Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Aslak Arnesson Aune

Perfluoroalkyl substances are positively correlated with triiodothyronine concentrations, but not metabolic rate in breeding arctic terns (Sterna paradisaea)

Master's thesis in Biology, physiology
Supervisor: Bjørn Munro Jenssen, NTNU
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Arctic tern trapping in Kongsfjorden. Photo by Geir W. Gabrielsen.

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Acknowledgments

First of all, I would like to thank my supervisors Prof. Bjørn M. Jenssen at NTNU and Prof. Geir W. Gabrielsen at NPI. Thank you for allowing me to work on this very exciting topic, answering all my questions and all the proofreading. It has been a pleasure to work with you both.

Several people have been involved in this project and would like to thank Hugh Ellis at the University of San Diego, for conducting the MR measurements in Ny-Ålesund and teaching me a lot about bird energetics. I would also like to Linda Hansen at NILU, for assisting me with the PFAS analysis, Grethe Stavik Eggen at NTNU for helping me out with the TH analysis, and all the field assistants and staff at the Sverdrup Station in Ny-Ålesund for making my stays there very enjoyable.

I also feel I need to thank the arctic terns of Kongsfjorden, for letting me kidnap you and do all kinds of scientific measurements, which probably were not too comfortable. However, considering the way I was treated I feel we can call it even and please remember it was all in the name of science.

Finally, I would like to thank my family and my girlfriend for being very supportive and helpful during the last two years.

Trondheim, May 2021

Aslak Aune

This master thesis was funded by the Norwegian Research Council and the Arctic Field Grant. The TH analysis and molecular sexing was founded by the Department of Biology at the Norwegian University of Science and Technology (NTNU) and the PFAS analyses were funded by the Norwegian Polar Institute (NPI).

Abstract

Perfluoroalkyl substances (PFAS) are ubiquitous environmental pollutants, many of which are highly persistent, and mounting evidence suggests this group of chemicals has thyroid hormone (TH) disruptive properties and possible effects on the metabolic rate of exposed animals, though the latter is poorly documented. This study aimed to investigate the concentrations and patterns of circulating PFASs in incubating arctic terns (*Sterna paradisaea*), and how the PFASs correlated with total thyroxine (TT4) and triiodothyronine (TT3) concentrations and resting metabolic rate (RMR). Arctic terns (n=20) were trapped and sampled in Kongsfjorden, Svalbard, during June/July 2019. RMR was measured in an open circuit respirometry chamber and PFASs were quantified in whole blood by liquid chromatography–mass spectrometry (LC-MS), whereas TT4 and TT3 were quantified in plasma by radioimmunoassay (RIA). The top three dominating PFASs in the arctic terns were, in decreasing order, linear perfluorooctane sulfonate (linPFOS), perfluoroundecanoate (PFUnDA), and perfluorotridecanoate (PFTrDA) which is consistent with PFAS compositions reported in other arctic seabird species in Kongsfjorden. This pattern indicates there could be some local sources in Kongsfjorden, although long-range transported (LRT) PFAS is likely the main exposure source. Males had significantly higher concentrations of most PFASs, compared to females. ∑PFAS, ∑perfluoroalkyl carboxylic acids (PFCA), ∑perfluoroalkyl sulfonic acids (PFSA), ∑PFOS, and seven specific PFASs (branched PFOS, linPFOS, and five PFCAs) were significantly positively correlated with TT3 in both sexes combined, whereas perfluorododecanoate (PFDoDA) and PFTrDA were significantly negatively correlated with the TT4/TT3 ratio. In females, ∑PFAS, ∑PFSA, ∑PFOS, and four specific PFASs (linPFOS and three PFCAs) were significantly positively correlated with TT3. No PFASs were correlated with TT3 in males and neither TT4 nor RMR was significantly correlated with any PFASs in neither sex. Further research is recommended to unravel the driving mechanisms behind the positive correlations between specific PFASs and THs, and thus the possible TH disruptive effects of PFAS and their potential ecological consequences.

Sammendrag

Perfluorerte alkylstoffer (PFAS) er allestedsnærværende miljøgifter, hvorav mange er svært persistente. Forskning tyder på at denne kjemikaliegruppen har tyroidhormonforstyrrende egenskaper og mulige effekter på metabolismen i eksponerte dyr, selv om sistnevnte er dårlig dokumentert. Formålet med dette studiet var å undersøke konsentrasjoner og sammensetning av sirkulerende PFASer i rugende rødnebbterner (*Sterna paradisaea*), og hvordan PFASene korrelerte med totalt nivå av tyroksin (TT4) og trijodtyronin (TT3) og hvilemetabolisme (RMR). Rødnebbterner (n=20) ble fanget og målt i Kongsfjorden, Svalbard, i perioden juni/juli 2019. RMR ble målt i et åpent respirometer og PFASer ble kvantifisert i helblod med væskekromatografi-massespektrometri (LC-MS), mens TT4 og TT3 ble kvantifisert i blodplasma med radioimmunoassay (RIA). De topp tre dominerende PFASene i rødnebbternene var, i synkende rekkefølge, linear perfluoroktan sulfonat (linPFOS), perfluorundekanoat (PFUnDA) og perfluortridekanoat (PFTrDA), hvilket er konsekvent med PFAS komposisjonen i andre arktiske sjøfugler i Kongsfjorden. Dette mønsteret indikerer at det kan være lokale kilder i Kongsfjorden, selv om langtransporterte (LRT) PFASer sannsynligvis er den største eksponeringskilden. Hanner hadde signifikante høyere konsentrasjoner av den fleste PFASer sammenlignet med hunner. ∑PFAS, ∑perfluorerte karboksylsyrer (PFCA), ∑perfluorerte sulfonsyrer (PFSA), ∑PFOS og sju spesifikke PFASer (forgreinet PFOS, linPFOS og fem PFCAer) var signifikant positivt korrelert med TT3 i begge kjønn kombinert, mens perfluordodekanoat (PFDoDA) og PFTrDA var signifikant negativt korrelert med ratioen av TT4/TT3. I hunner var ∑PFAS, ∑PFSA, ∑PFOS og fire spesifikke PFASer (linPFOS og tre PFCAer) signifikant positivt korrelert med TT3. Ingen PFASer var korrelert med TT3 i hanner, og hverken TT4 eller RMR korrelerte med noen PFASer i noen kjønn. Videre forskning er anbefalt for å kartlegge mekanismene bak den positive korrelasjonen mellom spesifikke PFASer og tyroidhormoner, og derfor mulige tyroidhormonforstyrrende effekter av PFASer og de mulige økologiske konsekvensene av dette.

Abbreviations

4:2 FTS	4:2 Fluorotelomer sulfonic acid
6:2 FTS	6:2 Fluorotelomer sulfonic acid
8:2 FTS	8:2 Fluorotelomer sulfonic acid
AFFF	Aqueous film-forming foams
BFR	Brominated flame retardant
BMR	Basal metabolic rate
brPFOS	Branched PFOS
CHD	Chromo-helicase-DNA-binding
DDT	Dichlorodiphenyltrichloroethane
FOSA	Perfluorooctane sulfonamide
FT3	Free T3
FT4	Free T4
HCB	Hexachlorobenzene
I	Iodine
LC-MS	Liquid chromatography-mass spectrometry
LCT	Lower critical temperature
linPFOS	Linear PFOS
LRT	Long-range transported
MOA	Mechanisms of action
NILU	Norwegian Institute for Air Research
NINA	Norwegian institute of nature research
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
PFAS	Per- and polyfluoroalkyl substance
PFBS	Perfluorobutane sulfonate
PFDA	Perfluorodecanoate
PFDoDA	Perfluorododecanoate
PFDS	Perfluorodecane sulfonate

PFHpA	Perfluoroheptanoate
PFHpS	Perfluoroheptane sulfonate
PFHxA	Perfluorohexanoate
PFHxDA	Perfluorohexadecanoate
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoate
PFNS	Perfluorononane sulfonate
PFOcDA	Perfluorooctadecanoate
PFOS	Perfluorooctane sulfonate
PFPS	Perfluoropentane sulfonate
PFTeDA	Perfluorotetradecanoate
PFTrDA	Perfluorotridecanoate
PFUnDA	Perfluoroundecanoate
POFA	Perfluorooctanoate
POP	Persistent organic pollutant
RMR	Resting metabolic rate
T3	Triiodothyronine
T4	Thyroxine
T_a	Ambient temperature
T_b	Body temperature
TH	Thyroid hormone
TNZ	Thermoneutral zone
TR	Thyroid receptor
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
TT3	Total T3
TT4	Total T4
UHPLC-MS/MS	Ultrahigh pressure liquid chromatography triple–quadrupole massspectrometry
WW	Wet Weight

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1 Introduction

1.1 Transportation and accumulation of contaminants in the arctic

The Arctic has always been regarded as a pristine and untouched region, sheltered from the discharge of chemicals from the industrial world. Despite this, a wide array of semi-volatile, toxic compounds have been detected there, mainly due to atmospheric, ocean-current, and river transport (Braune et al., 2005; de Wit et al., 2010, 2006). One dominating group of pollutants detected in the arctic are the persistent organic pollutants (POPs), a large group of man-made, organic chemicals. POPs are characterized by having a high level of persistence to biological and chemical degradation, the ability to be long-range transported (LRT), the capacity to bioaccumulate in organisms and biomagnify in the food chain, and toxic effects on wildlife (Dietz et al., 2019; Letcher et al., 2010). Many POPs are today regulated by the Stockholm convention. The Stockholm convention (Stockholm Convention, 2021a) is an international environmental treaty that entered into force in 2004, with 12 listed chemicals, today known as the dirty dozen (Kaiser and Enserink, 2000; Stockholm Convention, 2021b). These 12 chemicals are all aromatic, polychlorinated, hydrocarbon-based pesticides, industrial chemicals, and by-products. They are known as legacy POPs because the levels we detect in the environment today are mainly a legacy of past use (Cabrerizo et al., 2018). The low polarity of these compounds enables them to accumulate in the fatty tissues of exposed animals, and accumulated POPs are released into the bloodstream and other organs when fat stores are utilized during periods of stress. This is when they may elicit harmful effects (Henriksen et al., 1996; Jansen et al., 2017).

1.2 Per- and polyfluoroalkyl substances

Since the dirty dozen was established, many new persistent chemicals have been added to the Stockholm Convention. These are termed emerging compounds (Cabrerizo et al., 2018), and two examples are perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), which are listed under Annex A and B respectively (Stockholm Convention, 2021c). They belong to a group of chemicals known as per- and polyfluoroalkyl substances (PFASs). PFASs account

for a large group of molecules with a completely (per) or partially (poly) fluorinated, hydrophobic carbon-chain body and a hydrophilic head group, often a carboxylic acid or sulfonic acid (Buck et al., 2011). This gives PFASs water and fat repellent properties, making them excellent surfactants, even more so than classical hydrocarbons due to the incredible strength of the carbon - fluor bond. This also makes them very persistent to chemical, biological, thermal, and physical degradation (Key et al., 1997). PFASs have, and are being used in hundreds of different applications, ranging from electronic devices to water-repellent textiles, Teflon, and fire-fighting foams (Glüge et al., 2020). Many PFASs meet all the POPs criteria set by the Stockholm Convention, but unlike the classical organochlorine (OC) POPs, their amphiphilic nature gives them a high affinity for proteins, and PFASs are mainly distributed to and accumulated in the blood serum, liver, and kidneys (Giesy and Kannan, 2002; Lau et al., 2007). The most studied PFASs are PFOS and PFOA, which have documented toxic effects on the immune and endocrine system, embryonic development, and the liver (Lau et al., 2007). As of today, PFOS and PFOA are the only PFASs that have been listed on the Stockholm convention, but perfluorohexane sulfonic acid (PFHxS) is proposed for listing under the convention. Additionally, Norway and several other EU countries have called for a general ban of PFASs on the European market, as there exist more than 5000 PFASs (OECD, 2018), many of which we know nothing about, and may potentially have an equal or greater toxicity than that of PFOS and PFOA.

1.3 Endocrine disruptive effects

The Endocrine system is crucial in every aspect of organism development and function, spanning from reproductive development to regulation of body temperature (Hiller-Sturmhöfel and Bartke, 1998). The internal homeostasis is maintained through a well-balanced, and well-timed secretion of hormones, where small alterations may result in profound, organism-wide effects, making the endocrine system vulnerable to the effects of contaminants. Endocrine disruptive effects are regarded as chemically induced alterations to any aspect of the endocrine system, including hormone synthesis, secretion, circulatory transportation, receptor binding, and excretion (Diamanti-Kandarakis et al., 2009). Chemicals capable of exerting such effects are known as endocrine disruptive chemicals (EDCs) and many of the regulated POPs are classified as EDCs (Hormone Health Network, 2021). EDCs can have many different mechanisms of action (MOA), e.g. affecting the endocrine secreting

glands directly, blocking the uptake of chemicals needed for hormone synthesis, or mimicking endogenous hormones, allowing the chemicals to bind to the same transport proteins and receptors, resulting in disrupted homeostasis (Boas et al., 2006). EDCs that initiate a response in the cell are known as agonists, whereas EDCs that bind to receptors but do not initiate a response are known as antagonists (Matsui, 2008). One group of hormones documented to be affected by both legacy and emerging POPs in mammals and birds are the thyroid hormones (THs), the primary regulators of metabolism and thermogenesis (Howdeshell, 2002; Jenssen, 2006).

1.4 Thyroid hormones and basal metabolism

In addition to being the main regulators of metabolism and thermoregulation, THs modulate many other physiological functions such as reproduction, growth, molting, and brain development (McNabb, 2007; Zoeller et al., 2002). Secretion of THs is controlled by the hypothalamus secreting thyrotropin-releasing hormone (TRH), stimulating the release of thyroid-stimulating hormone (TSH) from the pituitary gland which stimulates the secretion of THs from the thyroid gland. This negative feedback system is known as the hypothalamic-pituitary-thyroid axis (HTP axis). THs include the precursor hormone thyroxine (T4) and the biological active triiodothyronine (T3). The vast majority of circulating TH is T4 bound to the transport proteins albumin and transthyretin (TTR). After entering the target cell, T4 is deiodinated to T3 by iodothyronine deiodonase enzymes, binds to the thyroid receptor (TR) in the cytoplasm, and initiates a physiological response (McNabb, 2007). Polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT), and chlordane, incl. metabolites have in several studies demonstrated negative associations with both total T3 (TT3) and T4 (TT4) concentrations, and free T3 (FT3) and T4 (FT4) concentrations in arctic seabirds (Blévin et al., 2017; Braune et al., 2011; Melnes et al., 2017; Verreault et al., 2004). However, other studies have not identified such effects (Nost et al., 2012; Verreault et al., 2013, 2007). PFASs on the other hand, have demonstrated a consistent positive correlation with several TH parameters in arctic seabirds incl. TT3, FT3, TT4 and FT4 (Ask, 2015; Braune et al., 2011; Melnes et al., 2017; Nost et al., 2012). In polar bears (*Ursus maritimus*), a negative correlation between PFASs and THs has been reported (Bourgeon et al., 2017), suggesting the response may be species-dependent. In addition to environmental pollutants, there are many other factors that many influence thyroid function

and TH balance including age, iodine (I) availability, season, body condition, activity, and ambient temperature (T_a) (McNabb, 2007).

Serving as the primary metabolic hormones, contaminant-driven alterations of the TH homeostasis can also indirectly affect an organism's basal metabolic rate (BMR). BMR is an organism's minimum energy expenditure needed to sustain vital bodily functions and can only be measured correctly in adult, non-digesting animals, resting within their thermoneutral zone (TNZ) (Bligh and Johnson, 1973; Ellis and Gabrielsen, 2001). Avian BMR can be affected by many factors, including life history stages such as breeding, but also geographical distribution (Ellis and Gabrielsen, 2001). Birds of high latitudes such as the arctic tern (*Sterna paradisaea*), generally have a higher BMR than birds of lower latitudes as an adaptation to the cold climate (Bech et al., 2002; Ellis, 1984; Gabrielsen et al., 1988). BMR is the most common measurement of animal energetics, and OCs, such as chlordane and PCBs have demonstrated a negative association with BMR in glaucous gulls (*Larus hyperboreus*) (Verreault et al., 2007) and metabolic rate (MR) in black-legged kittiwakes (*Rissa tridactyla*, hereafter "kittiwakes") (Blévin et al., 2017). Decreased BMR in relation to PCB exposure has also been reported by Tori and Mayer, (1981) in mourning doves (*Zenaida macroura*). Even though the scientific literature is scarce, PFASs appear to have an opposite effect on avian BMR compared to the investigated OCs, with perfluorotridecanoate (PFTrDA) having demonstrated a positive relationship with MR in female kittiwakes (Blévin et al., 2017).

1.5 The arctic tern

The arctic tern is a long-lived tern species in the *Laridae* family. Annually flying between Antarctica and the circumpolar regions of the northern hemisphere, it holds the world record for the longest migration of any known animal, more than 80 000 km for certain individuals (Egevang et al., 2010). This makes the arctic tern an interesting study species regarding contamination for several reasons. Firstly, its migration is an exceptional energy-demanding activity, and the arctic tern depends on good foraging and fat accumulation at the breeding ground, possibly making them vulnerable to metabolic effects of contaminants. Secondly, the arctic tern travels close to several industrialized areas in Europe, Africa, and South America during its migration (Egevang et al., 2010; Fijn et al., 2013), possibly exposing them to a cocktail of various POPs and other contaminants, additional to what they might accumulate at their breeding and wintering grounds.

The arctic tern is an opportunistic feeder, with a diet made up of crustaceans and fish, the latter being the most important prey (Anker-Nilssen et al., 2000; Watson et al., 1975). To my knowledge, there is no data regarding the trophic position of arctic terns available, but considering its diet and size, it is reasonable to assume its trophic position is lower than that of kittiwakes, northern fulmars (*Fulmarus glacialis*), and glaucous gulls, which are the seabirds previously investigated for TH-disrupting effects from PFAS (Ask et al., 2020; Blévin et al., 2017; Melnes et al., 2017; Nost et al., 2012). This implies that seeing TH alteration as a response to PFAS exposure in the arctic terns would suggest that many arctic seabirds could be impacted.

Data regarding the accumulation of contaminants in arctic terns are scarce and non-existing regarding effects. Several OCs and brominated flame retardants (BFRs) have been detected in arctic tern eggs, but the concentrations are substantially lower than in other arctic breeding seabirds such as great skuas (*Stercorarius skua*) and northern fulmars (Jenssen et al., 2007; Jörundsdóttir et al., 2010). PFASs have to my knowledge never been quantified in arctic terns before.

1.6 Aims of the study

This study aimed to investigate to what extent PFASs affect the TH balance and MR of arctic terns nesting at Svalbard, and also to survey the concentrations and patterns of PFASs in the arctic terns. This was accomplished by measuring and comparing whole blood concentrations of PFASs with plasma concentrations of TT3 and TT4 and measured MR. The hypothesis is that there will be a positive correlation between PFASs and THs, and possibly MR as this has been observed in several other arctic seabirds (Ask et al., 2020; Blévin et al., 2017; Braune et al., 2011; Melnes et al., 2017; Nost et al., 2012).

2 Materials and methods

2.1 Sampling area

The arctic tern trapping was conducted at three different colonies in Kongsfjorden. The sampling locations were Gerdøya, Observasjonsholmen and Innerholmen (Fig. 1), operating out of Ny-Ålesund (78°55'00'N, 11°56'00'E) in the period of 24th of June – 15th of July 2019. A total of 20 individuals were caught on their nest while egg brooding. This is a period of midnight sun at Svalbard, and the weather was unusually warm during the 2019 season with an average July temperature of 7,3 ºC (Min: 3 ºC – Max: 16 ºC), 2,4 ºC warmer than average (Meterologisk Institutt, 2021). The fieldwork was approved by the governor of Svalbard and the Norwegian Animal Research Authorities (reference no: 18/00746-3).

Figure 1: Map of the study area in Kongsfjorden. Sampling locations marked with a red dot. The figure is from Toposvalbard, Norsk Polarintitutt.

