baltic Sea, cod are among the predominant predators in this region (Österblom et al. 2007; Casini et al. 2008). Baltic cod are lean fish that store neutral lipids in their liver as an energy reserve; consequently, fat-soluble persistent organic pollutants such as dioxins and dioxin-like polychlorinated biphenyls also accumulate in the liver. Cod are an important sentinel species in the Baltic Sea and are also of high economic value. However, cod stocks have declined since the 1980s, mainly due to human impact and overfishing (Harvey et al. 2003; Zeller et al. 2011), which in turn has caused changes in Baltic Sea food web dynamics (Harvey et al. 2003; Casini et al. 2008).

As a result of the phase-out of PFOS by the 3M Company during 2000 and 2002, declines in PFOS and PFOA concentrations have been observed in human blood (Haug et al. 2009; Kato et al. 2011; Glynn et al. 2012; Olsen et al. 2012; Nøst et al. 2014; Gebbink et al. 2015a), human milk (Sundström et al. 2011; Nyberg et al. 2018), and food items including fish from the Baltic (Johansson et al. 2014; Gebbink et al. 2015b). However, increasing PFAS concentrations have been reported in some wildlife species from the Baltic region, for example in otters (Roos et al. 2013), herring, and white-tailed sea eagle (Faxneld et al. 2016). In contrast, gray seal from the Baltic showed decreasing trends for most PFAS except for long-chain PFCAs (Kratzer et al. 2011).

The present study aimed to evaluate levels and temporal trends of legacy and emerging PFAS, as well as total fluorine, in Baltic cod liver, over a time period covering important regulatory events. In addition, correlations between PFAS concentrations and several biological variables such as body condition and liver somatic index (LSI) of individual cod were assessed. These data provide insight into the response of PFAS temporal trends following reductions in production and use, as well as correlations of certain biomarkers with PFAS concentrations.

MATERIALS AND METHODS

Sample collection and selection

Baltic cod were caught south-east of Gotland in the Baltic Sea (~56° 53′N, 18° 38′E) during fall (September–December), after which they were brought to the Swedish Museum of Natural History for subsampling. After dissection, liver samples were stored in individual polypropylene tubes at –25 °C until analysis. Ten individuals per year were selected for analysis (16 yr for PFASs analysis [1981, 1990, 2000–2013], and 5 for EOF and total fluorine analysis [1981, 1990, 2000, 2007, and 2013]). Several biological variables were obtained for each individual, including age, sex, total length, body length (BL), body weight (BW), liver weight (LW), liver lipid content, and gonad weight. Condition factor, defined as $BW/BL^3 \times 100$ and LSI, defined as LW/BW, were calculated for each individual.

Sample preparation

Samples were extracted according to published methods (Gebbink et al. 2016). For target PFAS analysis, 0.5 g liver was fortified with 2.5 ng of individual internal standard solutions (see Supplemental Data, Table S1 for list of internal standards). The tissues were homogenized using a bead blender (SPEX SamplePrep 1600 MiniG) and 4.8-mm stainless steel beads in 4 mL acetonitrile for 4 min at 1500 rpm. Samples were extracted twice, centrifuged, and the combined supernatants reduced to approximately 1 mL under a stream of nitrogen. Weak anion exchange solid-phase extraction cartridges (150 mg, 6 mL) were conditioned with 6 mL of 2% ammonium

hydroxide in methanol, 6 mL methanol, and 6 mL Milli-Q water. The concentrated extract was diluted with 10 mL Milli-Q water and loaded onto the cartridge. Samples for targeted analysis were washed with 1 mL of 1% formic acid and 2 mL Milli-Q water. After drying the cartridges, neutral compounds were eluted with 1 mL methanol (fraction 1) followed by a wash with 2 mL methanol, which was discarded. Fraction 1 was fortified with 2.5 ng of individual recovery standards ($^{13}C_8$PFOA and $^{13}C_8$PFOS). Acidic compounds were eluted with 4 mL of 2% ammonium hydroxide in methanol (fraction 2), evaporated to dryness, reconstituted in 1 mL methanol, and fortified with 2.5 ng of individual recovery standards ($^{13}C_8$PFOA and $^{13}C_8$PFOS). Details about the extraction procedure for EOF are given in the Supplemental Data. For total fluorine analysis, aliquots of frozen neat material (60–130 mg) were placed directly into ceramic combustion boats and analyzed without further sample pretreatment.

