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Temporal Change and Effects of Perfluoroalkyl Substanses (PFASs) on Thyroid Hormone Levels in Mother-Cub Pairs of Polar Bear (Ursus maritimus) from Svalbard in 1998 and 2008

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Cover photo: Polar bear cub from Svalbard. Photo by Jenny Bytingsvik.

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Trondheim, May 2011
Else Mari Espseth Nilsen

Abstract

High levels of persistent organic pollutants (POPs) have been detected in the Arctic wildlife. While many of the traditional POPs have been reported to decline the last decades, emerging contaminants such as perfluoroalkyl substanses (PFASs) have been detected in increasing amounts in biota worldwide. PFOS has been the major PFASs detected and the leading manufacturer of PFOS announced a voluntarily phase-out of the perfluorooctanesulfonyl fluoride (POSF) chemistry and ended the production in 2002. However, other PFASs are still being used in the industry and no regulations have been established. The toxicological effects of PFASs are not fully elucidated, but they have been linked to hepatotoxicity, developmental toxicity, immunotoxicity and disruption of hormone homeostasis.

The aim of this study was to investigate temporal changes of PFASs and to evaluate possible relationships between PFASs and circulating thyroid hormone (TH) levels in polar bear (*Ursus maritimus*) mother-cub pairs from Svalbard sampled in 1998 (12 mother-cub pairs) and 2008 (9 mother-cub pairs). Plasma samples from all polar bears were extracted by liquid-liquid extraction (LLE) using methanol and analysed for perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTrDA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS) and perfluorooctane sulfonamide (PFOSA) using liquid chromatography – mass spectrometry (LC-MS). Total thyroxine (TT4), free thyroxine (FT4), total triiodothyronine (TT3) and free triiodothyronine (FT3) were quantified by the use of radioimmunoassay (RIA). Relationships between PFASs, THs, age (only mothers), capture day and biometric variables were investigated by the use of principle component analysis (PCA) and general linear models (GLM).

PFHpA, PFOA and PFOS showed significant decreasing levels in mothers, while the levels did not differ between the years in cubs. In contrast, the longest chain perfluorinated carboxylic acids (PFCAs) showed significantly increasing concentrations over time in both mothers (PFNA, PFUnDA, PFDoDA, PFTrDA) and cubs (PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA). Concentration of PFBA, PFPeA and PFHxA were not detected in mothers or cubs, PFBS was detected in one mother and PFOSA was only detected sporadically. A positive relationship was

observed between the longest chain PFCAs and TT3 in both mothers and their offspring, particularly with regard to PFTrDA in mothers and PFDoDA in cubs. In addition, TT3 and body mass were positively correlated in cubs. In contrast to the longest chain PFCAs, no relationships were observed between PFOS and THs in the polar bear plasma samples. The positive correlation between TT3 and the longest chain PFCAs may indicate a possible interaction between PFCAs and the TH homeostasis in polar bear mother-cub pairs. However, no final conclusions can be drawn based on the mechanism behind this finding and further research is needed on these emerging contaminants to elucidate their potential TH-disrupting effects.

Abbreviations

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
CD	Capture day
D1	Type 1 deiodinase
D2	Type 2 deiodinase
ECF	Electrochemical fluorination
ESI	Electrospray ionization
FT3	Free triiodothyronine
FT4	Free thyroxine
FTOH	Fluorotelomer alcohol
GLM	General linear model
HPT	Hypothalamus-pituitary-thyroid
I.S.	Internal standard
LC-MS	Liquid chromatography – mass spectrometry
LLE	Liquid-Liquid extraction
MeFBSA	Methyl perfluorobutane sulfonamide
MeOH	Methanol
MRM	Multiple reaction monitoring
OH-PCB	Hydroxylated polychlorinated biphenyl
PBSF	Perfluorobutanesulfonyl fluoride
PC	Principle component
PCA	Principle component analysis
PCBs	Polychlorinated biphenyls
PFAS	Perfluoroalkyl substanse
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutane sulfonate
PFCA	Perfluorinated carboxylic acid
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonate

PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFOSA	Perfluorooctane sulfonamide
PFPeA	Perfluoropentanoic acid
PFSA	Perfluorinated sulfonic acid
PFTeDA	Perfluorotetradecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFUnDA	Perfluoroundecanoic acid
POPs	Persistent organic pollutants
POSF	Perfluorooctanesulfonyl fluoride
PPAR	Peroxisome proliferator activated receptor
RIA	Radioimmunoassay
SD	Standard deviation
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroid-binding globulin
TSH	Thyroid-stimulating hormone
TT3	Total triiodothyronine
TT4	Total thyroxine
TTR	Transthyretin
UGT1A1	Uridin diphoshoglucuronosyl transferase
UGTs	UDP-glucuronosyltransferases
US EPA	United States Environmental Protection Agency
UV	Unit variance
w.w.	Wet weight

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1. Introduction

The Arctic environment is characterized by long winters, low temperatures, seasonal variation in solar energy, long food chains and low species diversity (AMAP, 1998, Borgå et al., 2004). Even in remote areas like the Arctic, high levels of pollutants have been detected, although recently a decline in many of the traditional persistent organic pollutants (POPs), e.g. polychlorinated biphenyls (PCBs) have been reported (Henriksen et al., 2001, Dietz et al., 2004, Braune et al., 2005, Braune, 2007). However, emerging contaminants with different physio-chemical properties, such as perfluoroalkyl substanses (PFASs) have been detected in increasing amounts in the Arctic biota (Bossi et al., 2005, Dietz et al., 2008). In combination with increased levels in the Arctic biota, these compounds are of increasing concern due to toxic effects reported in laboratory studies (Lau et al., 2007) and general lack of regulations (Jensen and Leffers, 2008).

1.1 PFASs

PFASs are synthetic chemicals that have been utilized in the industry for more than 50 years and are characterized by a hydrophobic fluorinated carbon chain and a hydrophilic functional group (Giesy et al., 2009). In addition to their stability caused by the C-F-bonds, the PFASs amphiphatic character give the compounds qualities like oil and water repellence and stain resistance (Conder et al., 2008). PFASs have therefore been used in products such as lubricants, adhesives, paper coatings, insecticides and fire-fighting foams (Houde et al., 2006).

The most common PFASs found in nature can be divided into two major groups, perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs) (AMAP, 2009). Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) (Figure 1.1) are the most known PFCA and PFSA, respectively (AMAP, 2009).

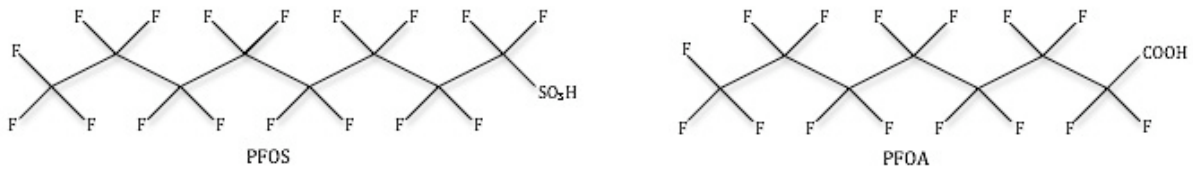

Figure 1.1. Chemical structure of PFOS and PFOA.

The major producer of PFOS, the 3M Company, voluntarily started a phase-out of the perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$) chemistry, inclusive PFOS, in 2000, ended the production in 2002 and replaced the production with shorter-chain PFASs using the perfluorobutanesulfonyl fluoride (PBSF, $C_4F_9SO_2F$) chemistry (Olsen et al., 2009, 3M, 2011). In 2009 PFOS was listed under annex B in the Stockholm Convention, meaning that the use is restricted and only permitted in specific cases (Stockholm Convention, 2009). Despite the phase-out of PFOS by the 3M Company, the chemical industry in China has been producing increasing amounts of PFOS since 2003 (Butt et al., 2010). Furthermore, no regulations have been introduced for the remaining PFASs (Jensen and Leffers, 2008).

Based on the PFASs non-volatile nature, these compounds are not expected to be transported to Arctic regions through the atmosphere in their original form (Young and Mabury, 2010). However, these compounds can be transported to the Arctic with ocean currents or through atmospheric transport of volatile precursors such as fluorotelomer alcohols (FTOHs) and perfluorinated sulfonamides (Ellis et al., 2004, Martin et al., 2005, D'Eon et al., 2006). FTOHs are the precursors of PFCAs (Ellis et al., 2004), while perfluorinated sulfonamides are the precursors for PFCAs and PFSAs (D'Eon et al., 2006). Evidence for the oceanic and atmospheric transport are supported by the detection of PFASs in seawater (Ahrens et al., 2010), Arctic snow (Young et al., 2007) and the Arctic atmosphere (Dreyer et al., 2009).

In contrast to the traditional POPs (eg. PCBs), PFAS are not lipophilic (Conder et al., 2008), but they have been reported to bind to proteins (Luebker et al., 2002, Jones et al., 2003, Bischel et al., 2010). Due to their proteinophilic nature, the highest concentration has been measured in protein-rich tissue like blood and liver (Ahrens et al., 2009). PFOS is the most abundant PFAS detected in the Arctic wildlife (Smithwick et al., 2005b, Haukås et al., 2007). However, the levels and patterns of the various PFASs in biota vary depending on location and specie (Bossi et al., 2005, Kannan et al., 2005, Smithwick et al., 2005b, Butt et al., 2008, Powley et al., 2008). A circumpolar study of polar bears (*Ursus maritimus*) reported higher concentration of PFOS in bears from Greenland, the Canadian Arctic and Norwegian Arctic compared to Alaska (Smithwick et al., 2005a). Although previous studies have reported an increasing trend of PFSAs like PFOS and the longest chain PFCAs in the Arctic biota (Bossi et al., 2005, Smithwick et al., 2006, Dietz et al., 2008), it should be noted that some recent studies on ringed seals (*Phoca hispida*) (Butt et al., 2006) and northern sea otters (*Enhydra lutris kenyoni*) (Hart et al., 2009) have reported a decreasing trend of PFOS after the phase-out by the 3M company (3M, 2011).

1.1.1 Maternal transfer of PFASs

Previous studies on humans have reported the presence of PFASs in maternal blood (Kärrman et al., 2007), cord blood (Inoue et al., 2004), maternal milk (Kärrman et al., 2007) and in infants (Toms et al., 2009). Thus, PFASs seem to be transferred from mother to fetus through placenta and to nursing offspring through maternal milk (Kärrman et al., 2007, Needham et al., 2010). PFAS have been detected in low concentrations in human milk compared to maternal serum and cord serum, indicating that placenta can be the major contamination pathway for human infants (Kärrman et al., 2007, Needham et al., 2010). In polar bears, levels of PFASs do not differ between genders in adult animals (Smithwick et al., 2005b), indicating that transfer of PFASs through maternal milk is a less important route of elimination. This is in contrast to previous reports on trans-generation transfer of PCBs and other lipophilic POPs in polar bears (Gebbink et al., 2007). To my knowledge, no studies have investigated the mother-cub transfer of PFASs in polar bears and the actual uptake and levels of PFASs in cubs recently after den emergence is so far unknown.

1.1.2 Effects of PFASs

Toxicological effects of PFASs on wildlife have been an increasing concern due to PFASs persistent nature and ability to bioaccumulate in biota (Kelly et al., 2009). PFASs have close structural resemblance to free fatty acids and have the ability to activate peroxisome proliferator activated receptors (PPARs) (Wolf et al., 2008). These fully-fluorinated compounds have been linked to hepatotoxicity, developmental toxicity, immunotoxicity and change in hormone levels such as thyroid hormones (THs) and sex hormones (Lau et al., 2007).

THs have a broad specter of actions and have important effects on growth, development and metabolism (McNabb, 1992). Toxicants can interfere with the TH homeostasis by disrupting one or more of the steps in the hypothalamus-pituitary-thyroid (HPT) axis. The HPT axis regulates the production of the THs, thyroxine (T4) and triiodothyronine (T3), from the thyroid gland by a negative feedback mechanism (Zoeller et al., 2007). POPs, such as PCBs, have been reported to affect the TH homeostasis in polar bears (Skaare et al., 2001, Braathen et al., 2004). The last decade, several studies have reported that non-aromatic compounds like PFASs also may disrupt the TH homeostasis in cynomolgus monkeys (*Macaca fasicularis*) (Seacat et al., 2002) and Sprague-Dawley rats (*Rattus norvegicus*) (Chang et al., 2008).

Circulating THs are mainly bound to transport proteins, while a minor proportion are detected as free hormones in the plasma. In humans the thyroxine-binding globulin (TBG), transthyretin

(TTR) and albumin binds 75 %, 15 % and 10 % of the THs, respectively (Zoeller et al., 2007). Only albumin and TTR have been detected in polar bears (Sandau, 2000). PFASs have been reported to have a weak binding to TTR (Weiss et al., 2009) and a stronger binding to albumin (Jones et al., 2003, Bischel et al., 2010). Replacements of natural ligands by PFASs through competitive binding can potentially disrupt the TH system in organisms (Weiss et al., 2009).

The HPT axis is strictly regulated through a negative feedback mechanism, and a decrease in THs levels should theoretically result in an increase of thyroid-stimulating hormone (TSH) (Zoeller et al., 2007). Laboratory studies on rodents have reported decreased levels of serum THs after *in vitro* exposure to PFOS. However, this decrease was not followed by an increase in TSH (Thibodeaux et al., 2003, Luebker et al., 2005, Chang et al., 2008). On the contrary, Seacat et al (2002) reported decreased concentration of total T3 (TT3) and a slight increase in TSH after PFOS-treatment in cynomolgus monkeys. In addition to a decrease in total T4 (TT4) levels in PFOS-exposed rats, Yu et al (2008) reported increased activities of uridine diphosphoglucuronosyl transferase (UGT1A1) and type 1 deiodinase (D1) in liver and thyroida, respectively. This increased enzyme activity can explain some of the decrease since 80 % of the circulating T3 is produced by deiodination of T4 in peripheral tissue and UDP-glucuronosyltransferases (UGTs) catalyse conjugation reactions in the liver that increase the excretion of THs through the bile (Klaassen, 2001, Zoeller et al., 2007, Yu et al., 2009). In summary, PFASs may potentially affect circulating levels of THs in several mammals and possible also polar bears through several different mechanisms.