2.2 Field procedures, blood sampling and biometrics collection

Incubating arctic terns were caught on their nest with an automatically triggered nest trap. Prior to trapping, the eggs were swapped out with fake, chalk-infused eggs, to avoid damage to the eggs during the capture. Once two birds, from two different nests were caught, they were immediately transported back to Ny-Ålesund, in cardboard boxes with air holes. Following the capture, the real eggs were placed back in the nest, allowing for the partner to continue brooding. Back in Ny-Ålesund, the birds were weighed to the nearest 0.1 g with an electronic balance and placed in a respiratory chamber for BMR measurements (Paragraph 2.3). After the measurements, the birds were weighed once more, and the body temperature (T_b) was measured with a Schultheis fast-reading reptile mercury thermometer. The wing length, beak length, beak height, and skull length were measured with a sliding caliper and 1 mL of blood was collected from the brachial vein using a heparinized syringe (1 mL syringe, 25G needle). 20 individuals were sampled, but the amount of blood sample collected varied considerably, and hence many sample volumes were lower than 1 mL. When the bleeding had stopped, the bird was immediately released. The blood was transferred in equal amounts into two Eppendorf tubes (0,5 mL in each tube). One Eppendorf tube was centrifuged for 9 min at 7000 rpm and the plasma was transferred to yet another tube. The three tubes now containing whole blood (500 µL for PFAS analysis), plasma (250 µL for TH analysis), and blood cells (250 µL for molecular sexing), were frozen at -20 ºC until further analysis.

2.3 BMR measurements

BMR was measured by open-circuit respirometry on 18 arctic terns, which were kept for a minimum of 2 hours and 30 minutes after capture, to allow for complete digestion of ingested food. The birds were placed in a 25 L plexiglass chamber, connected to a Sable Systems FoxBox analyzer® (Sable Systems International, Las Vegas, USA), drawing outside air into the chamber at a flow rate of 1.7 L/min. The air was dried in indicator silica gel, drawn through the bird chamber, and into the FoxBox which measured CO_2 concentration. The air was then scrubbed of CO_2 with indicator lime soda and dried a second time before O_2 concentration was measured in the FoxBox. The measurement of each tern was conducted for at least 2 hours. The amount of O_2 consumed (mL) in a given unit of time is an indirect

measurement of metabolic activity, and BMR was calculated with the following equation (H Ellis 2021, personal communication):

$$BMR = FR * \frac{dO2}{dO2 + 0.7905} \quad \textbf{(Equation 1)}$$

FR is the flow rate (mL/min), and dO2 is the difference in O_2 content of the excurrent air stream (what the animal has consumed) and incurrent air stream (0.2095). The outcome of this equation (BMR) will be ml O_2/min. BMR values in the present study are presented as mL O_2/g*h, which was accomplished by multiplying the BMR with 60 min and diving it by the body mass.

The ambient temperature (T_a) in the respiratory chamber during the procedure was continuously measured by a probe connected to the FoxBox and it averaged 19,29 ºC ± 1,68. This was later discovered to be below a proposed lower critical temperature (LCT) of the TNZ for the arctic tern (C Bech 2021, personal communication), possibly making our measurements invalid as true BMRs. The reported values will hereby be referred to as resting metabolic rates (RMR), as this measurement does not require the animal to be in thermoneutrality (Ellis and Gabrielsen, 2001). The plexiglass chamber was covered with a towel, to prevent the arctic terns from being stressed by the surroundings. The FoxBox made readings of O_2, T_a, and flowrate every 20 s and stored the data on a connected laptop.

2.4 PFAS analysis

22 PFASs (Table 1) were analyzed in 19 arctic tern whole blood samples at the Norwegian Institute for Air Research (NILU) in Tromsø, September 2020. Separation and analysis were conducted using liquid chromatography-mass spectrometry (LC-MS), following the method described by Powley et al., (2005) and modified for blood by Hanssen et al., (2013). Together with the samples, a control sample (AMSY 2006) with known concentrations of 9 different PFASs (PFHxS, PFHpS, PFOS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA (See Table 1 for full names)) and blank samples were analyzed. The limit of detection (LOD) was set at three x signal-to-noise ratio for each compound (Table 1). For PFOS, both the linear (linPFOS) and the branched isomer (brPFOS) was analyzed in the sample.

Table 1: List of all the PFASs analyzed in the arctic tern (*Sterna paradisaea*) whole blood samples. The PFASs are assigned into one of the following three groups depending on the polar head group: Perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs), and perfluoroalkane sulfonamides (FASAs).

Group	Acronym	Compound	Carbon chain length	LOD (ng/g)
PFSA	4:2 FTS	4:2 Fluorotelomer sulfonic acid	6	0.10
	6:2 FTS	6:2 Fluorotelomer sulfonic acid	8	0.10
	8:2 FTS	8:2 Fluorotelomer sulfonic acid	10	0.10
	PFBS	Perfluorobutane sulfonate	4	0.05
	PFPS	Perfluoropentane sulfonate	5	0.05
	PFHxS	Perfluorohexane sulfonate	6	0.05
	PFHpS	Perfluoroheptane sulfonate	7	0.05
	PFOS	Perfluorooctane sulfonate	8	0.05
	PFNS	Perfluorononane sulfonate	9	0.05
	PFDS	Perfluorodecane sulfonate	10	0.05
PFCA	PFHxA	Perfluorohexanoate	6	0.10
	PFHpA	Perfluoroheptanoate	7	0.05
	PFOA	Perfluororoctanoate	8	0.05
	PFNA	Perfluorononanoate	9	0.075
	PFDA	Perfluorodecanoate	10	0.05
	PFUnDA	Perfluoroundecanoate	11	0.05
	PFDoDA	Perfluorododecanoate	12	0.075
	PFTrDA	Perfluorotridecanoate	13	0.05
	PFTeDA	Perfluorotetradecanoate	14	0.05
	PFHxDA	Perfluorohexadecanoate	16	0.05
	PFOcDA	Perfluorooctadecanoate	18	0.10
FASA	FOSA	Perfluorooctane sulfonamide	8	0.05

200 µL of whole blood sample was transferred to Eppendorf-centrifuge tubes and spiked with 10 ng internal standard, with known concentrations of ^{13}C labeled PFASs (Appendix D). The assumption is that the loss of ^{13}C-PFASs in the internal standard and ^{12}C-PFASs in the samples is equal, so the correct amount of ^{12}C-PFASs in the sample can be calculated. Three

samples had low volumes or a coagulated consistency, so the amount of blood was calculated by weighing the tubes with the sample and subtracting the empty tube weight. 500 µL of methanol (LiChrosolv, Merck, Darmstadt, Germany) was added to each tube, and the content was mixed by shaking and vortex. The tubes were placed in an ultrasonic bath for 3 x 10 minutes, and vortexed in between, followed by centrifugation for 10 min at 10 000 rpm. 300 µL supernatant was transferred from the centrifuged tubes to new corresponding 1.7 mL Eppendorf tubes which had been added 25 mg ENVI-Carb (graphitized carbon absorbent) and 50 µL glacial acetic acid. All tubes were centrifuged for 10 min at 10 000 rpm. 200 µL supernatant was then transferred to 2 mL vials and added 2 ng recovery standard (RSTD in methanol(3,7-diMeo-PFOA), 2 ng), to determine the performance of the analytical method. The vials were vortexed and kept in the fridge until further analysis.

For analysis, as describes by Hanssen et al., (2013), 50 µL of sample was transferred to an autosampler vial which was added 25 µL of buffer containing ammonium acetate (NH_4OAc, 2mM), water, and methanol (90:10). This mixture was vortexed before analysis and the quantification was performed by ultrahigh-pressure liquid chromatography triple–quadrupole mass spectrometry (UHPLC-MS/MS). Analysis was performed using a Thermo Scientific quaternary Accela 1250 pump (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a PAL Sample Manager (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled to a Thermo Scientific Vantage MS/MS (Thermo Fisher Scientific Inc., Waltham, MA, USA). 10 µL of the sample was injected into a Waters Acquity UPLC HSS 3 T column (2.1 x 100 mm, 1.8 µm, Waters Corporation, Milford, MA, USA) with a Waters Van guard HSS T3 guard column (2.1 x 5 mm, 1.8 µm, Waters Corporation, Milford, MA, USA). Separation was accomplished with 2 mM NH_4OAc in 90:10 methanol/water and 2 mM NH_4OAc in methanol serving as the mobile phases. The known ^{13}C labeled PFASs in the internal standard and the unknown PFAS concentrations in the blood samples were quantified using the LCQuan software (version 2.6, Thermo Fisher Scientific Inc., Waltham, MA, USA).

For quality assurance, all samples were analyzed in duplicates, and the reported values are the average of the two injections. The average recovery of the internal standards ranged between 56-101 % (Appendix E), and the PFAS concentrations measured in the control sample were within an acceptable range of the assigned values (89-123 %) (Appendix C). All blank samples had concentrations below the instrument's detection limits.

2.5 Thyroid hormone analysis

TT4 and TT3 concentrations were analyzed in plasma by radioimmunoassay (RIA) at the Norwegian University of Science and Technology (NTNU) in Trondheim, October 2020. Plasma from 28 arctic terns was analyzed, however only the results from 15 (TT4), and 16 (TT3) arctic terns were included in the dataset, as these were the individuals where RMR and PFAS data had been obtained. TH analysis was performed with ^{125}I-TT4 and -TT3 RIA kits (MP Biomedical, New York, USA). The principle of the assay is that ^{125}I-labeled TH in a tracer solution will compete for a limited number of binding sites in an antibody-coated tube with an unlabeled and unknown amount of TH in the sample. The level of gamma radiation emitted by the tube is therefore inversely related to the concentration of the analyte, and the concentration may be calculated from a standard curve based on serum standards (STD 1-6, T4 or T3 in human serum). The concentrations of the standards ranged from 0-800 ng/dL (TT3) and 0-20 µg/dL (TT4). These RIA kits have been used on plasma samples from birds in previous studies (Hovden, 2018; Svendsen et al., 2018).

For the TT3 analysis, 100 µL of plasma and serum standard was transferred to T3 antibody-coated tubes (T3 rabbit antiserum). 1 mL of radioactive traces solution was added to each tube, and all tubes were incubated in 37 ºC water for 60 min. After the incubation period, the content was discharged, and the tubes were rinsed once with 1 mL deionized water and dried. The procedure was almost identical for the TT4 analysis, but 25 µL of the sample was used, the tubes were incubated at room temperature, and not rinsed with deionized water before measuring the radioactivity. Also, the tubes for the TT4 analysis were coated with mouse T4 antiserum and not rabbit antiserum. The radioactivity of the tubes was counted with a Packard Cobra-II Auto gamma counter (GMI inc. Minneapolis, USA)

For quality assurance and assay validation, standard reference material (SRM level 1-3, BIO-RAD, Immunoassay plus control, California, USA), and internal reference material of chicken (*Gallus gallus domesticus*) and bull (*Bos taurus*) plasma was analyzed in addition to the samples. It was also strived for all the samples to be run in duplicates, but the sample volume did not always allow for this. For the TT3 analysis, the coefficient of variance (%CV) of all the samples analyzed in duplicates (n = 10) averaged at 3.2 ± 1.7 (Appendix F), suggesting that the reported values for the single run samples are accurate. For the TT4 analysis, however, the %CV of the duplicates (n = 17) averaged at 12.3 ± 13.1 (Appendix F), indicating

large variance and high uncertainty in the measured TT4 values. The average %CV for the reference material was 2.5 ± 2.0 (TT3) and 8.7 ± 3.7 (TT4).

2.6 Molecular sexing

Molecular sexing was performed by the Norwegian Institute of nature research (NINA) in Trondheim, February 2021. The sexing was performed on blood cells following the method developed by Griffiths et al., (1998) and modified by Bantock et al., (2008). It includes polymerase chain reaction (PCR) amplification of two conserved chromo-helicase-DNA-binding (CHD) genes located on the sex chromosomes of most avian species. CHD-W is a gene unique to the female W-chromosome, whereas CHD-Z is located on the Z-chromosome, and thus found in both males and females. The presence of the genes was revealed by gel-electrophoresis and the sex could be determined by the number of bands present (1 band for males, 2 bands for females).

10 µL of blood cells (n=17) was transferred to a 2 mL Eppendorf tube containing 280 µL lysis buffer (Qiagen, Hilden, Germany) and 20 µL proteinase K (Qiagen, Hilden, Germany). The tubes were incubated for 1 hour at 56 °C and pulse-vortexed twice in this period. Genomic DNA was extracted with a semi-automated system (Maxwell® 16 Research Instrument, Promega, Madison, WI, USA) and a Maxwell 16 tissue DNA Purification Kit. The sex was determined using the primers M5 (6FAM fluoro-labelled) (Griffiths et al., 1998) and M8 (Bantock et al., 2008). PCR was performed with Qiagen's Multiplex PCR Kit and a reaction volume of 8,4 µL. 1 µL of PCR product was mixed with GeneScan 500 LIZ (Applied Biosystems) size standard (0.14 µL) and Hi-Di formamide (6.86 µL). The genes were separated with capillary electrophoresis on an ABI 3500*xl* Genetic Analyzer, and size determined using GeneMapper v.6.0 software (Applied Biosystems)

2.7 Data treatment and statistical analysis

A total of 20 arctic terns were caught during the fieldwork of 2019, however, we only obtained RMR values from 18, PFAS concentrations from 19, and TH concentrations from 15 (TT4), and 16 (TT3) individuals, respectively. Molecular sexing was performed on 17 individuals. For safety and ethical reasons, we could only obtain 1 mL of blood from each arctic tern (≤ 1 % of total body mass), which proved to be quite difficult, due to the very small brachial veins. When sample volumes were low (< 1 mL), blood for PFAS analysis was prioritized (n = 19), and analysis of TT3 (n = 16), being the primary metabolic hormone, was prioritized before TT4 (n = 15). The reason for the two missing BMR values was a malfunction of the FoxBox analyzer, which was discovered after the two first measurements.

PFASs with concentrations below the LOD in more than 30 % of the individuals were not included in the dataset, which subsequently eliminated the 14 following PFASs; 4:2 FTS, 6:2 FTS, 8:2 FTS, PFBS, PFPS, PFHpS, PFNS, PFDS, PFHxA, PFHpA, PFOA, PFHxDA, PFODcA, and FOSA. The PFASs with concentrations below the LOD in less the 30 % in the individuals were included, and a random number between 0 and the LOD was generated in Excel's random number generator (function "= RAND()*0,05"). Due to the low sample size (n = 15), no individuals were excluded from the TT4-dataset despite having some duplicates with very high % CV, and the fact that many samples were not analyzed in duplicates (Appendix F). These results should therefore be treated with care.

In contrast to BMR, RMR measurements do not require the animal to be post-absorptive (Ellis and Gabrielsen, 2001). Regardless, we considered the birds to be post-absorptive, due to the time interval between capture and measurement. For extra assurance, we tested with the Pearson`s correlation coefficient ($\alpha = 0.05$) if the time interval between capture and RMR measurement was correlated with the obtain RMR value. This did not reveal any significant correlation (r: -0.269, $p = 0.281$).

All statistical analyses were performed using SPSS (Version 27, IBM, SPSS Inc., Chicago, IL, USA). The data were tested for normality with a Shapiro-Wilk test ($\alpha = 0.05$) (Appendix G). Variables that were normally distributed were tested for sex differences with a two-tailed Student`s t-test ($\alpha = 0.05$) (Appendix J), whereas the non-parametric Mann-Whitney U test was used when testing for sex differences in non-normally distributed variables ($\alpha = 0.05$)

(Appendix I). Levene's test was used to determine whether the variance of males and females was equal (Appendix J).

A principal component analysis (PCA) was performed to reduce all the variables down to a few principal components (PCs), for exploring how the variables correlate, and how the individuals relate with each other. PCs with eigenvalues > 1 were retained in the model (Appendix H) and a loading plot was made to show which variables influenced the model, and how the variables were correlated. The distance from the variables to the origin explains how much the variables influence the PCs, and the angle between the variables, relative to the origin, indicates how they are correlated. A small angle indicates a positive correlation, a 90º angle indicates no correlation, and a large angle indicates a negative correlation. A score plot, plotting the PC values of each observation/arctic tern, was also created to investigate how the variables differ between the individuals and sexes.

To validate if correlations indicated by the PCA were significant, a correlation matrix was made. This was done for all individuals combined and for males and females separately. Pearson's correlation coefficient was used for normally distributed variables ($\alpha = 0.05$) (Appendix K), and Spearman`s rank coefficient was used for non-normally distributed variables ($\alpha = 0.05$) (Appendix L). Individual linear regressions with 95%-confidence intervals were produced for 6 significantly correlated variables, determined by the correlation matrix.

For all the statistical models, PFAS and TH concentrations are given in pmol/mL wet weight (ww), because the number of molecules might be what initiates an effect, and not the total mass of the compound. In the tables, however, the unit of measurement was ng/mL ww, as this is more appropriate in terms of comparing the results to other studies. All biometric variables, PFAS concentrations, and energetic parameters are presented with the mean values ± standard deviation, median, range, number of individuals, and significance values, respectively.

3 Results

3.1 Biological variables

All biometric variables were normally distributed except for T_b (Table 2). Skull length was significantly longer in males than in females ($p \leq 0.05$). There were no sex differences in any other biometric variables ($p \geq 0.097$). Skull length was not measured in one male and two females, resulting in a lower number of individuals for this variable. See Appendix A for the individual biometric variables.

Table 2: Biometric variables of male and female arctic terns (*Sterna paradisaea*). Normally distributed variables were tested for sex differences with a two-tailed T-test (α = 0.05), and the non-normally distributed variables (*) were tested for sex differences with a Mann-Whitney U test (α = 0.05). Significant values are in bold.

	Males				**Females**				Sign.
Biometrics	Mean ± SD	Median	Range	n	Mean ± SD	Median	Range	n	*p*-value
Skull length (mm)	73.7 ± 1.9	73.8	71.3 – 76.5	8	70.5 ± 1.6	71.0	67.5 – 72.0	6	**0.006**
Beak length (mm)	33.4 ± 1.5	33.4	31.6 – 35.4	9	32.3 ± 1.1	32.2	31.2 – 34.5	8	0.13
Beak height (mm)	7.28 ± 0.57	7.00	6.75 – 8.40	9	7.15 ± 0.50	7.15	6.20 – 8.00	8	0.63
Wing length (cm)	27.2 ± 0.59	27.2	26.1 – 28.2	9	27.4 ± 0.54	27.4	26.8 – 28.2	8	0.43
Weight (g)	101 ± 4.8	102	95.8 – 112	9	105 ± 7.8	105	93.0 – 119	8	0.26
T_b (ºC)*	40.8 ± 0.37	40.8	40.4 – 41.7	9	41.5 ± 0.9	41.5	40.2 – 42.8	8	0.097

3.2 PFAS concentrations

9 PFASs were above the LOD in > 70 % of the individual samples. All PFAS variables were normally distributed, except for PFHxS, brPFOS, and PFNA (Table 3). Males had significantly higher concentrations of all detected PFAS ($p \leq 0.05$) except for PFHxS and PFNA ($p \geq 0.172$). In males the most dominating PFASs in decreasing order were linPFOS (41 % of ∑PFAS), PFUnDA (23 % of ∑PFAS), PFTrDA (11 % of ∑PFAS), brPFOS (7 % of ∑PFAS), PFDA (6 % of ∑PFAS), PFDoDA (5 % of ∑PFAS), PFNA (4 % of ∑PFAS), PFTeDA (2 % of ∑PFAS) and PFHxS (1 % of ∑PFAS). In females the most dominating PFASs in decreasing order were linPFOS (36 % of ∑PFAS), PFUnDA (24 % of ∑PFAS), PFTrDA (11 % of ∑PFAS), brPFOS (7 % of ∑PFAS), PFDA (7 % of ∑PFAS), PFNA (6 % of ∑PFAS), PFDoDA (4 % of ∑PFAS), PFHxS (3 % of ∑PFAS) and PFTeDA (2 % of ∑PFAS). A pie chart representation of the results is presented in Fig. 2. For simplicity, whenever a percentage is given after a PFAS throughout the rest of the thesis, this represents

that PFASs average abundance relative to the ∑PFAS if nothing else is specified. PFAS concentrations of individual arctic terns are presented in Appendix B.

Table 3: PFASs concentrations in male and female arctic terns (*Sterna paradisaea*). Normally distributed variables were tested for sex differences with a two-tailed T-test (α = 0.05), and the non-normally distributed variables (*) were tested for sex differences with a Mann-Whitney U test (α = 0.05). Significant values are in bold.