Target analytes

For the 28 target PFASs, 2 levels of data quality were defined based on the availability of standards. For level 1 compounds, having both the native standard and the exactly matched isotopically labeled internal standard, high accuracy quantification was obtained. For level 2 compounds, an exactly matched isotopically labeled internal standard was unavailable, and their quantification was based on other internal standards with similar retention times. Level 1 was assigned to the following 20 compounds: perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluorundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), PFHxS, PFOS, 1H,1H,2H,2H-perfluorooctane sulfonate (6:2 FTSA), FOSA, *N*-methyl perfluorooctane sulfonamide (MeFOSA), *N*-ethyl perfluorooctane sulfonamide (EtFOSA), *N*-methyl perfluorooctane sulfonamidoacetic acid (MeFOSAA), *N*-ethyl perfluorooctane sulfonamidoacetic acid (EtFOSAA), 1H,1H,2H,2H-perfluorooctylphosphate (6:2 monoPAP), 1H,1H,2H,2H-perfluorodecylphosphate (8:2 monoPAP), bis(1H,1H,2H,2H-perfluorooctyl)phosphate (6:2/6:2 diPAP), and bis(1H,1H,2H,2H-perfluorodecyl)phosphate (8:2/8:2 diPAP). Level 2 was assigned to the following 8 compounds: perfluorotridecanoate (PFTrDA), perfluorotetradecanoate (PFTeDA), perfluorodecane sulfonate (PFDS), *N*-methyl perfluorobutane sulfonamide (MeFBSA), 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS), bis(1H,1H,2H,2H-perfluorohexyl) phosphate (4:2/4:2 diPAP), (1H,1H,2H,2H-perfluorooctyl 1H,1H,2H,2H-perfluorodecyl) phosphate (6:2/8:2 diPAP), and bis(1H,1H,2H,2H-perfluorododecyl) phosphate (10:2/10:2 diPAP). More information on target analytes, internal standards, and recovery standards can be found in the Supplemental Data, Table S1.

Sample analysis

For target PFAS analysis, 5 µL of extract was injected onto a ultra-high performance liquid chromatograph (UHPLC; Acquity, Waters) coupled to a triple quadrupole mass spectrometer (Xevo TQS, Waters), which was operated in negative electrospray ionization mode. Chromatographic separation was achieved on a BEH C18 column (1.7 µm, 50 × 2.1 mm, Waters). Fraction 1 was analyzed for PFCAs, PFSAs, FOSAAs, FTSAs, and 9Cl-PF3ONS, whereas fraction 2 was analyzed for FOSAs and PAPs. The composition and gradient of the mobile phases are given in the Supplemental Data, Tables S2 and S3. More detailed information about monitored transitions and mass spectrometry (MS) parameters are provided in the Supplemental Data, Table S1. Analytes were quantified using a 9-point linear calibration curve (0.03–300 ng/mL). Solvent blanks and quality control (QC) standards were run intermittently between samples to monitor instrument stability and carry-over. Limits of detection (LOD) were based on extraction blanks and were defined as the average concentration of the respective analyte in the blank plus 3 times the standard deviation of the blank concentration. Method accuracy and precision were assessed via replicate spike/recovery experiments. Briefly, native PFASs were fortified into cod liver ($n = 5$) and extracted as described above. Quality control samples (unspiked cod liver) and blanks were processed and analyzed with every batch ($n = 12$) to monitor blank contamination and inter-day variability.

Extractable organic fluorine and total fluorine contents were measured by combustion ion chromatography (CIC) (AQF-2100H, Mitsubishi coupled to Dionex ICS-2100 Integrion; Thermo Fisher Scientific) according to published methods, with small modifications (Schultes et al. 2018). Briefly, sample extracts/neat samples were placed in ceramic boats and combusted slowly with argon as carrier and oxygen as oxidation gas. Two mL of the absorption solution (a total of 10 mL Milli-Q water) were loaded onto a concentrator column, which was then eluted onto an ion-exchange column (AS19 Dionex IonPac; Thermo Fisher Scientific). Fluoride was quantified using a linear calibration curve (2.5–250 ng F). To monitor background contamination and signal drift, instrumental blanks and QC standards were run intermittently. Extractable organic fluorine method accuracy and precision were assessed through replicate spike-recovery experiments. In brief, cod liver was fortified with 1) PFOS, 2) sodium fluoride (NaF), and 3) PFOS +NaF, each in triplicate, and extracted as described above. Details on spiking levels are provided in the Supplemental Data. Method accuracy and precision for total fluorine measurements were assessed by fortifying neat cod liver (100 mg) with NaF at 2 levels (high spike = 10 ng; low spike = 100 ng), each in triplicate.

