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Interactions between Pollutant Exposure and the Physiology in Adult Kittiwakes (*Rissa tridactyla*) at Svalbard

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Environmental Toxicology and Chemistry
Submission date: May 2015
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Cover photo: Adult kittiwake (*Rissa tridactyla*) by Niels Borup Svendsen

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Acknowledgements

This master's thesis has been written at the Norwegian University of Science and Technology (NTNU) in collaboration with the Norwegian Polar Institute (NPI), and the Norwegian Institute of Air Research (NILU). The Arctic Field Grant from Svalbard science forum, which I am grateful to have received, financed the fieldwork in Kongsfjorden. I am appreciative for the received grant from the Society of Arctic Research and Technology (SAFT) as well.

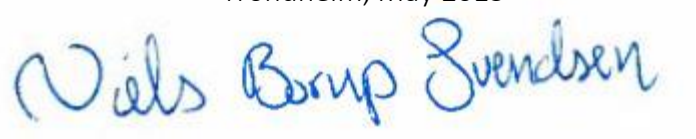
First, I would like to express my sincere gratitude to Veerle Jaspers, my main supervisor, for letting me in on this exciting project, and all your help during the writing of my thesis. It has been truly appreciated. Thanks to Geir W. Gabrielsen, my external supervisor, for useful feedback, and approving and partly founding this project.

Several persons helped during fieldwork in Kongsfjorden, but I would like to direct a sincere gratitude to Claus Bech for teaching me everything there is to know about kittiwakes and Svalbard, showing me how to do sampling of kittiwakes in the field, and sharing your coffee during lunch each day at the top of Blomstrandhalvøya. Pierre-Axel Monternier, I have really appreciated your assistance in the field every day, your daily lessons in how to curse in French, and all the fun we had in Ny-Ålesund.

Thanks to Dorte Herzke for letting me do my lab work at the Norwegian Institute for Air Research (NILU). Line Christoffersen, Mikael Harju, and Arntraut Götsch you have been very helpful during my stay in Tromsø - thank you.

Last but not least, spending almost two years in Trondheim and Longyearbyen together with inspirational and fantastic people has been truly amazing; I would not change it for anything in the world.

Trondheim, May 2015



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Abstract

The present study investigated the use of feathers as a useful non-destructive biomonitoring tool for novel organic pollutants in black-legged kittiwakes (*Rissa tridactyla*), and evaluated the interaction of both novel and legacy pollutants on body condition and thyroid hormones. In July and August 2014, feather and blood samples were collected from 20 black-legged kittiwakes (*Rissa tridactyla*) at two colonies (Blomstrandhalvøya and Krykkjefjellet) in Kongsfjorden, Svalbard. Samples were analyzed for polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), organochlorine pesticides (OCPs) and phosphorous flame retardants (PFRs).

All compound classes were detected and quantified in feathers ranging from <2.38 to 36.2 ng/g wet weight (ww) for sum PCBs, <1.23 to 7.81 ng/g ww for sum PBDEs, <0.99 to 14.2 ng/g ww for sum OCPs, and <1 to 15.6 ng/g feather for sum PFRs. This confirms the suitability of kittiwake feathers for quantification of both legacy and novel pollutants. Strong significant differences ($p < 0.001$) in organic pollutant levels were found in plasma, but not in feathers, between the colonies (total POP load in plasma mean±SE: 72.9±8.63 ng/g ww for Blomstrandhalvøya; 29.6±1.67 ng/g ww for Krykkjefjellet). None of the investigated compounds in feathers correlated with plasma levels, which indicate that kittiwake feathers reflect contamination from the wintering areas where the feathers were grown, whereas plasma levels reflect pollution at the breeding areas at Svalbard.

Significant negative correlations between the ratio of total triiodothyronine (TT3) to free triiodothyronine (fT3), and CB 28, -138, and -187, BDE 47, and sum PBDE (mainly BDE 47) were found for kittiwakes from Blomstrandhalvøya (all $r \geq -0.60$ and $p \leq 0.05$). The endocrine disrupting abilities of PCBs and PBDEs seem to be of concern in the kittiwakes, since only the kittiwakes with the higher levels of circulating pollutants from Blomstrandhalvøya were significantly affected. In addition, body condition of birds from both colonies was significantly negatively correlated with most OCPs, and PCBs. Altogether, these results show an additional stress factor to kittiwakes caused by exposure to organic pollutants during the already stressful breeding period at Svalbard.

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Resumé

Dette studie har undersøgt brugbarheden af fjer fra rider (*Rissa tridactyla*), som et ikke-destruktivt biomoniteringsredskab for nye organiske miljøgifte, samt efterforsket hvordan nye og gamle miljøgifte interagerer med både thyroideahormoner og kropsvægt hos rider. I perioden juli-august 2014 blev der i alt taget 20 fjer- og blodprøver fra rider fra to kolonier (Blomstrandhalvøya og Krykkjefjellet) ved Kongsfjorden, Svalbard. Alle prøver blev analyseret for polyklorerede bifenyler (PCBer), polybromerede bifenyl ethere (PBDEer), klorede organiske pesticider (OCPer) og fosfat flammehæmmere (PFRer).

Alle undersøgte typer af miljøgifte blev både detekteret og kvantificeret i fjerene i niveauer fra <2,38 til 36,2 ng/g vådvægt (ww) for summen af PCBer, <1,23 til 7,81 ng/g ww for summen af PBDEer, <0,99 til 14,2 ng/g ww for summen af OCPer, og <1 til 15,6 ng/g fjer for summen af PFRer. Dette bekræfter egnetheden af fjer for detektering og kvantificering af både nye og gamle miljøgifte. En stærk signifikant forskel ($p < 0.001$) blev fundet i den totale mængde af miljøgifte i plasma, men ikke i fjerene, mellem kolonier (gennemsnitlig mængde af miljøgifte±SE var for rider fra Blomstrandhalvøya 72,7±8,62 ng/g ww, mens kun 29,6±1,67 ng/g ww for rider fra Krykkjefjellet). Ingen af de undersøgte miljøgifte i fjer korrelerede med niveauer i plasma, hvilket tyder på at ridernes fjer reflekterer forureningen fra overvintringsområderne, hvor de blev groet, mens plasma niveauer reflekterer forureningen på Svalbard.

