Temporal Trends of Per- and Polyfluoroalkyl Substances in Baltic Cod

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Introduction

Per- and polyfluoroalkyl substances (PFASs) are anthropogenic substances, some of which pose a considerable risk to humans and wildlife [1,2]. Production of the two most prominent PFASs, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), started in the 1950s and was only reduced in the 2000s following a phase-out by the major fluorochemical manufacturer and introduction of a voluntary Stewardship Program by the USEPA. It was not until 2007 when PFOS and its precursors were added to the Stockholm Convention on Persistent Organic Pollutants. Despite these initiatives, production of legacy PFASs continues in some developing countries, while elsewhere alternative fluorinated substances have been introduced which display considerable persistence but less potential for bioaccumulation [3].

The Baltic Sea is highly contaminated with anthropogenic pollutants compared to other seas, exhibiting long residence times of contaminants due to slow water exchange and low temperatures. Contaminants can enter the Baltic Sea through rivers, atmospheric deposition or point sources. PFASs are taken up by biota where they can bind to protein and accumulate mainly in liver and blood and biomagnify along the food web. Despite its phase-out, PFOS concentrations are still increasing in wildlife in the Baltic Sea [4].

The Baltic Cod (*Gadus morhua*) is a bottom-dwelling predatory fish which preys on herring and sprat, and small cod [5]. It is an important sentinel species in the Baltic Sea and of high economic importance, with Cod stocks declining since the 1980s due to e.g. overexploitation. The role of contaminants as additional confounding factor in this development is still unclear. As diet – in particular fish consumption – is among the principal sources of PFAS exposure in humans [6], investigating PFAS levels in Baltic Cod is important to characterize health risks associated with cod consumption [7]. The European Food Safety Authority's Contaminant Panel established a TDI for PFOS of 150 ng/kg body weight per day and for PFOA of 1.5 µg/kg body weight per day based on toxicity data available in the scientific literature [8].

The objectives of this study were to analyze current levels of legacy and emerging PFASs in Baltic Cod liver tissue at a site uninfluenced by local sources. In addition, we examined time trends and detected significant changes in PFAS concentrations over the last two decades.

Materials and methods

All fish were caught south-east of Gotland in the Baltic Sea during fall between 1981 and 2013. Subsampling of liver was conducted at the Swedish Museum of Natural History and all samples were stored in individual polypropylene tubes at -25°C prior to analysis. For analysis of 39 target PFAS (10 PFCAs (C4-C13), 4 PFSAs (C4, C6, C8, C10), 3 fluorotelomer alcohols (4:2, 6:2, 8:2), FOSA, MeFOSA, EtFOSA, FOSAA, MeFOSAA, EtFOSAA, F53-B, 4 monoPAPs (4:2, 6:2, 8:2, 10:2) and 11 diPAPs), 16 time points were chosen (years 1981, 1990, 2000-2013). For each year, 10 individuals of random sex and age were selected to ensure an unbiased analysis. All concentrations are reported on a wet-weight basis.

The tissues were homogenized using a SPEX SamplePrep 1600 MiniG® bead blender with 4.8 mm stainless steel beads and 4 ml acetonitrile for 4 minutes at 1500 rpm. After a second consecutive extraction, supernatants were combined, centrifuged and reduced to 1 ml under a stream of nitrogen. Weak anion exchange solid phase extraction cartridges (150 mg, 6 ml) were conditioned with 6 ml 2% ammonium hydroxide in methanol, 6 ml methanol and 6 ml MilliQ water. The concentrated extracts were diluted with 10 ml MilliQ water, loaded onto the cartridge and washed with 1 ml 1% formic acid and 2 ml MilliQ water. After drying the cartridges, analytes were collected in two fractions: fraction one was eluted with 1 ml methanol; fraction two with 4 ml 2% ammonium hydroxide in methanol.

Quantitative analysis was achieved using a UPLC (Acquity, Waters) coupled to a triple quadrupole mass spectrometer (Xevo TQS, Waters) which was operated in negative ion mode electrospray ionization. Analytes were separated on a BEH C18 column (1.7 µm, 50 × 2.1mm, Waters) and a binary gradient of ammonium acetate-buffered methanol and water. Multiple reaction monitoring was used for identification of the targets which were quantified using an internal standard method.