Statistical data analysis

Concentrations above the LOD were used directly for statistical analysis, whereas concentrations below the LOD were imputed based on the log-normal distribution of the concentrations above the LOD for the respective compound before statistical analysis (John, 1998). This approach reduces potential data bias introduced by substitution of non-detects with a fixed value. Potential outliers were detected using

Tukey's outer fence method (Foreman, 2014), wherein the inter-quartile range (IQR) was achieved from a window of 5 yr. A less sensitive outlier detection was attained by widening the outer fence from the suggested 3 × IQR to 6 × IQR, excluding only extreme values. The numbers of outliers per compound that were detected and excluded from analysis are given in the Supplemental Data, Table S11. For all time-trend analyses, log-linear regression analysis using individual values was carried out. Non-linear trends were tested for significance using change-point detection, aimed at detecting 2 log-linear regressions with different slopes (Sturludottir et al. 2017). Power analyses were carried out and used to calculate the minimum slope that could be detected with a power of 80% during a 10-yr period (Bignert et al. 2004). Multiple regression analyses were carried out to find potential confounding variables: for example age, body length, condition, and so on. The concentrations of all PFAS and total fluorine (i.e. the dependent variables) were adjusted for all variables in the model, except year, by using the partial regression coefficients and the intercept, i.e. the adjusted concentrations were estimated as if all confounding variables were constant at their mean values. Significance level for all statistical analyses was set to 5%.

Fluorine mass balance calculations

To compare PFAS concentrations (ng/g) derived from UHPLC-MS/MS analysis to EOF and total fluorine (ng F/g) measured by CIC, molecular PFAS concentrations were converted to fluorine equivalents (ng F/g) as described in the Supplemental Data (Equation S1). Calculations for amount of total fluorine not accounted for by ΣPFAS concentrations are also provided in the Supplemental Data (Equation S2).

RESULTS AND DISCUSSION

Method performance

Spike-recovery experiments revealed percentage recoveries (i.e. accuracy) of 53 to 96% for most PFASs, with the exception of 6:2 FTSA (recovery of 129%), PFTrDA, PFTeDA, and 6:2/8:2 diPAP (recoveries as low as 40%; Supplemental Data, Table S4). Although recoveries were generally lower than expected, precision (assessed via relative standard deviation [RSD] of replicate spiked samples) was excellent, averaging 6% over all target compounds. Internal standard recoveries, which provide an indication of procedural losses and matrix-induced ionization effects using $^{13}C_8$PFOS or $^{13}C_8$PFOA as recovery standard, averaged 88% with an average precision of 18%. Detailed recoveries and RSD values are given in the Supplemental Data, Table S5. Reproducibility, assessed via analysis of replicate control samples (unspiked cod liver) included in each batch, averaged 17% (Supplemental Data, Table S6).

Recovery for total fluorine analysis, as assessed via NaF-spiked neat liver, averaged 91.3% with 3.9% relative standard deviation (Supplemental Data, Table S8). Instrumental blanks for total fluorine analysis were below detection limit at all times. Spike/recovery experiments for EOF demonstrated an average recovery of 71% for PFOS, and efficient removal of inorganic fluorine (3% recovery for NaF) with an average precision of 8.5% relative standard deviation (Supplemental Data, Table S7). However, concentrations of fluorine in the extraction blanks ($n = 10$), which were processed and analyzed together with the samples, displayed average fluorine concentrations that were significantly higher ($p < 0.001$; t-test) and more variable ($p < 0.001$; F-test) than the mean fluorine concentration in $n = 50$ samples, for both fractions (Supplemental Data, Figures S1 and S2, for fractions 1 and 2, respectively). Subsequent attempts at obtaining representative method blanks were unsuccessful. Due to the absence of appropriate method blanks, leading to high uncertainty in the accuracy of the EOF concentrations, we consider the EOF data uninterpretable. The EOF data are therefore not used further for fluorine mass balance calculations, and are only presented in the Supplemental Data (Figures S1 and S2, Table S14). All EOF data should be considered semi-quantitative and interpreted cautiously.

PFAS concentrations and profiles

Ten out of 28 target PFASs were detected above the method detection limit (PFOS, PFHxS, and PFCAs of chain length C8 up to C13) as well as the 2 precursor compounds (6:2 FTSA and FOSA). Geometric means, concentration ranges as well as detection frequencies are provided in the Supplemental Data, Table S9. Perfluorooctane sulfonate was the dominant compound in all but 2 samples from 1981 (in which FOSA was the dominant PFAS), with geometric mean concentrations ranging from 2.58 to 19.1 ng/g (sum of linear and branched isomers, ΣPFOS), accounting for 42 to 80% of Σ_{28}PFASs (Figure 1). The highest individual PFOS concentration was found in a sample from 2005 (35.5 ng/g), whereas 2012 showed the highest PFOS geometric mean concentrations (19.1 ng/g). The ratio of branched to linear PFOS isomers averaged 0.09 over all years, with no significant trend over time. Besides PFOS, PFNA was also detected in all samples, while PFOA, PFDA, and PFUnDA were detected in more than 90% of the samples. Lower detection frequencies were found for PFDoDA (55%), PFHxS (67%), and 6:2 FTSA (17%). The detection frequency for FOSA averaged 18% over the entire time period, with more frequent occurrence in the early years (90 and 40% in 1981 and 1990, respectively) compared with later years. No short-chain PFAA or other precursors were detected in any of the samples, consistent with their low bioaccumulation potential in fish (Gebbink et al. 2016). Among the PFCAs, PFUnDA was detected in the highest geometric mean concentrations (0.18–1.85 ng/g) followed by PFNA (0.28–1.52 ng/g), PFDA (0.11–0.98 ng/g), PFOA (0.10–0.52 ng/g), PFDoDA (0.02–0.30 ng/g), and PFTrDA (0.04–0.16 ng/g). Two PFAA precursors were detected above the LOD: FOSA (0.59–1.73 ng/g; sum of linear and branched isomers) and 6:2 FTSA (0.20–0.30 ng/g). Branched and linear isomers of FOSA were detected in an average ratio of 0.24, which did not change significantly over the years.