Signifikant negative korrelationer blev fundet for rationen mellem frit trijodthyronin (fT3) og den totale mængde af trijodthyronin (TT3), og CB 28, -138, -187, BDE 47, og summen af PBDEer (hovedsageligt BDE 47), men kun for rider fra Blomstrandhalvøya (alle $r \geq -0.60$ og $p \leq 0.05$). De hormonforstyrrende egenskaber hos PCBer og PBDEer må tages alvorligt, da specielt riderne fra Blomstrandhalvøya, med højt niveau af miljøgifte i blodkredsløbet, blev signifikant påvirket. Derudover korrelerede ridernes kropsvægt negativt med næsten alle PCBer og OCPer. Samlet set viser dette studie at mængden miljøgifte i rider udgør en yderligere stressfaktor i en allerede udfordrende yngleperiode på Svalbard.

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List of abbreviations

$(NH_4)_2SO_4$	Ammonium sulphate
ACN	Acetonitrile
AMAP	Arctic monitoring and assessment programme
BCI	Body condition index
CHD	Chromobox-helicase-DNA-binding gene (W or Z)
DCM	Dichloromethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
EDC	Endocrine disrupting compound
fT3	Free triiodothyronine
GC-MS	Gas chromatography mass spectrometry
GPC	Gel permeation chromatography
HCB	Hexachlorobenzene
HCl	Hydrogen chloride
HPLC	High-pressure liquid chromatography
HPT	hypothalamic-pituitary-thyroid (axis)
HRGC	High-resolution gas chromatography
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of detection
LOQ	Limit of quantification
LRAT	Long-range atmospheric transport
$MgSO_4$	Magnesium sulphate
NA	Not available
NBFR	Novel brominated flame retardant
ND	Not detected
NILU	Norwegian Institute for Air Research

OCP	Organochlorine pesticide
PAH	Polycyclic aromatic hydrocarbon
PBB	Polybrominated biphenyl
PBDE	Polybrominated diphenylether
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PFA	Perfluorinated acids
PFAS	Per- and polyfluoroalkyl sulfate
PFR	Phosphorous flame retardant
POP	Persistent organic pollutant
PSA	Primary-secondary amine
SE	Standard error
SPE	Solid phase extraction
SRM	Standard reference material
T3	triiodothyronine
T4	Thyroxine
TBME	Tert-butyl methyl ether
TCN	1,2,3,4-tetrachloronaphtalene
TMB	3, 3', 5, 5'-tetramethylbenzidine
TSH	Thyroid stimulating hormone (thyrotropin)
TT3	Total triiodothyronine
TTR	Transthyretin
UPLC	Ultrahigh pressure liquid chromatography
ww	Wet weight

IUPAC names of detected compounds are listed in Appendix I.

Table of contents

1. Introduction

Ever since Rachel Carson published “Silent Spring” in 1962 (Carson, 1962), the environmental movement has been scientifically addressing environmental issues regarding ecology and health. Due to increasing human activity and the introduction of new chemicals into the environment, these two issues are more important today than ever. Some of these chemicals degrade slowly in the environment and this persistency causes the unfortunate detection in wildlife, decades after their application. Moreover, the Arctic wildlife is no exception.

1.1 The polluted Arctic

The harsh environment of the Arctic is characterized by low air and water temperatures, permafrost, sea ice, long periods without sunlight, and lack of nutrients (Gordeev, 2002). Long food chains and low species diversity are some of several unique attributes (Borgå et al., 2004). Natural variations in biotic factors like food availability, predation rate and competition, as well as fluctuations in abiotic factors such as sea ice coverage and temperature are greatly affecting and challenging the Arctic wildlife (Bustnes et al., 2008; Moe et al., 2009). Furthermore, the Arctic wildlife is faced with both emerging and legacy pollutants from anthropogenic sources. Legacy pollutants are described as pollutants that have been regarded as an environmental concern for a long period by being banned or regulated (Rigét et al., 2010; McKinney et al., 2011). Emerging contaminants are newly detected chemicals in the environment or wildlife, and are known or suspected to cause adverse effects (Petrovic et al., 2004).

The best known group of legacy contaminants are the notorious persistent organic pollutants (POPs). Most POPs originate from industrial production of dielectric fluids such as polychlorinated biphenyls (PCBs), pesticides such as dichlorodiphenyltrichloroethane (DDT) and mirex, or as unwanted byproducts (Ritter et al., 1995; Smital, 2008). Emerging contaminants include phosphorous flame retardants (PFRs), novel brominated flame retardants (NBFRs) as well as polybrominated diphenylethers (PBDEs) although some of the PBDE congener mixtures used as flame retardants are now banned, or voluntarily phased-out (de Wit et al., 2010; Malik et al., 2011). The phase-out of the lesser brominated penta- and octaBDE flame retardants may have increased the production and use

of PFRs (Reemtsma et al., 2008; Stapleton et al., 2009; van der Veen and de Boer, 2012). Although legacy and emerging pollutants have diverse chemical structures, they share some physiochemical characteristics such as halogenation of aliphatic and aromatic rings. These attributes decrease water solubility and vapor pressure, increase lipophilicity, and cause resistance to both chemical and microbial degradation (Borgå et al., 2004). Therefore, these compounds can accumulate in lipid rich tissues and increase in concentration from low to high trophic level resulting in the highest levels of POPs in apex predators such as polar bear (*Ursus maritimus*) and glaucous gull (*Larus hyperboreus*) (Bernhoft et al., 1997; Fisk et al., 2001; Gabrielsen, 2007; Borgå et al., 2012).

1.2 Transport of pollutants to the Arctic

The first reports of contaminated Arctic wildlife were published in the early 1970's (AMAP, 1998), and now the Arctic is considered as an important indicator region for assessing persistence and bioaccumulative abilities of emerging contaminants (de Wit et al., 2010). Most of the pollutants and chemicals found in the Arctic environment cannot be related to any local sources and must originate from anthropogenic sources on lower latitudes, where mostly North America and Western Europe are indicated as source regions (Burkow and Kallenborn, 2000; Gordeev, 2002; de Wit et al., 2010). The major pathways and transportations routes of contaminants to the Arctic are long-range atmospheric transport (LRAT), inflowing ocean currents, sea-ice drift, and northward flowing rivers (Burkow and Kallenborn, 2000; Gordeev, 2002; de Wit et al., 2010). Seabirds as a biological transport vector for POPs is not to be neglected since it has been evaluated to be thirty times more efficient compared to LRAT (Blais et al., 2005; Evenset et al., 2007). As the temperature drops towards the Arctic, semi-volatile POPs condensate in the air, where more than 80 % of the atmospheric deposition happens by snow scavenging (Burkow and Kallenborn, 2000). Therefore, the atmospheric transport is believed to be the main source of semi-volatile POPs to the Arctic (Wania et al., 1998; Gordeev, 2002; Halsall, 2004).