1.2 Aim of the study

The aim of this present study was to investigate temporal changes of PFASs and to evaluate possible relationships between PFASs and circulating TH levels in polar bear mother-cub pairs from Svalbard sampled in 1998 and 2008.

2. Material and methods

2.1 Sampling

In 1998 and 2008, blood samples were collected during March/April from 51 polar bears at Svalbard, Norway. Blood samples were obtained from the femoral vein of mother-cub pairs located at Edgeøya and Hopen (Latitude: 76.46 – 78.15, Longitude: 22.00 – 25.45) in 1998, and Spitzbergen, Edgeøya and Barentsøya (Latitude: 77.05 – 79.49, Longitude: 13.93 – 21.94) in 2008. In 1998, 28 polar bears consisting of 12 mothers and 16 cubs (4 pairs of twins, 8 single cubs) were sampled. In 2008, a total number of 23 polar bears consisting of 9 mothers and 14 cubs (5 pairs of twins, 4 single cubs) were sampled. One of the cubs from each twin pair was randomly selected for the data analysis since the contaminant levels in twins are dependent on each other. Due to this, the number of individuals used in the data analysis was 42 (21 mothers and 21 cubs). All cubs sampled were approximately 4 months old (cubs of the year) and are referred to as cubs.

The adult individuals were sedated by the remote injection of a drug-filled dart (drug: Zoletil® 200 mg/mL; Virbac Laboratories, Carros, France, dart: Palmer Cap-Chur Equipment, Douglasville, Georgia) fired from a helicopter. For the cubs, sedation was given by manual injection. The drug was injected intra-muscular and the dose was 5-10 mg/kg body mass. The amount of drug for each bear was determined based on an estimation of the polar bear weight observed from the helicopter, or based on the excact body weight of the cubs. The blood was collected in Venoject® tubes containing sodium heparin (10 mL, Terumo Medical Corporation, Somerset, NJ, USA). Details on immobilization of the polar bears are described in Stirling et al. (1989) and Derocher and Wiig (Derocher and Wiig, 2002). Straight length, head length and axilliary girth were measured for mothers, and straight length, head length and body mass for cubs during the sampling. Body mass was estimated for mothers using the formula given by Derocher and Wiig (2002). Definitions of the biometric measurements are given by Derocher et al. (2005). All capture and handling methods were approved by the National Animal Research Authority (The National Animal Research Authority (NARA), P.O. Box 8147 Dep., N-0033 Oslo, Norway).

After sampling the blood was centrifuged (Labofuge 200, Termo Electron Corporation, Germany) and the plasma was transferred to cryogenic vials and kept at -20 °C. All samples were stored at -70 °C prior to analysis.

2.2 Radioimmunoassay (RIA)

Radioimmunoassay (RIA) was applied to measure TT4, Free T4 (FT4), TT3 and free T3 (FT3) in polar bear plasma. The hormone analysis was conducted at Department of Biology at the Norwegian University of Science and Technology (NTNU) and details regarding the analysis and validation of the analysis is presented by Bertinussen (2009).

Briefly, the RIA-method is based on the competitive binding to the antibody by unlabeled THs (T4, T3) and radioactive-labelled THs (T4*, T3*). Quantification of bound radioactive-labelled THs is then converted to the concentration of THs in the samples by using a calibration curve (Sigmond et al., 1992).

Thyroid hormone kits (Coat-A-Count Total T4, Free T4, Total T3 and Free T3, Siemens Medical Solutions, Diagnostics, Los Angeles, CA, USA) were applied and quantification was performed with a gammacounter (Cobra Auto-Gamma; Packard Instruments Company, Dowers Grove, IL, USA). Polar bear samples were analysed for TT4, TT3 and FT3 using 2 parallels, while FT4 had 3 parallels. Two parallels were used for the calibrators. The detection limit for TT4, FT4, TT3 and FT3 ranged from 0.135-0.444 nmol/L, 0.002-0.025 pmol/L, 0.005-0.081 nmol/L and 0.007-0.057 pmol/L, respectively.

2.3 Chemical analysis

The chemical analysis of PFASs in the polar bear plasma was conducted at the Institute for Environmental Studies (IVM) at the Vrije University (UV) in Amsterdam. The samples were analysed for 15 PFASs, consisting of 11 PFCAs, 3 PFSAs and 1 other (PFOSA) (Table 2.1). The chemical analysis was based on the extraction described by Powley et al. (2005) with modifications. The analysis included liquid-liquid extraction (LLE) with methanol (MeOH), Envi-Carb®clean-up procedure and quantification by liquid chromatography – mass spectrometry (LC-MS).

Table 2.1. Analysed PFASs in mother-cub pairs of polar bear at Svalbard, their abbreviation, molecular formula and number of carbons.

Compound	Abbreviation	Molecular formula	Number of carbons
PFCAs			
Perfluorobutanoic acid	PFBA	$CF_3(CF_2)_2COOH$	4
Perfluoropentanoic acid	PFPeA	$CF_3(CF_2)_3COOH$	5
Perfluorohexanoic acid	PFHxA	$CF_3(CF_2)_4COOH$	6
Perfluoroheptanoic acid	PFHpA	$CF_3(CF_2)_5COOH$	7
Perfluorooctanoic acid	PFOA	$CF_3(CF_2)_6COOH$	8
Perfluorononanoic acid	PFNA	$CF_3(CF_2)_7COOH$	9
Perfluorodecanoic acid	PFDA	$CF_3(CF_2)_8COOH$	10
Perfluoroundecanoic acid	PFUnDA	$CF_3(CF_2)_9COOH$	11
Perfluorododecanoic acid	PFDoDA	$CF_3(CF_2)_{10}COOH$	12
Perfluorotridecanoic acid	PFTrDA	$CF_3(CF_2)_{11}COOH$	13
Perfluorotetradecanoic acid	PFTeDA	$CF_3(CF_2)_{12}COOH$	14
PFSAs			
Perfluorobutane sulfonate	PFBS	$CF_3(CF_2)_3SO_3H$	4
Perfluorohexane sulfonate	PFHxS	$CF_3(CF_2)_5SO_3H$	6
Perfluorooctane sulfonate	PFOS	$CF_3(CF_2)_7SO_3H$	8
Others			
Perfluorooctane sulfonamide	PFOSA	$CF_3(CF_2)_7SO_2NH_2$	8

Prior to the extraction, the polar bear plasma (150 μL) was added to a polypropylen tube (15 mL, Greiner Bio-One GMBH, Frickenhausen, Germany) and the weight noted. An exact volume of internal standard (I.S.) (100 μL) consisting of $^{13}C_n$-analogues of PFBA, PFHxA, PFOA, PFNA, PFDA, PFUnDA and PFOS (Wellington Laboratories, Guelph, Ontario, Canada) and $^{18}O_2$–analogues of PFHxS and PFOSA (RTI International, NC, USA) was then added to the same polypropylen tube.

2.3.1 Liquid-Liquid Extraction (LLE) with MeOH

The mixture of plasma and I.S. was added MeOH (5 mL) and the tubes were mixed at maximum deflection for 30 minutes (Shaker SM-30, Edmund Bühler-GmbH, Hechingen, Germany). The samples were centrifuged (10 minutes, 3000 rpm) (Centrifuge, FirlabO SW-12, Meyzieu, France) before the supernatant was transferred to new polypropylen tubes (15 mL, Greiner Bio-One

GMBH, Frickenhausen, Germany). The extraction stage was repeated twice before the supernatant from the first and second extraction stage were merged and evaporated to 2 mL using a water bath (40 °C) and a gentle flow of nitrogen (Nitrogen 5.0, Praxair, Schoten, Belgium).

2.3.2 Clean-up

A clean-up stage was carried out on the extract to remove unwanted constituents occurring in the plasma that potentially could interfere with the quantification by LC-MS. Envi-Carb graphitized carbon adsorbent (100 mg)(Supelclean ENVI-Carb 120/400, Supelco, Bellefonte, PA, USA) was added to each tube and the content was mixed on a whirlmix before centrifugation (10 min, 3000 rpm). The supernatant was evaporated to dryness using a water bath (40 °C) and a gentle flow of nitrogen (Nitrogen 5.0, Praxair, Schoten, Belgium). MeOH (100 μL) and H_2O (100 μL) were added to each tube, and vortexed prior to centrifugation (10 min, 13000 rpm)(Centrifuge, Biofuge Stratos, Heraeus Kendro, Osterode, Germany). Finally, the supernatant was transferred to polypropylen vials ready for quantification.

2.3.3 Quantification

The extracted samples were analysed for PFASs using an LC-MS (6410 Triple Quad LC-MS, Agilent Technologies, Palo Alto, USA) with electrospray ionization (ESI) and multiple reaction monitoring (MRM) was utilized in the determination.

20 μL of extract was injected into A Fluorosep-RP octyl column (2.1x150mm, particle size = 5 μm, catalog #132211-FO, ES industries, West Berlin, New Jersey, USA). A second column, symmetry C18 column (2.1x50mm, particle size = 5 μm, catalogue # 186000206, Waters, Dublin, Ireland) was placed between the pump and the auto sampler to remove unwanted PFASs from the system. Mobil phase A was 5mM ammonium format and mobile phase B was MeOH. The mobile phase consisted of 35 % MeOH during the first 35 minutes before it was increased to 75 % until 45 minutes. Then it was increased to 95 % until 55 minutes and decreased to 35 % again from 55 to 65 minutes. The plasma concentration of PFASs is given as ng/g wet weight (w.w.).

2.3.4 Quality assurance

Equipment and solvents

To determine whether the sampling tubes (10 mL, Venoject® tubes, Terumo Medical Corporation, Somerset, NJ, USA) or storage tubes (Nalgener® Cryogenic vials, 1 mL and 5 mL Thermo Fisher Scientific, Rochester, USA) were potential sources of PFASs, these tubes were tested using a simple extraction with MeOH and final quantification using LC-MS. PFASs were not quantified in the test and it is reasonable to assume that the tubes did not leach PFASs to the blood neither during sampling or storage.

Due to the potential ability of PFASs binding to glassware, only plastic equipment was used in direct contact with the plasma or extracted plasma during the chemical analysis to minimize errors (van Leeuwen and de Boer, 2007). Glass equipment, on the contrary, was used in measurement of solvent volumes and this equipment was rinsed with MeOH before use to remove potential contamination. The nitrogen gas used in the evaporation was tested as a potential source for PFASs and blank samples were used as a potential indicator of PFASs contamination during the analysis and therefore an important part of the validation. None of the compounds were detected in the blank samples.

Batches and I.S.

All samples were divided into four batches. Each batch consisted of two parallels of blank and reference material (human plasma from 2001) and one parallel of polar bear plasma. I.S was used in all samples and increased the reliability of the method. Nevertheless, a identical but labelled I.S. was not available for all of the analysed compounds at the time the chemical analysis was performed. Thus, the concentration of PFPeA, PFHpA, PFDoDA, PFTrDA, PFTeDA and PFBS was determined using another I.S. (Table 2.2). In these cases, the I.S. used was selected based on compound similarity to optimize the quantification accuracy. The recoveries for the different I.S. are presented in table C.1 (Appendix C).

Table 2.2. Analysed compounds and I.S used in the chemical analysis. *Compounds quantified with another I.S.

Target molecule	I.S.
PFBA	$^{13}C_4$ PFBA
PFPeA*	$^{13}C_4$ PFBA
PFHxA	$^{13}C_2$ PFHxA
PFHpA*	$^{13}C_2$ PFHxA
PFOA	$^{13}C_4$ PFOA
PFNA	$^{13}C_5$ PFNA
PFDA	$^{13}C_2$ PFDA
PFUdA	$^{13}C_2$ PFUdA
PFDoA*	$^{13}C_2$ PFUdA
PFTrA*	$^{13}C_2$ PFUdA
PFTeA*	$^{13}C_2$ PFUdA
PFBS *	$^{18}O_2$ PFHxS
PFHxS	$^{18}O_2$ PFHxS
PFOS	$^{13}C_4$ PFOS
PFOSA	$^{18}O_2$ PFOSA

Calibration lines

Since technical mixtures used in the industry consist of a mixture of linear (70 %) and branched PFOS (30 %) (Stockholm Convention, 2006), two different calibration lines were used in the chemical analysis to quantify the concentration of PFOS. The first calibration line used linear standards to calculate both linear and branched PFOS. A second calibration line was therefore used to check if there would be differences in concentrations if the quantification was just based on the linear value or also based on a standard containing branched isomers. The calibration range for the first calibration line with linear standards was 0.1-200 ng/mL, while the calibration range for the second calibration line with linear and branched standards was 0.05-250 ng/mL. Small differences were reported when calculating the results based on the two different calibrators, and the final PFOS-results are calculated based on the linear standard.

Quantification

In addition to the analytical column, a second column was used in the LC-MS to remove PFASs from the system. This column was placed between the pump and the auto sampler, and was therefore not in contact with the samples. This step was necessary because parts of the LC-MS consist of Teflon® which can be a considerable source of contamination with regard to PFASs.

During the quantification by LC-MS, sensitivity drift was monitored as an intra-assay control and done by injecting one standard repeatedly during the quantification. In addition, MeOH was injected at least for every fifth sample to monitor carry over. The detection limit for the LC-MS was set to three times the noise level.