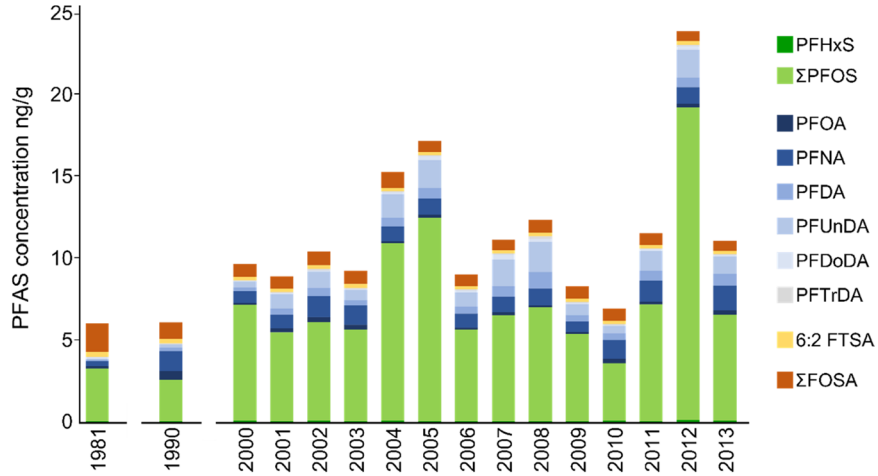

FIGURE 1: Per- and polyfluoroalkyl substance (PFAS) concentrations (geometric means, ng/g wet wt) in Baltic cod liver. PFHxS = perfluorohexane sulfonate; ΣPFOS = perfluorooctane sulfonate, sum of linear and branched isomers; PFOA = perfluorooctanoate; PFNA = perfluorononanoate; PFDA = perfluorodecanoate; PFUnDA = perfluorundecanoate; PFDoDA = perfluorododecanoate; PFTrDA = perfluorotridecanoate; 6:2 FTSA = 1H,1H,2H,2H-perfluorooctane sulfonate; ΣFOSA = perfluorooctane sulfonamide, sum of linear and branched isomers.

In general, the findings reported here are consistent with PFAS levels found in other species from the Baltic Sea. For instance, PFOS is usually the most abundant PFAS in samples from the Baltic Sea, including edible fish (Berger et al. 2009; Gebbink et al. 2016), guillemot eggs (Mochizuki et al. 2017), otters (Roos et al. 2013), herring and white-tailed sea eagle (Faxneld et al. 2016), as well as seals and salmon (Kannan et al. 2002). This trend has also been described in wildlife outside of the Baltic Sea (Martin et al. 2004b; Bossi et al. 2005; Hart et al. 2008; Houde et al. 2011; Valdersnes et al. 2017). Among the PFCAs, we observed PFUnDA and PFNA as the major homologues, followed by PFDA. The accumulation of long-chain PFCAs, in particular C11 and C9 homologues, has been reported frequently for fish (Martin et al. 2004a, 2004b; Hart et al. 2008; Berger et al. 2009; Faxneld et al. 2016; Gebbink et al. 2016), but also other wildlife (Bossi et al. 2005). However, some studies report PFTrDA in the highest concentrations for certain fish (Martin et al. 2004a; Berger et al. 2009), including a study on Atlantic cod from the Norwegian coast (Valdersnes et al. 2017). Similarly, the dominance of odd-numbered chain length PFCAs over adjacent even-numbered homologues has been consistently reported in wildlife (De Silva and Mabury, 2004; Martin et al. 2004a; Houde et al. 2005; Smithwick et al. 2005; Butt et al. 2007; Verreault et al. 2007; Rotander et al. 2012; Mochizuki et al. 2017) including fish from the Baltic (Berger et al. 2009; Faxneld et al. 2016), but also in other matrices such as human milk (Fujii et al. 2012) and dust (Liu et al. 2011). This pattern is hypothesized to originate either directly from production sources (Prevedouros et al. 2006) or indirectly through a combined effect of the degradation of fluorotelomer alcohols (FTOHs), yielding equal amounts of odd- and even-chain lengths (Ellis et al. 2004), and the increasing bioaccumulation potential of longer-chain PFCAs (Martin et al. 2004a, 2003).

