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Diet and metabolic state are the main factors determining concentrations of perfluoroalkyl substances in female polar bears from Svalbard☆

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ABSTRACT

Perfluoroalkyl substances (PFASs) have been detected in organisms worldwide, including Polar Regions. The polar bear (*Ursus maritimus*), the top predator of Arctic marine ecosystems, accumulates high concentrations of PFASs, which may be harmful to their health. The aim of this study was to investigate which factors (habitat quality, season, year, diet, metabolic state [i.e. feeding/fasting], breeding status and age) predict PFAS concentrations in female polar bears captured on Svalbard (Norway). We analysed two perfluoroalkyl sulfonates (PFSAs: PFHxS and PFOS) and C_8-C_{13} perfluoroalkyl carboxylates (PFCAs) in 112 plasma samples obtained in April and September 2012–2013. Nitrogen and carbon stable isotope ratios ($\delta^{15}N$, $\delta^{13}C$) in red blood cells and plasma, and fatty acid profiles in adipose tissue were used as proxies for diet. We determined habitat quality based on movement patterns, capture position and resource selection functions, which are models that predict the probability of use of a resource unit. Plasma urea to creatinine ratios were used as proxies for metabolic state (i.e. feeding or fasting state). Results were obtained from a conditional model averaging of 42 general linear mixed models. Diet was the most important predictor of PFAS concentrations. PFAS concentrations were positively related to trophic level and marine diet input. High PFAS concentrations in females feeding on the eastern part of Svalbard, where the habitat quality was higher than on the western coast, were likely related to diet and possibly to abiotic factors. Concentrations of PFSAs and C_8-C_{10} PFCAs were higher in fasting than in feeding polar bears and PFOS was higher in females with cubs of the year than in solitary females. Our findings suggest that female polar bears that are exposed to the highest levels of PFAS are those 1) feeding on high trophic level sea ice-associated prey, 2) fasting and 3) with small cubs.

1. Introduction

Perfluoroalkyl substances (PFASs) are a group of anthropogenic chemicals that have been manufactured for more than 50 years. PFASs are commonly used in the production of stain repelling agents, fluoropolymers, pesticides, lubricants, paints, medicines and fire-fighting foams due to their ability to repel both water and oils (Key et al., 1997; Prevedouros et al., 2006). PFAS are thermally and chemically stable, have no route of degradation and cannot be metabolized under normal environmental conditions, which makes them extremely persistent in the environment (Muir and de Wit, 2010). PFASs have been detected in blood and tissues of wildlife and humans worldwide, including remote regions (Haukås

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et al., 2007; Houde et al., 2011; Lau et al., 2007; Martin et al., 2004).

In contrast to persistent lipophilic pollutants, such as polychlorinated biphenyls (PCBs), PFASs have a high affinity towards plasma proteins, in particular albumin, and tend to accumulate in protein-rich compartments such as blood, liver and kidneys (Buck et al., 2011). Retention of PFASs in these organs and tissues may be toxicologically significant. In laboratory mammals, the effects of PFAS include disrupted steroid hormone and lipid homeostasis, reduced body weight, increased liver weight and a steep dose–response curve for mortality (Guruge et al., 2006; Jensen and Leffers, 2008; Lau et al., 2007).

The degree of bioaccumulation of PFASs generally increases with chain length (Martin et al., 2003a, 2003b). For instance, perfluorooctanesulfonic acid (PFOS) and C_9-C_{13} perfluoroalkyl carboxylate (PFCA, C_n refers to the carbon chain length) concentrations increase with trophic position thus, several PFASs can reach very high levels in top predators (Martin et al., 2004; Tomy et al., 2009; Van de Vijver et al., 2003). In addition, PFAS are transported by air and ocean currents to remote Arctic regions (Armitage et al., 2009; Shoeib et al., 2006; Wania, 2007). Polar bears (*Ursus maritimus*), as Arctic top predators are therefore highly exposed to PFASs (Kelly et al., 2009; Tomy et al., 2004).

Polar bears are among the most polluted species in the Arctic (Letcher et al., 2010). Quantitatively, PFAS is the most important contaminant group found in polar bear blood in wet weight concentrations (Bytingsvik et al., 2012a, 2012b). Among polar bears subpopulations, the concentrations of both lipophilic and proteinophilic pollutants are higher in the Barents Sea (i.e. Svalbard) than in most other subpopulations (McKinney et al., 2011; Smithwick et al., 2005a). Polar bears are seasonal feeders, their preferred prey being ringed (*Pusa hispida)* and bearded seals (*Erignathus barbatus*) especially in spring and early summer. Polar bears also feed opportunistically on a large range of land-based and marine species (Iversen et al., 2013; Tartu et al., 2016; Thiemann et al., 2008). Because of bioaccumulation up the food chain, bears feeding on seals may have higher pollutant concentrations than bears that feed on species lower in the food web. Moreover, pollutant exposure may also be affected by life history traits, during prolonged fasts, which can last up to 6–8 months for pregnant females (Andersen et al., 2012; Ramsay and Stirling, 1988) polar bears can lose over 40% of their body mass and the energy is drawn primarily from fat tissue (Atkinson and Ramsay, 1995).