2.4 Data analysis

The statistical analysis was performed using SPSS Statistical software (Version 18.0 for Windows, SPSS Inc., Chicago, IL) and SIMCA P12+ (Umetrics, Umeå, Sweden).

The data were tested for normal distribution using the Shapiro-Wilk test ($n \leq 50$). Variables not normally distributed were $\log_{10}$ transformed to obtain normality. Between-year differences of PFASs, THs, capture day, age (only mothers) and biometric variables were tested by Student's t-test and Mann-Whitney U test dependent on whether data was normally distributed or not, respectively. Correlation between capture day, age (only mothers) and biometric variables were tested for both years together using Pearson's correlation test (two-tailed) or Spearman's rank correlation test (two-tailed), depending on whether data was normally distributed or not, respectively. Missing data was replaced by half the limit of detection (LOD) prior to analysis. This counts for PFHpA (LOD: 0.05 ng/g) in one mother from 2008 (Appendix E. Table E.1) and PFTeDA (LOD: 0.025 ng/g) in two cubs from 2008 (Appendix E. Table E.2). The significant level was set to $p \leq 0{,}05$ for all the tests.

2.4.1 Multivariate analysis

Multivariate analysis was performed using SIMCA P12+ (Umetrics, Umeå, Sweden) and intercorrelations between variables were investigated using principle component analysis (PCA). The main goal was to investigate if the plasma TH levels in polar bear mothers and cubs were correlated to capture day, age (only mothers), biometric variables or PFSAs and PFCAs concentrations. R^2 describes the degree of fit in the model (explained variance) and Q^2 describes the predictive ability of the model (predicted variance) (Eriksson et al., 2006) .

In PCA, the data included in the model are orthogonally transformed to convert the original dataset of possible correlated variables into a dataset of uncorrelated variables called principle components (PCs) that explain as much as possible of the variance in the dataset (Eriksson et al., 2006). In datasets where the numerical values for variables vary on a great scale, it is important

to choose scaling to unit variance (UV). Variables will therefore contribute equally to the final model independent on their absolute values (Eriksson et al., 2006). Mean-centering was also performed on the data to increase the interpretability of the model and involved the subtraction of the variables mean to each observation in the dataset (Eriksson et al., 2006). In addition, variables not normal distributed were $\log_{10}$ in order to improve the model.

PCA loading bi plots, which combines the variables loadings (loading plot) with the individuals scores (score plot), were created for cubs and mothers to investigate the intercorrelations between the selected variables PFCAs, PFSAs, THs, capture day, age (only mothers) and biometric variables. Due to high correlation between biometric variables (Appendix B. Table B.1), only body mass was included in the model for mothers and cubs. In the present study, variables with similar or dissimilar loadings ≥ 0.65 to a PC were regarded as positively or negatively correlated, respectively.

2.4.2 General linear model (GLM)

General linear model (GLM, type III sum of squares)(SPSS Statistical software) was used to further investigate the relationship between the different THs and the other variables included in the PCA. This linear regression technique is based on analysis of variance (ANOVA), but is extended to also include covariates (Analysis of covariance (ANCOVA)) in an attempt to explain the dependent variable (Field, 2005). A univariate model was used meaning that only one dependent variable was included in each analysis (Field, 2005).

The different THs were selected as dependent variable, sampling year as fixed factor and the selected covariates were age (only mothers), body mass, capture day, PFCAs and PFSAs. Backward elimination was used meaning that all variables were included at the beginning and then variables not significant were excluded one by one until only significant variables remained (Sokal and Rohlf, 1995).

3. Results

3.1 Biometric variables

Biometric variables, age (only mothers) and capture day for mothers and cubs are presented in table 3.1. In mothers, none of the biometric variables or age were significantly different between the years (Student's t-test, age: p = 0.975; straight length: p = 0.254; axillary girth: p = 0.911; head length: p = 0.239; body mass: p = 0.705). In contrast, all biometric variables were significantly higher in cubs from 2008 compared to 1998 (Student's t-test, straight length: p<0.010; head length: p<0.010; body mass: p=0.010). In addition, the capture day was significantly later in 2008 compared to 1998 (Mann-Whitney U test, capture day: p= 0.017).

Table 3.1. Mean (± SD), median and range (min-max) of capture day, age and biometric variables measured in mother-cub pairs of polar bear sampled in 1998 (Mothers: n=12, cubs: n=12) and 2008 (Mothers: n=9, cubs: n=9) at Svalbard.

	1998			**2008**		
	Mean ± SD	**Median**	**Range (min-max)**	**Mean ± SD**	**Median**	**Range (min-max)**
Mothers						
Capture day* [a]	104 ± 7	105	91 - 113	112 ± 7	115	99 - 121
Age (years)	13 ± 3	12	7 - 19	13 ± 5	12	6 - 22
Straight length (cm)	197 ± 8	194	187 - 211	193 ± 4	191	189 - 201
Axillary girth (cm)	114 ± 10	115	99 - 132	113 ± 10	112	99 - 136
Head length (mm)	346 ± 13	341	330 - 370	340 ± 9	342	325 - 351
Body mass (kg)**	187 ± 38	179	141-264	181 ± 34	180	139 - 260
Cubs						
Straight length (cm)[a]	72 ± 6	74	61 - 81	86 ± 7	85	76 - 95
Head length (mm)[a]	162 ± 8	161	150 - 180	180 ± 13	182	157 - 202
Body mass (kg)[a]	10.2 ± 2.6	10.4	5.3 – 14.3	16.3 ± 5.4	14.8	9.5 – 25.0

* Capture day is the same for cubs as for mothers.

** Body mass for mothers were calculated based on the following formulae: Body mass = 0.00003377 * Axillary girth$^{1.7515}$ * Straight length$^{1.3678}$ (Derocher and Wiig, 2002).

[a] Statistical significant difference between the years (p≤0.05).

3.2 Levels of THs

In mothers, plasma concentration of TT4 and FT4 were significantly higher in 2008 than in 1998, while there was no significant difference with respect to the plasma concentration of TT3 and FT3 (Student's t-test, TT4: $p<0.010$; FT4: $p<0.010$; TT3: $p=0.084$; FT3: $p=0.633$) (Figure 3.1). In cubs, the plasma concentration of TT3 was significantly higher in 2008 than in 1998 (Student's t-test, TT3: $p<0.010$) (Figure 3.1). However, plasma concentrations of TT4, FT4 and FT3 did not differ between the years in cubs (Student's t-test, Log_{10} TT4: $p= 0.381$; FT4: $p=0.991$; FT3: $p=0.242$) (Figure 3.1).

The plasma concentrations of TT4 and FT4 were significantly higher in cubs compared to mothers in 1998 (Student`s t-test, TT4: $p<0.010$; FT4: $p<0.010$). However, there was no significant difference in plasma concentrations of TT3 and FT3 between mothers and cubs sampled in 1998 (Student`s t-test, TT3: $p=0.777$; FT3: $p=0.053$). Cubs from 2008 had significantly higher concentration of all THs compared to their mothers (Student`s t-test, Log_{10} TT4: $p<0.010$; FT4: $p=0.011$; TT3: $p<0.010$; FT3: $p<0.010$).

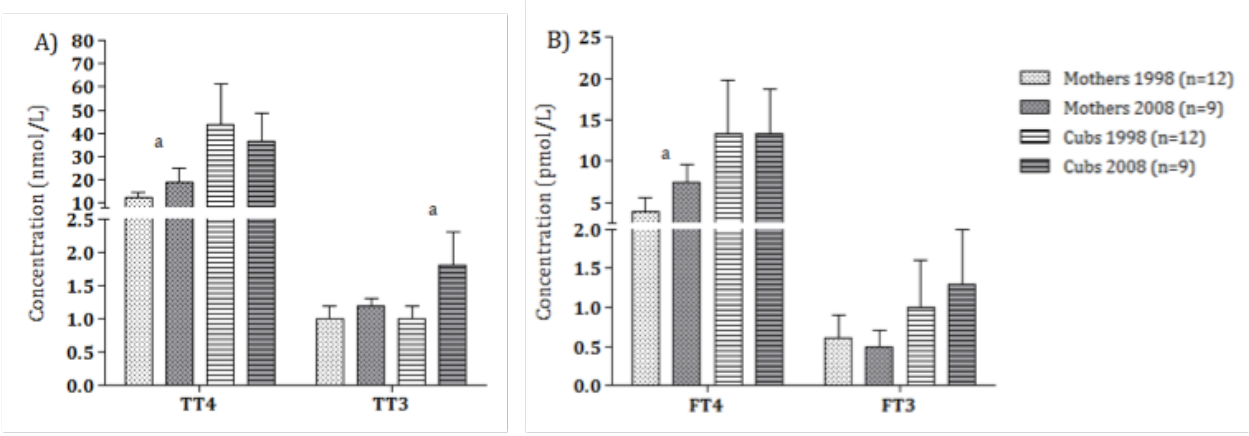

Figure 3.1. Mean (± SD) plasma concentrations of (A) TT4 (nmol/L) and TT3 (nmol/L), (B) FT4 (pmol/L) and FT3 (pmol/L) in mother-cub pairs of polar bear sampled at Svalbard in 1998 (Mothers: n=12, cubs: n=12) and 2008 (Mothers: n= 9, cubs: n=9). a: Significant difference between the years (Student`s t-test. $p\leq 0.05$). The gaps are from (A) 2.5 to 8 nmol/L and (B) 2.0 to 2.5 pmol/L.

3.3 Prevalence and levels of PFASs

Of the 15 PFASs analysed in plasma of polar bear mother-cub pairs sampled in 1998 and 2008, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFHxS and PFOS were quantified in all individuals from both years. PFHpA was detected in 98 % of the individuals and PFTeDA was detected in 95 % of the individuals. PFBS was detected in one mother from 1998 and PFOSA was detected in 31 % of the individuals. The shortest chain PFCAs (PFBA, PFPeA, PFHxA) were not detected in any of the samples. Average plasma concentrations of PFASs in polar bear mother-cub pairs sampled in 1998 and 2008 are presented in table E.1 and E.2 (Appendix E).

The compound measured in the highest concentration in all samples was PFOS. PFOS constituted 65-80 % of sum PFASs (PFCAs + PFSAs) in mothers and cubs (Appendix E. Table E.3). PFNA and PFUnDA were the dominant PFCAs in both mothers and cubs with concentrations from 25-41 % and 27-36 % of total PFCAs, respectively (Appendix E. Table E.3). Generally, mean concentrations of odd-chain PFCAs were higher than even-chain PFCAs with one less carbon (PFNA>PFOA, PFUnDA>PFDA, PFTrDA>PFDoDA) in both mothers (Figure 3.2A) and cubs (Figure 3.2B).

Both in 1998 and 2008, plasma concentration of all compounds were significantly higher in mothers then in cubs (Student's t-test, $p < 0.010$), except for the plasma concentration of PFHpA that did not differ between mothers and cubs in 1998 (Student's t-test, Log_{10} PFHpA: $p=0.865$) and that was significantly higher in cubs than their mothers in 2008 (Student's t-test, Log_{10} PFHpA: $p< 0.010$).

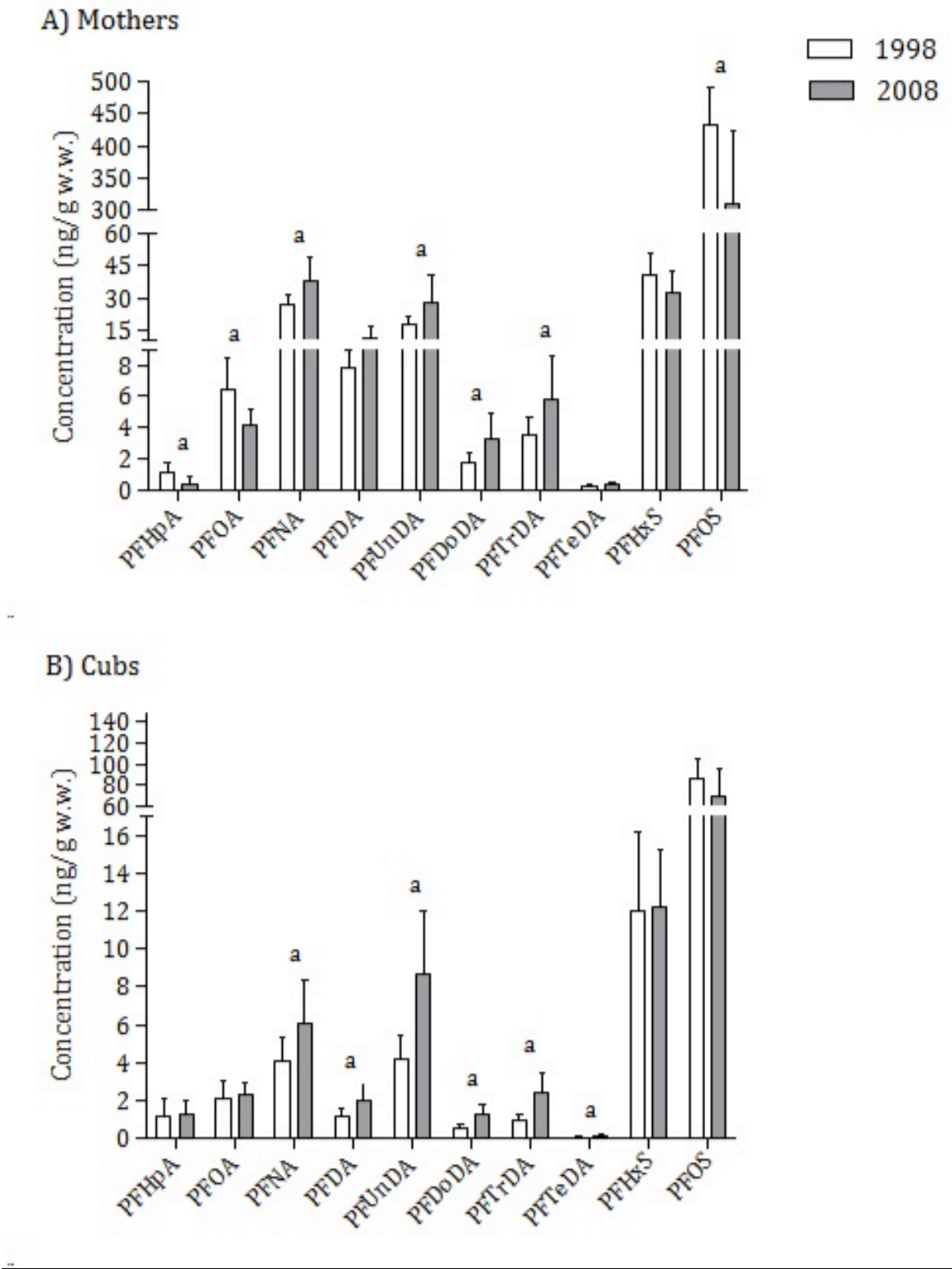

Figure 3.2. Mean (± SD) plasma PFASs concentrations (ng/g w.w.) in polar bear (A) mothers and (B) cubs sampled at Svalbard in 1998 (mothers: n=12, cubs: n=12) and 2008 (mothers: n=9, cubs: n=9). a= statistically significant difference between the years (Student's t-test. p≤ 0.05). Gabs from (A) 9 to 11 ng/g w.w. and 60 to 300 ng/g w.w. and (B) 17 to 60 ng/g w.w. Note the different range of the y-axis in mothers and cubs.