A few other studies have analyzed PFAS levels in cod caught at different geographical locations. Levels of PFOS in liver from polar cod *(Boreogadus saida)* from the Barents Sea in 2004 were much lower than those found in the present study (2.02 ng/g vs 10.9 ng/g, respectively; Haukås et al. 2007). Perfluorooctane sulfonate was also the major PFAS observed in Arctic cod (*Gadus morhua*) caught along the Norwegian coast in 2008 to 2009, with geometric mean concentrations of up to 7.0 ng/g, similar to levels found in the present study (PFOS [2009] = 5.36 ng/g). However, in those samples, PFOS was only detected in 72% of all liver samples (Valdersnes et al. 2017). In a recent report by the Swedish Museum of Natural History (NRM), PFAS levels in Baltic cod caught along the west coast of Sweden in 2011 were very similar to those from the present study's 2011 samples (e.g., PFOS: up to 6.8 ng/g in the NRM report vs 7.14 ng/g in the present study; PFUnDA: up to 1.6 ng/g in the NRM report vs 1.19 ng/g in the present study), which in turn are comparable to levels in eelpout but lower than respective concentrations in herring and perch liver analyzed in the same report (Danielsson et al. 2014).

Correlations between PFAS and biological variables

A short summary of the biological variables is provided in the Supplemental Data, Table S10. Condition factor and liver lipid content decreased significantly over time ($p < 0.05$), whereas other variables showed no significant trends. A significant negative correlation was observed between concentrations of individual PFASs and LSI ($p < 0.01$ for PFOA and PFDoDA; $p < 0.001$ for PFNA, PFDA, PFUnDA, and FOSA). Body length was significantly negatively correlated with PFOA and PFNA ($p < 0.001$) and significantly positively correlated with PFDoDA ($p < 0.05$) and FOSA ($p < 0.01$). Furthermore, a significant negative correlation between the condition factor of cod and PFOA was observed. Age, gender, body weight, total length, and gonad weight of the fish were uncorrelated with PFAS concentrations. Alterations in condition factor and LSI can indicate stress in fish in response to changes in feed or presence of pollutants (Adams and McLean, 1985; Valdersnes et al.

2017). Although PFOS has been shown to cause an increase in LSI in rodents (Lau et al. 2003; Seacat et al. 2003), we observed the opposite correlation in our study—decreasing LSI with certain PFAAs and FOSA, however, not with PFOS. Similarly, Valdersnes et al. (2017) reported negative correlations of PFOS with liver fat, LSI, and condition factor in cod from the Norwegian coast. Also, Oakes et al. (2005) observed decreasing LSI and condition factors in exposed freshwater fish species. As reviewed by Ahrens and Bundschuh (2014), species, gender, developmental stage, and duration of PFAS exposure are relevant for effects in aquatic organisms. It is important to note that the correlations reported above are not causal relationships, and therefore do not indicate a direct link between PFAS occurrence and biomarker response. Indeed, a comparison of the highest concentration of PFOS observed in the present study (35.5 ng/g) is orders of magnitude below the tissue residue–based toxicity reference value of 87 mg/kg reported by Beach et al. (2006). The occurrence of other persistent, bioaccumulative, and potentially toxic substances in cod, which may display the same or similar correlations, represent a significant potential confounding factor for these results. Clearly, additional toxicological studies are needed to investigate the effects of co-exposure to the diverse range of contaminants that exist in the environment.

Temporal trends

Eight compounds (PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFOS, PFHxS, and FOSA) were selected for time-trend analysis due to their high detection frequency. Perfluorotridecanoate and 6:2 FTSA were detected in only 21 and 17% of the samples, respectively; therefore they were excluded from time-trend analysis. Two different time spans were subjected to trend analysis in order to 1) evaluate overall trends from 1981 to 2013, and 2) identify changes in response to compound phase-out as well as reductions in use and production that occurred in more recent years (2000–2013). The detected temporal trends of both time periods are visualized in Figure 2, and additional summary statistics are given in the Supplemental Data, Tables S11 and S12. Supplemental Data Table S13 summarizes the best fit of the multiple regression model for each compound, including β-coefficients and confounding variables that were used to adjust the data. Plots of the data before and after adjustment for confounding variables for each compound can be found in the Supplemental Data, Figures S3 to S10.

