Cod: 8.6013

INCREASING PFAS CONCENTRATIONS IN OTTERS AND RINGED SEALS FROM SWEDEN, 1970-2015

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Introduction

Otters (Lutra lutra) and ringed seals (Pusa hispida botnica) are important top predators in aquatic ecosystems in and around Sweden. The otter is found primarily in limnic areas and feeds mainly on fish, but also small mammals, birds, amphibians and cray fish. The Baltic ringed seal is mostly found in Bothnian Bay and the Bothnian Sea, and feeds on small fishes and crustaceans. As aquatic top predators, otters and seals are subjected to elevated concentrations of contaminants from their diet. Population declines in the 1900s in ringed seals were attributable to high frequencies of female sterility and pathological alternations connected to environmental pollution [1-3]. International bans of PCBs and DDT have led to improved reproductive success in otters and other aquatic top predators, resulting in increases in population sizes [4]. Nowadays otters are found in most parts of Sweden, while ringed seals numbers are increasing but not fully recovered in the Baltic.

Despite improvements in population health over the last decade, emerging contaminants, such as per- and polyfluoroalkyl substances (PFASs), pose an ongoing risk to top predators. PFASs encompass a diverse class of substances which have been globally produced since the 1950s. Several PFASs are subject to international restrictions due to their persistence, toxicity, and potential to bioaccumulate. In 2004, a pilot study revealed very high concentrations (up to 16000 ppb ww) of perfluorooctanesulfonic acid (PFOS) in the livers of 14 otters from Sweden. This led to a larger time trend study of PFASs in otters from southern Sweden (1972-2011), where a rapid increase in concentrations was observed for most substances, in particular for perfluoroalkyl carboxylates (PFCAs) over the last ten years of the study [5]. Rapidly increasing PFAS levels in these animals provided considerable impetus to expand the time trend, and to investigate PFAS contamination in otters from other parts of Sweden.

The objectives of this study were to evaluate whether PFASs were continuing to increase in otters from Sweden and to use otters to try to identify contamination 'hotspots' through investigation of spatial trends. We also aimed to compare PFAS levels and trends in otters with those in Baltic ringed seals. Our hypothesis was that otters and ringed seals - both being top predators in a similar region – would display similar temporal trends.

Materials and methods

Specimens
Otters: Liver samples from 184 sub-adult and adult otters from Sweden (69 from southern and 115 from northern Sweden) were analyzed and combined with data from 123 otters that were analyzed previously [5]. Time trends were investigated in otters from Sweden after they were grouped based on geographical location. Most of the otters were killed either by traffic or drowning in fishing gear.
Ringed seals: Twenty two pooled liver samples from 77 juveniles collected between 1974 and 2015 were analyzed. The seals were either bycaught in fishing gear or shot.

Target analytes:
The present work reports concentrations of seven PFCAs, (PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA), 2 perfluoroalkyl sulfonic acids (PFSAs; PFHxS and PFOS) and perfluorooctane sulfonamide (FOSA), a PFOS-precursor. In all cases concentrations of PFCAs and PFSAs are reported as the sum of all homologues.

Chemical analysis

Samples were extracted and analyzed using a previously reported method [6]. A portion (~0.5g) of liver sample was fortified with a mixture of isotopically labelled internal standards and acetonitrile before homogenizing with an Ultra-Turrax hand blender and then sonicating for 15min. The supernatant was removed and the extraction procedure was repeated once, after which all extracts were combined, reduced in volume, and then cleaned-up using dispersive carbon. After spiking with recovery standard, a portion of the extract was subjected to instrumental analysis using a Waters ultra-performance liquid chromatograph coupled to a Waters triple quadrupole mass spectrometer (UPLC-MS/MS) operated under negative ionization, multiple reaction monitoring mode. Quantification was carried out by isotope dilution using a 5-point calibration curve. In each batch of samples, blanks and replicate control fish tissue were analyzed to assess lab contamination and ongoing accuracy and precision of the method. Method detection limits were calculated based on the concentration producing a signal-to-noise ratio of 3.

Statistical analysis
Values below the MDL (non-detects) were replaced by MDL/√2 prior to statistical treatment in less than 10% of the samples. Temporal trend analyses were carried out on otters from northern and southern Sweden and on ringed seals using ordinary log#linear regression analysis. Prior to statistical analysis concentration values were log-transformed in order to approach the assumptions of normal distribution and variance homogeneity. P-values below 0,05 were considered statistically significant.