	Males				**Females**				Sign.
PFAS (ng/mL)	Mean ± SD	Median	Range	n	Mean ± SD	Median	Range	n	*p*-value
PFHxS*	0.109 ± 0.043	0.0943	0.0688 – 0.185	8	0.137 ± 0.074	0.104	0.0580 – 0.240	8	0.345
BrPFOS*	0.645 ± 0.17	0.600	0.451 – 0.973	8	0.349 ± 0.096	0.308	0.285 – 0.563	8	**0.003**
LinPFOS	4.11 ± 0.80	3.84	3.32 – 5.87	8	1.67 ± 0.96	1.36	0.383 – 3.18	8	**0.000**
∑PFOS	4.71 ± 0.96	4.38	3.85 – 6.85	8	2.02 ± 1.04	1.66	0.675 – 3.60	8	**0.000**
∑PFSA	4.86 ± 0.98	4.51	3.94 – 7.03	8	2.15 ± 1.04	1.79	0.915 – 3.79	8	**0.000**
PFNA*	0.365 ± 0.15	0.291	0.244 – 0.638	8	0.298 ± 0.12	0.251	0.154 – 0.505	8	0.172
PFDA	0.592 ± 0.13	0.571	0.402 – 0.774	8	0.327 ± 0.16	0.315	0.135 – 0.532	8	**0.003**
PFUnDA	2.32 ± 0.41	2.24	1.85 – 3.05	8	1.12 ± 0.69	0.943	0.263 – 2.15	8	**0.001**
PFDoDA	0.465 ± 0.091	0.502	0.299 – 0.594	8	0.202 ± 0.16	0.161	0.0324 – 0.439	8	**0.001**
PFTrDA	1.11 ± 0.29	1.08	0.711 – 1.64	8	0.503 ± 0.39	0.384	0.0271 – 1.05	8	**0.003**
PFTeDA	0.225 ± 0.072	0.246	0.110 – 0.303	8	0.0888 ± 0.060	0.0778	0.0202 – 0.176	8	**0.001**
∑PFCA	5.08 ± 1.1	4.82	3.94 – 7.00	8	2.54 ± 1.5	2.17	0.73 – 4.53	8	**0.002**
∑PFAS	9.90 ± 1.9	9.67	7.88 – 14.0	8	4.70 ± 2.5	3.92	1.65 – 8.29	8	**0.000**

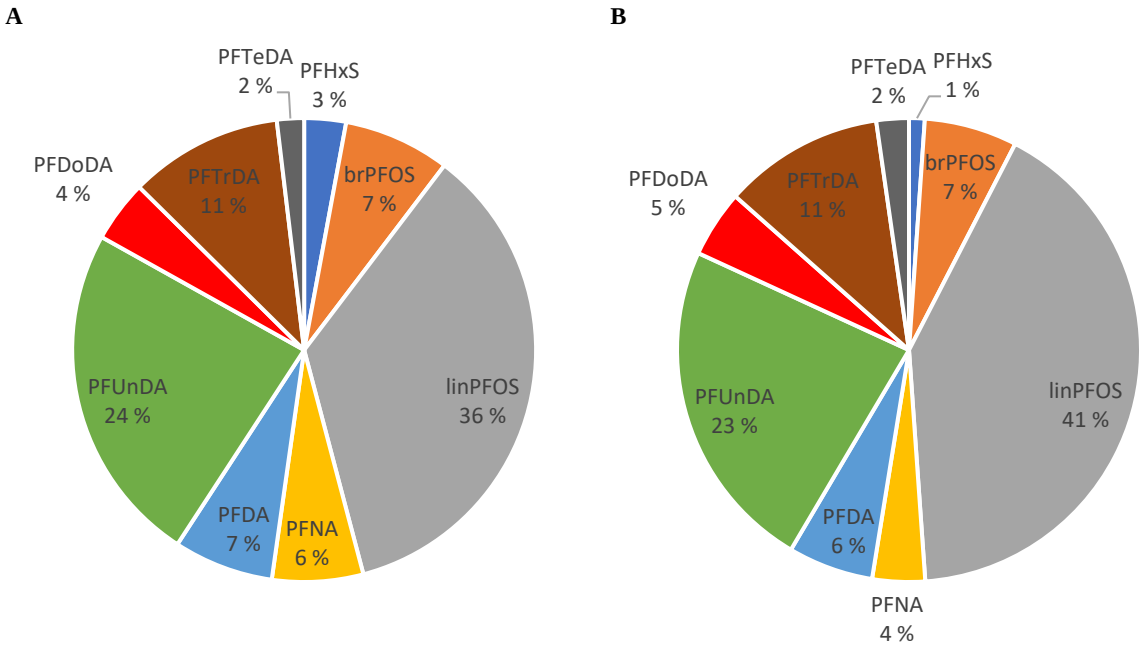

Figure 2: Pie chart representation of average relative abundance of the different PFASs in female (a) and male (b) arctic terns (*Sterna paradisaea*).

3.3 Thyroid hormones and RMR

All energetic variables were normally distributed, except for the TT4/TT3 ratio (Table 4). There were no significant sex differences between any of the variables, although the sex difference in RMR was close to being significant ($p \leq 0.058$). Individual TH concentrations and RMR values are given in Appendix F.

Table 4: Measured RMR, TT4 and, TT3 concentrations and the TT4/TT3 ratio in male and female arctic terns (*Sterna paradisaea*). Normally distributed variables were tested for sex differences with a two-tailed T-test ($\alpha = 0.05$), and the non-normally distributed variables (*) were tested for sex differences with a Mann-Whitney U test ($\alpha = 0.05$). Significant values are in bold.

	Males				**Females**				Sign.
Energetic parameter	Mean ± SD	Median	Range	n	Mean ± SD	Median	Range	n	*p*-value
TT4 (ng/mL)	24.0 ± 2.8	25.5	19.7 – 26.9	7	25.3 ± 6.2	25.4	16.8 – 34.1	8	0.388
TT3 (ng/mL)	2.53 ± 0.76	2.71	1.24 – 3.38	8	2.05 ± 1.3	1.80	0.358 – 4.47	8	0.623
TT4/TT3 ratio*	11.1 ± 5.9	8.12	5.83 – 20.8	6	18.2 ± 14	16.9	6.28 – 49.1	8	0.245
RMR (mL O_2/g*h)	3.00 ± 0.40	3.08	2.45 – 3.86	9	2.59 ± 0.31	2.61	2.16 – 3.02	6	0.058

3.4 Relationships between PFASs, THs, and RMR

3.4.1 Principle component analysis

The PCA identified three PCs with eigenvalues > 1, which was sufficient to explain the variance in the data set (Appendix H). The PCs described 59.8 % (PC1), 19.0 % (PC2) and 8.5 % (PC3) of the variation, respectively. Only the two most important components (PC1 and PC2) were displayed in the loading plot (Fig. 3), as all the relationships were explained by these two components. The loading plot suggest positive associations among PFNA, brPFOS, linPFOS, PFDA, PFTrDA, PFTeDA, PFUnDA and PFDoDA due to the tight cluster off these variables (PC1 = 0.828 – 0.992). The small angle suggests a positive association between the same PFASs and TT3 (PC1 = 0.630), and the large angle between the same PFASs and the TT4/TT3 ratio (PC1 = -0.647) suggests a negative relationship. The large angle between RMR (PC2 = 0.696) and mass (PC2 = -0.726) along PC2 suggest a negative relationship between these two variables, which could also be the case for PFHxS (PC2 = 0.765), TT3 (PC2 = -0.642) and TT4 (PC2 = -0.257). The small angle between RMR (PC1 = 0.482) and PFNA (PC1 = 0.828) along PC1 could indicate a positive association. The loading plot indicates no significant association between RMR and TT3 or TT4.

The score plot (Fig. 4), shows that males have a PC1 value of 0.785 ± 0.59 vs. a PC1 value of -0.654 ± 0.77 in females and that these differed significantly ($p = 0.008$). There is no difference in PC2 between the sexes ($p = 0.659$). The profound differences in PC1 suggest that variables with high PC1 values, such as several PFASs, are significantly different between the sexes. This can be verified in Table 3, where all PFASs except PFNA and PFHxS had higher concentrations in males, as compared to females. TT3 and RMR also had high PC1 values but did not significantly differ between the sexes, even though RMR was close ($p \leq 0.058$). Based on the PCA all PFASs, the TH variables, RMR, and mass were further tested in a correlation matrix. The variables ∑PFAS, ∑PFSA, ∑PFCA, and ∑PFOS were also included in this analysis.

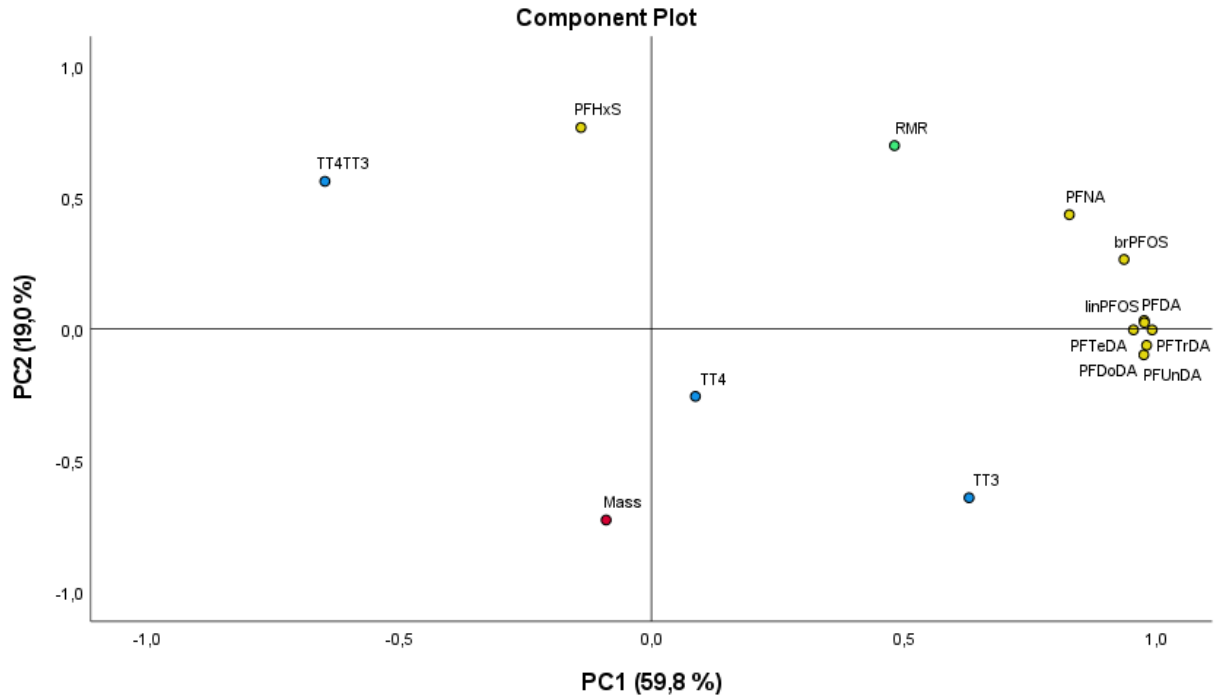

Figure 3: Loading plot of PC1 and PC2, showing how individual variables of the arctic terns (*Sterna paradisaea*) influence the principal components, and how the variables correlate with each other. PC1 and PC2 explain 59.8 % and 19.0 % of the variation in the data set.

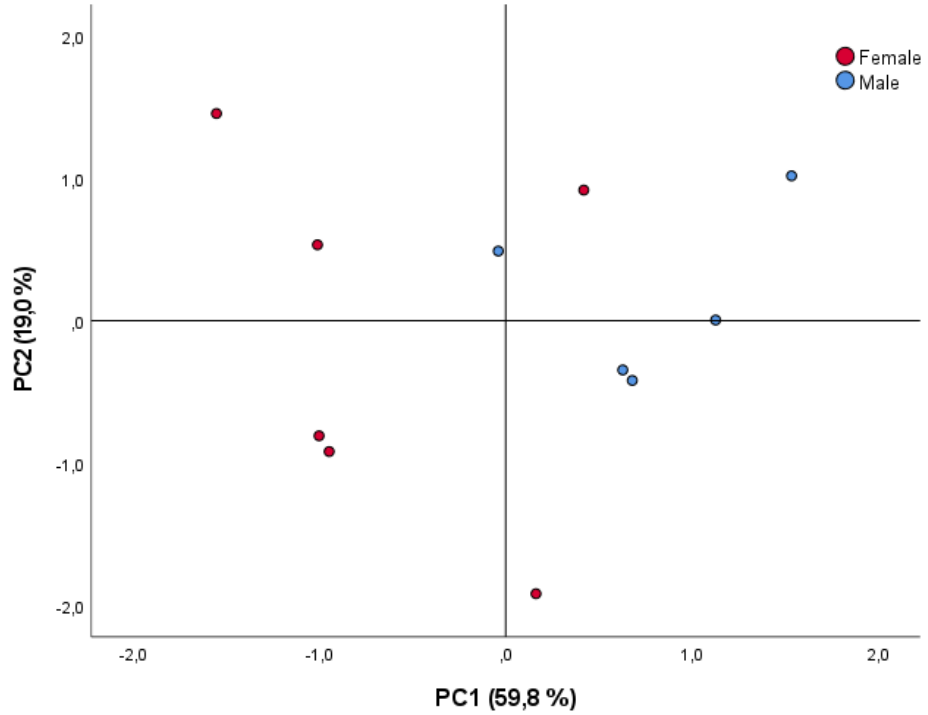

Figure 4: Score plot of PC1 and PC2, demonstrating how the individual arctic terns (*Sterna paradisaea*) affect the principal components, and how they relate to each other. PC1 and PC2 explain 59.8 % and 19.0 % of the variation in the data set. Males are marked by blue markers and females by red markers, respectively.

3.4.2 Correlation matrices

Based on the PCA, variables that appeared to correlate were tested with the Pearson's or Spearman's rank correlation coefficient (Appendix K and L). All significant correlations are presented in Table 5. For both sexes combined, there was a significant positive correlation between TT3 and brPFOS, linPFOS, ∑PFOS, ∑PFSA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, ∑PFCA, and ∑PFAS. The TT4/TT3 ratio was significantly negatively correlated with PFDoDA and PFTrDA.

In female artic terns, TT3 had a significant positive relationship with linPFOS, ∑PFOS, ∑PFSA, PFUnDA, PFDoDA, PFTrDA, and ∑PFAS. There were no significant associations between TT3 and PFASs in males. RMR and TT4 were not significantly correlated to any PFASs, nor was RMR correlated with any TH parameter in any sex, or the sexes combined. A negative association between RMR and mass was only demonstrated in females (r: -0.832, p = 0.040), but this is not shown in the correlation matrix.

Table 5: Correlations between the different PFASs, TT3 and TT4/TT3 ratio in male and female arctic terns (*Sterna paradisaea*), and all individuals combined. Correlations between normally distributed variables were tested with the Pearson correlation coefficient (α = 0.05), and the non-normally distributed variables (*) were tested with the Spearman`s rank coefficient (α = 0.05).

	Both sexes				**Females**				**Males**			
	TT3		**TT4/TT3***		**TT3**		**TT4/TT3***		**TT3**		**TT4/TT3***	
PFAS	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
PFHxS*	-	-	-	-	-	-	-	-	-	-	-	-
Branched PFOS*	0.593	0.020	-	-	-	-	-	-	-	-	-	-
Linear PFOS	0.595	0.019	-	-	0.870	0.005	-	-	-	-	-	-
∑PFOS	0.578	0.024	-	-	0.860	0.006	-	-	-	-	-	-
∑PFSA	0.566	0.028	-	-	0.833	0.010	-	-	-	-	-	-
PFNA*	-	-	-	-	-	-	-	-	-	-	-	-
PFDA	0.546	0.035	-	-	-	-	-	-	-	-	-	-
PFUnDA	0.634	0.011	-	-	0.708	0 .049	-	-	-	-	-	-
PFDoDA	0.700	0.004	-0.555	0.049	0.748	0.033	-	-	-	-	-	-
PFTrDA	0.610	0.016	-0.577	0.039	0.729	0.040	-	-	-	-	-	-
PFTeDA	0.557	0.031	-	-	-	-	-	-	-	-	-	-
∑PFCA	0.605	0.017	-	-	-	-	-	-	-	-	-	-
∑PFAS	0.594	0.020	-	-	0.755	0.030	-	-	-	-	-	-

The relationships between TT3 and PFDoDA, PFUnDA, PFTrDA, ∑PFOS, ∑PFCA, and ∑PFAS are presented in Fig. 5. Linear regressions are constructed for both sexes combined and females alone. Note that brPFOS, linPFOS, ∑PFSA, PFDA, and PFTeDA also had significant positive correlations with TT3 in the correlation matrix (Table 5), but these are not presented as linear regressions in Fig. 5. The same applies to the significant negative correlations between PFDoDA, PFTrDA, and the TT4/TT3 ratio.

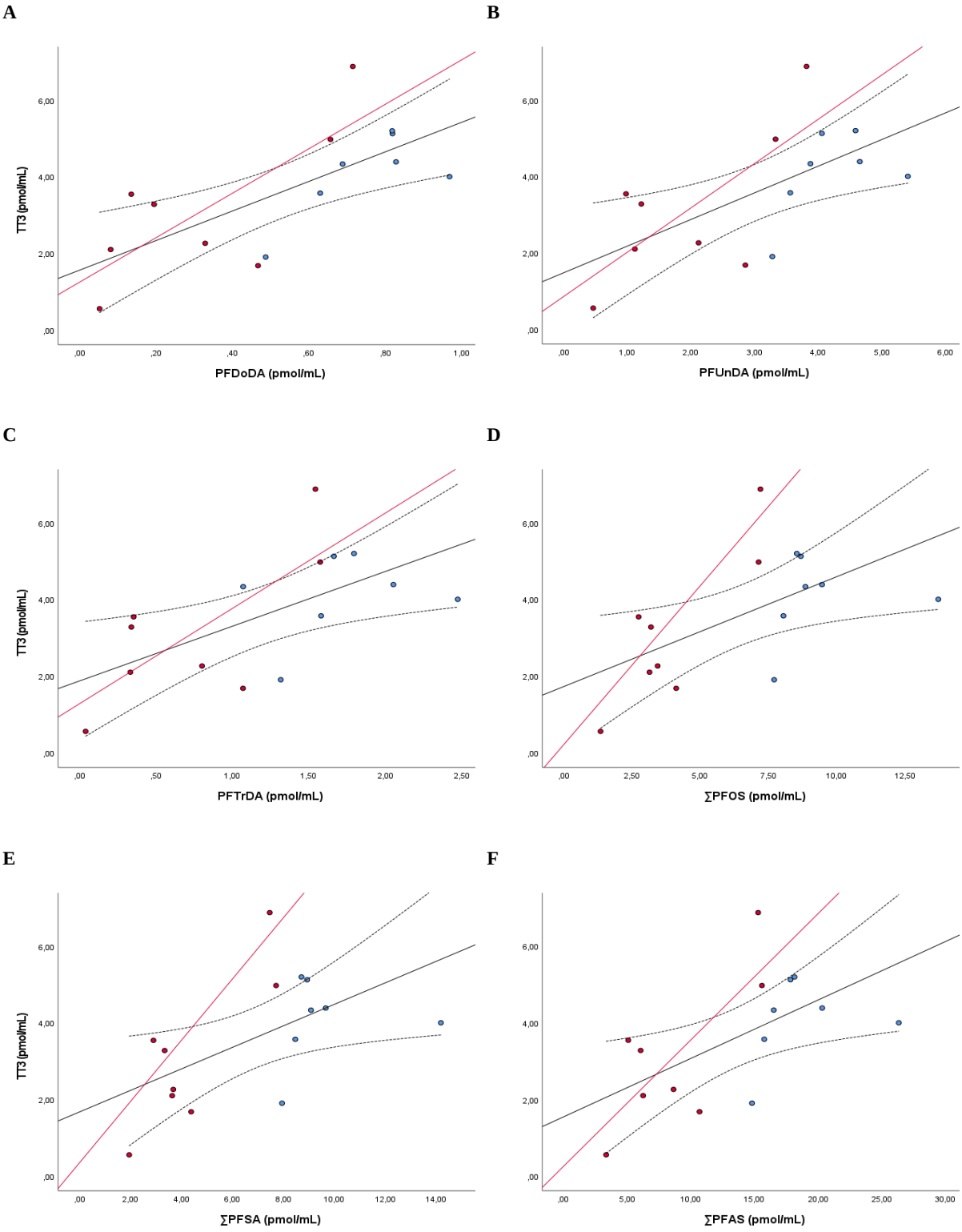

Figure 5: Linear regressions (± 95 % confidence interval) with TT3 (pmol/mL) plotted against PFDoDA (**A**, R^2: 0.490), PFUnDA (**B**, R^2: 0.402), PFTrDA (**C**: R^2: 0.372), ∑PFOS (**D**, R^2: 0.335), ∑PFSA (**E**, R^2: 0.320) and ∑PFAS (**F**, R^2: 0.353) in arctic terns (*Sterna paradisaea*) breeding in Kongsfjorden. Males are marked by blue markers, and females by red markers. The red regression line shows the significant correlation for females independently.