Log-linear regression analysis over 32 yr (1981–2013) revealed significantly increasing trends for PFOS, PFHxS, PFNA, PFDA, PFUnDA, and PFDoDA. Change-point detection did not explain significantly more of the variation than the log-linear regressions, and is therefore not reported. The most prevalent compound, PFOS, increased at a rate of 3.4 ± 1.3% per year, which would lead to a doubling time of approximately 20 yr relative to the initial concentration, assuming a continuous change. Interestingly, the sum of branched isomers displayed a steeper trend than the linear isomer (4.9 ± 1.3% compared to 3.3 ± 1.2% increase per year; Supplemental Data, Table S11). More alarming trends were detected for the long-chain PFCAs, PFDA, PFUnDA, and PFDoDA, with annual changes of 5.9 ± 1.2%, 7.2 ± 1.4%, and 7.3 ± 2.1%, respectively. At these rates, a two-fold increase in concentration could be expected after only approximately 12, 10, and 10 yr respectively. Perfluorononanoate concentrations increased at a rate of 3.9 ± 1.2% per year, whereas PFHxS concentrations increased at a rate of 3.0 ± 1.3% per year, leading to doubling times of 18 and 24 yr, respectively. Perfluorooctanoate was the only analyzed compound that did not show any significant changes in concentration over time. The only precursor subjected to time-trend analysis, FOSA, showed a declining trend, with concentrations decreasing at a rate of –4.4 ± 1.0% per year. At this rate, FOSA concentrations would be reduced by 50% after approximately 15 yr, with slightly faster decreasing trends for the branched isomers than the linear isomer (for details, see Supplemental Data, Table S11). Although temporal-trend analysis over the entire time period is useful for assessing overall changes in concentration, an analysis limited to more recent years can provide a better measure of an environmental response to changes in production and regulatory decisions, as for example 3M's phase-out of PFOS and its precursor FOSA in 2000, as well as the Stewardship program for long-chain PFCA (3M Company 2000; US Environmental Protection Agency, 2006). Results of the temporal-trend analysis from 2000 to 2013 are presented in Figure 2 and the Supplemental Data, Table S12. Perfluorodecanoate, PFUnDA, and PFDoDA displayed slower increasing trends over the last 13 yr as compared with the entire time period, whereas the trends for PFNA and PFOS became nonsignificant over the shorter time period. In contrast, PFHxS displayed a steeper increase (annual change of 3.7 ± 3.0% per year), reducing the doubling time from 24 to 19 yr, possibly reflecting the recent increase in production and use of C6-based compounds. The trend for FOSA declined more rapidly from 2000 to 2013, shortening the halving time from 15 to 10 yr.

Increasing time trends of PFOS and long-chain PFCAs have been observed in many other species in the Baltic Sea, despite the phase-out in 2000. The most prominent case was reported by Roos et al. (2013) in otters from Sweden, in which PFOS and PFCA (C8-C14) concentrations increased at rates of 5.5 to 13.0% between 1972 and 2011, and some compounds at an even faster rate during the most recent 10 yr (Roos et al. 2013). Faxneld et al. (2016) reported increasing PFOS (4–7% per year) and C8-C13 PFCA concentrations (3–10% per year) in herring liver from different sites in the Baltic Sea. Perfluorooctane sulfonamide concentrations showed decreasing trends, at rates of approximately 4 to 9% per year, similar to rates reported in the present study. White-tailed sea eagles analyzed in the same study showed similar trends for most PFASs, at a rate of up to 15% per year. In contrast, long-chain PFCA concentrations in herring analyzed by Bignert et al. (2016) mostly decreased over the last 10 yr, at rates of 5.6 to 9.7% per year (Bignert et al. 2016). Several other studies reported decreasing time trends in the Baltic, such as Kratzer et al. (2011) for C8-C11 PFCAs (1–13%) since 1997 in gray seals. Perfluorooctane sulfonate did not show a significant trend, whereas C12-C14 PFCA concentrations increased at 19 to 38% between 1972 and 2008 (Kratzer et al. 2011). Perfluorooctane sulfonate and long-chain PFCA (C10-C13) concentrations in guillemot eggs from the