3.3.1 Temporal changes

From 1998 to 2008, the plasma concentrations of PFHpA, PFOA and PFOS showed a significant decrease in mothers (29-64 % decrease) (Student's t-test, $p<0.010$)(Figure 3.2A). In contrast, a significant increase in plasma levels of PFNA, PFUnDA, PFDoDA and PFTrDA were reported from 1998 to 2008 (39-83 % increase) (Student's t-test, PFNA: $p=0.005$; PFUnDA: $p=0.045$; PFDoDA: $p=0.018$; PFTrDA: $p=0.034$)(Figure 3.2A). Plasma concentrations of PFDA, PFTeDA and PFHxS did not differ significantly between the years in mothers (Student's t-test, PFDA: $p=0.056$; PFTeDA: $p=0.327$; PFHxS: $p=0.075$)(Figure 3.2A).

In cubs, the plasma concentration of PFCAs from PFNA to PFTeDA increased (46-180 % increase) from 1998 to 2008 (Student's t-test, PFNA: $p=0.021$; PFDA: $p<0.010$; PFUnDA: $p<0.010$; PFDoDA: $p<0.010$; PFTrDA: $p<0.010$; PFTeDA: $p<0.010$) (Figure 3.2B). Plasma concentration of PFHpA, PFOA, PFHxS and PFOS did not differ significantly between the years in cubs (Student's t-test, Log_{10} PFHpA: $p=0.832$; PFOA: $p=0.667$; Log_{10} PFHxS: $p=0.791$; PFOS: $p=0.070$) (Figure 3.2B).

3.4 Principle component analysis (PCA)

Correlations between contaminants, THs, capture day, age (only mothers) and biometric variables were investigated in mothers and cubs in two different PCA models.

3.4.1 Mothers

The model for mothers resulted in one PC that explained 37 % of the variation in the dataset (R^2X=0.373, Q^2=0.223) and is presented as a loading bi plot (Figure 3.3. Appendix F. Table F.1). The eigenvalue for the PC was 6.34.

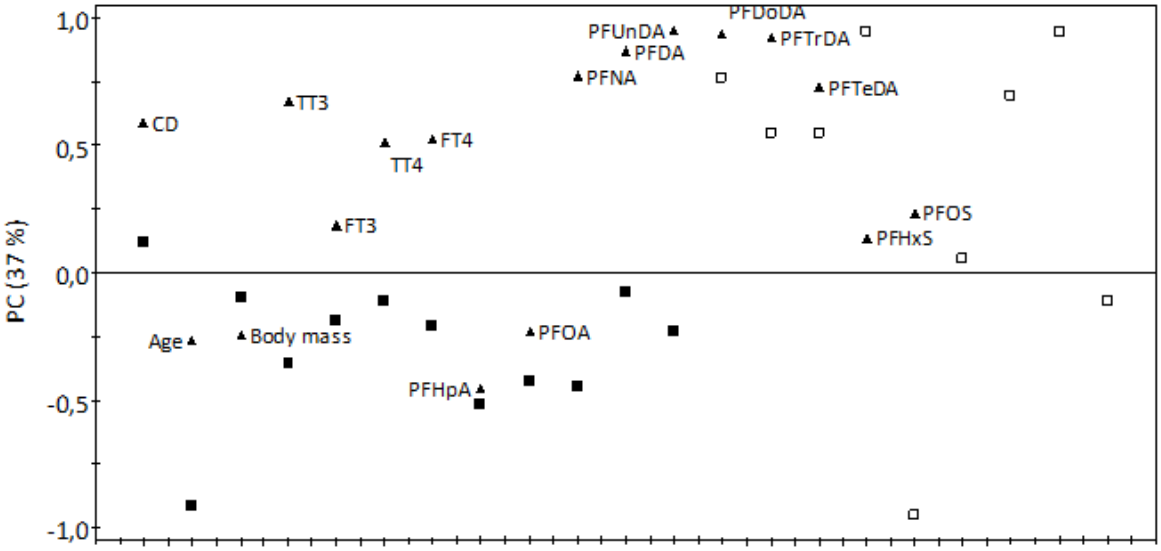

Figure 3.3. PCA Loading bi plot (R^2X= 0.373 Q^2=0.223) for polar bear mothers sampled in 1998 (n=12, filled squares) and 2008 (n=9, open squares) at Svalbard. Variables included in the plot were body mass, age, capture day (CD), PFCAs, PFSAs and THs (filled triangles).

The longest chain PFCAs (PFNA to PFTeDA) were positively correlated, with the strongest correlation between PFUnDA, PFDoDA and PFTrDA and were placed apart from the other compounds (PFHpA, PFOA, PFHxS and PFOS) in the plot. The PCA plot showed a positive correlation between TT3 and the longest chain PFCAs (PFNA to PFTeDA). Age, body mass, capture day, FT3, TT4, FT4, PFHpA, PFOA, PFHxS and PFOS had low loading values and showed a more central location in the plot.

3.4.2 Cubs

The PCA model for cubs resulted in two PCs that together explained 68 % of the variation in the dataset (R^2X=0.675 Q^2=0.44) and is presented as a loading bi plot (Figure 3.4. Appendix F. Table F.2). The eigenvalues of PC1 and PC2 were 6.35 and 4.46, respectively.

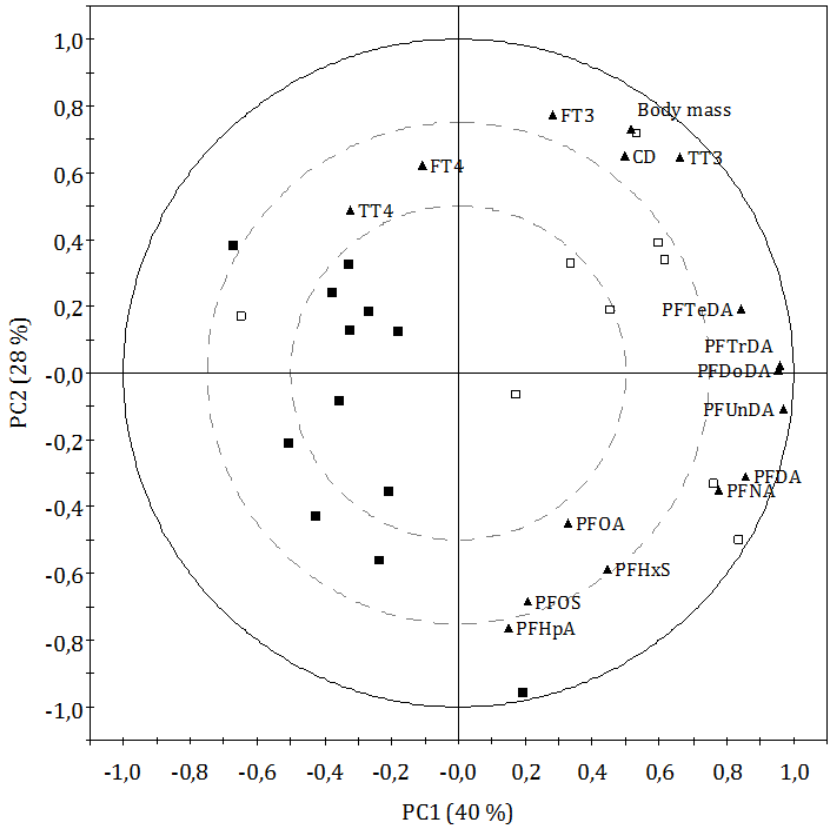

Figure 3.4. PCA Loading bi plot (R^2X= 0.675 Q^2=0.44) for polar bear cubs sampled at Svalbard in 1998 (n=12, filled squares) and 2008 (n=9, open squares). Variables included in the plot were body mass, capture day, PFCAs, PFSAs and THs (filled triangles). PC1 and PC2 explained 40 % and 28 % of the variation, respectively.

The plot in figure 3.4 showed a distinct separation of the individuals from 1998 and 2008, except from one individual from 2008. The longest chain PFCAs (PFNA to PFTeDA) and TT3 showed a positive correlation with respect to PC1, with the strongest correlation between the longest chain PFCAs. TT3 was in contrast to the longest chain PFCAs also explained by PC2. In addition, the PCA plot showed a positive correlation between TT3, FT3, capture day and body mass with regard to PC2. In addition, a positive relationship was observed between PFOS and PFHpA with

regard to PC2. TT4, FT4, PFHxS and PFOA had low loadings and showed a more central location in the plot.

3.5. General linear model (GLM)

Relationships between the same variables as included in the PCA model were investigated further by GLM with TH as dependent variable and capture day, age (only mothers), body mass, PFCAs and PFSAs as predictor variables (Table 3.2).

Table 3.2. Significant predictor variables of THs in polar bear mother-cub pairs from Svalbard sampled in 1998 and 2008. THs not explained by any of the predictor variables are not included in the table (mothers: FT3; cubs: TT4 and FT4).

Dependent variable	**Significant variables**	**ß**	**Std.error**	**F**	***p***	**r^2 (adjusted)**	**Levenes test (p-value)**
Mothers (n=21)							
Log_{10} TT4	Sampling year	-0.155	0.052	8.849	0.009		
	Log_{10} PFHpA	-0.122	0.053	5.371	0.034		
	Age	-0.012	0.005	5.314	0.035		
	Capture day	-0.007	0.003	5.008	0.040	0.554	0.746
FT4	Sampling year	-3.419	0.799	18.303	0.000	0.464	0.275
TT3	Log_{10} PFTrDA	0.754	0.185	16.686	0.001		
	Log_{10} Body mass	1.047	0.498	4.430	0.050	0.435	0.495
Cubs (n=21)							
Log_{10}TT3	Body mass	0.014	0.003	19.000	0.001		
	Log_{10} PFHxS	-0.459	0.133	11.807	0.004		
	Sampling year	-0.101	0.034	8.540	0.011		
	Log_{10} PFDoDA	0.162	0.068	5.681	0.031		
	PFOA	0.044	0.018	5.568	0.032	0.883	0.586
FT3	Capture day	0.064	0.016	16.097	0.001		
	PFOA	0.433	0.187	5.377	0.033		
	Log_{10} PFNA	-1.771	0.822	4.637	0.046	0.404	0.432

3.5.1 Mothers

In mothers, plasma concentration of TT3 was explained by PFTrDA and body mass, of which PFTrDA was the major predictor (Table 3.2). However, a linear relationship was only observed between TT3 and PFTrDA when examining TT3 against the individual predictors (Figure 3.5A and figure 3.5B). The plasma concentration of TT4 was explained by sampling year, PFHpA, age and capture day with sampling year being the major predictor (Table 3.2). TT4 and PFHpA showed a linear relationship when examining TT4 against the individual predictors (Figure 3.6A). However, lack of linear relationship was observed between TT4 and age (Figure 3.6B) and TT4 and capture day (Figure 3.6C). Sampling year was the only significant predictor variable explaining the FT4 concentration in mothers (Table 3.2). None of the predictor variables explained the plasma concentration of FT3 in mothers.