1.3 Pollutant stress during the reproductive period in Arctic Biota

Most pollutants enter the food web in aquatic living organisms by diffusion over the gills or in the gastrointestinal tract, where they cross the gut membrane and accumulate in lipid rich tissue (AMAP, 1998). The main entrance for POPs in seabirds, like the black-legged kittiwake (*Rissa tridactyla*,

hereafter just 'kittiwake'), is through their diet, where quick distribution to lipid rich tissue is reported (AMAP, 1998). When POPs are stored in energy-rich fat reserves such as adipose tissue, they exert no harm. However, during the reproductive period when birds are believed to function close to their physiological limit (Fyhn et al., 2001; Bech et al., 2002), they rely on the energy stored as fat. Mass loss during breeding period is common in birds (Moreno, 1989) and kittiwakes are no exception (Bech et al., 2002; Moe et al., 2002). The release of lipids to the blood will inevitably lead to a redistribution of lipophilic contaminants in an already stressful period, and cause the concentration of pollutants to increase. Hence, in periods of poor body condition, kittiwakes may be at higher risk for negative effects associated with POPs, than the mean concentration of POPs would suggest (Macdonald and Bewers, 1996).

1.4 Health effects associated with contaminant exposure

Although levels of pollutants in Arctic biota are considered generally low compared to industrialized areas in North America and Europe (AMAP, 1998; Gabrielsen, 2007), their effects can be detrimental. In Arctic seabirds several effects are related to POP exposure. Changed reproductive behavior (Bustnes et al., 2001), reduced adult survival rate (Bustnes et al., 2003), wing feather asymmetry (Bustnes et al., 2003), suppressed immune function (Grasman et al., 1996), reduced offspring performance (Verboven et al., 2009), and lowered levels of circulating thyroid hormones (Verreault et al., 2004; Nøst et al., 2012) are all reported effects. Chemicals that exert effects on the endocrine system are known as endocrine disrupting compounds (EDCs). In this study, all investigated legacy POPs, including PBDEs, have the potential to be EDCs (Petersen et al., 2007). The modes of action for EDCs include (but are not limited to) divergent pathways as mimicking or antagonizing the effect of the natural hormone by binding or blocking the hormone receptor, altering synthesis or metabolism of the hormone, or interfering with signaling to-and-from hypothalamus-pituitary endocrine gland (Dawson, 2000; Diamanti-Kandarakis et al., 2009). Consequently, EDCs may have adverse effects on the thyroid hormone system, which is vital for seabirds to adapt, reproduce, and survive in the cold Arctic climate (Gabrielsen, 2007).

1.5 The thyroid hormone system

The function of the thyroid system in birds appears to be similar to those of other vertebrates in general (Dawson, 2000; McNabb, 2007). As a part of the hypothalamic-pituitary-thyroid (HPT) axis, all thyroid gland activities such as iodine uptake, thyroxine (T4) release, and gland growth are controlled by the pituitary gland. Releases of T4 is regulated by the thyroid stimulating hormone (TSH; thyrotropin) released by the pituitary gland. T4 is derived from tyrosine and due to its iodine richness it is lipid soluble and transported in blood mainly by the transport proteins transthyretin (TTR) and albumin (McNabb, 2007; Hill et al., 2008). Avian TTR has four times higher affinity for triiodothyronine (T3) than mammalian TTR (Chang et al., 1999). T4 is mostly converted to the active form T3 by hepatic type 1 deiodinase (Dawson, 2000). Active thyroid hormones exert a wide range of effects and are required for growth, differentiation and maturation of several body systems, central nervous system development, and reproductive activity (Dawson, 2000; McNabb, 2007). Thyroid hormones do also induce molt and regulate heat production in order to maintain a constant body temperature, which is crucial for Arctic seabirds (McNabb, 2007).

Circulating levels of T4 are several times higher than T3, but numerous factors affect this relationship and their function. Iodine availability, food availability and composition, temperature, season, age, breeding status, and time of sampling all affect thyroid status (McNabb, 1999; McNabb, 2007), where food availability and temperature appear to be the most important. A reduction in circulating T3 levels is associated with both short-term fasting and long-term starvation (McNabb, 2007), whereas cold temperatures increase T3 concentrations and vice versa since thyroid hormones are vital in thermoregulation. Therefore, the presence of EDCs that alter the levels of thyroid hormones could result in adverse effects.

Thyroid hormones are considered as one of the most vulnerable endocrine variables to pollution in Arctic biota (Jenssen, 2006). Changed thyroid levels and abnormal thyroid gland structure are among associated effects of thyroid hormone interference, and these effects have been reported in several species as glaucous gull (Verreault et al., 2004), kittiwake chicks (Nøst et al., 2012), ribbon seals (Chiba et al., 2001), and polar bears (Braathen et al., 2004). The degree of effect depends on the concentration of the EDC, the disrupting potency of the EDC, and the mode of action (Zoeller, 2007).

PCBs and metabolites may act through numerous mechanisms as inhibiting the deiodinase, bind transthyretin, or exert a direct action on the thyroid hormone receptor (Zoeller, 2007).

Since the Arctic summer is short, proper timing of breeding, molting, and migration is essential for survival. Exposure to EDCs could disrupt abilities of the endocrine system to regulate these events and lead to less successful breeding and in the worst case reduced survival (Jenssen, 2006). Thus, the importance of timing in a changing environment is vital.

1.6 The use of feathers in biomonitoring pollutants

Feathers are only connected to the bloodstream through small blood vessels during the 3-6 weeks of growth (González-Solís et al., 2011). When the feathers are fully formed, the blood vessels atrophy and the feathers become inert. Thus, the exposure of contaminants in feathers will reflect the time of growth, when feathers are connected to the bloodstream and its circulating contaminants (Burger, 1993; Jaspers et al., 2004; van den Steen et al., 2007). The feathers remain in place as an inert archive of pollutant exposure until moult. Several studies have used feathers for measuring heavy metals (Burger, 1993; García-Fernández et al., 2013), whereas the use of feathers for measuring organic pollutants and novel pollutants is less common. Studies that have investigated this issue have evaluated feathers as a useful biomonitoring tool for non-destructive detection and quantification of organic pollutants in both adult and nestling birds (Dauwe et al., 2005; Jaspers et al., 2006; Jaspers et al., 2007b; van den Steen et al., 2007; Eulaers et al., 2011; García-Fernández et al., 2013).