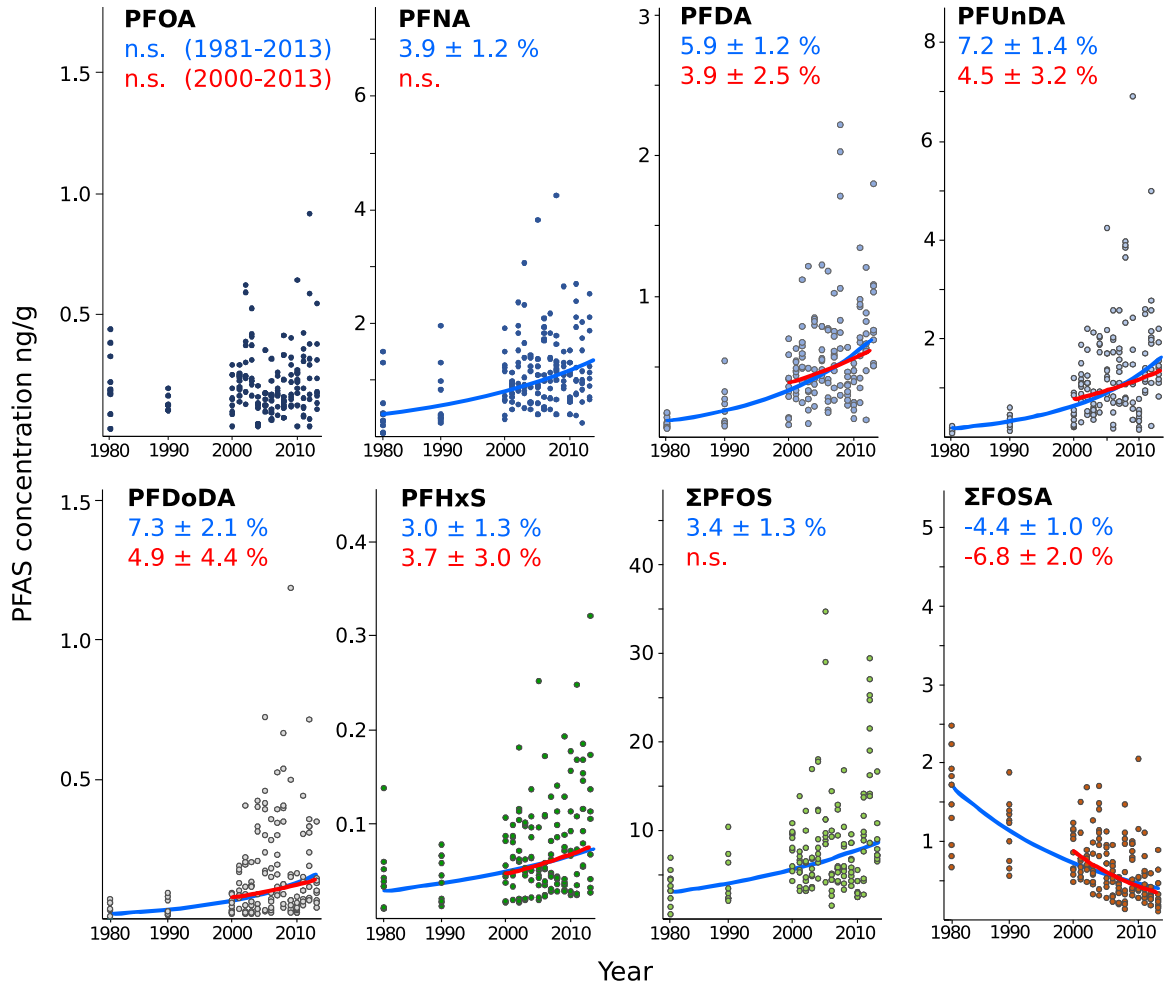

FIGURE 2: Per- and polyfluoroalkyl substance (PFAS) concentrations (ng/g wet wt) in Baltic cod liver from 1981 to 2013. Temporal trends are based on log-linear regression and displayed in blue for the time period 1981 to 2013, and in red for 2000 to 2013. The percental changes in concentrations per year with 95% confidence internal are given in blue for 1981–2013 and red for 2000–2013. Only significant trends at the level $p < 0.05$ are reported. PFOA = perfluorooctanoate; PFNA = perfluorononanoate; PFDA = perfluorodecanoate; PFUnDA = perfluorundecanoate; PFDoDA = perfluorododecanoate; PFHxS = perfluorohexane sulfonate; ΣPFOS = perfluorooctane sulfonate, sum of linear and branched isomers; ΣFOSA = perfluorooctane sulfonamide, sum of linear and branched isomers; n.s. = non-significant.

Baltic have also displayed downward trends in the recent years of analysis (Bignert et al. 2016). Rüdel et al. (2011) reported decreasing PFOS concentrations since 2000 (10% per year) in eelpout, but increasing concentrations (8%) in herring gull eggs from the Baltic (Rüdel et al. 2011). Swedish peregrine falcon eggs revealed increasing PFCA concentrations (7–12%) but decreasing PFOS and PFHxS concentrations after the mid-1980s (Holmström et al. 2010). In summary, the trends observed in cod from the present study align well with other trends of Baltic wildlife reported in the literature.