4 Discussions

4.1 PFAS concentrations

The present study reports PFAS concentrations in whole blood, whereas most other studies report it in plasma. When comparing results, this should be corrected for and according to Ehresman et al., (2007), the ratio between PFAS concentrations in human plasma/serum and whole blood is approximately 2:1. It is reasonable to assume it is the same for avian species. The corrected concentrations (whole blood concentrations x 2) of PFASs detected in the arctic tern males (19.8 ± 3.8 ng/mL) and females (9.40 ± 5.0 ng/mL) are relatively low compared to other seabird species in Kongsfjorden such as glaucous gulls, northern fulmars and kittiwakes (Blévin et al., 2017; Melnes et al., 2017; Nost et al., 2012). The exceptions are the PFAS concentrations reported in kittiwake chicks by Nost et al., (2012) (Fig. 6), and the levels of linPFOS reported in kittiwake adults by Ask et al., (2020) (Fig. 7), which are more similar to the concentrations reported herein. Kittiwakes occupy a trophic level of 3.3 – 3.8 (Hop et al., 2002) and have a diet similar to that of arctic terns, mainly comprised of caplin, polar cod, and crustaceans (Vihtakari et al., 2018). This fact, and the similar blood concentrations of PFASs, could imply that arctic terns occupy a trophic level similar to kittiwakes, but there is no direct evidence of this in the literature.

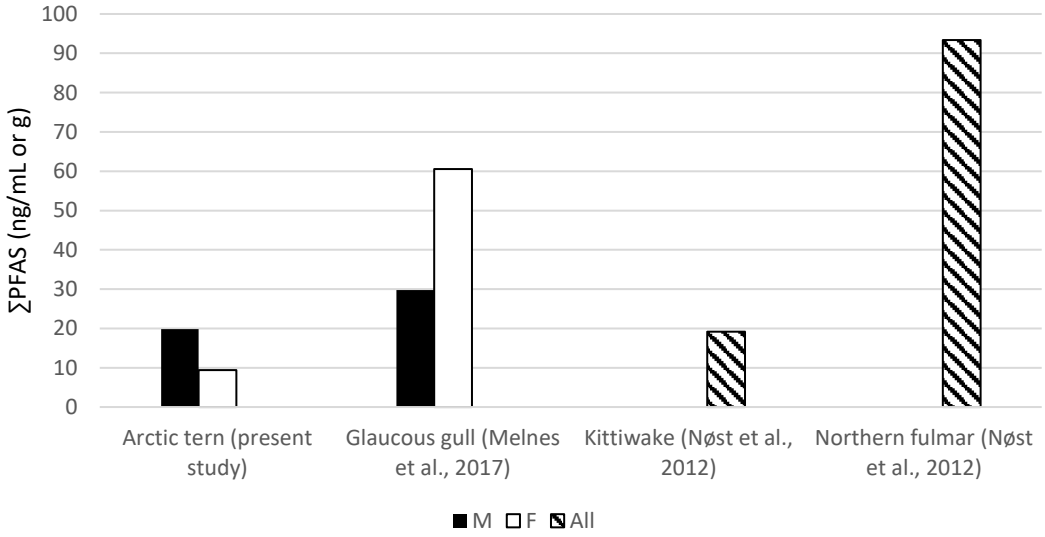

Figure 6: Column chart visualizing concentration differences of ∑PFAS reported in arctic terns (*Sterna paradisaea*), glaucous gull (*Larus hyperboreus*), black-legged kittiwakes (*Rissa tridactyla*), and northern fulmars (*Fulmarus glacialis*). Males are represented by black columns, females by white columns, and all individuals by black and white columns, respectively. Arctic tern concentrations are corrected for whole blood. The unit of measurement is ng/mL or g because some studies reported concentrations as ng/mL while some reported ng/g.

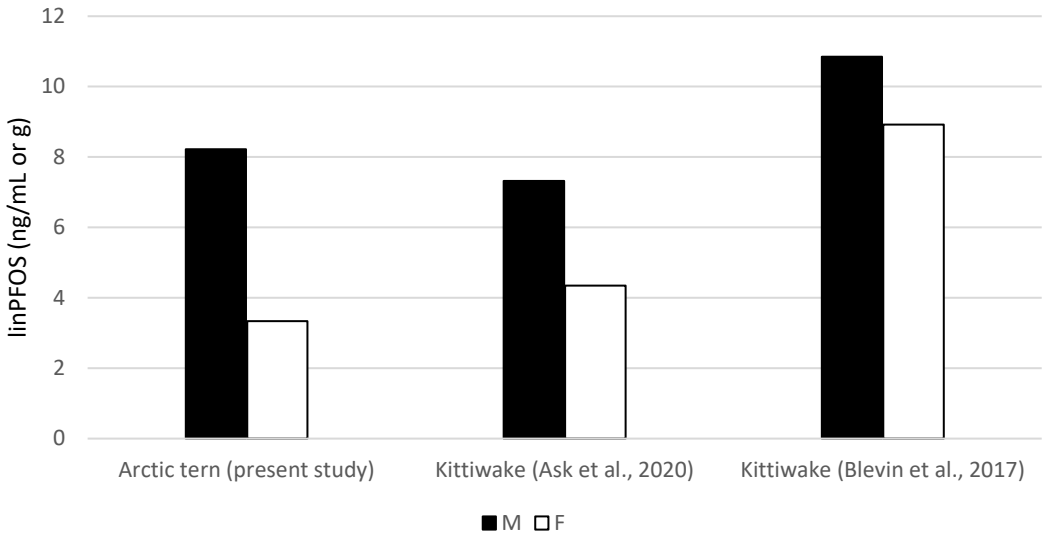

Figure 7: Column chart visualizing concentration differences of linPFOS reported in arctic terns (*Sterna paradisaea*) and black-legged kittiwakes (*Rissa tridactyla*). Males are represented by black columns and females by white columns, respectively. Arctic tern concentrations are corrected for whole blood. The unit of measurement is ng/mL or g because some studies reported concentrations as ng/mL while some reported ng/g.

The present study also shows significant sex differences in concentrations, for most of the measured PFASs. Males had twice as high concentrations of ∑PFASs compared to females and substantially higher concentrations of most individual PFASs. Although this may be due to differences in diet or biotransformation capacity, the most likely explanation is that females deposit PFASs in their eggs during reproduction. Maternal transfer of PFAS is demonstrated in several arctic sea birds including great skuas and glaucous gulls (Leat et al., 2013; Verreault et al., 2005), and has also been documented for PCBs and DDT in arctic terns (Lemmetyinen and Rantamäki, 1980). Elevated PFAS concentrations in males, as compared to females, have also been documented for other arctic seabirds such as kittiwakes (Ask et al., 2020; Blévin et al., 2017). Considering maternal transfer is the reason for this sex difference, detecting it in wildlife will probably depend on if the sampling is done before, during, or after the breeding season.

4.2 PFAS patterns

To my knowledge, there has only been one other study related to PFASs in terns (Van der Schyff et al., 2020), where the egg concentrations of PFASs in fairy terns (*Gygis alba*) and sooty terns (*Onychoprion fuscatus*) on St. Brandon's atoll (Western Indian ocean) were investigated. Comparing relative PFAS abundance between different matrixes, such as egg and whole blood is not ideal, as different PFASs might have a different accumulation potential in the different matrixes. Besides, when eggs are investigated, the results will only reflect the female body burden of contaminants. In the egg yolk of the fairy terns and sooty terns, the dominating PFASs were PFUnDA (31,9 %), PFOS (28,2 %), PFNA (12,9 %) and PFOS (28,9 %), PFUnDA (27,1 %), PFNA (10,8 %) respectively (van der Schyff et al., 2020). In the present study, the dominating PFASs in the female arctic tern whole blood, which is most appropriate to compare to the fairy and sooty tern egg content, were ∑PFOS (42 %), PFUnDA (24 %), and PFTrDA (11 %). These differences in relative abundance, particularly concerning PFOS could indicate different accumulation potential of the PFASs between the different matrixes, but it could also be evidence for differences in PFAS occurrence at the locations where the studies have been conducted, West Indian ocean and the Barents-sea, respectively. It is established that the southern hemisphere will have lower concentrations of POPs compared to the northern hemisphere. This is primarily because the production and usage of such compounds is, and has historically, been much lower in the southern hemisphere (Kallenborn et al., 1998, 2013). Hence, these data imply that there will be a difference in, not only PFAS concentrations but also PFAS composition in animals between the two hemispheres. With this information, it is reasonable to assume that the arctic terns acquire most of their accumulated POPs at the breeding grounds (northern hemisphere) and possibly during migration.

Different PFASs will have different origins, and the relative abundance of the different PFASs can be used to identify the main contamination sources (Langberg et al., 2021, submitted paper). PFOS is used in many applications, incl. fire-fighting aqueous film-forming foams (AFFF) (Leeson et al., 2021), and detection of high PFOS concentrations can often indicate proximity to airports where AFFF is utilized and discharged during fire-fighting drills and accidents (Awad et al., 2011; Kwadijk et al., 2014). Ny-Ålesund has an airport, where such drills are conducted, and this could help to explain the high relative abundance of ∑PFOS in the arctic terns (48 % in males, 42 % in females). To my knowledge, there is no

data available on the utilization and discharge of chemicals from the airport in Ny-Ålesund, but it is very likely PFAS containing AFFF has been used here, and it can also have been used in relation to mining activity (Granberg et al., 2017).

On St. Brandon`s atoll, where the fairy and sooty terns were sampled, there is no airport (Wild on the fly, 2021) and this could help to explain the low relative abundance of PFOS (28,2 % in fairy terns, 28,9 % in sooty terns) compared to the arctic terns sampled in Kongsfjorden. Long chained PFCAs, such as PFUnDA, PFNA and PFTrDA are more associated with LRT (Langberg et al., 2021, submitted paper), and this could suggest that the tropical terns on St. Brandon`s atoll are primarily exposed to LRT PFAS, whereas the arctic terns in Kongsfjorden are exposed to local sources in addition to LRT compounds.

Another parameter that can be useful in determining the source of PFOS, is the composition of linear and branched isomers. A recently discharged mixture of PFOS will have a linPFOS fraction similar to that of a freshly produced mixture (76 – 79 % of ∑PFOS) (Kärrman et al., 2007). LRT PFOS will have a higher fraction of linPFOS because the brPFOS fraction will decline more rapidly in the biosphere (Houde et al., 2008). The linPFOS percentage in the present study (87 % ∑PFOS for males, 83 % ∑PFOS for females), implies that the PFOS probably originate from LRT, but local sources cannot be excluded.

Identical to the present study, the dominating PFASs in male and female kittiwakes, glaucous gulls, and arctic skuas sampled in Kongsfjorden between 2011 and 2014 were PFOS, PFUnDA, and PFTrDA (Ask, 2015; Ask et al., 2020; Melnes et al., 2017). PFOS was also dominating in chicks of kittiwakes and northern fulmars sampled in Kongsfjorden in 2006 (Nost et al., 2012). However, in contrast to this, Blévin et al., (2017) reported a higher abundance of PFUnDA and PFTrDA than PFOS in male and female kittiwakes. Note, that in the study of Blévin et al., (2017), only the concentration of linPFOS was reported, and the concentration of ∑PFOS could potentially be higher than PFUnDA and PFTrDA. Regardless, the pattern of relative PFAS abundance in blood from sea birds in the Kongsfjorden appear to be very consistent, and independent of species, sex, and sampling year. The same pattern was seen in lesser black-backed gulls (*Larus fuscus*) sampled in Helgeland in 2005 (Bustnes et al., 2008).

The fact that PFOS is the dominating PFAS in almost all the seabirds in Kongsfjorden, could potentially reflect the presence of the nearby airport or another local source. This pattern also reflects the present regulation of PFAS. PFOA, the only PFAS listed under Annex A

(Stockholm Convention, 2021d) and therefore completely banned, is detected in very low amounts. PFOS, which was detected in high amounts, is also listed on the Stockholm convention, but under Annex B, and is thus still allowed to use in applications, where no other technically feasible alternatives exist (Stockholm Convention, 2021e). Regarding AFFF however, Norway banned the use of AFFF containing PFOS in 2007, but remains from earlier use may still be present, and the AFFF may contain other PFASs (Miljødirektoratet, 2021). The other dominating PFASs, PFUnDA, and PFTrDA are not listed on the Stockholm convention. All in all, the PFAS pattern in seabirds in Kongsfjorden are consistent and could indicate some local source, though LRT likely is the main exposure source.

4.3 TH concentrations and RMR

Plasma concentrations of THs have, to my knowledge never been reported in arctic terns. Evaluating if the measured concentrations of TT3 and TT4 are within the normal range of arctic terns is therefore impossible. Regardless, circulating THs are subject to great fluctuations, and the concentrations will depend on season, body condition, age, iodine content in the diet, time of day, etc. According to McNabb, (2007), normal avian plasma concentrations of THs are usually between 5-15 ng/mL for T4 and 0,5-4 ng/mL for T3. The TT4 concentrations measured in the arctic terns in this study (24.0 ± 2.8 for males, 25.3 ± 6.2 for females) can therefore be regarded as relatively high, but still similar to concentrations reported in kittiwakes and glaucous gulls (Melnes et al., 2017; Nost et al., 2012; Verreault et al., 2007). The TT3 concentrations (2.53 ± 0.76 for males, 2.05 ± 1.3 for females) are within the normal range of other birds (McNabb, 2007). The TT4 results should be treated with care, as the average %CV for the duplicates was very high (12.3 ± 13.1, n = 17) (Appendix F), indicating large variation and therefore large uncertainty. However, the TT3/TT4 ratio was 0.08 ± 0.048 for females and 0.11 ± 0.046 for males, which is similar to concentrations reported in Japanese quail (*Coturnix japonica*) and ring doves (*Streptopelia risorii*), where T3 made up around 8% of gland hormone content, during mid to late incubation was (McNabb et al., 1985; McNichols and McNabb, 1988). This provides some support that the measured TT4 values are reliable, despite the high %CV.

According to unpublished results, the BMR of the arctic tern is reported to be 2.06 mL O_2/g*h (n = 4), with a LCT of 28-30 ºC (Appendix M) (C Bech 2021, personal communication). This

indicates that our MR values of 3.00 ± 0.04 (males) and 2.59 ± 0.31 (females) mL O_2/g*h, measured at 19.29 ºC ± 1.68, might not be actual BMR values and should rather be regarded as RMR. This is likely because the T_a (19.29 ± 1.68) during the measurements, was below the arctic terns LCT of 28-30 ºC (C Bech 2021, personal communication). A rough analysis of the unpublished graph by Claus Bech and Marcel Klaassen (Appendix M), displaying MRs at different T_a, clearly demonstrates that the MRs measured at 18-20 ºC, are in the range of 2,5 – 3,0 mL O_2/g*h. This agrees with our results at the same T_a. Compared to other arctic seabird species, such as kittiwakes (Blévin et al., 2017) and glaucous gulls (Verreault et al., 2007), the mass-specific MR in arctic tern is relatively high, reflecting the small body mass. Body mass is a parameter known to be inversely correlated with BMR because animals with a higher body mass will have a smaller surface area to volume ratio, and correspondingly lower thermal conductance (Ellis and Gabrielsen, 2001; Gabrielsen, 2009). In the present study, females demonstrated a negative correlation between body mass and RMR, which is an expected result for the same reasons mentioned above.

4.4 Relationship between PFASs, THs, and RMR

Plasma concentrations of TT3 showed a strong positive association with whole blood concentrations of linPFOS, brPFOS, PFDA, PFUnDA, PFDoDA, PFTrDA, and PFTeDA in the arctic terns, but this was not the case for TT4. This relationship was also documented for plasma concentrations of PFOS and TT3 in female glaucous gulls by Melnes et al., (2017), and for hepatic concentrations of PFCAs and plasma concentrations of TT3 in northern fulmars (Braune et al., 2011). This could suggest a higher sensitivity toward effects from PFASs for T3 than for T4. However, in chicks of northern fulmars and kittiwakes, Nost et al., (2012), also documented positive correlations between PFHpS, PFNA, ∑PFOS, and PFOA and TT4 (in some cases also FT4), but this could potentially be related to age. Also, in the study of Ask et al., (2020), adult male kittiwakes had positive correlations between linPFOS and PFDA and TT4, whereas in adult female kittiwakes PFDoDA, PFTrDA, and PFTeDA was positively correlated with TT3. In conclusion, there is no obvious trend in the existing literature on how PFASs correlate with THs in arctic seabirds. Relationships can be observed because of exposure to both PFSAs and/or PFCAs, and both T3 and/or T4 can be altered. However, only T3 was altered in the present study. Considering sex specificity, correlations

appear to be more frequent in females as is demonstrated in this study, but relationships between THs and specific PFASs have also been reported in males only (Ask et al., 2020).

It could be that PFASs have an equal sensitivity for both T3 and T4, but that T4-effects are not seen in the present study due to a high variance in the TT4 measurements (12.3 ± 13.1), providing inaccurate values. A possible explanation as to why no significant relationships between PFASs and THs were seen in males alone, could be the lower variation in most detected PFASs, compared to the females. For correlation studies, where the relationship between two variables is investigated, a large variance in the explanatory variable (x-axis) is favorable, as this will make a potential relationship more visual.

The TT4/TT3 ratio is also a common biomarker of exposure (Peakall, 1992), and this was negatively associated with PFDoDA and PFTrDA in both sexes combined. This is to my knowledge the first study detecting a negative correlation between the TT4/TT3 ratio and PFAS, but it has been observed as a response to OC exposure in male glaucous gulls on Bear island (Verreault et al., 2004). The relationship observed in the present study is likely a result of the increased concentrations of TT3 and relatively unchanged concentrations of TT4. Therefore, the negative relationship between PFDoDA, PFTrDA, and TT4/TT3, and the positive relationship between the same PFASs and TT3, are probably explained by the same underlying MOA.

There have been conducted several studies regarding the effects of OCs on avian TH homeostasis, and the most evident pattern is a clear negative correlation between OCs and THs (Blévin et al., 2017; Braune et al., 2011; Melnes et al., 2017; Verreault et al., 2004). The MOA explaining this effect is largely understood and the theory is that OCs and BFRs, such as PCBs and PBDEs, including their hydroxylated metabolites, will bind to TH transporting plasma proteins, particularly TTR, displacing bound T3 and T4. This binding affinity to TTR is a result of a high structural resemblance to T3 and T4. This is particularly relevant for the hydroxylated metabolites of the mentioned compounds as they will have a bound OH-group identical to T3 and T4 (Ucán-Marin et al., 2010; Ucán-Marín et al., 2009). This results in increased concentrations of unbound hormone, which is easily excreted by the kidneys and will lead to decreased concentrations of total TH. This MOA has been documented in several studies both experimentally (Ucán-Marin et al., 2010; Ucán-Marín et al., 2009) and in silico (Mortensen et al., 2020). Other MOA, includes chemically induced inhibition of iodothyronine deiodinase enzymes, subsequently resulting in a skewed T4/T3 ratio.

Correlations between PBDE concentrations and hepatic iodothyronine deiodinase type 1 activity, has been observed in ringed billed gulls (*Larus delawarensis*) (François et al., 2016).