Polybrominated biphenyls (PBBs), PCBs, PBDEs, per- and polyfluoroalkyl sulfonates (PFASs), and organochlorine pesticides (OCPs) have been quantified in feathers from several bird species (García-Fernández et al., 2013) and significant correlations to levels in internal tissues have been reported as well (Jaspers et al., 2007b; Eulaers et al., 2011; Jaspers et al., 2011; Eulaers et al., 2014a). However, only one study, to the author's knowledge, has shown that PFRs can be measured in feathers (Eulaers et al., 2014b). Although PFRs have been detected in the Arctic environment (Salamova et al., 2014), only one study so far has investigated their occurrence in Arctic wildlife (Evenset et al., 2009). The present study will address this issue by examining the occurrence of PFRs in the Arctic kittiwake.

1.7 Objectives and aims of the study

Kittiwakes are ideal as study species for pollution since they are long-lived and have a relatively high trophic position. The present study will investigate the extent of pollution exposure of kittiwakes at Svalbard regarding traditional POPs, PBDEs, and PFRs by non-destructive sampling of feathers and blood.

The main objectives of the study are: (I) to assess whether traditional POPs, PBDEs, and PFRs can be quantified in feathers of kittiwakes on Svalbard, (II) to investigate the relationship between POPs, PBDEs, and PFRs quantified in feathers and in blood to verify the reliability of using feathers as a biomarker, and (III) to evaluate the effect of the different (legacy and novel) pollutants on thyroid hormones.

2. Materials and methods

2.1 Study area

Sampling was conducted in July and August 2014 during the breeding season of the kittiwake. Two colonies located close to Ny-Ålesund, Kongsfjorden, Svalbard (78°55'N, 11°55'E), were studied, respectively 'Krykkjefjellet' approximately 7 km east of Ny-Ålesund, and 'Blomstrandhalvøya' on the north side of Blomstrandhalvøya (fig. 1). Eight birds from Krykkjefjellet were sampled (four in the middle of July and four in the early August), and twelve birds from Blomstrandhalvøya were sampled (all in early August). In these colonies, kittiwakes constitute most of the breeding birds, but breeding northern fulmars (*Fulmarus glacialis*) are also found in Blomstrandhalvøya and breeding black guillemots (*Cepphus grylle*) are likewise found in Krykkjefjellet.

Figure 1. An overview of Kongsfjorden situated on the west side of the Arctic archipelago Svalbard. The two colonies are marked with an asterisk. All map data are from the Norwegian Polar Institute. Map design: Niels Borup Svendsen.

2.2 Study species

The kittiwake is a medium sized gull and has a circumpolar distribution with breeding areas in the arctic and boreal zones of the northern hemisphere (Strøm, 2006). It is the most numerous bird at Svalbard with more than 270.000 breeding pairs, and the estimated population in the Barents Sea region is over 2.2 million individuals (Gabrielsen, 2009). Kittiwakes often forage in flocks either on or just beneath the sea surface. Their dominant food source is polar cod (*Boreogadus saida*) but amphipods, cephalopods, and copepods also constitute a substantial part of their food (Mehlum and Gabrielsen, 1993; Coulson, 2011). The nests are built on steep rock outcrops close to the sea, and consist of plant material held together with feces. Abandoned buildings as in Pyramiden, Svalbard, are also cherished fundament for nest building (own observation). Egg laying takes place in the middle of June and the incubation period is approximately 4 weeks, where both parents nurture the eggs and chicks (Strøm, 2006). Chicks are fed regurgitated food and leave the nest 5-6 weeks after hatching. Kittiwakes reach sexual maturity after 3-5 years, and often return to their natal colony to breed (Coulson, 1966).

2.3 Sampling methods

By using a long fishing rod with a nylon noose at its end, adult kittiwakes were caught either on their nest or on adjacent cliffs. Biometric measurements of weight, skull-, tarsus- and wing length, and blood and feather sampling were carried out immediately after capture. Feathers from the back, the head, and the sixth primary feather (both wings) were sampled. Most birds were released within 10 minutes of capture. Preliminary sex determination in the field was estimated by assuming that skull length exceeding 92.0 mm corresponded to a male, which correctly determines sex in 87 % of the cases according to Barrett et al. (1985). Birds were dyed either red for female or blue for male on their head to avoid recapture. Sex was finally determined by DNA-based sex identification (see 2.5).

Nine females and eleven males kittiwakes were caught, where eight were breeding. Of the eight breeding birds, half of them were incubating and the other half were in early chick rearing.

2.4 Contaminant analysis

PFR and POP analyses were conducted at the Norwegian Institute for Air Research (NILU) in Tromsø in the period 25/8-2014 to 25/9-2014. The analyses were supervised by NILU personnel. Skilled and certified chemists from NILU did GC-MS (gas chromatography mass spectrometry) and UPLC/MSMS (ultrahigh pressure liquid chromatography mass spectrometry) analysis and quantification. All glassware used for PFRs and POP analysis was washed, rinsed once with *n*-hexane and once with dichloromethane (DCM), before being burnt at 450 °C for 8 h. In addition, all glassware used for PFR analysis was washed, immersed in acetone and put in an ultrasonic bath (Branson 5510, Branson Ultrasonics Corp., Conneticut, USA) for 10 min right before use.

2.4.1 POPs extraction and cleanup

2.4.1.1 POPs extraction from plasma

Approximately 0.5-1.1 g of frozen plasma samples were thawed and gently agitated with Vortex (VWR, Pennsylvania, USA) to assure a uniform sample, before spiking it with 100 µL internal standard in an iso-octane keeper solution. 2 mL deionized water saturated ammonium sulphate ($(NH_4)_2SO_4$) and 2 mL of ethanol were added. Extractions were carried out by adding 6 mL of *n*-hexane and allowing for phase separation. Supernatant was transferred to a 15 mL glass tube and the addition of *n*-hexane was repeated two more times for extracting sufficient amount of POPs for quantification. Sample extracts were kept at 5 °C until further processing.

2.4.1.2 Gravimetrically lipid determination for plasma

The extract from plasma was evaporated to approximately 0.2 mL with RapidVap® Vacuum Dry Evaporation System (Labconco, Kansas City, USA), and thereafter transferred to a 15 mL glass-centrifuge tube with a known weight and left over night to dry completely. Dry tubes with extract were weighed and lipid percentages were calculated. Extracts were re-dissolved in 0.5 mL of *n*-hexane after weighing.

2.4.1.3 POPs extraction from feathers

Prior to extraction, feathers were washed with Milli-Q® Advantage A10 Ultrapure Water Purification System (Merck KGaA, Darmstadt, Germany) to remove particles and covered with aluminum foil and Kimtech wipes (Kimberly-Clark Proffesional, Roswell, USA), and left to dry overnight in a fume hood.