Results and discussion

Otters: PFOS was observed in by far the highest concentrations of all PFASs (over 80%), consistent with numerous prior studies of fish from lakes in Sweden [7]. PFNA, PFDA and PFUnDA accounted for more than 75% of the PFCAs in animals from northern and southern Sweden. All but FOSA showed prominent increasing trends (FOSA decreased during the study period) and PFCAs increased with higher rates compared to PFSAs. When only looking at the last ten years of the time trend, PFCAs continued to increase in concentrations; PFOA and PFTeDA in particular displayed even higher increasing rates compared to the full period in southern Sweden. This is in contrast to time trends of grey seals from the Baltic (1969-2008), where PFOA, PFNA, PFDA and PFUnDA all started to decrease in the late 1990s-early 2000s [8]. While the sum PFCA concentrations in northern and southern Sweden were similar, the rate of increase for sum PFCA concentrations in otters from less urban areas of northern Sweden (7,8%) was somewhat less than for otters from the more populated southern Sweden (9,5%; see Figure 1).

The pattern was the opposite for PFSAs. PFOS increased with a higher rate in northern Sweden (4,5% yearly) compared to southern Sweden (1,8%, Figure 2). No trend was observed during the last ten years even though decreasing concentrations of PFOS have been reported since approximately 1995 in, for example, arctic char from the north, and perch from the south of Sweden [7]. As observed in fish, the concentrations of PFOS were generally higher in otters from southern Sweden. However, the range was large: in southern Sweden, PFOS concentrations ranged 18-16000 ppb (ww) for the full time period with a mean of 1000 ppb ww over the last 10 years (2006-2015). In northern Sweden the range for the full period was 11-3660 ppb ww and mean for the last ten years was 313 ppb ww.
FOSA was the only compound that declined during the study period, and it decreased with a higher rate in the last ten years. In northern Sweden FOSA decreased by 10% annually and in southern Sweden it decreased by 14,5% over the last ten years.

As for otters from northern Sweden, all PFAS (except for FOSA) increased significantly during the study period (i.e. same trend as in the south). However, the increasing rates are not as strong as seen for southern Sweden and in contrast to otters from the south, and stabilized over the last 10 year period.

Ringed seals: Sum PFCA concentrations ranged from 2,5 to 164 ppb ww (mean over the last 10 years was 120 ppb ww). PFOS ranged from 9,4 to 400 ppb (ww), with a mean of 232 ppb ww over the last 10 years, i.e. similar mean as observed for grey seals in the Baltic (220 ppb ww, range 9.6-1444 ppb ww) [8]. The ringed seals showed similar increasing trends for PFOS as the otters (Figure 3), with stabilized concentrations over the last ten years of the study period. Concentrations were generally lower or much lower in seals compared to otters. And, as for the otters, FOSA was the only compound that showed a statistically decreasing trend (-3,1%).
In conclusion, while concentrations of several PFASs have ceased to increase over the last ten years, a significant decrease in concentrations was not observed. Some otters have very high concentrations

of PFOS and many but not all of the otters with elevated concentrations were found in the vicinity of firefighting practicing areas or airports. Although no pathological changes can be correlated to the elevated PFAS concentrations, a negative effect on otter health due to these chemicals cannot be ruled out. The high prevalence of cysts on the deference duct could be a result of exposure to endocrine disrupting chemicals [9] and several PFASs are known endocrine disruptors. For example, laboratory tests on rodents given PFOS and PFOA revealed lower testosterone levels and higher estradiol levels in the adult rats [10].

The ongoing increasing trends for PFCAs are of concern, as well as the fact that PFOS still does not show decreasing concentrations in otters and ringed seals.

Acknowledgements

This study was funded by numerous county boards in Sweden, Swedish EPA and World Wide Fund for Nature, WWF. As for ringed seal analysis: EPA Grant number 2213-15-022. Malin Stenström, Katarina Loso and Charlotta Moraeus and coworkers in the lab at SMNH are acknowledged for help with sampling prior to chemical analysis. Anne-Sofie Kärsryd and Thomas Alsberg (ACES) are acknowledged for their assistance in sample processing and instrumental analysis.

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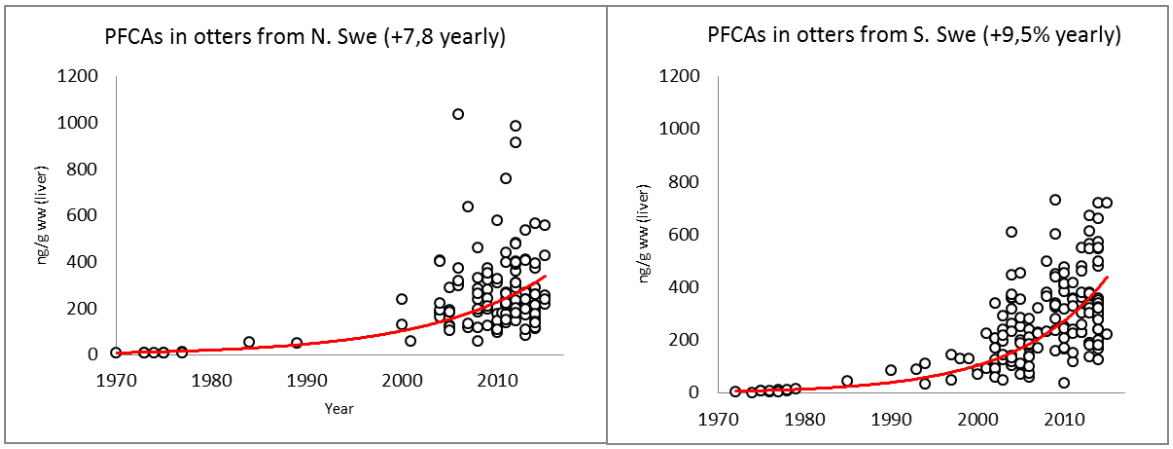