Endocrine-disrupting effects from PFASs have received much less attention, and there are still large knowledge gaps considering the MOA behind the apparent thyroid disrupting effect. It is established that PFASs can bind to TH transporting plasma proteins. This is not due to structural resemblance as is the case with many OCs and BFRs (Ucán-Marin et al., 2010; Ucán-Marín et al., 2009), but because of their amphiphilic nature and correspondingly high protein affinity. PFASs have, experimentally and *in silico*, been documented to bind to TTR (Mortensen et al., 2020; Ren et al., 2016), but the main transport protein, at least for PFOS, PFOA, PFNA, PFHxS, and PFDA, is likely albumin (Forsthuber et al., 2020). This MOA, i.e., TH displacement from transport proteins by competitive binding, seems remarkably similar to that of many OCs and BFRs. Paradoxically, the measured endpoint is the opposite. If THs were displaced, this should lead to an increased renal excretion, and therefore a negative correlation, as has been demonstrated with many legacy POPs (Blévin et al., 2017; Braune et al., 2011; Melnes et al., 2017; Verreault et al., 2004). This leaves the question if THs are being displaced from the transport proteins. One possibility, suggested by Ask et al., (2020), is that birds with naturally high TH concentrations (which can vary a lot between individuals), may also have a higher concentration of albumin, and therefore more substrate for PFASs to bind with. If this is the case, the elevated concentrations of TT3 due to PFAS exposure in the arctic terns, cannot be regarded as an effect, but rather a predisposition. If T3 concentrations are linked to albumin concentrations, birds with naturally higher concentrations of T3 will have a predisposition to accumulate more PFASs than birds with naturally low concentrations of T3, and therefore you would see a positive correlation between T3 and PFASs. Unfortunately, albumin, or any other TH transporting protein, was not analyzed in the present study, due to a lack of blood sample volume, so this is only speculative.

A variable that was not measured in this study due to a lack of sample but could have provided valuable information is the level of unbound T3 and T4. If THs are displaced from albumin by PFASs, there would be an increase in the concentration of FT3 and/or FT4. This would not necessarily assist in explain the elevated concentrations of TT3 detected in this study, but it could provide some understanding of the MOA. An increased fraction of FT3 (additional to TT3) has been detected as a response to PFOS in glaucous gulls (Melnes et al., 2017), and an increase in FT4 (additional to TT4) has been detected as a response to PFHpS,

PFNA, and linPFOS in chicks of kittiwakes (Nost et al., 2012). An increased level of unbound TH could indicate that THs are displaced from TTR and albumin by competitive binding with PFAS, but it does not explain why there would be an increase in the level of total TH in the mentioned studies. It could also imply that birds with a naturally higher level of total TH, also would have a naturally higher level of free TH.

Herein, RMR was not correlated with any PFASs in arctic terns, nor was it correlated to TT3 or TT4. This is in contrast with the positive relationship between MR and PFTrDA documented in female kittiwakes (Blévin et al., 2017), to my knowledge the only study demonstrating this particular relationship in any vertebrate. In the study of Blévin et al., (2017), PFTrDA did not correlate with T3, nor did T3 correlate with the MR. This is surprising, considering that T3 is the primary metabolism-regulating hormone, and has been documented to be correlated with BMR in many avian species (Bobek et al., 1977; Chastel et al., 2003; Welcker et al., 2013). Why MR and T3 did not correlate in the present study, nor the study of Blévin et al., (2017), could be because these studies only reported MR values, and only true BMR will have a strong association with T3 (Bobek et al., 1977; Chastel et al., 2003; Welcker et al., 2013). The fact that BMR was not measured in this study could also be a reason as to why no correlation was seen between MR and any PFASs, but other confounding factors could also be responsible for this. Also, considering that only one other study (Blévin et al., 2017) has documented a relationship between one specific PFAS and MR, one cannot claim for certain that this is a physiological effect of PFASs on all avian species.

5 Conclusion

PFASs were detected in whole blood of brooding arctic terns in Kongsfjorden, and males had significantly higher concentrations than females. The pattern of PFASs was similar to previously investigated seabird species in Kongsfjorden. The composition of PFASs indicates the arctic terns could be exposed to some local sources additional to LRT, though this needs to be further investigated. Several PFASs demonstrated a significant positive correlation with TT3 in both sexes combined and in females alone, whereas TT4 and RMR did not correlate with any PFASs in either of the sexes. The MOA of PFASs on the thyroid hormone system is poorly understood in the literature, but there are implications that high PFAS concentrations could be a result of high plasma protein concentrations. For future research, it would therefore be recommended to measure plasma protein concentrations in addition to TH concentrations, as this would provide clues on the MOA, but experimental studies should also be conducted. The ecological consequences elevated TH concentrations might impose on the arctic terns are not known. In the present study, RMR was not affected, but THs are hormones with multiple physiological functions, and prolonged elevation could potentially have other life-history impacts not yet investigated.

References

Anker-Nilssen, T., Bakken, V., Strøm, H., Golovkin, A., Bianki, V., Tatarinkova, I., 2000. The Status of Marine Birds Breeding in the Barents Sea Region, Waterbirds: The International Journal of Waterbird Biology. https://doi.org/10.2307/1522196

Ask, A.V., 2015. Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs) Affect the Thyroid Hormone System, Body Condition, and Body Mass in Two Arctic Seabird Species. NTNU.

Ask, A.V., Jenssen, B.M., Tartu, S., Angelier, F., Chastel, O., Gabrielsen, G.W., 2020. Per- and polyfluoroalkyl substances (PFASs) are positively associated with thyroid hormones in an arctic seabird. Environ. Toxicol. Chem. https://doi.org/10.1002/etc.4978

Awad, E., Zhang, X., Bhavsar, S.P., Petro, S., Crozier, P.W., Reiner, E.J., Fletcher, R., Tittlemier, S.A., Braekevelt, E., 2011. Long-term environmental fate of perfluorinated compounds after accidental release at Toronto airport. Environ. Sci. Technol. 45, 8081–8089. https://doi.org/10.1021/es2001985

Bantock, T.M., Prys-Jones, R.P., Lee, P.L.M., 2008. New and improved molecular sexing methods for museum bird specimens. Mol. Ecol. Resour. 8, 519–528. https://doi.org/10.1111/j.1471-8286.2007.01999.x

Bech, C., Langseth, I., Moe, B., Fyhn, M., Gabrielsen, G.W., 2002. The energy economy of the arctic-breeding Kittiwake (Rissa tridactyla): A review. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 133, 765–70. https://doi.org/10.1016/S1095-6433(02)00153-8

Blévin, P., Tartu, S., Ellis, H.I., Chastel, O., Bustamante, P., Parenteau, C., Herzke, D., Angelier, F., Gabrielsen, G.W., 2017. Contaminants and energy expenditure in an Arctic seabird: Organochlorine pesticides and perfluoroalkyl substances are associated with metabolic rate in a contrasted manner. Environ. Res. 157, 118–126. https://doi.org/10.1016/j.envres.2017.05.022

Bligh, J., Johnson, K.G., 1973. Glossary of terms for thermal physiology. J. Appl. Physiol. 35, 941–961. https://doi.org/10.1152/jappl.1973.35.6.941

Boas, M., Feldt-Rasmussen, U., Skakkebæk, N.E., Main, K.M., 2006. Environmental chemicals and thyroid function. Eur. J. Endocrinol. 154, 599–611. https://doi.org/10.1530/eje.1.02128

Bobek, S., Jastrzebski, M., Pietras, M., 1977. Age-related changes in oxygen consumption and plasma thyroid hormone concentration in the young chicken. Gen. Comp. Endocrinol. 31, 169–174. https://doi.org/10.1016/0016-6480(77)90014-4

Bourgeon, S., Riemer, A.K., Tartu, S., Aars, J., Polder, A., Jenssen, B.M., Routti, H., 2017. Potentiation of ecological factors on the disruption of thyroid hormones by organo-halogenated contaminants in female polar bears (Ursus maritimus) from the Barents Sea. Environ. Res. 158, 94–104. https://doi.org/10.1016/j.envres.2017.05.034

Braune, B.M., Outridge, P.M., Fisk, A.T., Muir, D.C.G., Helm, P.A., Hobbs, K., Hoekstra, P.F., Kuzyk, Z.A., Kwan, M., Letcher, R.J., Lockhart, W.L., Norstrom, R.J., Stern, G.A., Stirling, I., 2005. Persistent organic pollutants and mercury in marine biota of the Canadian Arctic: An overview of spatial and temporal trends. Sci. Total Environ., Contaminants in Canadian Arctic Biota and Implications for Human Health 351–352, 4–56. https://doi.org/10.1016/j.scitotenv.2004.10.034

Braune, B.M., Trudeau, S., Jeffrey, D.A., Mallory, M.L., 2011. Biomarker responses associated with halogenated organic contaminants in northern fulmars (Fulmarus

glacialis) breeding in the Canadian Arctic. Environ. Pollut., Nitrogen Deposition, Critical Loads and Biodiversity 159, 2891–2898. https://doi.org/10.1016/j.envpol.2011.04.036

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P., 2011. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. Integr. Environ. Assess. Manag. 7, 513–541. https://doi.org/10.1002/ieam.258

Bustnes, J.O., Borgå, K., Erikstad, K.E., Lorentsen, S.-H., Herzke, D., 2008. Perfluorinated, brominated, and chlorinated contaminants in a population of lesser black-backed gulls (Larus fuscus). Environ. Toxicol. Chem. 27, 1383–1392. https://doi.org/10.1897/07-473.1

Cabrerizo, A., Muir, D.C.G., De Silva, A.O., Wang, X., Lamoureux, S.F., Lafrenière, M.J., 2018. Legacy and Emerging Persistent Organic Pollutants (POPs) in Terrestrial Compartments in the High Arctic: Sorption and Secondary Sources. Environ. Sci. Technol. 52, 14187–14197. https://doi.org/10.1021/acs.est.8b05011

Carravieri, A., Burthe, S.J., de la Vega, C., Yonehara, Y., Daunt, F., Newell, M.A., Jeffreys, R.M., Lawlor, A.J., Hunt, A., Shore, R.F., Pereira, M.G., Green, J.A., 2020. Interactions between Environmental Contaminants and Gastrointestinal Parasites: Novel Insights from an Integrative Approach in a Marine Predator. Environ. Sci. Technol. 54, 8938–8948. https://doi.org/10.1021/acs.est.0c03021

Chastel, O., Lacroix, A., Kersten, M., 2003. Pre-breeding energy requirements: Thyroid hormone, metabolism and the timing of reproduction in house sparrows Passer domesticus. J. Avian Biol. - J AVIAN Biol. 34, 298–306. https://doi.org/10.1034/j.1600-048X.2003.02528.x

de Wit, C.A., Alaee, M., Muir, D.C.G., 2006. Levels and trends of brominated flame retardants in the Arctic. Chemosphere, Brominated Flame Retardants (BFRs) in the Environment 64, 209–233. https://doi.org/10.1016/j.chemosphere.2005.12.029

de Wit, C.A., Herzke, D., Vorkamp, K., 2010. Brominated flame retardants in the Arctic environment — trends and new candidates. Sci. Total Environ., Levels, trends and effects of legacy and new persistent organic pollutants in the Arctic: An AMAP Assessment 408, 2885–2918. https://doi.org/10.1016/j.scitotenv.2009.08.037

Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. Endocr. Rev. 30, 293–342. https://doi.org/10.1210/er.2009-0002

Dietz, R., Letcher, R.J., Desforges, J.-P., Eulaers, I., Sonne, C., Wilson, S., Andersen-Ranberg, E., Basu, N., Barst, B.D., Bustnes, J.O., Bytingsvik, J., Ciesielski, T.M., Drevnick, P.E., Gabrielsen, G.W., Haarr, A., Hylland, K., Jenssen, B.M., Levin, M., McKinney, M.A., Nørregaard, R.D., Pedersen, K.E., Provencher, J., Styrishave, B., Tartu, S., Aars, J., Ackerman, J.T., Rosing-Asvid, A., Barrett, R., Bignert, A., Born, E.W., Branigan, M., Braune, B., Bryan, C.E., Dam, M., Eagles-Smith, C.A., Evans, M., Evans, T.J., Fisk, A.T., Gamberg, M., Gustavson, K., Hartman, C.A., Helander, B., Herzog, M.P., Hoekstra, P.F., Houde, M., Hoydal, K., Jackson, A.K., Kucklick, J., Lie, E., Loseto, L., Mallory, M.L., Miljeteig, C., Mosbech, A., Muir, D.C.G., Nielsen, S.T., Peacock, E., Pedro, S., Peterson, S.H., Polder, A., Rigét, F.F., Roach, P., Saunes, H., Sinding, M.-H.S., Skaare, J.U., Søndergaard, J., Stenson, G., Stern, G., Treu, G., Schuur, S.S., Víkingsson, G., 2019. Current state of knowledge on biological effects from contaminants on arctic wildlife and fish. Sci. Total Environ. 696, 133792. https://doi.org/10.1016/j.scitotenv.2019.133792

Egevang, C., Stenhouse, I.J., Phillips, R.A., Petersen, A., Fox, J.W., Silk, J.R.D., 2010. Tracking of Arctic terns Sterna paradisaea reveals longest animal migration. Proc. Natl. Acad. Sci. 107, 2078–2081. https://doi.org/10.1073/pnas.0909493107

Ehresman, D.J., Froehlich, J.W., Olsen, G.W., Chang, S.-C., Butenhoff, J.L., 2007. Comparison of human whole blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals. Environ. Res. 103, 176–184. https://doi.org/10.1016/j.envres.2006.06.008

Ellis, H.I., 1984. Energetics of Free-Ranging Seabirds. https://doi.org/10.1007/978-1-4684-4859-7_10

Ellis, H.I., Gabrielsen, G.W., 2001. Energetics of Free-Ranging Seabirds. Biol. Mar. Birds. https://doi.org/10.1201/9781420036305.ch11

Fijn, R., Hiemstra, D., Phillips, R., van der Winden, J., 2013. Arctic Terns Sterna paradisaea from the Netherlands Migrate Record Distances Across Three Oceans to Wilkes Land, East Antarctica. Ardea -Wagening.- 101, 3–12. https://doi.org/10.5253/078.101.0102

Forsthuber, M., Kaiser, A.M., Granitzer, S., Hassl, I., Hengstschläger, M., Stangl, H., Gundacker, C., 2020. Albumin is the major carrier protein for PFOS, PFOA, PFHxS, PFNA and PFDA in human plasma. Environ. Int. 137, 105324. https://doi.org/10.1016/j.envint.2019.105324

François, A., Técher, R., Houde, M., Spear, P., Verreault, J., 2016. Relationships between polybrominated diphenyl ethers and transcription and activity of type 1 deiodinase in a gull highly exposed to flame retardants. Environ. Toxicol. Chem. 35, 2215–2222. https://doi.org/10.1002/etc.3372

Gabrielsen, G.W., 2009. Seabirds in the Barents Sea, in: Ecosystem Barents Sea. pp. 415–452.

Gabrielsen, G.W., Mehlum, F., Karlsen, H., 1988. Thermoregulation in four species of Arctic seabirds. J. Comp. Physiol. B 157, 703–708. https://doi.org/10.1007/BF00691000

Giesy, J.P., Kannan, K., 2002. Perfluorochemical surfactants in the environment. Environ. Sci. Technol. 36, 146A-152A. https://doi.org/10.1021/es022253t

Glüge, J., Scheringer, M., Cousins, I.T., DeWitt, J.C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C.A., Trier, X., Wang, Z., 2020. An overview of the uses of per- and polyfluoroalkyl substances (PFAS). Environ. Sci. Process. Impacts 22, 2345–2373. https://doi.org/10.1039/D0EM00291G

Granberg, M.E., Ask, A., Gabrielsen, G.W., 2017. Local contamination in Svalbard: overview and suggestions for remediation actions, 51. Norsk Polarinstitutt.

Griffiths, R., Double, M., Griffiths, K., Dawson, R., 1998. A DNA test to sex most birds. Mol. Ecol. 7, 1071–5. https://doi.org/10.1046/j.1365-294x.1998.00389.x

Hanssen, L., Dudarev, A.A., Huber, S., Odland, J.Ø., Nieboer, E., Sandanger, T.M., 2013. Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. Sci. Total Environ. 447, 430–437. https://doi.org/10.1016/j.scitotenv.2013.01.029

Henriksen, E.O., Gabrielsen, G.W., Skaare, J.U., 1996. Levels and congener pattern of polychlorinated biphenyls in kittiwakes (Rissa tridactyla), in relation to mobilization of body-lipids associated with reproduction. Environ. Pollut. 92, 27–37. https://doi.org/10.1016/0269-7491(95)00087-9

Hiller-Sturmhöfel, S., Bartke, A., 1998. The Endocrine System. Alcohol Health Res. World 22, 153–164.

Hop, H., Borgá, K., Gabrielsen, G.W., Kleivane, L., Skaare, J.U., 2002. Food web magnificaton of persistent organic pollutants in poikilotherms and homeotherms. Environ. Sci. Technol. 36, 2589–2597. https://doi.org/10.1021/es010231l

Hormone health Network, 2021, *Endocrine-Disrupting Chemicals EDCs*. Available from: https://www.hormone.org/your-health-and-hormones/endocrine-disrupting-chemicals-edcs (accessed 4.29.21).

Houde, M., Czub, G., Small, J.M., Backus, S., Wang, X., Alaee, M., Muir, D.C.G., 2008. Fractionation and Bioaccumulation of Perfluorooctane Sulfonate (PFOS) Isomers in a Lake Ontario Food Web. Environ. Sci. Technol. 42, 9397–9403. https://doi.org/10.1021/es800906r

Hovden, T.S., 2018. Thyroid hormone status and thyroid gland histology in PFAS- and mercury contaminated glaucous gulls (Larus hyperboreus) from Svalbard. NTNU.

Howdeshell, K.L., 2002. A model of the development of the brain as a construct of the thyroid system. Environ. Health Perspect. 110, 337–348.