Approximately 500 mg of feathers were transferred to a 100 mL centrifugation glass, cut into 1 mm pieces and covered with 30 mL of cyclohexane/acetone 3:1 (v:v). The samples were spiked with 100 µL of internal standard and put in ultrasonic bath (Branson 5510) for 15 min. The organic solvent was filtered to a TurboVap® 500 Evaporation System (Caliper Life Sciences, Mountain View, USA) glass through a pipette with a small piece of Kimtech wipes inside it. 0.2 mL of isooctane were added as a keeper to avoid total evaporation of samples. After concentration of samples to 0.5 mL, extracts were transferred to 4 mL vials and TurboVap® glasses were rinsed twice, once with *n*-hexane and once with DCM. Rinsing solvents were added to the extracts. Sample extracts were kept at 5 °C until further processing. Lipid determination for feathers was not feasible due to low levels.

2.4.1.4 Initial extraction cleanup for feathers

The extraction procedure retains pollutants of interest but also lipids and other cellular components from the matrix. To ensure a clean sample and to reduce disturbing signals on the analytical spectrograms, further purifying is needed. Waters (Waters Corporation, Milford, USA) gel permeation chromatography (GPC) is a convenient first step technique for separating analytes from most of the unwanted lipids by size exclusion chromatography. The Waters GPC cleanup system used for sample cleanup consisted of two EnviroGel™ GPC Cleanup Columns run by a high-pressure liquid chromatograph (HPLC) pump. The columns were packed with a stationary phase of porous beads with 100 Å (Ångstrøm) pore size, which increased the retention time of our small analytes of interest, whereas larger molecules, as lipids, did not enter the porous beads and eluted faster. Approximately 1.5 mL of sample was injected and DCM was used as mobile phase. Eluent was collected after 17 min and 50 µL of isooctane was added as a keeper as the extract was concentrated to 0.5 mL in TurboVap® 500 Evaporation System. The concentrated extracts were transferred to 15 mL glass-centrifuge tubes. TurboVap® glass were rinsed once with hexane, and rinsing solvents were added to the extracts. Sample extracts were kept at 5 °C until further processing.

2.4.1.5 Solid phase extraction (SPE) cleanup for plasma and feathers

The next cleanup step was performed on both plasma and feathers to ensure an almost lipid free sample by using the Zymark RapidTrace SPE Workstation (Caliper Life Sciences, Mountain View, USA). The RapidTrace Workstation is automated and loads each sample onto a separate SPE column. The SPE columns were prewashed in *n*-hexane, and 1.0 g florisil® (burnt at 450 °C for 8 hours) was packed

between two glass fiber frits. A 10 % DCM/*n*-hexane solvent was used as mobile phase as florisil retains unwanted polar compounds from the matrix in the SPE column. The eluents were collected in 15 mL glass-centrifuge tubes and evaporated to 0.2 mL by RapidVap® using isooctane as keeper. Lastly, the concentrated extracts were transferred to gas chromatography (GC) vials with insert and by N_2-evaporation further reduced to approximately 30 µL. As recovery standard 10 µL of ^{13}C-PCB 159 (213 pg/µL) was added. Vials were kept at 5 °C until GC-MS analysis.

2.4.2 PFR extraction and cleanup

Only four individuals were used in the PFR quantification, due to insufficient sample size of the remaining individuals.

2.4.2.1 PFR extraction and cleanup from plasma

The working bench was cleaned with isopropanol prior to use to minimize external contamination. Approximately 1 mL of plasma was transferred to a 15 mL glass-centrifuge tube and the samples were spiked with 10 µL of internal standard (PFR I, 5ng/µL). 6 mL of acetonitrile (ACN), 2 mL Oasis® HLB cleaned MilliQ water (Waters Corporation, Milford, USA) and 1 mL of saturated ammonium-sulphate water solution were added. The samples were thoroughly mixed and centrifuged at 4400 rpm for 5 min at 4 °C. Two layers were clearly separated and the top layer of ACN was carefully transferred to new 15 mL glass-centrifuge tubes, which were prepared with 200 mg of primary-secondary amine (PSA) and 400 mg of $MgSO_4$. PSA has a strong affinity for fatty acids and other organic acids, whereas $MgSO_4$ is added to bind water. After 5 min of centrifugation at 4400 rpm (4 °C) the supernatant from the samples was transferred to new 15 mL glass-centrifuge tubes and reduced to 200 µL in RapidVap®. Following evaporation samples were pipetted to GC vials with insert. The tubes were rinsed with 100 µL DCM which were added to the analytical vials. Finally, 20 µL of d21-TPrP (deuterated tripropyl phosphate; 206 pg/µL) was added as a recovery standard. The vials were kept at 5 °C until analyzed in UPLC/MSMS.

2.4.2.2 PFR extraction and cleanup from feathers

Prior to extraction, all feathers were washed with Milli-Q water to remove particles and dust, covered with aluminium foil and Kimtech wipes, and left to dry overnight in a fume hood. Approximately 1 g of feathers cut into 1 mm pieces were transferred to a 15 mL glass-centrifuge tube for each sample,

and covered with 6 mL of hydrogen chloride (HCl, 1 M) and 6 mL of hexane:DCM (4:1; v:v). The samples were spiked with 10 µL of internal standard (PFR I, 5 ng/µL), mixed thoroughly and incubated at 45 °C for 5 h. All fluid from incubated samples was transferred to new 15mL glass-centrifuge tubes and 4 mL of hexane:DCM (4:1; v:v) was added. The organic phase was pipetted to new 15 mL glass-centrifuge tubes, and addition of hexane:DCM and pipetting of organic layer was repeated once before the samples were reduced to 100 µL using RapidVap®.

Glass SPE columns with 0.8 g of PSA were washed with 6 mL of tert-butyl methyl ether (TBME):methanol (1:1; v:v), 4 mL of TBME, and 6 mL of hexane before the concentrated samples were loaded onto the PSA SPE column. The sample was loaded onto the SPE column and the tubes were rinsed once with hexane, which also was added to the column. After loading the column, all POPs were eluted with 10 mL of hexane. The POP fraction was not analyzed, as the SPE fraction mentioned in section 2.4.1.5 was used for analysis of POPs. New 15 mL glass-centrifuge tubes were placed under the column to collect the next fraction including PFRs which were eluted with 10 mL of TBME. The PFR containing eluents were reduced to 100 µL using iso-octane as a keeper by RapidVap®, and transferred to GC-vials with insert, where 20 µL of d21-TPrP (deuterated tripropyl phosphate; 206 pg/µL) was added as a recovery standard. The vials were kept at 5 °C until analyzed in UPLC.