Results and discussion

PFAS concentrations. Of the 39 target PFASs included in this study, 8 were detected. PFOS was detected in all samples and was the dominant compound in all but two samples from 1981 in which FOSA was found at the highest concentrations. PFOS concentrations ranged from 2.58 – 19.13 ng/g (all ranges reported in the following section are geometric means), constituting 42-80% of ΣPFAS and about 98% of ΣPFSAs. This is in accordance with other studies that have reported PFOS as the most abundant PFAS in wildlife [6,10]. The only other PFSA detected was PFHxS, at low concentrations from LOD (0.03 ng/g) to 0.11 ng/g. Five PFCAs (C8-C12) were observed at high detection frequencies above LOQ. No short-chain acids were detected in any of the samples. Amongst the PFCAs, PFUnDA showed the highest concentrations (0.18 – 1.85 ng/g; 94% detection frequency) followed by PFNA (0.28 – 1.52 ng/g; 100% detection frequency), PFDA (0.11 – 0.98 ng/g; 97% detection frequency), PFOA (0.10 – 0.52 ng/g; 93% detection frequency) and PFDoDA (0.02 – 0.30 ng/g; 44% detection frequency). The higher concentrations of odd-numbered relative to even-numbered homologues is also a pattern often observed in wildlife [11,12]. The only PFAA-precursor that was detected above LOD was FOSA, with a detection frequency of 100% and concentrations ranging from 0.2 – 1.8 ng/g.

PFAS time trends. Time trend analysis was carried out on all eight compounds reported above. Before statistical analysis, all concentrations below limit of detection were imputed using a model based on the log-normal distribution of all data above LOD. To assess whether changes over the studied time were significant, linear regression was carried out on log-transformed data. All trends reported in the following section are significant at $p < 0.001$. Multiple regression was used to check for correlations between compound concentrations and any biological variables available for the sample set. In case of significant correlations, the time trends were adjusted in

order to eliminate changes not attributed to the contaminants themselves.

Increases in concentrations over the entire time span were observed for PFOS, PFHxS, PFDoDA, PFUnDA, PFDA and PFNA (see figure 1). Only PFOA levels did not show any significant changes over time. PFOS showed an increase of 3.5% per year, which equates to a doubling time of ~20 years, relative to the initial concentration. Alarmingly, increases were considerably more rapid for PFDA, PFUnDA and PFDoDA, with annual changes of 6.7, 7.6 and 7.7% respectively. At these rates, it would take approximately 10 years to double the concentrations. FOSA was the only compound with a declining trend (see figure 1); its proportion of the ΣPFAS concentration decreased from 27% to 2% between 1981 and 2013 at a rate of -4.4% per year. At this rate, FOSA concentrations would be halved after about 16 years.

Increasing trends in PFOS concentrations have been observed in many other species in the Baltic Sea including herring and white-tailed sea eagle [13], otters [4] and grey seals [14].

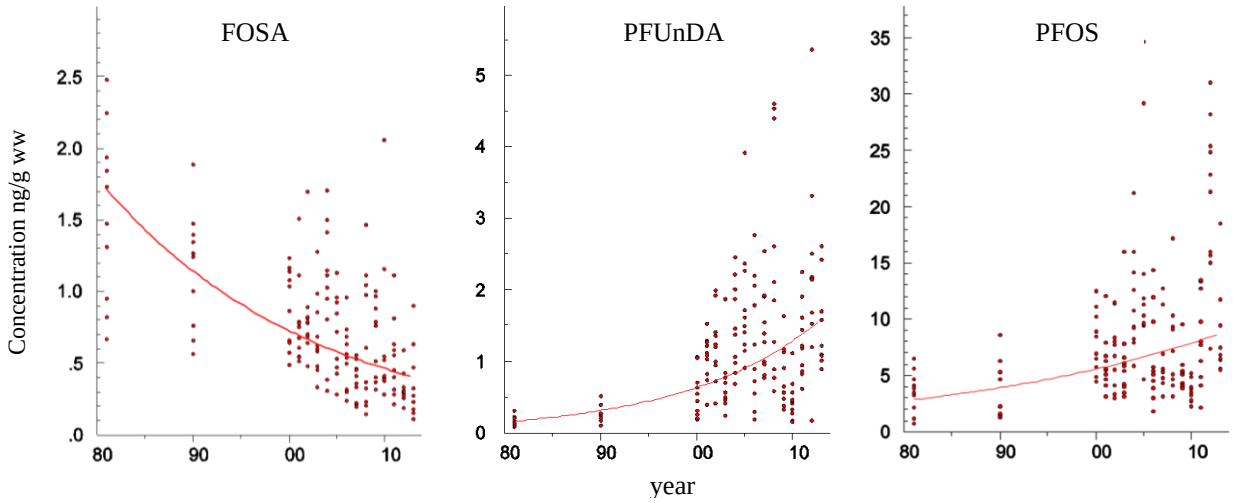

Figure 1: Time trends of FOSA, PFUnDA and PFOS in Baltic Cod from 1981 – 2013.

Correlation with biological variables. Age, gender and body weight of the fish were uncorrelated with PFASs concentration. However, a significant negative correlation was observed between concentrations of individual PFASs (with the exception of PFOS) and liver somatic index (LSI; ratio of liver weight to body weight). Body length was significantly negatively correlated with PFOA, PFNA and significantly positively correlated with PFDoDA and FOSA. According to these results, compound time trends were adjusted for the correlations with body length and LSI.

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