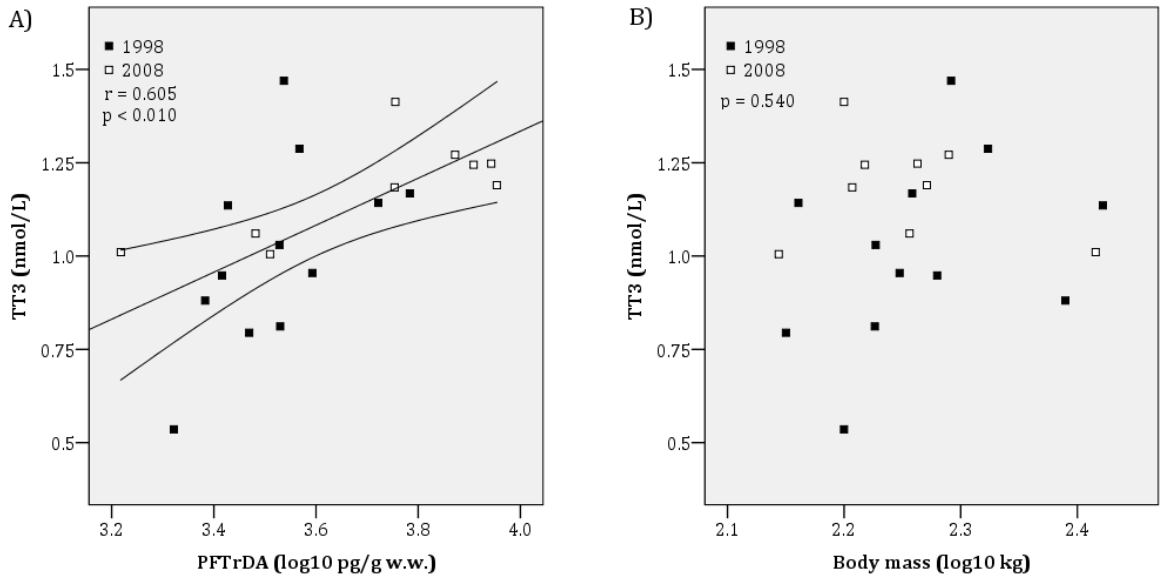

Figure 3.5. Relationship between TT3 (nmol/L) and A) PFTrDA (log_{10} pg/g w.w.) and B) body mass (log_{10} kg) in polar bear mothers from Svalbard sampled in 1998 (n= 12, filled squares) and 2008 (n=9, open squares).

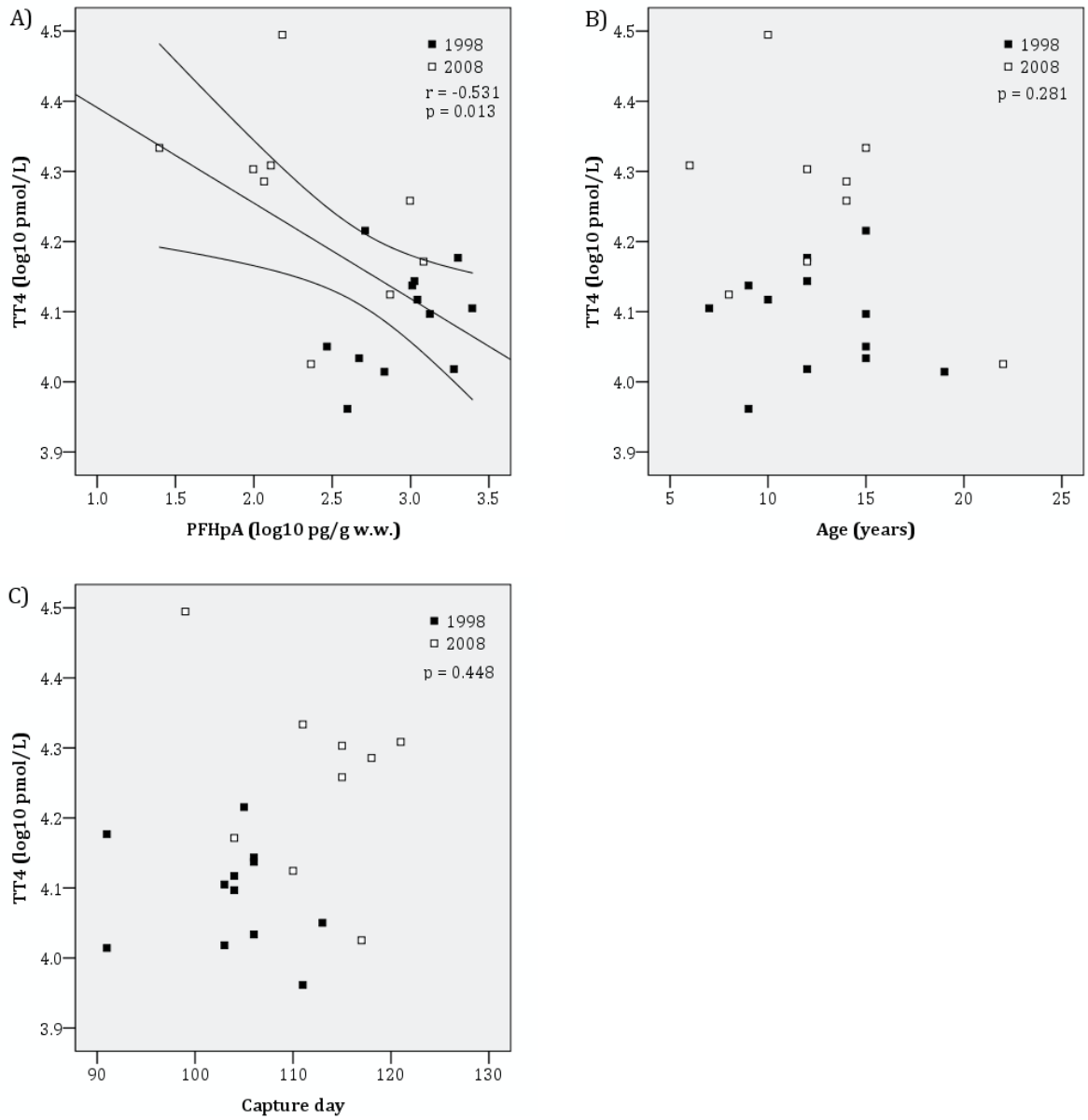

Figure 3.6. Relationship between TT4 (log_{10} pmol/L) and A) PFHpA (log_{10} pg/g w.w.), B) age (years) and C) capture day in polar bear mothers from Svalbard in 1998 (n= 12, filled squares) and 2008 (n=9, open squares).

3.5.2. Cubs

In cubs, the plasma concentration of TT3 was explained by body mass, PFHxS, sampling year, PFDoDA and PFOA, with body mass being the major predictor (Table 3.2). This is in accordance with the position of TT3 and body mass in the loading bi plot (Figure 3.4.). A linear relationship was observed between TT3 and body mass (Figure 3.7A) and between TT3 and PFDoDA (Figure 3.7B), when TT3 was examined against the individual predictors. However, a linear relationship was not observed between TT3 and PFHxS (Figure 3.7C) and between TT3 and PFOA (Figure

3.7D). FT3 concentration in the plasma was explained by capture day, PFOA and PFNA (Table 3.2). However, a linear relationship was only observed between FT3 and capture day (Figure 3.8A) and not for FT3 and PFNA (Figure 3.8B) and FT3 and PFOA (Figure 3.8C), when FT3 was examined against the individual predictors. The plasma concentration of TT4 and FT4 was not explained by any of the predictor variables (Table 3.2) and is in accordance with the low loadings of these two hormones in the loading bi plot (Figure 3.4. Appendix F. Table F.2.).

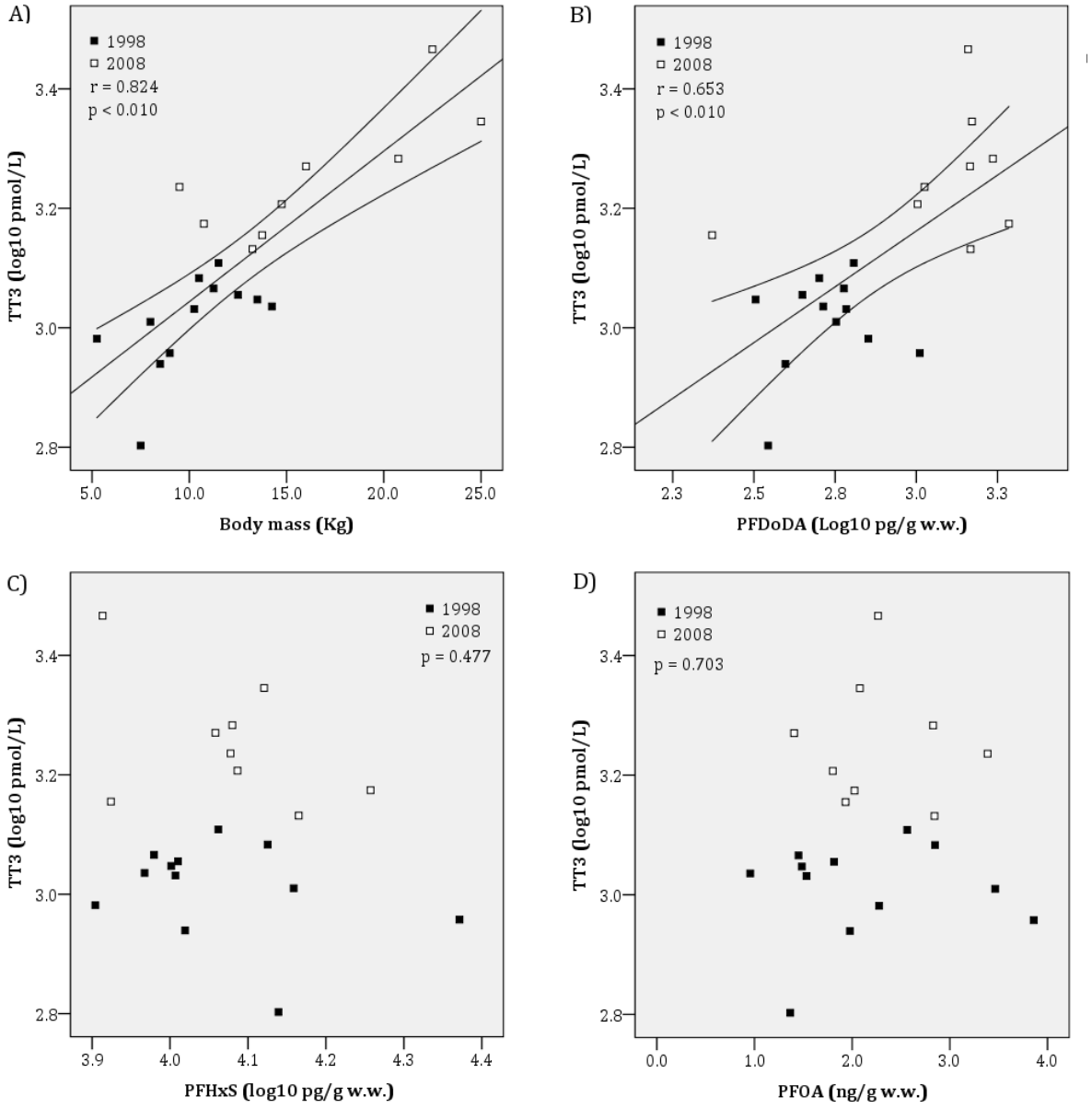

Figure 3.7. Relationship between TT3 (log_{10} pmol/L) and A) body mass (kg), B) PFDoDA (log_{10} pg/g w.w.), C) PFHxS (log_{10} pg/g w.w.) and D) PFOA (ng/g w.w.) in polar bear cubs from Svalbard sampled in 1998 (n=12, filled squares) and 2008 (n=9, open squares).

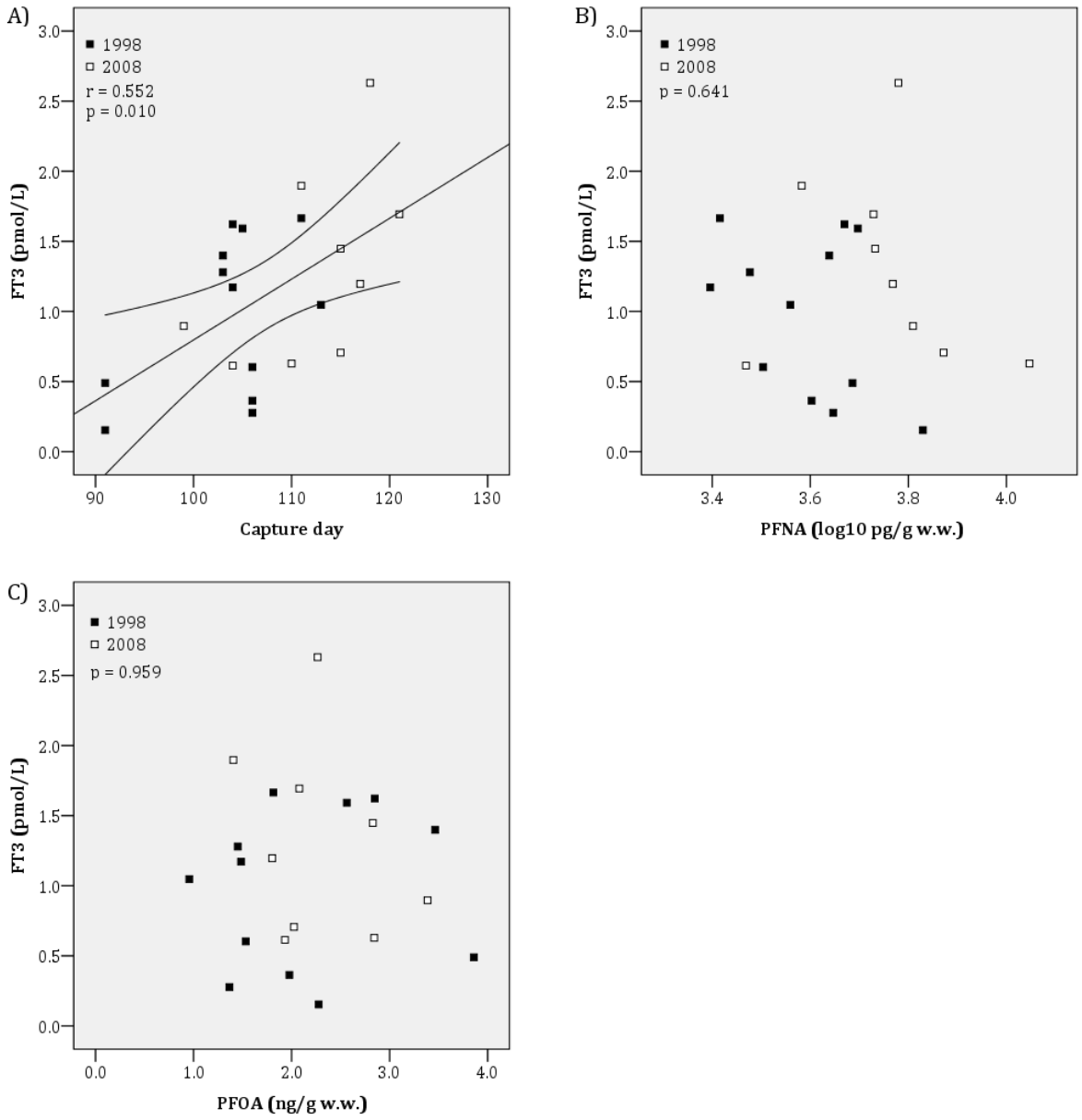

Figure 3.8. Relationship between FT3 (pmol/L) and A) capture day, B) PFNA (log_{10} pg/g w.w.) and C) PFOA (ng/g w.w.) in polar bear cubs from Svalbard sampled in 1998 (n=12, filled squares) and 2008 (n=9, open squares).