2.4.3 Quality assurance

For every tenth sample, one blank sample was included. Blank samples were treated in the same way as regular samples to identify external contamination. Four blanks were included in PFR analyses due to very fluctuating background levels. The standard reference material (SRM) used for plasma samples was SRM 1958 human serum from National Institute of Standards and Technology (NIST), Gaithersburg, USA. No SRM was available for feather samples. The internal standard method was used and a mixture was added to all samples to compensate for any loss of analyte during extraction and cleanup of the samples. Lastly, recovery standards were added to assess the recovery of internal standards in each sample as well. Together, internal standards and recovery standards ensure a precise estimation of the concentration of analytes of interest in the samples.

2.4.4 Analyte identification and quantification

All compounds of interest were identified and quantified at the Norwegian Institute of Air Research (NILU), Tromsø, Norway. In all samples 8 PBDE congeners (28, 47, 99, 100, 138, 153, 154 and 184),

12 PCB congeners (28, 52, 99, 101, 105, 118, 138, 153, 180, 183, 187 and 194), hexachlorobenzene (HCB), *oxy*-, *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, mirex, *α*-, *β*-, and *γ*-hexachlorocyclohexane (HCH), both *o,p'*-DDT and *p,p'*-DDT and metabolites (*p,p'*-DDD, *o,p'*-DDD, *p,p'*-DDE and *o,p'*-DDE) were analyzed. In four feather and blood samples the following 13 phosphorous flame retardants were analyzed as well: tris(2-chloroethyl) phosphate (TCEP), tripropyl phosphate (TPrP), tri(chloropropyl) phosphate (TCPP), tris isobutyl phosphate (TiBP), tri-*n*-butyl phosphate (TnBP), butyl diphenyl phosphate (BdPhP), triphenyl phosphate (TPP), dibutyl phenyl phosphate (DBPhP), tris(1,3-dichloro-2-propyl)phosphate (TDCPP), tris(2-butoxyethyl)phosphate (TBEP), 2-ethylhexyl diphenyl phosphate (EHDP), sum of tricresyl phosphates (sumTCPs), and tris(2-ethyl hexyl) phosphate (TEHP).

The analysis of PCBs, PBDEs, and pesticides was performed by high-resolution gas chromatography (HRGC) on an Agilent 7890A gas chromatograph equipped with an Agilent 7683B automatic injector and an Agilent 5975C mass spectrometer (Agilent, Folsom, USA). The GC was fitted with a 30 m DB-5 MS column (J&W Scientific, Folsom, USA). Separation was achieved by splitless injection of 1 µL aliquot of the sample extract with helium as a carrier gas at a constant flow of 1.5mL/min, where the temperature program was as follows: initial temperature 70 °C held for 2 min, 15 °C/min to 180 °C, 5 °C/min to 280 °C held for 5 min. The MS was running in the negative ion chemical ionisation mode (NICI) for the pesticides and in electron impact mode (EI) for PCBs and PBDEs. The instrument was operated in single ion monitoring mode (SIM).

Analysis of PFRs was performed using UPLC on an Accella 1250 quaternary pump fitted to a Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, USA). The analytical column was an Acquity BEH Phenyl 100mm x 2.1mm, 1.7µm particle size. The mobile phase was 0.1 % formic acid in water and methanol using a gradient of 0.3 to 0.4 mL/min. The analysis was achieved using electrospray ionization (ESI) and selective reaction monitoring mass spectrometry.

2.5 Sex determination

DNA was isolated from blood samples by using the Chelex method as described by Walsh et al. (1991). 2-4 µL of sample were transferred to a 5 % Chelex solution and heated to 96 °C. Chelex chelates with metal ions and thereby prevents any DNA degradation. DNA was then isolated in the supernatant

after centrifugation at 12000 rpm for 3 min. Polymerase Chain Reaction (PCR) was performed in GeneAMP® PCR System 9700 thermal cycler (PE Applied Biosystems, Life Technologies, New York, USA). Chromobox-helicase-DNA-binding genes (CHD-W and CHD-Z) were amplified since CHD-W is the only avian sex chromosome gene that has been discovered and only occurs in females (ZW) and not in males (ZZ) as detailed in Griffiths et al. (1998). The PCR kit used was Qiagen Taq PCR Core Kit (Düsseldorf, Germany). An initial denaturation step at 94 °C for 3 min was followed by 35 cycles of 94 °C for 30 s, 46 °C for 45 s, and 70 °C for 45 s. The PCR was ended with a cycle at 70 °C for 10 min.

PCR products were separated by electrophoresis for 45-50 min at 75 V in a 1 % agarose gel stained with ethidium bromide. Products were visualized under ultraviolet light, since the fluorescence from ethidium bromide intensifies when binding DNA. PCR products from males showed one line due to amplification of only the CHD-Z gene, whereas females showed two lines since both the CHD-Z and CHD-W gene were amplified.

The skull length measurements correctly determined sex in 79 % of the kittiwakes (see 2.3).

2.6 Thyroid hormone analysis

Triiodothyronine (T3) and free triiodothyronine (fT3) were quantified in plasma by a competitive enzyme immunoassay from MP Biomedicals, LLC, Ohio, USA. Two blank samples and either human T3 or fT3 standard reference sets were used as quality assurance of the quantification.

For fT3 quantification 50 µL of plasma was added to monoclonal fT3 antibody coated wells on a microtiter plate together with 100 µL of fT3-enzyme labeled conjugate, where these compete for available binding sites. After 60 min of incubation, unbound fT3-enzyme conjugate was washed off, and 3, 3', 5, 5'-tetramethylbenzidine (TMB) was added. A further 20 min of incubation developed a blue color, which was halted and turned yellow by addition of 50 µL of 3N HCl. Absorbance was measured at 450 nm, where color intensity is proportional to the amount of enzyme present and therefore inversely related to the amount of unlabeled fT3 in the sample.

For T3 quantification, 50 µL of plasma was added to antibody (goat anti-mouse IgG) coated wells on a microtiter plate, together with mouse anti-T3 antibody and T3 conjugate. Mouse anti-T3 antibody binds the coated wells and the T3 from plasma and T3 conjugate compete for the limited binding sites on the mouse anti-T3 antibody. After 60 min of incubation TMB reagent was added and

incubated for another 20 min. The development of the resulting blue color was halted by addition of 100 µL of 1N HCl, which turned the solution yellow. Absorbance was measured at 450 nm, where color intensity is proportional to the amount of enzyme present and therefore inversely related to the amount of unlabeled T3 in the sample.