Jansen, A., Lyche, J.L., Polder, A., Aaseth, J., Skaug, M.A., 2017. Increased blood levels of persistent organic pollutants (POP) in obese individuals after weight loss-A review. J. Toxicol. Environ. Health B Crit. Rev. 20, 22–37. https://doi.org/10.1080/10937404.2016.1246391

Jenssen, B.M., 2006. Endocrine-Disrupting Chemicals and Climate Change: A Worst-Case Combination for Arctic Marine Mammals and Seabirds? Environ. Health Perspect. 114, 76–80. https://doi.org/10.1289/ehp.8057

Jenssen, B.M., Sørmo, E.G., Baek, K., Bytingsvik, J., Gaustad, H., Ruus, A., Skaare, J.U., 2007. Brominated flame retardants in North-East Atlantic marine ecosystems. Environ. Health Perspect. 115 Suppl 1, 35–41. https://doi.org/10.1289/ehp.9355

Jörundsdóttir, H., Löfstrand, K., Svavarsson, J., Bignert, A., Bergman, Å., 2010. Organochlorine Compounds and Their Metabolites in Seven Icelandic Seabird Species - a Comparative Study. Environ. Sci. Technol. 44, 3252–3259. https://doi.org/10.1021/es902812x

Kaiser, J., Enserink, M., 2000. Treaty Takes a POP At the Dirty Dozen.(persistent organic pollutants treaty to eliminate twelve toxic chemicals)(Statistical Data Included). Science 290, 2053–2053. https://doi.org/10.1126/science.290.5499.2053

Kallenborn, R., Breivik, K., Eckhardt, S., Lunder, C., Manø, S., Schlabach, M., Stohl, A., 2013. Long-term monitoring of persistent organic pollutants (POPs) at the Norwegian Troll station in Dronning Maud Land, Antarctica. Atmospheric Chem. Phys. 13, 6983–6992. https://doi.org/10.5194/acp-13-6983-2013

Kallenborn, R., Oehme, M., Wynn-Williams, D.D., Schlabach, M., Harris, J., 1998. Ambient air levels and atmospheric long-range transport of persistent organochlorines to Signy Island, Antarctica. Sci. Total Environ. 220, 167–180. https://doi.org/10.1016/S0048-9697(98)00257-5

Kärrman, A., Langlois, I., Bavel, B. van, Lindström, G., Oehme, M., 2007. Identification and pattern of perfluorooctane sulfonate (PFOS) isomers in human serum and plasma. Environ. Int. 33, 782–788. https://doi.org/10.1016/j.envint.2007.02.015

Key, B.D., Howell, R.D., Criddle, C.S., 1997. Fluorinated Organics in the Biosphere. Environ. Sci. Technol. 31, 2445–2454. https://doi.org/10.1021/es961007c

Kwadijk, C.J.A.F., Kotterman, M., Koelmans, A.A., 2014. Partitioning of perfluorooctanesulfonate and perfluorohexanesulfonate in the aquatic environment after an accidental release of aqueous film forming foam at Schiphol Amsterdam Airport. Environ. Toxicol. Chem. 33, 1761–1765. https://doi.org/10.1002/etc.2602

Langberg, H., Hale, S., Jenssen, B.M., Jartun, M., 2021. PFAS fingerprints in fish from Norwegian freshwater bodies subject to different source inputs. Environ. Sci. Technol.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl Acids: A Review of Monitoring and Toxicological Findings. Toxicol. Sci. Off. J. Soc. Toxicol. 99, 366–94. https://doi.org/10.1093/toxsci/kfm128

Leat, E.H.K., Bourgeon, S., Eze, J.I., Muir, D.C.G., Williamson, M., Bustnes, J.O., Furness, R.W., Borgå, K., 2013. Perfluoroalkyl substances in eggs and plasma of an avian top predator, great skua (Stercorarius skua), in the north Atlantic. Environ. Toxicol. Chem. 32, 569–576. https://doi.org/10.1002/etc.2101

Leeson, A., Thompson, T., Stroo, H.F., Anderson, R.H., Speicher, J., Mills, M.A., Willey, J., Coyle, C., Ghosh, R., Lebrón, C., Patton, C., 2021. Identifying and Managing Aqueous Film-Forming Foam-Derived Per- and Polyfluoroalkyl Substances in the Environment. Environ. Toxicol. Chem. 40, 24–36. https://doi.org/10.1002/etc.4894

Lemmetyinen, R., Rantamäki, P., 1980. DDT and PCB residues in the arctic tern (Sterna paradisaea) nesting in the archipelago of southwestern Finland. Ann. Zool. Fenn. 17, 141–146.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ., Levels, trends and effects of legacy and new persistent organic pollutants in the Arctic: An AMAP Assessment 408, 2995–3043. https://doi.org/10.1016/j.scitotenv.2009.10.038

Matsui, S., 2008. Endocrine Disruptors, in: Jørgensen, S.E., Fath, B.D. (Eds.), Encyclopedia of Ecology. Academic Press, Oxford, pp. 1259–1260. https://doi.org/10.1016/B978-008045405-4.00402-X

Mcnabb, A., 2007. The Hypothalamic-Pituitary-Thyroid (HPT) Axis in Birds and Its Role in Bird Development and Reproduction. Crit. Rev. Toxicol. 37, 163–93. https://doi.org/10.1080/10408440601123552

McNabb, F.M.A., Blackman, J.R., Cherry, J.A., 1985. The effects of different maternal dietary iodine concentrations on Japanese quail I. Thyroid status of hens. Domest. Anim. Endocrinol. 2, 25–34. https://doi.org/10.1016/0739-7240(85)90023-2

McNichols, M.J., McNabb, F.M.A., 1988. Development of thyroid function and its pituitary control in embryonic and hatchling precocial Japanese quail and altricial Ring doves. Gen. Comp. Endocrinol. 70, 109–118. https://doi.org/10.1016/0016-6480(88)90099-8

Melnes, M., Gabrielsen, G.W., Herzke, D., Sagerup, K., Jenssen, B.M., 2017. Dissimilar effects of organohalogenated compounds on thyroid hormones in glaucous gulls. Environ. Res. 158, 350–357. https://doi.org/10.1016/j.envres.2017.06.007

Meterologisk Insititutt, 2021, *Historiske værdata for Ny-Ålesund*. Available from: https://www.yr.no/nb/historikk/graf/1-2813634/Norge/Svalbard/Svalbard/Ny-Ålesund?q=2019-07 (accessed 1.21.21).

Miljødirketoratet, 2021, *Perfluorerte stoffer (PFOS, PFOA og andre PFAS-er) - Miljøstatus for Norge.* Available from: https://miljostatus.miljodirektoratet.no/tema/miljogifter/prioriterte-miljogifter/perfluorerte-stoffer-pfos-pfoa-og-andre-pfas-er/ (accessed 1.19.21).

Mortensen, Å.-K., Mæhre, S., Kristiansen, K., Heimstad, E.S., Gabrielsen, G.W., Jenssen, B.M., Sylte, I., 2020. Homology modeling to screen for potential binding of contaminants to thyroid hormone receptor and transthyretin in glaucous gull (Larus hyperboreus) and herring gull (Larus argentatus). Comput. Toxicol. 13, 100120. https://doi.org/10.1016/j.comtox.2020.100120

Nost, T.H., Helgason, L.B., Harju, M., Heimstad, E.S., Gabrielsen, G.W., Jenssen, B.M., 2012. Halogenated organic contaminants and their correlations with circulating thyroid hormones in developing Arctic seabirds. Sci. Total Environ. 414, 248–256. https://doi.org/10.1016/j.scitotenv.2011.11.051

OECD, 2018. *Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFASs): Summary report on updating the OECD 2007 list of per- and*

polyfluoroalkyl substances (PFASs). (Series on Risk Management No. 39, ENV/JM/MONO(2018)7). Available from: https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/5099062 (accessed 3.24.21).

Peakall, D.B. (Ed.), 1992. Animal Biomarkers as Pollution Indicators, Chapman & Hall Ecotoxicology Series. Springer Netherlands. https://doi.org/10.1007/978-94-011-2346-4

Powley, C.R., George, S.W., Ryan, T.W., Buck, R.C., 2005. Matrix Effect-Free Analytical Methods for Determination of Perfluorinated Carboxylic Acids in Environmental Matrixes. Anal. Chem. 77, 6353–6358. https://doi.org/10.1021/ac0508090

Ren, X.-M., Qin, W.-P., Cao, L.-Y., Zhang, J., Yang, Y., Wan, B., Guo, L.-H., 2016. Binding interactions of perfluoroalkyl substances with thyroid hormone transport proteins and potential toxicological implications. Toxicology 366–367, 32–42. https://doi.org/10.1016/j.tox.2016.08.011

Stockholm Convention, 2021a, *The Stockholm Convention - Home page*. Available from: http://www.pops.int/ (accessed 4.20.21).

Stockholm Convention, 2021b, *The 12 Initial POPs*. Available from: http://chm.pops.int/TheConvention/ThePOPs/The12InitialPOPs/tabid/296/Default.aspx (accessed 4.5.21).

Stockholm Convention, 2021c, *The New POPs under the Stockholm Convention*. Available from: http://www.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx (accessed 1.19.21).

Stockholm Convention, 2021d, *Perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds*. Available from: http://chm.pops.int/Implementation/Alternatives/AlternativestoPOPs/ChemicalslistedinAnnexA/PFOA/tabid/8292/Default.aspx (accessed 3.24.21).

Stockholm Convention, 2021e, *Overview*. Available from: http://chm.pops.int/Implementation/IndustrialPOPs/PFOS/Overview/tabid/5221/Default.aspx (accessed 3.24.21).

Svendsen, N.B., Herzke, D., Harju, M., Bech, C., Gabrielsen, G.W., Jaspers, V.L.B., 2018. Persistent organic pollutants and organophosphate esters in feathers and blood plasma of adult kittiwakes (Rissa tridactyla) from Svalbard - associations with body condition and thyroid hormones. Environ. Res. 164, 158–164. https://doi.org/10.1016/j.envres.2018.02.012

Tori, G.N., Mayer, L.P., 1981. Effects of polychlorinated biphenyls on the metabolic rates of mourning doves exposed to low ambient temperatures. Bull. Environ. Contam. Toxicol. 27, 678–682.

Ucán-Marín, F., Arukwe, A., Mortensen, A., Gabrielsen, G.W., Fox, G.A., Letcher, R.J., 2009. Recombinant transthyretin purification and competitive binding with organohalogen compounds in two gull species (Larus argentatus and Larus hyperboreus). Toxicol. Sci. Off. J. Soc. Toxicol. 107, 440–450. https://doi.org/10.1093/toxsci/kfn240

Ucán-Marin, F., Arukwe, A., Mortensen, A.S., Gabrielsen, G.W., Letcher, R.J., 2010. Recombinant albumin and transthyretin transport proteins from two gull species and human: chlorinated and brominated contaminant binding and thyroid hormones. Environ. Sci. Technol. 44, 497–504. https://doi.org/10.1021/es902691u

van der Schyff, V., Kwet Yive, N.S.C., Polder, A., Cole, N.C., Bouwman, H., 2020. Perfluoroalkyl substances (PFAS) in tern eggs from St. Brandon's Atoll, Indian

Ocean. Mar. Pollut. Bull. 154, 111061. https://doi.org/10.1016/j.marpolbul.2020.111061
Verreault, J., Bech, C., Letcher, R.J., Ropstad, E., Dahl, E., Gabrielsen, G.W., 2007. Organohalogen contamination in breeding glaucous gulls from the Norwegian Arctic: associations with basal metabolism and circulating thyroid hormones. Environ. Pollut. Barking Essex 1987 145, 138–145. https://doi.org/10.1016/j.envpol.2006.03.049
Verreault, J., Helgason, L.B., Gabrielsen, G.W., Dam, M., Braune, B.M., 2013. Contrasting retinoid and thyroid hormone status in differentially-contaminated northern fulmar colonies from the Canadian Arctic, Svalbard and the Faroe Islands. Environ. Int. 52, 29–40. https://doi.org/10.1016/j.envint.2012.12.001
Verreault, J., Houde, M., Gabrielsen, G.W., Berger, U., Haukås, M., Letcher, R.J., Muir, D.C.G., 2005. Perfluorinated alkyl substances in plasma, liver, brain, and eggs of glaucous gulls (Larus hyperboreus) from the Norwegian arctic. Environ. Sci. Technol. 39, 7439–7445. https://doi.org/10.1021/es051097y
Verreault, J., Skaare, J.U., Jenssen, B.M., Gabrielsen, G.W., 2004. Effects of organochlorine contaminants on thyroid hormone levels in Arctic breeding glaucous gulls, Larus hyperboreus. Environ. Health Perspect. 112, 532–537. https://doi.org/10.1289/ehp.6756
Vihtakari, M., Welcker, J., Moe, B., Chastel, O., Tartu, S., Hop, H., Bech, C., Descamps, S., Gabrielsen, G.W., 2018. Black-legged kittiwakes as messengers of Atlantification in the Arctic. Sci. Rep. 8, 1178. https://doi.org/10.1038/s41598-017-19118-8
Watson, G.E., Angle, J.P., Harper, P.C., 1975. Birds of the Antarctic and Sub-Antarctic. Wiley.
Welcker, J.O., Chastel, O., Gabrielsen, G.W., Guillaumin, J., Kitaysky, A.S., Speakman, J.R., Tremblay, Y., Bech, C., 2013. Thyroid hormones correlate with basal metabolic rate but not field metabolic rate in a wild bird species. 8. https://doi.org/10.1371/journal.pone.0056229
Wild on the fly, 2021, *St. Brandon's Atoll*: Available from: https://wildonthefly.com/destinations/st-brandons-atoll (accessed 4.21.21).
Zoeller, T.R., Dowling, A.L.S., Herzig, C.T.A., Iannacone, E.A., Gauger, K.J., Bansal, R., 2002. Thyroid hormone, brain development, and the environment. Environ. Health Perspect. 110, 355–361.

Appendix A: Biometric measurements

Table 6: Sample ID, capture location, sex, and biometric measurement of each individual arctic tern (*Sterna paradisaea*).

ID	Sample location	Sex	Body mass (g)	Wing length (cm)	Beak length (mm)	Beak height (mm)	Scull length (mm)	Body temperature (ºC)
AT15	Innerholmen	F	99,3	27,7	32,1	7,2	67,5	42,8
AT16	Innerholmen	F	105,1	27,1	32,9	6,2	71,3	42,6
AT17	Observasjonsholmen	M	111,9	27,2	35,1	6,9	74,0	40,8
AT18	Observasjonsholmen	M	95,8	27,5	32,0	7,0	71,5	40,8
AT19	Observasjonsholmen	F	102	26,9	34,5	7,4	-	41,7
AT20	Observasjonsholmen	M	102,1	26,5	31,6	8,4	-	40,5
AT21	Observasjonsholmen	F	93,0	26,9	32,9	8,0	-	40,7
AT22	Observasjonsholmen	F	111,2	26,8	31,2	7,1	72,0	41,0
AT23	Observasjonsholmen	M	101,5	27,6	32,0	7,1	72,2	40,8
AT24	Observasjonsholmen	M	99,3	27,0	34,3	7,5	75,8	40,8
AT25	Gerdøya	M	102,8	27,6	35,4	8,0	76,5	41,0
AT26	Gerdøya	F	119,2	28,2	31,4	7,0	70,9	41,2
AT27	Observasjonsholmen	-	104,8	27,9	30,7	6,6	71,4	40,0
AT28	Observasjonsholmen	F	105,8	27,7	32,6	7,3	71,0	40,2
AT29	Observasjonsholmen	M	102,9	26,1	33,4	6,8	74,6	41,7
AT30	Observasjonsholmen	F	104,1	27,9	31,8	7,0	70,2	41,8
AT31	Observasjonsholmen	-	-	26,2	30,3	7,3	69,4	40,8
AT32	Observasjonsholmen	M	96,0	27,1	32,0	6,9	71,3	40,4
AT33	Gerdøya	-	111,4	27,2	31,0	6,9	71,9	41,0
AT34	Gerdøya	M	100	28	34,4	7,0	73,6	40,8

Appendix B: PFAS concentrations

Table 7: Detected concentrations of PFASs in each individual arctic tern (*Sterna paradisaea*). The full names and LODs are described in Table 1.

ng/mL/g	4:2 FTS	6:2 FTS	8:2 FTS	PFBS	PFPS	PFHxS	PFHpS	Br PFOS	PFOSlin	sum PFOS	PFNS	PFDS
AT 15	<0.10	<0.10	<0.10	<0.05	<0.05	0,105	0,061	0,330	1,73	2,06	<0.05	<0.05
AT 16	<0.10	<0.10	<0.10	<0.05	<0.05	0,096	<0.05	0,306	1,41	1,72	<0.05	<0.05
AT 17	<0.10	<0.10	<0.10	<0.05	<0.05	0,080	<0.05	0,778	4,10	4,72	<0.05	<0.05
AT 19	<0.10	<0.10	<0.10	<0.05	<0.05	0,240	<0.05	0,293	0,383	0,675	<0.05	<0.05
AT 20	<0.10	<0.10	<0.10	<0.05	<0.05	0,093	<0.05	0,524	3,89	4,42	<0.05	<0.05
AT 21	<0.10	<0.10	<0.10	<0.05	<0.05	0,230	0,050	0,563	3,00	3,56	<0.05	<0.05
AT 22	<0.10	<0.10	<0.10	<0.05	<0.05	0,063	<0.05	0,285	1,09	1,37	<0.05	<0.05
AT 23	<0.10	<0.10	<0.10	<0.05	<0.05	0,185	<0.05	0,973	5,87	6,84	<0.05	<0.05
AT 24	<0.10	<0.10	<0.10	<0.05	<0.05	0,168	<0.05	0,450	3,57	4,02	<0.05	<0.05
AT 25	<0.10	<0.10	<0.10	<0.05	<0.05	0,099	<0.05	0,662	3,76	4,33	<0.05	<0.05
AT 26	<0.10	<0.10	<0.10	<0.05	<0.05	0,104	<0.05	0,416	3,18	3,60	<0.05	<0.05
AT 27	<0.10	<0.10	<0.10	<0.05	<0.05	0,163	<0.05	0,294	0,947	1,24	<0.05	<0.05
AT 28	<0.10	<0.10	<0.10	<0.05	<0.05	0,058	<0.05	0,290	1,31	1,60	<0.05	<0.05
AT 29	<0.10	<0.10	<0.10	<0.05	<0.05	0,096	<0.05	0,522	3,32	3,85	<0.05	<0.05
AT 30	<0.10	<0.10	<0.10	<0.05	<0.05	0,198	<0.05	0,309	1,26	1,57	<0.05	<0.05
AT 31	<0.10	<0.10	<0.10	<0.05	<0.05	0,118	<0.05	0,269	1,73	2,00	<0.05	<0.05
AT 32	<0.10	<0.10	<0.10	<0.05	<0.05	0,081	<0.05	0,710	4,55	5,20	<0.05	0,095
AT 33	<0.10	<0.10	<0.10	<0.05	<0.05	0,068	<0.05	0,437	2,24	2,68	<0.05	0,069
AT 34	<0.10	<0.10	<0.10	<0.05	<0.05	0,069	<0.05	0,537	3,78	4,26	<0.05	<0.05
	PFHxA	**PFHpA**	**PFOA**	**PFNA**	**PFDA**	**PFUnDA**	**PFDoDA**	**PFTrDA**	**PFTeDA**	**PFHxDA**	**PFODcA**	**FOSA**
AT 15	<0.10	<0.05	0,051	0,423	0,446	1,61	0,287	0,710	0,090	<0.05	<0.10	<0.05
AT 16	<0.10	<0.05	<0.05	0,342	0,371	1,20	0,202	0,532	0,176	<0.05	<0.10	0,190
AT 17	<0.10	<0.05	<0.05	0,566	0,771	2,62	0,508	1,36	0,286	0,075	<0.10	<0.05
AT 19	<0.10	<0.05	0,116	0,251	0,135	0,263	<0.05	<0.05	<0.05	<0.05	<0.10	<0.05
AT 20	<0.10	<0.05	<0.05	0,258	0,457	2,19	0,423	0,711	0,245	<0.05	<0.10	<0.05
AT 21	<0.10	<0.05	0,084	0,505	0,518	1,88	0,403	1,05	0,151	<0.05	<0.10	<0.05
AT 22	<0.10	<0.05	<0.05	0,154	0,154	0,554	0,083	0,236	0,052	<0.05	<0.10	<0.05
AT 23	<0.10	<0.05	<0.05	0,638	0,774	3,05	0,594	1,64	0,303	0,061	<0.10	0,141
AT 24	<0.10	<0.05	<0.05	0,260	0,402	2,01	0,387	1,05	0,130	<0.05	<0.10	<0.05
AT 25	<0.10	<0.05	<0.05	0,371	0,595	2,29	0,503	1,10	0,279	<0.05	<0.10	<0.05
AT 26	<0.10	<0.05	<0.05	0,243	0,532	2,15	0,439	1,02	0,135	<0.05	<0.10	<0.05
AT 27	<0.10	<0.05	<0.05	0,215	0,223	0,443	<0.05	0,118	0,037	<0.05	<0.10	<0.05
AT 28	<0.10	<0.05	<0.05	0,215	0,204	0,690	0,120	0,226	0,066	<0.05	<0.10	<0.05
AT 29	<0.10	<0.05	<0.05	0,262	0,541	1,85	0,299	0,874	0,110	<0.05	<0.10	<0.05
AT 30	<0.10	<0.05	<0.05	0,252	0,259	0,632	0,050	0,221	0,020	<0.05	<0.10	<0.05
AT 31	<0.10	<0.05	0,055	0,238	0,245	0,742	0,143	0,273	<0.05	<0.05	<0.10	<0.05
AT 32	<0.10	<0.05	<0.05	0,244	0,548	1,98	0,501	0,971	0,246	<0.05	<0.10	<0.05
AT 33	<0.10	<0.05	<0.05	0,326	0,508	1,64	0,281	0,664	0,059	<0.05	<0.10	<0.05
AT 34	<0.10	<0.05	<0.05	0,321	0,646	2,59	0,502	1,19	0,202	<0.05	<0.10	<0.05

Appendix C: PFAS concentrations in control samples

Table 8: Detected PFAS concentrations and assigned values of the control sample (AMSY 2006).

ng/mL/g	4:2 FTS	6:2 FTS	8:2 FTS	PFBS	PFPS	PFHxS	PFHpS	Br PFOS	PFOSlin	sum PFOS	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA
AMSY 2006-1	<0.10	<0.10	<0.10	<0.05	<0.05	6,01	1,09	23,08	58,5	81,6	0,962	2,27	4,63	3,07	1,56	0,956
AMSY 2006-2	<0.10	<0.10	<0.10	0,074	<0.05	6,00	1,31	21,80	57,7	79,5	0,906	2,22	4,67	2,80	1,59	0,796
AMSY 2006-3	<0.10	<0.10	<0.10	0,064	<0.05	5,88	1,04	23,36	58,8	82,2	0,940	2,20	4,41	3,21	1,58	0,936
AMSY 2006-4	<0.10	<0.10	<0.10	0,071	<0.05	6,13	0,88	24,90	60,7	85,6	1,01	2,25	4,79	2,74	1,65	0,994
Snitt						6,00	1,08	23,29	58,9	82,2	0,953	2,23	4,62	2,96	1,59	0,920
Stdev						0,103	0,175	1,27	1,27	2,532	0,042	0,032	0,159	0,221	0,040	0,087
%RSD						1,71	16,2	5,47	2,15	3,08	4,39	1,41	3,43	7,48	2,49	9,42
Assigned value						6,06	1,08	21,3	60,0	78,9	0,985	2,25	4,37	3,46	1,67	0,777

Appendix D: Internal standards

PFASs in the internal standard:

^{13}C 6:2 FTS

^{13}C PFHxS

^{13}C PFOS

^{13}C FOSA

^{13}C PFBA

^{13}C PFPeA

^{13}C PFHxA

^{13}C PFHpA

^{13}C PFOA

^{13}C PFNA

^{13}C PFDA

^{13}C PFUnDA

^{13}C PFTeDA

Appendix E: Recovery of internal standard

Table 9: Percentage recovery of the ^{13}C labeled internal standard (PFAS ISTD ALLE) in each individual arctic tern (*Sterna paradisaea*).