Information on the effects of PFAS in polar bears is scarce. Modelling and correlative field studies suggest that concentrations of PFASs in polar bears are associated with increased steroid hormone concentrations in the brain, impaired reproduction and immunity (Dietz et al., 2015; Pedersen et al., 2016). There is currently little knowledge of the intrinsic or extrinsic factors that determine individual variation in PFAS concentrations in Arctic wildlife. For example, trophic level is a likely factor to influence PFAS exposure in marine mammals (Van de Vijver et al., 2003). Furthermore, PFAS concentrations have been related to body condition in Arctic foxes (*Vulpes lagopus*), harbor seals (*Phoca vitulina*) and Arctic breeding black-legged kittiwakes (*Rissa tridactyla*) (Aas et al., 2014; Tartu et al., 2014; Van de Vijver et al., 2003).

Breeding status in mammals may also be a source of variation as PFAS can be transferred from mother to young during pregnancy and lactation. Placental transfer is the dominant pathway for PFASs in hooded seals (*Cystophora cristata*) and polar bears (Bytingsvik et al., 2012b; Grønnestad et al., 2016). In polar bears, maternal transfer of PFASs via lactation is relatively low (Bytingsvik et al., 2012b). Finally, space-use patterns may also influence exposure to PFAS and other contaminants in polar bears through abiotic or biotic factors (Olsen et al., 2003; van Beest et al., 2015). The aim of this study was to investigate which factors (habitat quality, season, year, diet, metabolic state [i.e. feeding/fasting], breeding status and age) predict PFAS concentrations in female polar bears from Svalbard. This information is highly valuable for management to identify which individuals are the most vulnerable to PFAS exposure and how ongoing climate change might alter PFAS exposure in polar bears.

2. Material and methods

2.1. Field sampling

Adult female polar bears (age 4–28 years) from the Barents Sea subpopulation were captured non-selectively throughout Svalbard in April and September 2012 and 2013. The 112 samples collected (April 2012, n = 33, age: 12.9 ± 1.1 years (mean ± standard deviation), September 2012, n = 24, 13.2 ± 1.4 years, April 2013, n = 29, 13.4 ± 1.0 years and September 2013, n = 26, 12.8 ± 1.2 years) represented 78 females. Twenty-six females were captured more than once, specifically, we captured 19 females twice, six females three times and one female four times. However, females were not recaptured within the same fieldwork season.

Females were immobilized by remote injection of tiletamine hydrochloride and zolazepam hydrochloride (Zoletil Forte Vet®; Virbac, France), delivered by a dart fired from a helicopter (Eurocopter AS350 Ecureuil). We collected 50–100 ml of blood from the femoral vein using vacutainers (9–10 ml) with Lithium-Heparine to avoid clotting. We kept samples cool and out of sunlight until centrifuged within 10 h (3500 rpm, 10 min). Red blood cells and plasma were transferred to two separate cryotubes and frozen at −20 °C. Adipose tissue samples were collected using an 8 mm biopsy punch taken approximately 15 cm lateral to the base of the tail. In the field, adipose tissue samples were stored in a dry-shipper then kept at −80 °C until analyses. Immobilization and handling procedures followed standard protocols (Derocher and Wiig, 2002; Stirling et al., 1989), and were approved by the National Animal Research Authority (Norwegian Animal Health Authority, P.O. Box 8147 Dep., N-0033 Oslo, Norway).

Females were classified in three groups according to their breeding status: solitary (i.e., alone or together with a male in spring), with 1 or 2 cubs of the year (COYs; cubs younger than 1 year old) or with 1 or 2 yearlings (cubs aged between 1 and 2 years). No females with older cubs were captured as part of the current project. Female polar bears were aged using a vestigial premolar tooth (P1) following a method described previously (Calvert and Ramsay, 1998). The age of the females was not significantly different between groups ($p > 0.25$). Body condition index (BCI) was calculated as described for polar bears (Cattet et al., 2002) based on body mass (BM) and straight-line body length (SL): BCI=(lnBM-3.07 × lnSL+10.76)/(0.17 + 0.009 x lnSL).

2.2. Analysis of PFASs

Plasma samples (n = 112) were analysed for PFASs at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences (NMBU), Oslo, Norway. The plasma samples were analysed for six perfluoroalkyl carboxylic acids (PFCAs: perfluorooctanoate PFOA, perfluorononanoate PFNA, perfluorodecanoate PFDA, perfluoroundecanoate PFUnDA, perfluorododecanoate PFDoDA and perfluorotridecanoate PFTrDA) and two perfluoroalkyl sulfonic acids (PFSAs: perfluorohexane sulfonate PFHxS and PFOS). The methods were described in another study (Grønnestad et al., 2016).

Plasma samples (1 ml) were weighed in 15 ml Falcon centrifuge tubes (VWR International, LLC Radnor, USA). All tubes and pipettes used were made of plastic. Internal standards (^{13}C-labeled