2.7 Statistical analyses

For statistical analyses, Microsoft Excel® 2013, Microsoft Excel® 2013 add-in XLSTAT (version 2015, 1.01), JMP 12.0.0®, SigmaPlot 13.0, and the free statistical software R (version 3.1.2) were used. Statistics were only performed on compounds if more than 50 % of the measurements were above LOD (limit of detection). Levels below LOD were assigned a value of $p \times$ LOQ (limit of quantification), were 'p' is the proportion of measurements with a value above LOQ (Voorspoels et al., 2002; Jaspers et al., 2007b). Cumulative probability plots for PCBs and OCPs have been carried out to visualize the proportion of data below LOD in feathers. The cumulative probability plot is a reverse Kaplan-Meier method as described in Gillespie et al. (2010). Median concentrations, quantiles, and LOD can be read from the plots. The level of significance was set to $p \leq 0.05$ throughout the study.

A body condition index (BCI) was made to reduce the difference between sexes. BCI is expressed as residual mass from linear regression relating body mass to skull length (r^2=0.65, n=19, p<0.001) as described in Chastel et al. (2005). Skull length was used due to its high correlation with body mass (r=0.82, p<0.001). Sex-specific regressions did not vary (ANCOVA p=0.46).

Only 19 samples were available for statistical analyses although 20 birds were sampled. One sample from a female kittiwake simply contained too little blood to extract sufficient plasma for chemical analysis.

The concentrations of the majority of the pollutants were not normally distributed according to the Shapiro-Wilk test of normality. Common logarithmic (base 10) transformation of all POP concentrations were performed in order to approximate normal distribution. Pearson product-moment coefficients were carried out to evaluate correlations between levels in feathers and blood. Principal component analysis (PCA) was performed to investigate relations between POPs, thyroid hormones, and BCI.

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3. Results

3.1 Physiological condition

Body mass of the 19 studied kittiwakes ranged from 300 to 432.5 g, and an overall significant difference were found between the sexes (p<0.001). When comparing sexes within the two colonies significant differences were found as well (fig. 2).

An overview of the body condition is seen in fig. 3, showing that BCI does not differ between sexes for Blomstrandhalvøya (p=0.057), Krykkjefjellet (p=0.609), nor between colonies (p=0.10). Breeding status did not affect neither body mass nor body condition. All biometric measurements are found in Appendix II.

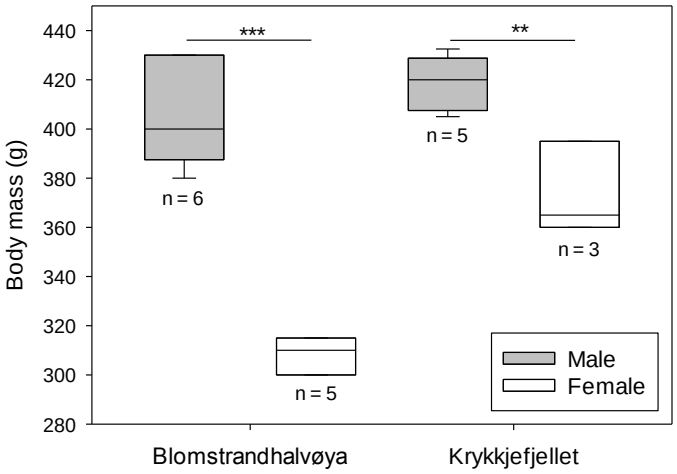

Figure 2. Body mass (g) of male and female kittiwakes from Blomstrandhalvøya (n = 11) and Krykkjefjellet (n = 8). First and third quartiles form bottom and top of the box, and whiskers are minimum and maximum values. Median is given as the horizontal line inside the box. **: denote significant difference between the sexes, p<0.01. ***: denote significant difference between the sexes, p<0.001.

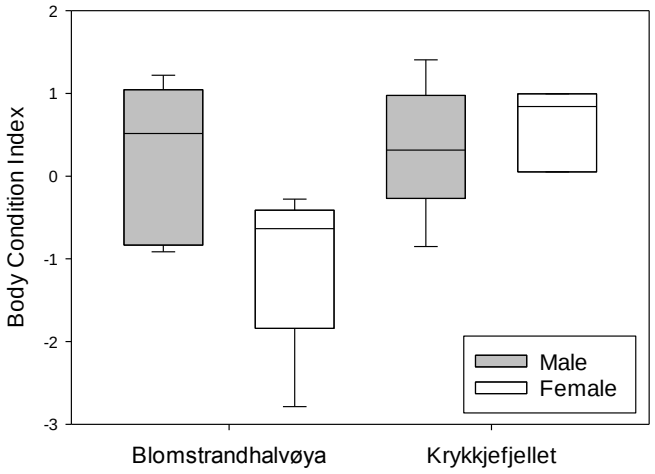

Figure 3. Centered body condition index (BCI) of male and female kittiwakes from Blomstrandhalvøya (n = 11) and Krykkjefjellet (n = 8). First and third quartiles form bottom and top of the box, and whiskers are minimum and maximum values. Median is given as the horizontal line inside the box.

3.2 Levels of pollutants

Of the 36 different POPs that were investigated the following compounds were detected in plasma in more than 50 % of the samples: *p,p'*-DDE, HCB, *β*-HCB, *oxy*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, mirex, CB -28, -99, -105, -118, -138, -153, -180, -183, -187, and BDE 47. In feathers, *p,p'*-DDE, HCB, *oxy*-chlordane, *trans*-nonachlor, and PCB 153 were detected in more than 50 % of the 19 samples. Of the thirteen investigated PFRs, seven were detected in feathers (TCEP, TnBP, TPP, TBEP, sum TCP, EHDP, and TEHP) and one in plasma (TCPP).

Table 1 provides an overview of plasma concentrations of all detected compounds for each colony, whereas table 2 shows feather concentrations. Mean concentration of ΣPOPs for Blomstrandhalvøya and Krykkjefjellet were respectively 72900±8630 pg/g ww and 29600±1670 pg/g ww in plasma, and 13400±3630 pg/g ww and 7080±1580 pg/g ww in feathers. The biggest contributor to mean ΣPOPs, for plasma and feathers respectively were ΣPCBs (Blomstrandhalvøya 80.1±1.41 %, 51.1±10.5 %; Krykkjefjellet 75.7±1.90 %, 41.8±11.5 %).

Only the ΣPFRs concentrations have been included in table 1 and 2 due to low sample size (N=4 individuals). In plasma samples, only one sample had levels above LOQ, and only for TCPP. Mean concentration of ΣPFR in feathers ranged from <LOQ to 15.6 ng/g feather. LOQ for blank values ranged from 0.03 to 5.83 ng/g ww for plasma, and from 0.02 to 26.5 ng/g ww for feathers. Only CB 153, *p,p'*-DDE, HCB, *oxy*-chlordane, and *trans*-chlordane were detected in more than 50 % of the feather samples.