ID	13C 6:2 FTS	13C PFHxS	13C PFOS	13C PFHxA	13C PFHpA	13C PFOA	13C PFNA	13C PFDA	13C PFUnDA	13CPFDoDA	13C PFTeDA	13C FOSA
AT 15	80	90	83	92	105	94	94	91	88	77	66	84
AT 15	85	91	85	93	109	97	103	92	89	76	62	86
AT 16	83	93	84	94	101	96	103	92	89	75	63	83
AT 16	85	94	85	95	100	99	98	91	89	76	59	84
AT 17	78	88	82	90	97	92	92	88	85	73	59	85
AT 17	80	90	80	90	98	93	91	89	85	72	53	84
AT 19	74	85	79	84	98	89	85	86	88	76	70	84
AT 19	73	85	80	84	97	87	93	88	85	74	67	87
AT 20	81	90	80	90	111	95	96	84	76	61	35	78
AT 20	80	89	78	90	111	92	97	84	76	60	32	79
AT 21	80	89	80	89	97	92	94	90	87	73	58	84
AT 21	84	90	86	91	101	96	92	92	87	72	56	83
AT 22	100	113	101	114	132	116	117	108	102	78	47	101
AT 22	104	116	102	118	137	122	118	108	100	79	44	106
AT 23	82	90	83	90	100	95	96	89	85	75	59	88
AT 23	84	91	84	91	99	96	89	91	86	72	53	85
AT 24	81	89	82	88	99	93	93	89	89	75	59	86
AT 24	80	90	83	90	100	92	93	90	88	73	58	85
AT 25	81	90	81	88	98	93	92	88	87	74	57	83
AT 25	79	90	84	88	97	92	94	89	87	74	56	87
AT 26	86	94	89	92	102	99	94	97	96	82	71	89
AT 26	83	91	86	89	99	93	93	94	95	79	69	88
AT 27	79	87	84	84	100	89	87	89	89	74	65	88
AT 27	80	87	84	85	103	90	90	88	91	75	66	88
AT 28	77	86	83	86	97	90	84	84	85	69	53	85
AT 28	81	93	89	90	100	94	91	88	87	70	52	85
AT 29	78	89	85	87	104	89	94	87	85	70	57	87
AT 29	81	87	84	86	104	90	88	87	85	71	53	85
AT 30	75	86	82	84	94	87	84	85	83	69	53	81
AT 30	79	88	83	87	96	88	92	87	86	69	49	83
AT 31	77	84	80	84	99	88	87	83	80	63	44	81
AT 31	80	85	80	85	101	87	83	84	82	63	39	80
AT 32	85	87	84	87	93	91	92	87	89	74	61	87
AT 32	84	85	85	85	93	88	93	88	88	73	57	85
AT 33	83	86	87	86	94	90	86	87	90	76	62	85
AT 33	81	87	87	88	98	90	85	87	88	73	60	83
AT 34	84	86	89	88	94	91	92	90	89	75	59	87
AT 34	84	86	90	89	95	89	95	89	90	74	58	85
	13C 6:2 FTS	13C PFHxS	13C PFOS	13C PFHxA	13C PFHpA	13C PFOA	13C PFNA	13C PFDA	13C PFUnDA	13CPFDoDA	13C PFTeDA	13C FOSA
Snitt	82	90	85	90	101	93	93	89	88	73	56	86
Stdev	5,6	6,4	5,0	6,9	9,1	7,0	7,4	5,2	5,1	4,8	9,0	5,0
%RSD	6,9	7,2	5,9	7,7	8,9	7,6	8,0	5,8	5,8	6,6	16,0	5,8
Min	73	84	78	84	93	87	83	83	76	60	32	78
Max	104	116	102	118	137	122	118	108	102	82	71	106

Appendix F: TH concentrations and RMRs

Table 10: Measured RMR, TT4, and TT3 concentration in each individual arctic tern (*Sterna paradisaea*). Only the specimens marked with red were used in the analysis, but the average %CV from all individuals was applied to determine the reliability of the samples not run in duplicates.

ID	RMR (mL O_2/g*min)	TT4 (ng/mL)	% CV (TT4)	TT3 (ng/mL)	%CV (TT3)
AT2	-	-	-	7.44	9.59
AT3	-	37.95	4.70	6.68	1.61
AT4	-	30.83	1.61	7.70	1.79
AT5	-	29.01	-	3.80	-
AT6	-	34.95	-	2.29	2.08
AT7	-	22.10	1.82	3.48	-
AT8	-	23.24	1.90	1.65	3.36
AT9	-	29.01	18.3	3.10	1.89
AT10	-	25.14	10.0	4.99	-
AT11	-	32.30	0.528	7.92	-
AT12	-	27.40	-	0.91	-
AT15	-	23.07	32.0	1.09	-
AT16	-	26.62	-	1.47	-
AT17	3.144	22.42	7.70	2.85	-
AT18	3.075	26.91	-	1.69	-
AT19	2.598	17.57	45.9	0.36	-
AT20	3.863	-	-	2.82	-
AT21	3.024	32.07	1.31	3.24	2.09
AT22	2.629	16.83	-	2.30	4.77
AT23	3.090	21.63	1.24	2.60	0.84
AT24	2.451	-	-	2.32	4.78
AT25	2.600	26.43	-	3.33	-
AT26	2.158	28.06	12.3	4.47	-
AT27	3.388	-	-	-	-
AT28	2.332	34.09	8.57	2.13	-
AT29	3.090	25.69	-	1.24	-
AT30	2.814	24.26	28.4	1.36	-
AT31	2.558	-	-	-	-
AT32	2.931	25.50	23.7	-	-
AT33	2.523	-	-	-	-
AT34	2.758	19.71	9.87	3.38	-

Appendix G: Shapiro Wilk`s test of normality

Table 11: Shapiro Wilk`s test of normality for each individual parameter of the arctic terns (*Sterna paradisaea*). The null hypothesis is that the parameter is normally distributed, therefore $p \leq 0.05$ means the parameter is not normally distributed.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Wing	,121	20	,200*	,963	20	,610
Scull	,182	17	,137	,958	17	,590
Bille lenght	,187	20	,064	,934	20	,181
Bille height	,159	20	,200	,923	20	,113
Tb	,239	20	,004	,888	20	,024
Mass	,151	20	,200*	,945	20	,296
BMR	,136	18	,200*	,957	18	,548
TT3	,093	16	,200*	,983	16	,983
TT4	,124	15	,200*	,971	15	,875
TT4/TT3	,225	14	,052	,735	14	,001
PFHxS	,248	19	,003	,867	19	,013
brPFOS	,183	19	,094	,877	19	,019
linPFOS	,162	19	,200*	,950	19	,393
∑PFOS	,166	19	,177	,944	19	,313
PFDoDA	,143	19	,200*	,919	19	,107
PFTrDA	,158	19	,200*	,952	19	,425
PFTeDA	,137	19	,200*	,919	19	,108
PFNA	,254	19	,002	,850	19	,007
PFDA	,137	19	,200*	,950	19	,403
PFUnDA	,163	19	,200*	,938	19	,248
∑PFAS	,152	19	,200*	,946	19	,343

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Appendix H: Principal component eigenvalues

Table 12: Eigenvalue of each individual principal component.

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	8,367	59,764	59,764	8,367	59,764	59,764
2	2,661	19,007	78,771	2,661	19,007	78,771
3	1,197	8,551	87,322	1,197	8,551	87,322
4	,647	4,618	91,941			
5	,433	3,094	95,035			
6	,367	2,620	97,654			
7	,198	1,418	99,072			
8	,091	,653	99,725			
9	,025	,180	99,905			
10	,013	,095	100,000			
11	2,829E-16	2,021E-15	100,000			
12	-7,123E-17	-5,088E-16	100,000			
13	-2,629E-16	-1,878E-15	100,000			
14	-9,016E-16	-6,440E-15	100,000			

Extraction Method: Principal Component Analysis.

Appendix I: Mann-Whitney U test

Table 13: Mann-Whitney U test – nonparametric comparison of means between the non-normally distributed variables of male and female arctic terns (*Sterna paradisaea*).

Test Statistics[a]

	Tb	TT4/TT3	PFHxS	brPFOS	PFNA
Mann-Whitney U	19,000	15,000	23,000	4,000	19,000
Wilcoxon W	64,000	36,000	59,000	40,000	55,000
Z	-1,658	-1,162	-,945	-2,941	-1,365
Asymp. Sig. (2-tailed)	,097	,245	,345	,003	,172
Exact Sig. [2*(1-tailed Sig.)]	,114[b]	,282[b]	,382[b]	,002[b]	,195[b]

a. Grouping Variable: Sex(nr)

b. Not corrected for ties.

Appendix J: Levene`s test for equality of variances and t-test for equality of means

Table 14: Levene`s test and student t-test, comparing means of normally distributed variables in male and female arctic terns (*Sterna paradisaea*).

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Wing	Equal variances assumed	,041	,842	-,807	15	,432	-,2222	,2753	-,8091	,3646
	Equal variances not assumed			-,812	14,987	,430	-,2222	,2737	-,8056	,3612
Scull	Equal variances assumed	,669	,429	3,317	12	,006	3,2042	,9661	1,0993	5,3090
	Equal variances not assumed			3,421	11,858	,005	3,2042	,9367	1,1606	5,2478
Bille lenght	Equal variances assumed	1,775	,203	1,602	15	,130	1,0431	,6511	-,3447	2,4308
	Equal variances not assumed			1,628	14,716	,125	1,0431	,6405	-,3245	2,4106
Bille height	Equal variances assumed	,578	,459	,490	15	,631	,12778	,26078	-,42807	,68363
	Equal variances not assumed			,494	15,000	,629	,12778	,25883	-,42390	,67946
Mass	Equal variances assumed	1,042	,324	-1,162	15	,264	-3,5958	3,0958	-10,1943	3,0027
	Equal variances not assumed			-1,129	11,318	,282	-3,5958	3,1860	-10,5843	3,3926
BMR	Equal variances assumed	,141	,713	2,076	13	,058	,4077901996	,1963865651	-,016477180	,8320575794
	Equal variances not assumed			2,190	12,572	,048	,4077901996	,1861706602	,0041974332	,8113829660
TT3	Equal variances assumed	1,618	,224	,891	14	,388	,7304500	,8194854	-1,0271714	2,4880714
	Equal variances not assumed			,891	11,208	,391	,7304500	,8194854	-1,0691561	2,5300561
TT4	Equal variances assumed	3,592	,081	-,503	13	,623	-1,6538589	3,2853208	-8,7513629	5,4436451
	Equal variances not assumed			-,528	9,944	,609	-1,6538589	3,1318275	-8,6373016	5,3295838
linPFOS	Equal variances assumed	,388	,544	5,524	14	,000	4,883214958	,8839990845	2,987225489	6,779204426
	Equal variances not assumed			5,524	13,578	,000	4,883214958	,8839990845	2,981685753	6,784744162
∑PFOS	Equal variances assumed	,164	,692	5,370	14	,000	5,383238305	1,002428807	3,233242343	7,533234266
	Equal variances not assumed			5,370	13,913	,000	5,383238305	1,002428807	3,231978699	7,534497910
PFDoDA	Equal variances assumed	3,436	,085	4,056	14	,001	,4274190701	,1053720870	,2014184205	,6534197197
	Equal variances not assumed			4,056	11,163	,002	,4274190701	,1053720870	,1959092039	,6589289363
PFTrDA	Equal variances assumed	1,564	,232	3,551	14	,003	,9194951265	,2589226405	,3641612939	1,474828959
	Equal variances not assumed			3,551	12,947	,004	,9194951265	,2589226405	,3598944148	1,479095838
PFTeDA	Equal variances assumed	,327	,577	4,130	14	,001	,1911943544	,0462928236	,0919061226	,2904825863
	Equal variances not assumed			4,130	13,514	,001	,1911943544	,0462928236	,0915702109	,2908184979
PFDA	Equal variances assumed	1,023	,329	3,568	14	,003	,5144218721	,1441850788	,2051756345	,8236681097
	Equal variances not assumed			3,568	13,584	,003	,5144218721	,1441850788	,2042844085	,8245593356
PFUnDA	Equal variances assumed	4,257	,058	4,227	14	,001	2,127958081	,5033709426	1,048334784	3,207581378
	Equal variances not assumed			4,227	11,277	,001	2,127958081	,5033709426	1,023358498	3,232557664
∑PFAS	Equal variances assumed	1,216	,289	4,661	14	,000	9,638370214	2,067947370	5,203064223	14,07367621
	Equal variances not assumed			4,661	13,204	,000	9,638370214	2,067947370	5,177840098	14,09890033

Appendix K: Correlation matrix with Pearson's correlation (excluding non-normally distributed variables)

Table 15: Pearson's correlation coefficient for all normally distributed variables (all individuals).

		BMR	Mass	TT3	TT4	linPFOS	∑PFOS	∑PFSA	PFDoDA	PFTrDA	PFTeDA	PFDA	PFUnDA	∑PFCA	∑PFAS
BMR	Pearson Correlation	1	-,345	-,118	-,154	,291	,301	,307	,176	,131	,375	,234	,216	,215	,264
	Sig. (2-tailed)		,161	,687	,616	,256	,241	,230	,499	,615	,138	,367	,405	,408	,305
	N	18	18	14	13	17	17	17	17	17	17	17	17	17	17
Mass	Pearson Correlation	-,345	1	,337	-,138	-,168	-,173	-,191	-,121	-,086	-,116	-,017	-,059	-,081	-,140
	Sig. (2-tailed)	,161		,202	,623	,492	,479	,433	,620	,727	,635	,945	,810	,742	,568
	N	18	20	16	15	19	19	19	19	19	19	19	19	19	19
TT3	Pearson Correlation	-,118	,337	1	,261	,595*	,578*	,566*	,700**	,610*	,557*	,546*	,634*	,605*	,594*
	Sig. (2-tailed)	,687	,202		,367	,019	,024	,028	,004	,016	,031	,035	,011	,017	,020
	N	14	16	16	14	15	15	15	15	15	15	15	15	15	15
TT4	Pearson Correlation	-,154	-,138	,261	1	,053	,047	,045	,100	,062	,045	,053	,052	,058	,052
	Sig. (2-tailed)	,616	,623	,367		,857	,872	,879	,732	,835	,879	,857	,860	,843	,859
	N	13	15	14	15	14	14	14	14	14	14	14	14	14	14
linPFOS	Pearson Correlation	,291	-,168	,595*	,053	1	,999**	,997**	,948**	,926**	,867**	,887**	,938**	,932**	,983**
	Sig. (2-tailed)	,256	,492	,019	,857		,000	,000	,000	,000	,000	,000	,000	,000	,000
	N	17	19	15	14	19	19	19	19	19	19	19	19	19	19
∑PFOS	Pearson Correlation	,301	-,173	,578*	,047	,999**	1	,999**	,944**	,926**	,869**	,889**	,934**	,932**	,984**
	Sig. (2-tailed)	,241	,479	,024	,872	,000		,000	,000	,000	,000	,000	,000	,000	,000
	N	17	19	15	14	19	19	19	19	19	19	19	19	19	19
∑PFSA	Pearson Correlation	,307	-,191	,566*	,045	,997**	,999**	1	,939**	,925**	,864**	,884**	,929**	,929**	,983**
	Sig. (2-tailed)	,230	,433	,028	,879	,000	,000		,000	,000	,000	,000	,000	,000	,000
	N	17	19	15	14	19	19	19	19	19	19	19	19	19	19
PFDoDA	Pearson Correlation	,176	-,121	,700**	,100	,948**	,944**	,939**	1	,961**	,893**	,930**	,978**	,975**	,974**
	Sig. (2-tailed)	,499	,620	,004	,732	,000	,000	,000		,000	,000	,000	,000	,000	,000
	N	17	19	15	14	19	19	19	19	19	19	19	19	19	19
PFTrDA	Pearson Correlation	,131	-,086	,610*	,062	,926**	,926**	,925**	,961**	1	,842**	,955**	,975**	,988**	,973**
	Sig. (2-tailed)	,615	,727	,016	,835	,000	,000	,000	,000		,000	,000	,000	,000	,000
	N	17	19	15	14	19	19	19	19	19	19	19	19	19	19
PFTeDA	Pearson Correlation	,375	-,116	,557*	,045	,867**	,869**	,864**	,893**	,842**	1	,836**	,866**	,879**	,887**
	Sig. (2-tailed)	,138	,635	,031	,879	,000	,000	,000	,000	,000		,000	,000	,000	,000
	N	17	19	15	14	19	19	19	19	19	19	19	19	19	19
PFDA	Pearson Correlation	,234	-,017	,546*	,053	,887**	,889**	,884**	,930**	,955**	,836**	1	,959**	,975**	,945**
	Sig. (2-tailed)	,367	,945	,035	,857	,000	,000	,000	,000	,000	,000		,000	,000	,000
	N	17	19	15	14	19	19	19	19	19	19	19	19	19	19
PFUnDA	Pearson Correlation	,216	-,059	,634*	,052	,938**	,934**	,929**	,978**	,975**	,866**	,959**	1	,992**	,977**
	Sig. (2-tailed)	,405	,810	,011	,860	,000	,000	,000	,000	,000	,000	,000		,000	,000
	N	17	19	15	14	19	19	19	19	19	19	19	19	19	19
∑PFCA	Pearson Correlation	,215	-,081	,605*	,058	,932**	,932**	,929**	,975**	,988**	,879**	,975**	,992**	1	,981**
	Sig. (2-tailed)	,408	,742	,017	,843	,000	,000	,000	,000	,000	,000	,000	,000		,000
	N	17	19	15	14	19	19	19	19	19	19	19	19	19	19
∑PFAS	Pearson Correlation	,264	-,140	,594*	,052	,983**	,984**	,983**	,974**	,973**	,887**	,945**	,977**	,981**	1
	Sig. (2-tailed)	,305	,568	,020	,859	,000	,000	,000	,000	,000	,000	,000	,000	,000	
	N	17	19	15	14	19	19	19	19	19	19	19	19	19	19

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 16: Pearson's correlation coefficient for all normally distributed variables (females only).