Figure 1. PFCAs concentrations in otters from northern (left) and southern (right) Sweden (ng/g ww in liver).

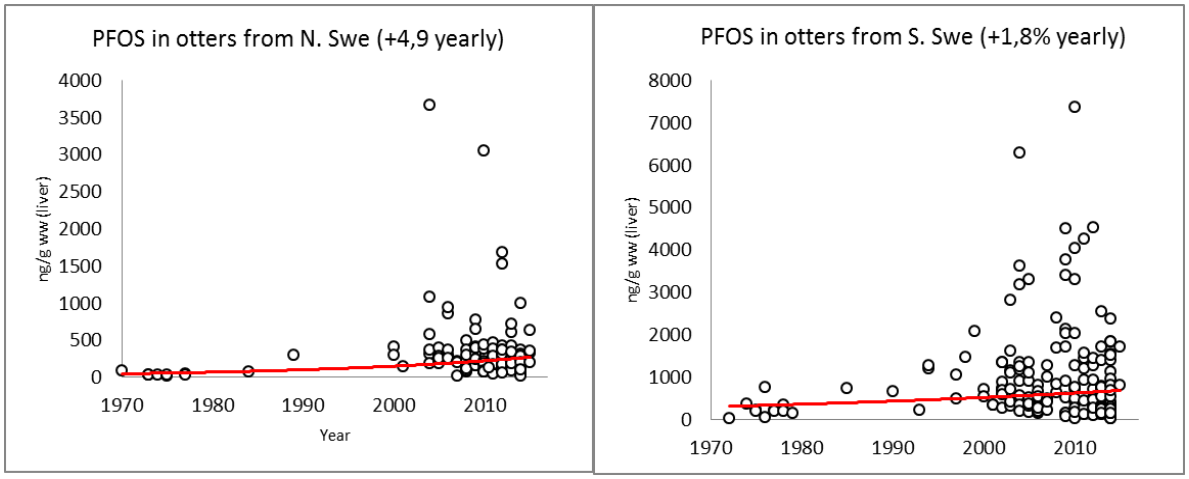

Figure 2. PFOS concentrations (ng/g ww in liver) in otters from northern (left) and southern (right) Sweden. One outlier (16000 ng/g) from southern Sweden 2004 was omitted in the diagram in order to maximize the y-axis.

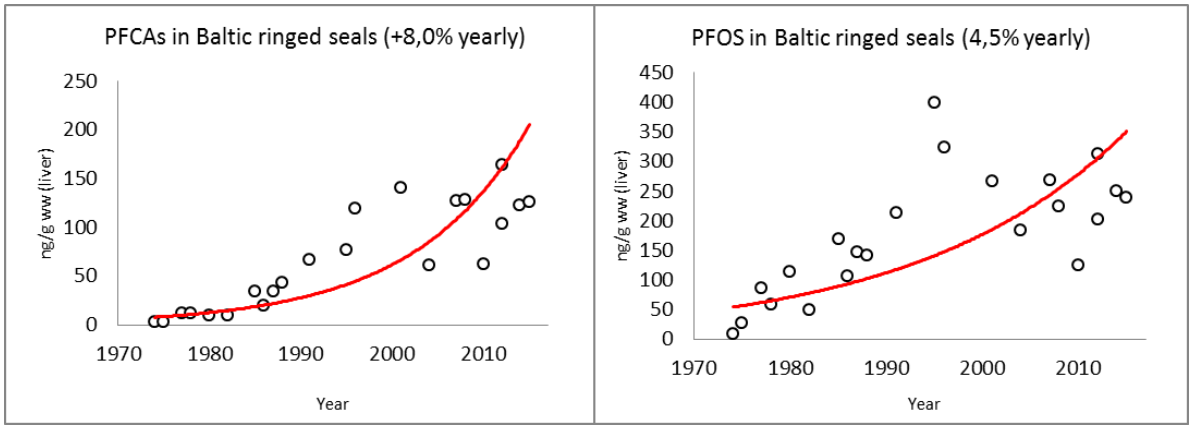

Figure 3. PFCAs (left) and PFOS (right) in juvenile ringed seals from the Baltic (ng/g ww in liver).