4. Discussion

4.1 Levels of THs

Few studies have examined the plasma levels of THs in polar bears and the majority of these studies have focused on endocrine disruption and reported associations between levels of THs and POPs (Skaare et al., 2001, Braathen et al., 2004). In the present study, the plasma concentration of TT3 and FT3 in mothers (Figure 3.1) is in accordance with previously reported hormone concentrations in female polar bears with approximately 4 months old cubs sampled at Svalbard in 1997 and 1998 (mean ± SD: TT3: 1.26 ± 0.26 nmol/L; FT3: 1.26 ± 0.75 pmol/L) (Braathen et al., 2004). However, well-established baseline levels for THs are lacking in polar bears. In addition, the TT4 and FT4 levels in the present study were significantly higher in mothers sampled in 2008 compared to mothers sampled in 1998 (Figure 3.1). The plasma TT4 and FT4 levels in mothers sampled in 1998 corresponded better then the mothers sampled in 2008 with the levels in mothers with cubs (approximately four months old) in Braathen et al. (2004) (mean ± SD: TT4: 15.3 ± 5.18 nmol/L; FT4: 4.38 ± 1.77 pmol/L). Another study on denning polar bears sampled at Svalbard from 1991 to 1994 reported plasma TT4 concentration (median: 21 nmol/L) in the same range as the mothers sampled in 2008 in the present study (Figure 3.1) and plasma TT3 concentration (median: 0.8 nmol/L) in the same range as mothers from both years in the present study (Figure 3.1) (Skaare et al., 2001). However, the same study by Skaare et al. (2001) reported higher levels of free THs (median: FT4: 28 pmol/L; FT3: 9.7 pmol/L) than the present study.

To my knowledge, only one study has investigated the levels of THs in polar bear cubs and this study was on cubs from the Canadian Arctic and the Norwegian Arctic in 1997 and 1998, respectively (Sandau, 2000). This particular study reported lower concentrations of TT4 and TT3 in both the Canadian Arctic (mean ± SD: TT4: 8.00 ± 2.75 nmol/L; TT3: 0.21 ± 0.21 nmol/L) and the Norwegian Arctic (mean ± SD: TT4: 1.15 ± 1.70 nmol/L; TT3: 0.23 ± 0.13 nmol/L) (Sandau, 2000) compared to the present study (Figure 3.1). However, the values reported by Sandau (2000) is somewhat uncertain due to the low number of cubs examined. In the present study, the concentration of THs in cubs were higher than in their mothers (except for TT3 in 1998) (Figure 3.1). The same pattern was only reported in cubs from the Canadian Arctic in the study by Sandau (2000), but have been reported in several other species such as grey seals (*Halichoerus grypus*) (Woldstad and Jenssen, 1999), hooded seals (*Cystophora cristata*)

(Gabrielsen, 2010) and humans (Corcoran et al., 1977). The higher TH levels in younger mammals is thought to be related to these hormones important physiological functions in metabolism, heat production, central nervous system, growth and development (Sand et al., 2001) .

4.2 Prevalence and levels of PFCAs and PFSAs

During the last decade several studies have investigated PFCAs and PFSAs in several trophic levels in the Arctic biota and a widespread distribution of these compounds have been reported (Giesy and Kannan, 2001, Smithwick et al., 2005a). Even though most of these compounds have been measured in adult polar bears, no earlier studies have to my knowledge measured PFCAs and PFSAs in polar bear cubs. Most previous polar bear studies measuring PFASs have analysed the concentrations of these compounds in liver tissue (Martin et al., 2003c, Smithwick et al., 2005b, Dietz et al., 2008) and not in plasma as in the present study. To my knowledge, only one previous study have measured blood concentrations of PFASs in polar bears (Bentzen et al., 2008). Due to the lack of a reliable liver-to-blood conversion factor for these compounds in polar bears, a direct comparison of levels between the present study (plasma) and previous studies (liver) is not appropriate.

4.2.1 Levels of PFCAs and PFSAs

Despite that absolute levels are not optimal to compare, due to analysis done in different tissues in different studies (plasma in the present study, liver in the previous studies), it may still be informative to compare the PFASs patterns between these studies. In addition, some levels can be compared to the study by Bentzen et al. (2008). PFOS was the dominant fluorinated compound in both mothers and cubs (Figure 3.2), and this is in accordance with earlier studies on polar bears (Smithwick et al., 2005a, Bentzen et al., 2008). Bentzen et al (2008) reported lower concentrations of PFOS in whole blood in Southern Beaufort Bay polar bears (mean ± SD: 60 ± 53.4 ng/g) than measured in the present study. In addition, the same particular study reported lower concentration of PFNA (mean ± SD: 8 ± 5.1 ng/g w.w.), PFDA (mean ± SD: 2 ± 2.1 ng/g w.w.) and PFUnDA (mean ± SD: 11 ± 39 ng/g w.w.) then reported herein. This is in accordance with earlier studies that have reported a higher contamination load of PCBs in polar bears from Svalbard (Bernhoft et al., 1997) compared to the Canadian and North American Arctic (Letcher et al., 1995).

After the phase-out of the POSF ($C_8F_{17}SO_2F$) chemistry by the 3M Company, PFOS area of application was replaced with shorter chain compounds by utilizing PBSF ($C_4F_9SO_2F$) (Olsen et al., 2009, 3M, 2011). PFBS have been shown to be a by-product of this new chemistry (3M, 2011). To my knowledge, no previous studies have analysed PFBS in polar bears (Martin et al., 2003c, Smithwick et al., 2005a, Smithwick et al., 2006, Bentzen et al., 2008, Dietz et al., 2008). The suggested non-bioaccumulative nature of PFBS (NICNAS, 2005) is in accordance with the findings in the present study which detected a low concentration of PFBS in only one mother (0.133 ng/g w.w.). That PFBS does not seem to bioaccumulate is supported by the marked higher elimination rate of PFBS in humans compared to PFHxS and PFOS (Olsen et al., 2007, Olsen et al., 2009) and that several studies have reported levels of PFBS to be non-detectible in Arctic species such as ringed seals from the Canadian Arctic (Butt et al., 2006) and Brünnich's guillemot (*Uria lomvia*) eggs from the Norwegian Arctic (Miljeteig and Gabrielsen, 2010). Furthermore, and maybe an even stronger indication on the theory that PFBS do not bioaccumulate is the low or non-detectible levels of PFBS in industrial regions in species such as Common Guillemot (*Uria aalge*) (Holmstrøm and Berger, 2008) and humans (Olsen et al., 2008). It should be noted that methyl perfluorobutane sulfonamide (MeFBSA) have been detected in the Svalbard atmosphere (Dreyer et al., 2009) and this compound may serve as a source for the shortest chain PFCAs and PFSAs such as PFBS (D'Eon et al., 2006). It is difficult to conclude on whether the non-detection of PFBS in the present study is due to lack of transportation to the Arctic or the lack of bioaccumulation in polar bears. Therefore, further research is needed to evaluate the fate of PFBS in the environment.

In addition to PFBS, the shortest chain PFCAs (PFBA, PFPeA and PFHxA) were not detected in any of the polar bear samples either. There are few reports including these compounds in their set of PFASs analysed and none of the previous studies in polar bears have analysed these compounds (Martin et al., 2003c, Smithwick et al., 2005a, Smithwick et al., 2006, Bentzen et al., 2008, Dietz et al., 2008). However, in a study on Brünnich's guillemot eggs from Svalbard, PFHxA was analysed but not found in detectible concentrations (Miljeteig and Gabrielsen, 2010). Further research is needed on the shortest chain PFCAs in the Arctic regions to confirm their assumed absence or minor presence with a higher certainty.

In contrast to the high levels of PFOS, PFOSA was only detected in 31 % of the individuals and in these cases in very low concentrations (Appendix E. Table E.1. Table E.2). The high concentration difference between PFOS and PFOSA in polar bears in this study and in previous studies (Martin et al., 2003c, Bentzen et al., 2008, Dietz et al., 2008), in addition to the polar bears high biotransformation capacity when it comes to PCBs and pesticides (Letcher et al.,

2000), may suggest that polar bears also have the ability to biotransform PFOSA to PFOS. Biotransformation of PFOSA to PFOS have been reported in rat liver slices (Xu et al., 2004). However, to my knowledge, the polar bears ability to biotransform PFASs has not yet been studied. In addition, low concentrations of PFOSA have been reported in other species in the Arctic biota like ringed seals (Butt et al., 2006) and Brünnich's guillemots eggs (Miljeteig and Gabrielsen, 2010), and PFOSA was not detected in the Svalbard atmosphere (Dreyer et al., 2009), indicating low exposure of Arctic wildlife in regard to PFOSA. However, the reason for the low detection of PFOSA in the present study is unknown, but may be due to low exposure or high biotransformation in polar bears. Further studies are needed to explain the low levels in polar bears observed in the present study.

The high levels of PFNA and PFUnDA compared to the other PFCAs, and the characteristic pattern of higher concentrations of odd-chain PFCAs than even-chain PFCAs with one less carbon (Figure 3.2), are in accordance with earlier studies on polar bears (Kannan et al., 2005, Bentzen et al., 2008) and seals (Butt et al., 2008). It has been suggested that this pattern could indicate that the volatile 8:2 FTOHs, which may degrade to PFCAs (Ellis et al., 2004), can be the major source of PFCAs in the Arctic biota (Martin et al., 2003c). This is further supported by the detection of 8:2 FTOHs and 10:2 FTOHs in the Canadian Arctic (Shoeib et al., 2006). The degradation of 8:2 FTOHs have been reported to give equal quantities of PFOA and PFNA (Wallington et al., 2005). The combined effect of this degradation pattern and the increased bioaccumulation potential with increased carbon length observed in fish (Martin et al., 2003a, Martin et al., 2003b), may explain the higher levels of PFNA compared to PFOA. Higher levels of PFUnDA compared to PFDA would be anticipated by the degradation of 10:2 FTOHs (Butt et al., 2010). Thus, the presence of the PFCAs in the Arctic environment may be due to the long-range transport of the volatile FTOHs.

The significantly higher concentrations of all PFCAs and PFSAs, except for PFHpA, in mothers than in cubs indicates a less efficient transfer of these compounds from mother to offspring compared to lipophilic compounds like PCBs. Higher concentration of PCBs have been reported in polar bear cubs compared to females with cubs in the Canadian Arctic (Polischuk et al., 1995, Sandau, 2000). In contrast, plasma levels of hydroxylated polychlorinated biphenyls (OH-PCBs) have been reported to be higher or not differ in concentrations when comparing concentrations in mothers to their cubs (Bertinussen, 2009). The lipophilic PCBs have been measured in high concentrations in polar bear milk (Polischuk et al., 1995, Bernhoft et al., 1997) and maternal milk is thought to be the main route of transfer for these compounds. However, PFASs are associated with proteins (Luebker et al., 2002, Jones et al., 2003, Bischel et al., 2010) and not

lipids, and therefore more similar to OH-PCBs than PCBs. Studies on humans have shown higher concentration of PFCAs and PFSAs in cord blood and maternal blood than in mothers milk (Needham et al., 2010), indicating that placenta can be the major contamination pathway. In addition, polar bears lipid-rich mothers milk will probably not facilitate the transfer of PFCAs and PFSAs, as is the case for lipophilic POPs, due to the hydrophilic and protein binding nature of the compounds. The lack of correlation between capture day and body mass with contamination load in the polar bear cubs (Figure 3.4) may also indicate that the major transfer of PFCAs and PFSAs from mother to offspring occur through placenta and not by ingestion of mothers milk.

4.2.2 Temporal changes of PFCAs and PFSAs

Increased focus on levels and temporal trends of PFASs, and especially PFOS, have been seen after the worldwide detection of PFOS in biota in 2001 (Giesy and Kannan, 2001) and the ended production of PFOS by the 3M Company in 2002. The PFOS concentration in the present study showed decreasing levels in mothers from 1998 to 2008, while the levels were equal between the years in cubs (Figure 3.2). The decrease observed in the present study is in accordance with studies in Brünnich`s guillemot eggs from the Norwegian Arctic (Miljeteig and Gabrielsen, 2010), ringed seals from the Canadian Arctic (Butt et al., 2006) and northern sea otters from South-Central Alaska (Hart et al., 2009). In addition, a decrease has been reported in industrial regions (Ahrens et al., 2009, Sundstrøm et al., 2011), thus supporting that the levels are declining in humans and wildlife after the phase-out. Furthermore, a continued decrease may be expected in the years to come due to the incorporation of PFOS in the Stockholm Convention (Stockholm Convention, 2009). In contrast, continuous emission from industry in China, which still is using PFOS (Butt et al., 2010), could potentially slow down the expected decrease in nature. Furthermore, there are pronounced differences between species in elimination half-life of PFOS (Seacat et al., 2002, Olsen et al., 2007). The half-life reported in monkeys is 200 days (Seacat et al., 2002), whereas in humans it is 4.6 years (Olsen et al., 2007). Thus, it is difficult to conclude on the elimination time in polar bears, and it is difficult to predict the future rate of decrease. However, the decrease seen in the present study may be due to the voluntarily phase-out of PFOS by the 3M Company in 2000.