		BMR	Mass	TT3	TT4	linPFOS	PFDoDA	PFTrDA	PFTeDA	PFDA	PFUnDA	∑PFOS	∑PFSA	∑PFCA	∑PFAS
BMR	Pearson Correlation	1	-,832*	-,317	-,102	-,060	-,117	,007	-,044	,010	-,116	-,020	,036	-,009	,011
	Sig. (2-tailed)		,040	,540	,847	,910	,826	,989	,934	,985	,827	,970	,946	,987	,983
	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mass	Pearson Correlation	-,832*	1	,461	-,148	,104	,036	-,023	-,024	-,067	,051	,065	,015	-,058	-,026
	Sig. (2-tailed)	,040		,250	,727	,806	,933	,958	,955	,876	,905	,878	,973	,891	,951
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
TT3	Pearson Correlation	-,317	,461	1	,502	,870**	,748*	,729*	,521	,613	,708*	,860**	,833*	,665	,755*
	Sig. (2-tailed)	,540	,250		,205	,005	,033	,040	,186	,106	,049	,006	,010	,072	,030
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
TT4	Pearson Correlation	-,102	-,148	,502	1	,598	,520	,502	,529	,515	,503	,597	,583	,517	,557
	Sig. (2-tailed)	,847	,727	,205		,117	,187	,205	,178	,191	,204	,118	,129	,190	,152
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
linPFOS	Pearson Correlation	-,060	,104	,870**	,598	1	,949**	,956**	,689	,909**	,939**	,999**	,991**	,930**	,976**
	Sig. (2-tailed)	,910	,806	,005	,117		,000	,000	,059	,002	,001	,000	,000	,001	,000
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
PFDoDA	Pearson Correlation	-,117	,036	,748*	,520	,949**	1	,988**	,781*	,955**	,987**	,948**	,938**	,984**	,982**
	Sig. (2-tailed)	,826	,933	,033	,187	,000		,000	,022	,000	,000	,000	,001	,000	,000
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
PFTrDA	Pearson Correlation	,007	-,023	,729*	,502	,956**	,988**	1	,790*	,973**	,988**	,957**	,951**	,993**	,993**
	Sig. (2-tailed)	,989	,958	,040	,205	,000	,000		,020	,000	,000	,000	,000	,000	,000
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
PFTeDA	Pearson Correlation	-,044	-,024	,521	,529	,689	,781*	,790*	1	,778*	,775*	,687	,666	,801*	,755*
	Sig. (2-tailed)	,934	,955	,186	,178	,059	,022	,020		,023	,024	,060	,072	,017	,030
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
PFDA	Pearson Correlation	,010	-,067	,613	,515	,909**	,955**	,973**	,778*	1	,982**	,907**	,905**	,989**	,970**
	Sig. (2-tailed)	,985	,876	,106	,191	,002	,000	,000	,023		,000	,002	,002	,000	,000
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
PFUnDA	Pearson Correlation	-,116	,051	,708*	,503	,939**	,987**	,988**	,775*	,982**	1	,934**	,922**	,993**	,980**
	Sig. (2-tailed)	,827	,905	,049	,204	,001	,000	,000	,024	,000		,001	,001	,000	,000
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
∑PFOS	Pearson Correlation	-,020	,065	,860**	,597	,999**	,948**	,957**	,687	,907**	,934**	1	,996**	,930**	,978**
	Sig. (2-tailed)	,970	,878	,006	,118	,000	,000	,000	,060	,002	,001		,000	,001	,000
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
∑PFSA	Pearson Correlation	,036	,015	,833*	,583	,991**	,938**	,951**	,666	,905**	,922**	,996**	1	,924**	,976**
	Sig. (2-tailed)	,946	,973	,010	,129	,000	,001	,000	,072	,002	,001	,000		,001	,000
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
∑PFCA	Pearson Correlation	-,009	-,058	,665	,517	,930**	,984**	,993**	,801*	,989**	,993**	,930**	,924**	1	,985**
	Sig. (2-tailed)	,987	,891	,072	,190	,001	,000	,000	,017	,000	,000	,001	,001		,000
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8
∑PFAS	Pearson Correlation	,011	-,026	,755*	,557	,976**	,982**	,993**	,755*	,970**	,980**	,978**	,976**	,985**	1
	Sig. (2-tailed)	,983	,951	,030	,152	,000	,000	,000	,030	,000	,000	,000	,000	,000	
	N	6	8	8	8	8	8	8	8	8	8	8	8	8	8

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 17: Pearson's correlation coefficient for all normally distributed variables (males only).

		BMR	TT3	TT4	linPFOS	∑PFOS	∑PFSA	PFDoDA	PFTrDA	PFTeDA	PFDA	PFUnDA	∑PFCA	∑PFAS
BMR	Pearson Correlation	1	-,128	-,028	,160	,162	,145	-,062	-,291	,265	,036	,078	-,014	,073
	Sig. (2-tailed)		,763	,953	,704	,702	,732	,883	,484	,525	,933	,855	,974	,864
	N	9	8	7	8	8	8	8	8	8	8	8	8	8
TT3	Pearson Correlation	-,128	1	-,542	,187	,161	,142	,728	,275	,669	,288	,527	,441	,299
	Sig. (2-tailed)	,763		,266	,688	,730	,761	,064	,551	,100	,531	,225	,322	,515
	N	8	8	6	7	7	7	7	7	7	7	7	7	7
TT4	Pearson Correlation	-,028	-,542	1	-,327	-,302	-,296	-,467	-,643	-,188	-,670	-,752	-,653	-,496
	Sig. (2-tailed)	,953	,266		,528	,561	,569	,350	,169	,721	,145	,085	,160	,318
	N	7	6	7	6	6	6	6	6	6	6	6	6	6
linPFOS	Pearson Correlation	,160	,187	-,327	1	,999**	,997**	,783*	,708*	,675*	,601	,716*	,751*	,938**
	Sig. (2-tailed)	,704	,688	,528		,000	,000	,013	,033	,046	,087	,030	,020	,000
	N	8	7	6	9	9	9	9	9	9	9	9	9	9
∑PFOS	Pearson Correlation	,162	,161	-,302	,999**	1	,999**	,773*	,716*	,675*	,617	,716*	,757*	,942**
	Sig. (2-tailed)	,702	,730	,561	,000		,000	,015	,030	,046	,077	,030	,018	,000
	N	8	7	6	9	9	9	9	9	9	9	9	9	9
∑PFSA	Pearson Correlation	,145	,142	-,296	,997**	,999**	1	,758*	,720*	,655	,598	,711*	,752*	,940**
	Sig. (2-tailed)	,732	,761	,569	,000	,000		,018	,029	,056	,089	,032	,020	,000
	N	8	7	6	9	9	9	9	9	9	9	9	9	9
PFDoDA	Pearson Correlation	-,062	,728	-,467	,783*	,773*	,758*	1	,746*	,878**	,708*	,821**	,844**	,855**
	Sig. (2-tailed)	,883	,064	,350	,013	,015	,018		,021	,002	,033	,007	,004	,003
	N	8	7	6	9	9	9	9	9	9	9	9	9	9
PFTrDA	Pearson Correlation	-,291	,275	-,643	,708*	,716*	,720*	,746*	1	,524	,839**	,870**	,939**	,883**
	Sig. (2-tailed)	,484	,551	,169	,033	,030	,029	,021		,148	,005	,002	,000	,002
	N	8	7	6	9	9	9	9	9	9	9	9	9	9
PFTeDA	Pearson Correlation	,265	,669	-,188	,675*	,675*	,655	,878**	,524	1	,654	,695*	,728*	,738*
	Sig. (2-tailed)	,525	,100	,721	,046	,046	,056	,002	,148		,056	,038	,026	,023
	N	8	7	6	9	9	9	9	9	9	9	9	9	9
PFDA	Pearson Correlation	,036	,288	-,670	,601	,617	,598	,708*	,839**	,654	1	,832**	,910**	,800**
	Sig. (2-tailed)	,933	,531	,145	,087	,077	,089	,033	,005	,056		,005	,001	,010
	N	8	7	6	9	9	9	9	9	9	9	9	9	9
PFUnDA	Pearson Correlation	,078	,527	-,752	,716*	,716*	,711*	,821**	,870**	,695*	,832**	1	,969**	,893**
	Sig. (2-tailed)	,855	,225	,085	,030	,030	,032	,007	,002	,038	,005		,000	,001
	N	8	7	6	9	9	9	9	9	9	9	9	9	9
∑PFCA	Pearson Correlation	-,014	,441	-,653	,751*	,757*	,752*	,844**	,939**	,728*	,910**	,969**	1	,932**
	Sig. (2-tailed)	,974	,322	,160	,020	,018	,020	,004	,000	,026	,001	,000		,000
	N	8	7	6	9	9	9	9	9	9	9	9	9	9
∑PFAS	Pearson Correlation	,073	,299	-,496	,938**	,942**	,940**	,855**	,883**	,738*	,800**	,893**	,932**	1
	Sig. (2-tailed)	,864	,515	,318	,000	,000	,000	,003	,002	,023	,010	,001	,000	
	N	8	7	6	9	9	9	9	9	9	9	9	9	9

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix L: Correlation matrix with Spearman's Rank correlation

Table 18: Spearman's rank correlation coefficient for the non-normally distributed variables (all individuals)

			TT4/TT3	brPFOS	PFHxS	PFNA
Spearman's rho	TT4/TT3	Correlation Coefficient	1,000	-,330	,451	,033
		Sig. (2-tailed)	.	,271	,122	,915
		N	14	13	13	13
	brPFOS	Correlation Coefficient	-,330	1,000	-,004	,711**
		Sig. (2-tailed)	,271	.	,989	,001
		N	13	19	19	19
	PFHxS	Correlation Coefficient	,451	-,004	1,000	,177
		Sig. (2-tailed)	,122	,989	.	,468
		N	13	19	19	19
	PFNA	Correlation Coefficient	,033	,711**	,177	1,000
		Sig. (2-tailed)	,915	,001	,468	.
		N	13	19	19	19
	BMR	Correlation Coefficient	,102	,487*	,120	,297
		Sig. (2-tailed)	,753	,048	,646	,247
		N	12	17	17	17
	Mass	Correlation Coefficient	-,270	-,257	-,461*	-,225
		Sig. (2-tailed)	,350	,288	,047	,355
		N	14	19	19	19
	TT3	Correlation Coefficient	-,921**	,593*	-,218	,200
		Sig. (2-tailed)	,000	,020	,435	,475
		N	14	15	15	15
	TT4	Correlation Coefficient	,081	,011	,007	-,042
		Sig. (2-tailed)	,782	,970	,982	,887
		N	14	14	14	14
	linPFOS	Correlation Coefficient	-,473	,884**	-,182	,530*
		Sig. (2-tailed)	,103	,000	,455	,020
		N	13	19	19	19
	∑PFOS	Correlation Coefficient	-,451	,900**	-,174	,556*
		Sig. (2-tailed)	,122	,000	,477	,013
		N	13	19	19	19
	∑PFSA	Correlation Coefficient	-,418	,918**	-,133	,586**
		Sig. (2-tailed)	,156	,000	,586	,008
		N	13	19	19	19
	PFDoDA	Correlation Coefficient	-,555*	,895**	-,161	,626**
		Sig. (2-tailed)	,049	,000	,509	,004
		N	13	19	19	19
	PFTrDA	Correlation Coefficient	-,577*	,867**	-,075	,679**
		Sig. (2-tailed)	,039	,000	,759	,001
		N	13	19	19	19
	PFTeDA	Correlation Coefficient	-,462	,872**	-,196	,632**
		Sig. (2-tailed)	,112	,000	,420	,004
		N	13	19	19	19
	PFDA	Correlation Coefficient	-,467	,921**	-,130	,686**
		Sig. (2-tailed)	,108	,000	,596	,001
		N	13	19	19	19
	PFUnDA	Correlation Coefficient	-,544	,872**	-,147	,633**
		Sig. (2-tailed)	,055	,000	,547	,004
		N	13	19	19	19
	∑PFCA	Correlation Coefficient	-,516	,896**	-,084	,686**
		Sig. (2-tailed)	,071	,000	,732	,001
		N	13	19	19	19
	∑PFAS	Correlation Coefficient	-,511	,932**	-,130	,635**
		Sig. (2-tailed)	,074	,000	,596	,003
		N	13	19	19	19

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 19: Spearman's rank correlation coefficient for the non-normally distributed variables (females only)

			TT4/TT3	PFHxS	brPFOS	PFNA
Spearman's rho	TT4/TT3	Correlation Coefficient	1,000	,429	-,143	,405
		Sig. (2-tailed)	.	,289	,736	,320
		N	8	8	8	8
	PFHxS	Correlation Coefficient	,429	1,000	,524	,595
		Sig. (2-tailed)	,289	.	,183	,120
		N	8	8	8	8
	brPFOS	Correlation Coefficient	-,143	,524	1,000	,738*
		Sig. (2-tailed)	,736	,183	.	,037
		N	8	8	8	8
	PFNA	Correlation Coefficient	,405	,595	,738*	1,000
		Sig. (2-tailed)	,320	,120	,037	.
		N	8	8	8	8
	BMR	Correlation Coefficient	,257	,429	,257	,600
		Sig. (2-tailed)	,623	,397	,623	,208
		N	6	6	6	6
	Mass	Correlation Coefficient	-,595	-,714*	-,429	-,833*
		Sig. (2-tailed)	,120	,047	,289	,010
		N	8	8	8	8
	TT3	Correlation Coefficient	-,952**	-,381	,286	-,214
		Sig. (2-tailed)	,000	,352	,493	,610
		N	8	8	8	8
	TT4	Correlation Coefficient	-,333	-,214	,405	,190
		Sig. (2-tailed)	,420	,610	,320	,651
		N	8	8	8	8
	linPFOS	Correlation Coefficient	-,429	-,048	,810*	,452
		Sig. (2-tailed)	,289	,911	,015	,260
		N	8	8	8	8
	∑PFOS	Correlation Coefficient	-,429	-,048	,810*	,452
		Sig. (2-tailed)	,289	,911	,015	,260
		N	8	8	8	8
	∑PFSA	Correlation Coefficient	-,357	,143	,905**	,643
		Sig. (2-tailed)	,385	,736	,002	,086
		N	8	8	8	8
	PFDoDA	Correlation Coefficient	-,500	-,143	,714*	,357
		Sig. (2-tailed)	,207	,736	,047	,385
		N	8	8	8	8
	PFTrDA	Correlation Coefficient	-,500	-,048	,714*	,452
		Sig. (2-tailed)	,207	,911	,047	,260
		N	8	8	8	8
	PFTeDA	Correlation Coefficient	-,214	-,190	,476	,452
		Sig. (2-tailed)	,610	,651	,233	,260
		N	8	8	8	8
	PFDA	Correlation Coefficient	-,405	,071	,881**	,524
		Sig. (2-tailed)	,320	,867	,004	,183
		N	8	8	8	8
	PFUnDA	Correlation Coefficient	-,429	-,048	,810*	,452
		Sig. (2-tailed)	,289	,911	,015	,260
		N	8	8	8	8
	∑PFCA	Correlation Coefficient	-,381	,024	,833*	,571
		Sig. (2-tailed)	,352	,955	,010	,139
		N	8	8	8	8
	∑PFAS	Correlation Coefficient	-,357	,143	,905**	,643
		Sig. (2-tailed)	,385	,736	,002	,086
		N	8	8	8	8

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 20: Spearman's rank correlation coefficient for the non-normally distributed variables (males only)

			TT4/TT3	PFHxS	brPFOS	PFNA
Spearman's rho	TT4/TT3	Correlation Coefficient	1,000	,700	-,100	-,100
		Sig. (2-tailed)	.	,188	,873	,873
		N	6	5	5	5
	PFHxS	Correlation Coefficient	,700	1,000	-,033	,267
		Sig. (2-tailed)	,188	.	,932	,488
		N	5	9	9	9
	brPFOS	Correlation Coefficient	-,100	-,033	1,000	,600
		Sig. (2-tailed)	,873	,932	.	,088
		N	5	9	9	9
	PFNA	Correlation Coefficient	-,100	,267	,600	1,000
		Sig. (2-tailed)	,873	,488	,088	.
		N	5	9	9	9
	BMR	Correlation Coefficient	,203	-,252	,311	,072
		Sig. (2-tailed)	,700	,548	,453	,866
		N	6	8	8	8
	TT3	Correlation Coefficient	-,943**	-,571	,464	,286
		Sig. (2-tailed)	,005	,180	,294	,535
		N	6	7	7	7
	TT4	Correlation Coefficient	,600	,429	-,314	-,314
		Sig. (2-tailed)	,208	,397	,544	,544
		N	6	6	6	6
	linPFOS	Correlation Coefficient	-,300	,067	,800**	,250
		Sig. (2-tailed)	,624	,865	,010	,516
		N	5	9	9	9
	∑PFOS	Correlation Coefficient	-,100	,067	,883**	,317
		Sig. (2-tailed)	,873	,865	,002	,406
		N	5	9	9	9
	∑PFSA	Correlation Coefficient	-,100	,150	,833**	,283
		Sig. (2-tailed)	,873	,700	,005	,460
		N	5	9	9	9
	PFDoDA	Correlation Coefficient	-,100	-,067	,900**	,733*
		Sig. (2-tailed)	,873	,865	,001	,025
		N	5	9	9	9
	PFTrDA	Correlation Coefficient	-,300	,100	,667*	,850**
		Sig. (2-tailed)	,624	,798	,050	,004
		N	5	9	9	9
	PFTeDA	Correlation Coefficient	-,100	,067	,917**	,567
		Sig. (2-tailed)	,873	,865	,001	,112
		N	5	9	9	9
	PFDA	Correlation Coefficient	-,300	-,117	,833**	,833**
		Sig. (2-tailed)	,624	,765	,005	,005
		N	5	9	9	9
	PFUnDA	Correlation Coefficient	-,300	,033	,650	,817**
		Sig. (2-tailed)	,624	,932	,058	,007
		N	5	9	9	9
	∑PFCA	Correlation Coefficient	-,300	-,083	,833**	,783*
		Sig. (2-tailed)	,624	,831	,005	,013
		N	5	9	9	9
	∑PFAS	Correlation Coefficient	-,300	,017	,900**	,617
		Sig. (2-tailed)	,624	,966	,001	,077
		N	5	9	9	9

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix M: Temperature-dependent MR in arctic terns (1990)

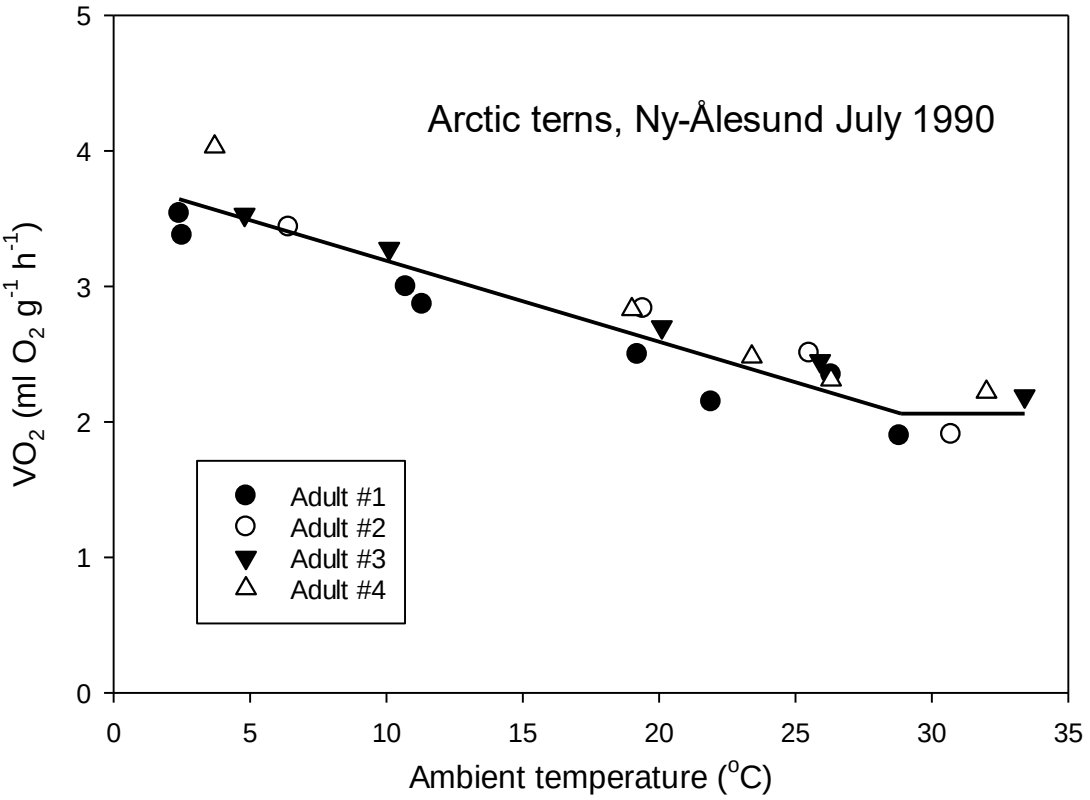

Figure 8: Graph by Claus Bech and Marcel Klaassen, showing the temperature-dependent MR of 4 adult arctic terns (*Sterna paradisaea*) in Kongsfjorden 1990. T_a (ºC) is shown on the x-axis and MR (mL O_2/g*h) is shown on the y-axis. Graph provided by Claus Bech, March 2021.

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