Total fluorine and fluorine mass balance

To estimate the amounts of total fluorine and EOF overlooked by targeted PFAS analysis, and how their proportions change with time, a subset of samples (5 time points, 10 individuals each) was analyzed using CIC. Due to poor data quality of the EOF, these data and the associated unidentified extractable organic fluorine are not reported in the present study. Concentrations of EOF are displayed in the Supplemental Data, Figures S1 and S2 for informative purposes only (these data should be considered semi-quantitative and interpreted cautiously). Geometric mean concentrations of total fluorine ranged from 102 to 369 ng F/g (Supplemental Data, Table S14); individual data are displayed in Figure 3. No statistically significant temporal trend or correlations with biological variables were observed in the data. Total PFAS concentrations were low in comparison with total fluorine quantities. According to Equations S1 and S2 in the Supplemental Data, the amount of total fluorine not accounted for by ΣPFAS concentrations amounted to 93.1% to 98.3% (range of yearly averages, Supplemental Data, Table S14). This quantity can be categorically divided into organic and inorganic fluorine species; however, because neither organic fluorine (as for example EOF) nor inorganic fluorine (as for example fluoride) were measured in this work, their relative proportions to the total fluorine remain unknown. Wright et al. (1974) reported concentrations of fluoride in cod liver from the North Sea of

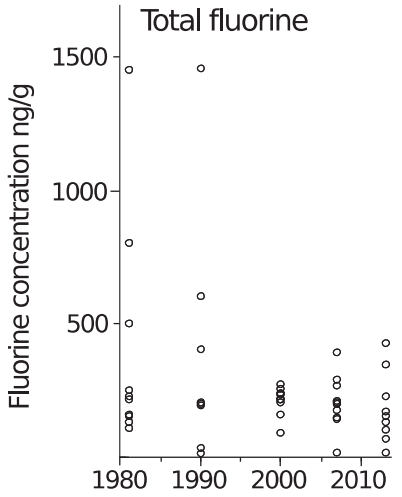

FIGURE 3: Total fluorine concentrations in ng F/g wet wt in Baltic cod liver.

3.18 ± 2.88 μg/g wet weight (Wright and Davison, 1974). These concentrations are considerably higher than the average total fluorine or non-extractable fluoride concentrations found in the present study (0.17 ± 0.32 μg/g wet wt), possibly due to the lower salinity of the Baltic Sea compared with the North Sea (Janssen et al. 1999).

CONCLUSIONS

The present study showed the occurrence of several long-chain PFAAs and 2 PFAA-precursors in Baltic cod liver. Perfluorooctane sulfonate was dominant and occurred in all samples, with geometric means of up to 19.1 ng/g wet weight. The pattern of long-chain PFCAs (C8-C13) was dominated by odd-numbered chain-length compounds (e.g. PFUnDA and PFNA) over their adjacent even-chain homologues, consistent with several other wildlife studies reported in the literature. No short-chain PFAAs were detected, presumably due to their low bioaccumulation potential compared to longer-chain-length PFCAs. Time-trend analysis revealed no significant trend for PFOA, which could be attributed to its lower bioaccumulation potential as well. Perfluorooctane sulfonate, on the other hand, increased significantly over the entire monitoring period, whereas the trend analysis starting in the same year as PFOS production started to decline (2000) revealed a nonsignificant trend only, possibly indicating the start of declining concentrations. To further assess this hypothesis, samples from more recent years should be analyzed and included in the temporal analysis. The shorter-chain homologue of PFOS, PFHxS, showed increasing trends for both time spans, with a steeper slope for the more recent years. Concentrations of the long-chain PFCAs (C9-C12) increased significantly over time, for both analyzed time periods, with the exception of PFNA. For PFNA, the trend of 3.9% increase per year turned non-significant when analyzed from 2000 to 2013, conceivably reflecting on a reduction in usage. The other long-chain PFCAs (C10-C12) increased at rates of up to 7.3% per year. At this rate, a two-fold increase in concentrations would occur in only 9 yr, assuming continuous change. Perfluorooctane sulfonamide was the only compound with declining trends, as commonly reported in relevant literature, indicating reductions in production and use. Due to the lack of EOF data, the amount of unidentified EOF could not be determined in the present samples, concealing information about the presence of other fluorinated organic substances such as PFAS-precursors or pharmaceuticals. An improved extraction procedure has been developed in-house to obtain reliable EOF data (Spaan et al. 2019). Analysis of total fluorine disclosed large amounts of unidentified fluorine, yet its composition remains unclear. Furthermore, the observed correlations of certain PFAS with health variables call for further ecotoxicological studies for a better understanding of how health outcomes are associated with individual PFAS in wildlife, as well as with mixtures of PFAS and other environmental contaminants.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4615.

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