From 1998 to 2008, the plasma levels of PFHpA and PFOA showed a significant decrease in polar bear mothers (Figure 3.2A). However, the concentrations in cubs did not differ between the years (Figure 3.2B), and the different temporal change observed for PFOA and PFHpA in cubs compared to their mothers may indicate different behaviour of these compounds in cubs

compared to their mothers. The comparison to other Arctic species is complicated due to the relatively few number of temporal trend studies on PFHpA and PFOA in the Arctic wildlife and the few studies existing have either not detected PFOA (Bossi et al., 2005, Hart et al., 2009) or the last year of sampling have been some years before the last sampling in the present study (Smithwick et al., 2006, Dietz et al., 2008). However, some previous reports from temperate regions are in accordance with the decrease observed in mothers in the present study. Concentrations of PFOA in harbor seals (*Phoca vitulina*) from the German Bight decreased from 1998 to 2008 (Ahrens et al., 2009), and after increasing from 1972 to 2000, the levels of PFOA in human milk from Swedish women were reported to decrease from 2001 to 2008 (Sundstrøm et al., 2011). The use of PFHpA and PFOA is not regulated, but in 2006 the United States Environmental Protection Agency (US EPA) invited the eight largest producers of fluoropolymers and telomers to join the PFOA Stewardship program with the goal to reduce the content of PFOA and related chemicals in emission and products by 95 % within 2010 and 100 % within 2015 (US EPA, 2006). The initiation of this program may not entirely explain the decreased levels of PFHpA and PFOA due to the short time interval from the initiation of the Stewardship program to the sampling in 2008 and the long PFOA elimination time (3,4 years) observed in humans (Olsen et al., 2007). However, pronounced species differences in elimination half-life have been reported for PFOA in rodents (Hundley et al., 2006), monkeys (Butenhoff et al., 2002) and humans (Olsen et al., 2007), and the exact elimination rate in polar bears is highly unknown. Another and more likely explanation for the observed decrease of PFHpA and PFOA in polar bear mothers may be the phase-out of the POSF chemistry. The electrochemical fluorination (ECF) process used by the 3M Company produced impure mixtures with contamination such as PFHpA and PFOA (Arsenault et al., 2008). The decrease observed in the present study may indicate that the major source of PFHpA and PFOA in the Norwegian Arctic are products from the ECF process used by 3M Company. Thus, the observed decline in levels in polar bear mothers in this present study may be due to a combined effect of the phase-out by the 3M Company and the initiation of the Stewardship program.

Unlike PFHpA and PFOA, the plasma levels of the majority of the PFCAs with the longest chains increased from 1998 to 2008 in both mothers (PFNA, PFUnDA, PFDoDA, PFTrDA)(Figure 3.2A) and cubs (PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA) (Figure 3.2B). This is in accordance with studies done between 1984 and 2006 on polar bears from Greenland (Dietz et al., 2008) and between 1972 and 2002 in the North American Arctic (Smithwick et al., 2006). Another study conducted on Greenland reported increasing levels of PFDA and PFUnDA in ringed seals until 2003 (Bossi et al., 2005). Furthermore, the concentration of PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA increased from 2003 to 2007 in the Norwegian Arctic in Brünnich's

guillemot eggs (Miljeteig and Gabrielsen, 2010). The opposite findings regarding temporal changes for the longest chain PFCAs (PFNA to PFTeDA) (increased) compared to PFHpA and PFOA (decreased) may indicate different sources to the Arctic region. To my knowledge, high quantities of the longest chain PFCAs are still being produced by the industry. Since no regulations are apparent for the longest chain PFCAs (Jensen and Leffers, 2008), the increasing levels observed in the present study may indicate a possible increased production rate in the industry. In addition, the observed increase may also be due to increased release from deposits or changes in the transport of PFCAs or PFCA precursor to the Arctic regions.

4.3 Effects of PFASs on thyroid hormone levels

The present study reported a positive relationship between TT3 and the longest chain PFCAs (PFNA to PFTeDA) in both mothers (Figure 3.3) and cubs (Figure 3.4). Several predictors included in the GLM explained the TT3 plasma concentration in mothers and cubs, however, only PFTrDA (mothers) (Figure 3.5), and body mass and PFDoDA (cubs)(Figure 3.7) showed a linear relationship with TT3 when examining TT3 against the individual predictors.

Little information is available for the longest chain PFCAs and their possible effects on the TH homeostasis. However, the positive correlation between TT3 and longest chain PFCAs observed in polar bear mothers (Figure 3.3. Table 3.2. Figure 3.5A) and cubs (Figure 3.4. Table 3.2. Figure 3.7B) is consistent with a recently published two-generation exposure study on zebrafish (*Danio rerio*) (Liu et al., 2011). The study by Liu et al. (2011) reported histopatological changes in the thyroid gland of F0 adults and significantly increased T3 levels in both F0 and F1 adults after exposure of both generations to PFNA for 180 days. The particular study assumed that TH transport and glucuronide conjugation elimination were the key targets for the TH-disrupting effects due to the increased and decreased expression of TTR and UGTs in the liver, respectively. UGTs are liver enzymes that increase the elimination of THs through the bile (Zoeller et al., 2007) and a down-regulation of these enzymes may possible increase the levels of THs in the blood. Neither TTR nor UGTs were measured in the present study. However, in the awareness of between-species differences, an explanation equal to the one suggested to take place in PFCA-exposed zebrafish may explain or partly explain the reported positive correlation between TT3 and the longest chain PFCAs observed in the present study.

T4 is both the major excreted TH thyroid hormone from the thyroid gland and the major circulating TH in blood (McNabb, 1992). The majority of T3 present in the organism is produced by deiodination by deiodinases in peripheral tissue (Kogai and Brent, 2005). Upregulation of the

deiodinases may explain the positive correlation observed between TT3 and the longest chain PFCAs in the present study. An upregulation of type II deiodinase (D2) mRNA was observed in PFNA exposed primary cultures of chicken (*Gallus domesticus*) embryonic neuronal cells (Vongphachan et al., 2011). The direct influence on circulating TH levels is unknown in that particular study due to the nature of *in vitro* experiments. However, the physiological role of D2 is T3 production in tissues like brain, pituitary, muscle and brown tissue, and an induction of D2 would increase the T3 levels in the affected tissue (Kogai and Brent, 2005). In the awareness of between-species and between-tissue differences, the same mechanism or effects on other deiodinase enzymes influencing circulating levels of T3 such as D1, may possible be reason for the increase in TT3 seen in the present study. D1 enzymes in liver, kidneys and thyroid gland is the major producer of T3 in peripheral tissue (Kogai and Brent, 2005), and in combination with the high concentrations of PFASs reported in liver and kidneys (Ahrens et al., 2009), this could potentially explain the positive relationship between PFCAs and plasma levels of TT3 in polar bear mothers and cubs.

A positive relationship between TT3 and body mass was observed in polar bear cubs in the present study (Figure 3.7A). This is in accordance with the higher TT3 levels (Figure 3.1), later sampling and consequently larger cubs in 2008 compared to 1998 (Table 3.1). In addition, polar bear cubs from 2008 had higher levels of the longest chain PFCAs (PFNA to PFTeDA) compared to cubs from 1998 (Figure 3.2). The positive relationship observed between the longest chain PFCAs and TT3 in the polar bear cubs could therefore be related to both later sampling and higher body mass or the higher levels of the longest chain PFCAs (PFNA to PFTeDA) observed in cubs from 2008.

Polar bears are exposed to a large number of environmental contaminants, and the positive correlation between the longest chain PFCAs and TT3 may be caused by other contaminants not included in the present study. At Svalbard, the levels of PCBs and OH-PCBs have decreased in polar bears (Bertinussen, 2009, Henriksen et al., 2001), and previous studies have reported that these compounds possibly may affect the TH homeostasis in this subpopulation of polar bears (Braathen et al., 2004, Bertinussen, 2009). Thus, the increased levels of THs and the positive correlation observed between TT3 and PFCAs could simply reflect the better health state of the bears in regards to THs in 2008 than in 1998, and not a direct cause of the increasing trend of the longest chain PFCAs in polar bears.

The plasma concentration of TT4 in polar bear mothers was explained by sampling year, PFHpA, age and capture day (Table 3.2). The association between sampling year and capture day with

plasma TT4 concentration is in accordance with the significantly higher TT4 concentration in the polar bears mothers (Figure 3.1) and the later sampling done in 2008 (Table 3.1). The negative relationship between PFHpA and TT4 (Figure 3.6A) is in contrast to the positive relationship reported for the longest chain PFCAs and TT3. To my knowledge, no studies have reported similar negative relationship between TT4 and PFHpA. However, a negative relationship has been reported between PFOS and THs (TT4 and TT3) in adult and newborn PFOS-exposed Sprague-Dawley rats (Luebker et al., 2005, Chang et al., 2008). In addition, Chang et al. (2008) observed increased liver UGT1A mRNA transcription in PFOS-exposed Sprague-Dawley rats, suggesting that the negative relationship between THs and PFOS may be due to increased elimination of THs. Despite the temporal decrease in levels of PFOS and temporal increase in levels of TT3 in cubs from 1998 to 2008, no relationship were reported between PFOS and TT3 in cubs examined in the present study. However, a negative relationship was observed between TT4 and PFHpA in mothers in the present study (Figure 3.6A). Even though no final conclusion can be drawn in relation to the mechanism behind this finding, it can be that an increased elimination may be the explanation as reported for the PFOS exposed rats (Chang et al., 2008). However, it should be noted that the levels of PFHpA were much lower than PFOS in the present study, and the exact reason for the observed relationship between TT4 and PFHpA, and not between TT4 and PFOS is difficult to conclude on. It should also be mentioned that the PFHpA was measured using PFOA as an I.S. which may cause some uncertainty in the final concentrations of PFHpA reported in the polar bears. Thus, further research is needed to elucidate the reported negative relationship between PFHpA and TT4 observed in the present study.

5. Conclusion

The present study is the first study examining the levels of PFASs in mother-cub pairs of polar bear. From 1998 to 2008, the plasma levels of PFHpA, PFOA and PFOS decreased in polar bear mothers, while the same compounds did not differ over time in cubs. The decreasing levels in mothers, particularly of PFOS, may indicate that levels in biota are declining due to the phase-out of the POSF chemistry by the 3M Company. In contrast, increasing plasma levels of the longest chain PFCAs in polar bears from Svalbard indicate another major source of these compounds to the Arctic environment and may indicate an increased use, release from deposits, or changes in the transport of PFCA or PFCA precursor to the Arctic.

The positive relationship between TT3 and the longest chain PFCAs reported in the present study, may indicate a possible interaction between these PFCAs and the TH homeostasis. However, few studies have investigated the toxicology of the longest chain PFCAs and no final conclusion can be drawn in relation to the mechanism behind this finding. The increasing levels of the longest chain PFCAs combined with the potential effects these compounds may have on the TH system emphasise the need for further research in the years to come.

6. References

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Appendix A: Sampling information

Table A.1. Age, sex, capture day, sampling year and location (latitude and longitude) in mother-cub pairs of polar bear from Svalbard sampled in 1998 and 2008.

ID	Age	Sex	Capture day	Sampling year	Latitude	Longitude
23190	12	F	91	1998	77.88	24.35
23193	19	F	91	1998	77.95	23.22
23226	12	F	103	1998	76.57	25.16
23229	7	F	103	1998	76.70	25.45
23242	15	F	104	1998	77.55	23.75
23246	10	F	104	1998	76.69	25.44
23252	15	F	105	1998	77.40	23.11
23256	15	F	106	1998	76.67	25.36
23265	12	F	106	1998	77.46	23.23
23271	9	F	106	1998	77.95	22.47
23288	9	F	111	1998	78.15	22.44
23294	15	F	113	1998	76.46	24.88
23343	6	F	121	2008	77.16	17.40
23634	14	F	118	2008	77.40	16.23
23703	15	F	111	2008	78.58	21.00
23781	8	F	110	2008	77.05	16.99
23909	12	F	104	2008	79.33	13.93
23924	10	F	99	2008	79.49	17.96
7757/23962	12	F	115	2008	77.34	17.77
23966	14	F	115	2008	77.75	18.48
7803	22	F	117	2008	77.25	17.76
23191	0	F	91	1998	77.88	24.35
23194	0	F	91	1998	77.95	23.22
23227	0	F	103	1998	76.57	25.16
23230	0	F	103	1998	76.70	25.45
23243	0	F	104	1998	77.55	23.75
23248	0	F	104	1998	76.69	25.44
23253	0	F	105	1998	77.40	23.11
23258	0	F	106	1998	76.67	25.36
23268	0	F	106	1998	77.46	23.23
23273	0	F	106	1998	77.95	22.47
23289	0	F	111	1998	78.15	22.44
23295	0	F	113	1998	76.46	24.88
23926	0	M	99	2008	79.49	17.96
23940	0	F	104	2008	79.33	13.93
23951	0	F	110	2008	77.05	16.99
23952	0	F	111	2008	78.58	21.00
23964	0	F	115	2008	77.34	17.77
23967	0	M	115	2008	77.75	18.48
23975	0	F	117	2008	77.25	17.76
23981	0	F	118	2008	77.40	16.23
23983	0	M	121	2008	77.16	17.4

Appendix B: Correlations between variables

Table B.1. Correlation between biometric variables, capture day and age (mothers only) in polar bear mothers and cubs sampled at Svalbard in 1998 (mothers: n=12, cubs: n=12) and 2008 (mothers: n=9, cubs= n=9).

Correlated variables		Correlation coeffisient	p-value	Test
Mothers				
Axillary girth	Log_{10} Body mass	0.986	<0.010	Pearson
Straight length	Head length	0.892	<0.010	Spearman`s rank
Head length	Log_{10} Body mass	0.811	<0.010	Pearson
Axillary girth	Head length	0.717	<0.010	Pearson
Straight length	Log_{10} Body mass	0.720	<0.010	Spearman`s rank
Straight length	Axillary girth	0.593	<0.010	Spearman`s rank
Straight length	Age	0.131	0.571	Spearman`s rank
Capture day	Log_{10} Body mass	0.118	0.612	Pearson
Capture day	Axillary girth	0.114	0.623	Pearson
Head length	Age	0.109	0.638	Pearson
Age	Head length	0.109	0.638	Pearson
Age	Log_{10} Body mass	0.109	0.637	Pearson
Age	Axillary girth	0.087	0.709	Pearson
Capture day	Head length	0.037	0.874	Pearson
Capture day	Age	-0.188	0.415	Pearson
Cubs				
Straight length	Head length	0.919	<0.010	Pearson
Straight length	Body mass	0.944	<0.010	Pearson
Head length	Body mass	0.925	<0.010	Pearson
Head length	Capture day	0.787	<0.010	Pearson
Body mass	Capture day	0.779	<0.010	Pearson
Straight length	Capture day	0.702	<0.010	Pearson

Appendix C: Recoveries

Table C.1. Measured recoveries for the different I.S. used in the chemical analysis. Recoveries are presented as mean ± SD in each batch, all batches together, in cubs and in mothers.

I.S.	Batch 1	Batch 2	Batch 3	Batch 4	All batches	Cubs	Mothers
PFOA (C8)	134 ± 14	89 ± 9	79 ± 5	78 ± 5	90 ± 21	98 ± 24	78 ± 4
PFNA (C9)	134 ± 19	86 ± 9	74 ± 6	62 ± 6	84 ± 26	97 ± 26	65 ± 7
PFDA (C10)	98 ± 15	95 ± 11	80 ± 6	76 ± 5	86 ± 13	92 ± 13	77 ± 5
PFUdA (C11)	107 ± 10	81 ± 9	66 ± 8	70 ± 7	78 ± 16	84 ± 18	69 ± 7
PFHxS (C6)	106 ± 6	76 ± 7	68 ± 3	66 ± 4	76 ± 15	82 ± 16	66 ± 4
PFOS (C8)	94 ± 5	74 ± 8	57 ± 12	47 ± 5	64 ± 19	77 ± 13	46 ± 6
PFOSA (C8)	-	-	54 ± 5	84 ± 6	70 ± 16	53 ± 3	75 ± 14

Appendix D: Plasma concentration of THs

Table D.1. Plasma concentrations of TT4 (nmol/L), FT4 (pmol/L), TT3 (nmol/L) and FT3 (pmol/L) in polar bear mothers and cubs from Svalbard sampled in 1998 (mothers: n=12, cubs: n=12) and 2008 (mothers: n= 9, cubs: n=9). The concentrations are presented as mean ± SD, median and range (min-max).

	TT4 (nmol/L)	**FT4 (pmol/L)**	**TT3 (nmol/L)**	**FT3 (pmol/L)**
Mothers 1998				
Mean ± SD	12.4 ± 2.1	3.9 ± 1.6	1.0 ± 0.2	0.6 ± 0.3
Median	12.6	3.6	1.0	0.6
Range (min-max)	9.2-16.4	1.6-7.2	0.5-1.5	0.1-1.3
Mothers 2008				
Mean ± SD	18.8 ± 5.9	7.4 ± 2.1	1.2 ± 0.1	0.5 ± 0.2
Median	19.3	7.6	1.2	0.6
Range (min-max)	10.6-31.2	5.1-11.3	1.0-1.4	0.3-0.8
Cubs 1998				
Mean ± SD	43.8 ± 17.4	13.3 ± 6.5	1.0 ± 0.2	1.0 ± 0.6
Median	41.7	11.8	1.1	1.1
Range (min-max)	20.0-74.9	2.7-23.7	0.6-1.3	0.2-1.7
Cubs 2008				
Mean ± SD	36.7 ± 11.9	13.3 ± 5.4	1.8 ± 0.5	1.3 ± 0.7
Median	32.5	11.4	1.7	1.2
Range (min-max)	26.1-59.1	7.4-21.8	1.4-2.9	0.6-2.6

Appendix E: Levels of PFASs

Table E.1. Mean (± SD), median and range (min-max) of plasma concentrations of PFASs (ng/g w.w.) in polar bear mothers from Svalbard sampled in 1998 (n=12) and 2008 (n=9). The percentage of individuals with measured concentrations above the limit of detection (LOD) is given. Mean ± SD, median, and range not given for compounds with detection in only one individual (PFBS in mothers 1998 and PFOSA in mothers 2008). * Missing data from one mother sampled in 2008 was replaced by half the limit of detection (LOD).

	Mothers 1998				Mothers 2008			
Compounds	Samples above LOD (%)	Mean ± SD	Median	Range (min-max)	Samples above LOD (%)	Mean ± SD	Median	Range (min-max)
PFBA	0	-	-	-	0	-	-	-
PFPeA	0	-	-	-	0	-	-	-
PFHxA	0	-	-	-	0	-	-	-
PFHpA*	100	1.1 ± 0.7	1.0	0.3-2.5	89	0.4 ± 0.4	0.2	0.03-1.2
PFOA	100	6.4 ± 2.0	6.0	3.4-9.8	100	4.1 ± 1.0	3.7	2.6-5.3
PFNA	100	27.5 ± 3.9	27.5	21.4-34.1	100	38.1 ± 10.8	37.1	26.7-60.1
PFDA	100	7.9 ± 1.1	8.1	5.9-9.3	100	11.9 ± 5.4	11.1	4.5-22.5
PFUnDA	100	18.1 ± 3.4	18.2	12.9-24.6	100	28.3 ± 12.8	27.3	8.7-48.3
PFDoDA	100	1.8 ± 0.6	1.7	1.2-3.1	100	3.3 ± 1.5	3.5	1.0-5.6
PFTrDA	100	3.5 ± 1.2	3.4	2.1-6.1	100	5.8 ± 2.7	5.7	1.7-9.0
PFTeDA	100	0.3 ± 0.1	0.3	0.2-0.5	100	0.4 ± 0.2	0.4	0.1-0.7
PFBS	8	-	-	-	0	-	-	-
PFHxS	100	40.8 ± 9.9	39.0	22.2-60.7	100	32.6 ± 10.1	28.5	22.5-52.5
PFOS	100	431.9 ± 58.9	455.4	297.4-502.0	100	309 ± 114.5	313.9	109.8-464.8
PFOSA	58	0.08 ± 0.04	0.07	0.03-0.14	11	-	-	-
ΣPFCAs	-	66.6 ± 9.7	66.44	52.8-84.4	-	92.4 ± 31.8	88.09	49.2-146.9
ΣPFSAs	-	472.8 ± 64.2	489.87	319.5-562.7	-	341.5 ± 122.1	343	138.2-517.3
ΣPFASs	-	539.3 ± 72.1	561.44	372.4-647.1	-	434.0 ± 151.9	419.64	187.5-653.5

Table E.2. Mean (± SD), median and range (min-max) of plasma concentrations of PFASs (ng/g ww) in polar bear cubs from Svalbard sampled in 1998 (n=12) and 2008 (n=9). The percentage of individuals with measured concentrations above the LOD are given. * Missing data from two cubs sampled in 1998 were replaced by half the LOD.

	Cubs 1998				Cubs 2008			
Compounds	**Samples above LOD (%)**	**Mean ± SD**	**Median**	**Range (min-max)**	**Samples above LOD (%)**	**Mean ± SD**	**Median**	**Range (min-max)**
PFBA	0	-	-	-	0	-	-	-
PFPeA	0	-	-	-	0	-	-	-
PFHxA	0	-	-	-	0	-	-	-
PFHpA	100	1.2 ± 0.9	0.7	0.5-3.4	100	1.2 ± 0.8	1.1	0.3-2.6
PFOA	100	2.1 ± 0.9	1.9	1.0-3.9	100	2.3 ± 0.6	2.1	1.4-3.4
PFNA	100	4.1 ± 1.2	4.2	2.5-6.8	100	6.0 ± 2.3	5.9	2.9-11.1
PFDA	100	1.2 ± 0.4	1.2	0.7-2.0	100	2.0 ± 0.9	1.9	0.5-3.7
PFUnDA	100	4.2 ± 1.2	4.0	2.5-6.7	100	8.7 ± 3.3	8.4	1.5-12.9
PFDoDA	100	0.6 ± 0.2	0.5	0.3-1.0	100	1.3 ± 0.5	1.5	0.2-1.9
PFTrDA	100	1.0 ± 0.4	0.9	0.5-1.9	100	2.5 ± 1.0	2.4	0.4-3.6
PFTeDA*	83	0.05 ± 0.03	0.05	0.01-0.11	100	0.14 ± 0.04	0.15	0.06-0.20
PFBS	0	-	-	-	0	-	-	-
PFHxS	100	12.0 ± 4.1	10.35	8.0-23.5	100	12.2 ± 3.0	12.0	8.2-18.1
PFOS	100	86.8 ± 18.2	83.4	67.7-129.7	100	68.2 ± 26.4	65.3	17.2-101.6
PFOSA	25	0.03 ± 0.02	0.02	0.02-0.05	22	0.03 ± 0.01	0.03	0.02-0.04
ΣPFCAs	-	14.4 ± 4.3	13.05	8.6-23.5	-	24.2 ± 8.0	23.60	8.7-37.4
ΣPFSAs	-	98.8 ± 21.6	95.72	77.7-153.3	-	80.4-28.9	76.77	25.6-119.1
ΣPFASs	-	113.2 ± 25.2	109.68	86.3-176.7	-	104.6 ± 36.2	97.09	34.3-153.7

Table E.3. The proportion (%) of the compounds to ΣPFASs and ΣPFCAs in mother-cub pairs of polar bear calculated based on mean values from each year (mothers: 1998: n=12 and 2008: n=9, cubs: 1998: n=12 and 2008: n=9).

	Mothers 1998	**Mothers 2008**	**Cubs 1998**	**Cubs 2008**
% of PFASs				
PFHpA (C7)	0.2	0.1	1.0	1.2
PFOA (C8)	1.2	0.9	1.9	2.2
PFNA (C9)	5.1	8.8	3.6	5.8
PFDA (C10)	1.5	2.7	1.1	2.0
PFUnDA (C11)	3.4	6.5	3.7	8.3
PFDoDA (C12)	0.3	0.8	0.5	1.3
PFTrDA (C13)	0.6	1.3	0.9	2.3
PFTeDA (C14)	0.1	0.1	0	0.1
PFHxS (C6)	7.6	7.5	10.6	11.7
PFOS (C8)	80.1	71.2	76.6	65.2
ΣPFCAs	12.3	21.3	12.7	23.1
ΣPFSAs	87.7	78.7	87.3	76.9
% of PFCAs				
PFHpA (C7)	1.7	0.4	8.2	5.1
PFOA (C8)	9.6	4.5	14.8	9.4
PFNA (C9)	41.3	41.2	28.3	25
PFDA (C10)	11.8	12.9	8.4	8.4
PFUnDA (C11)	27.2	30.6	29.3	35.9
PFDoDA (C12)	2.7	3.6	3.9	5.4
PFTrDA (C13)	5.2	6.3	6.7	10.1
PFTeDA (C14)	0.5	0.4	0.4	0.6

Appendix F: PCA loadings

Table F.1. Loadings in the PCA plot for polar bear mothers from Svalbard sampled in 1998 (n=12) and 2008 (n=9). The model resulted in one PC that explained 38 % of the variation. Loadings ≥ 0.65 are marked in grey.

Variable	**PC (38 %)**
Capture day	0.587
Age	-0.261
Body mass	-0.246
TT3	0.670
FT3	0.187
TT4	0.513
FT4	0.529
PFHpA	-0.451
PFOA	-0.231
PFNA	0.767
PFDA	0.869
PFUnDA	0.953
PFDoDA	0.935
PFTrDA	0.920
PFTeDA	0.732
PFHxS	0.137
PFOS	0.238

Table F.2. Loadings in the PCA plot for polar bear cubs from Svalbard in 1998 (n=12) and 2008 (n=9). The model resulted in two PC that together explained 68 % of the variation (PC1 =40 %, PC2 =28 %). Loadings ≥ 0.65 are marked in grey.

Variable	PC1 (40 %)	PC2 (28 %)
Capture day	0.495	0.651
Body mass	0.516	0.731
TT3	0.659	0.647
FT3	0.283	0.771
TT4	-0.324	0.486
FT4	-0.109	0.622
PFHpA	0.150	-0.765
PFOA	0.329	-0.450
PFNA	0.776	-0.353
PFDA	0.858	-0.309
PFUnDA	0.971	-0.106
PFDoDA	0.957	0.008
PFTrDA	0.960	0.021
PFTeDA	0.844	0.191
PFHxS	0.445	-0.586
PFOS	0.208	-0.682