Table 1. Plasma concentrations (pg/g wet weight) of detected compounds from Krykkjefjellet (n = 8) and from Blomstrandhalvøya (n = 11). Median is based on samples above limit of detection (LOD). Range is defined as minimum and maximum unless noted otherwise. ND = Not detected, NA = Not available due to few detections. [a] = LOD.

	Krykkjefjellet			Blomstrandhalvøya		
	Samples > LOD	Median	Range	Samples > LOD	Median	Range
p,p'-DDE	8/8	1710	946 - 6560	11/11	4620	874 - 10000
HCB	8/8	1780	100 - 2150	11/11	3140	1400 - 6210
β-HCH	0/8	ND	ND	2/11	NA	724 - 1110
Oxy-chlordane	8/8	1310	106 - 1580	11/11	2610	1000 - 4070
Trans-chlordane	7/8	151	69.6 - 203	6/11	281	52.7 - 453
Trans-nonachlor	8/8	84.4	32.8 - 180	10/11	115	31.7 - 250
Cis-nonachlor	8/8	71.7	44.8 - 133	11/11	72.8	33.6 - 246
Mirex	8/8	908	606 - 1300	11/11	2220	834 - 3850
ΣOCPs	8/8	6320	4510 - 11300	11/11	15700	4350 - 20900
PCB 28	8/8	72.8	36.0 - 91.7	11/11	155	64.2 - 262
PCB 99	8/8	1280	1140 - 1790	11/11	3330	1240 - 4320
PCB 105	5/8	492	254 - 644	10/11	1050	369 - 1620
PCB 118	8/8	1240	1170 - 2040	11/11	2930	1260 - 5290
PCB 138	8/8	6140	5270 - 8350	11/11	15100	6600 - 25200
PCB 153	8/8	7400	6010 - 10100	11/11	19800	8500 - 30500
PCB 180	8/8	3400	2330 - 4400	11/11	9100	426 - 18100
PCB 183	6/8	654	425 - 742	11/11	1780	874 - 3160
PCB 187	8/8	1300	1100 - 1840	11/11	3030	1140 - 5660
PCB 194	0/8	ND	ND	8/11	1040	474 - 2350
ΣPCBs	8/8	21900	18100 - 29800	11/11	58800	24900 - 95200
PBDE 47	8/8	257	135 - 509	10/11	633	40.6 - 1690
PBDE 99	4/8	72.2	112[a] - 303	3/11	NA	112[a] - 503
PBDE 100	1/8	NA	185	3/11	NA	182 - 323
PBDE 138	1/8	NA	101	1/11	NA	598
PBDE 153	3/8	NA	77.5[a] - 305	3/11	NA	77.5[a] - 324
PBDE 154	1/8	NA	114	2/11	NA	77.0 [a] - 301
ΣPBDEs	8/8	591	135- 960	10/11	871	40.6 - 3120
ΣPFRs	1/4	NA	48.3	0/0	NA	NA
ΣPOPs	8/8	28400	24600 - 37400	11/11	79700	30000 - 114000

Table 2. Feather concentrations (pg/g wet weight) of detected compounds from Krykkjefjellet (n = 8) and from Blomstrandhalvøya (n = 11). Median is based on samples above limit of detection (LOD). Range is defined as minimum and maximum unless noted otherwise. [a]=LOD, [b]=LOQ. [C]=highest LOD of compound group. ND = Not detected, NA = Not available due to few detections. ΣPFR is in pg/g feather weight.

	Krykkjefjellet			Blomstrandhalvøya		
	Samples > LOD	Median	Range	Samples > LOD	Median	Range
p,p'-DDE	6/8	1960	737[a] - 2290	5/11	NA	737[a] - 2800
HCB	6/8	578	281[a] - 1010	10/11	767	281[a] - 5180
β-HCH	1/8	NA	1110	1/11	NA	414
Oxy-chlordane	7/8	574	266[a] - 891	4/11	NA	266[a] - 6150
Trans-chlordane	2/8	NA	67[a] - 226	0/11	ND	ND
Cis-chlordane	0/8	ND	ND	6/11	22.0	<39.6[b]
Trans-nonachlor	8/8	126	78 [a] - 320	2/11	NA	78[a] - 236
Cis-nonachlor	3/8	NA	<199[b]	6/11	108	<199[b]
Mirex	0/8	ND	ND	1/11	NA	574
ΣOCPs	8/8	3500	2190 - 4400	11/11	1570	737[c] - 14200
PCB 28	3/8	NA	<438[b]	3/11	NA	146[a] - 889
PCB 99	1/8	NA	<2180[b]	4/11	NA	1490[a] - 3680
PCB 118	3/8	NA	<3360[b]	2/11	NA	1120[a] - 3730
PCB 138	3/8	NA	<5990[b]	5/11	NA	2000[a] - 9430
PCB 153	5/8	3080	<5030 - 5330	9/11	4100	1680[a] - 18500
ΣPCBs	5/8	5210	<2380[c] - 11000	9/11	6310	<2380[c] - 36200
PBDE 47	0/8	ND	ND	5/11	NA	<440[a] - 1410
PBDE 99	0/8	ND	ND	3/11	NA	<1070[a] - 2830
PBDE 153	0/8	ND	ND	1/11	NA	3570
ΣPBDEs	0/8	ND	ND	6/11	619	1230[c] - 7810
ΣPFRs	2/4	4170	<1000[c] - 15600	0/0	NA	NA
ΣPOPs	8/8	7500	1550 - 14900	11/11	12200	<7130[c] - 45800

The cumulative probability plot is a reverse Kaplan-Meier plot, as explained in section 2.7. The two figures (fig. 4) show cumulative probability plots of PCBs (n=19) and OCPs (n=19) in feathers respectively. For PCBs, CB 153 was the most abundant PCB congener, and it was the only congener detected in more than 50 % of the samples. This might be caused by the high detection limits as seen on the graph. CB -138 and -153 contributed the most to the total ΣPCB in feathers.

For the pesticides, oxychlordane was found in the highest concentration, although HCB was the most abundant. Highest mean levels were found for *p,p'*-DDE. Only HCB, *p,p'*-DDE, and *oxy*-chlordane were found in more than 50 % of the samples.

Levels of PFRs were only investigated in four individuals from Krykkjefjellet, since only their sample amount of plasma and feathers where sufficient for PFR analyses. Two birds had no detectable levels of any of the investigated compounds in feathers after blank correction. Main contributors to ΣPFRs in feathers were EHDP and TPP, where only TPP was detected in both samples.

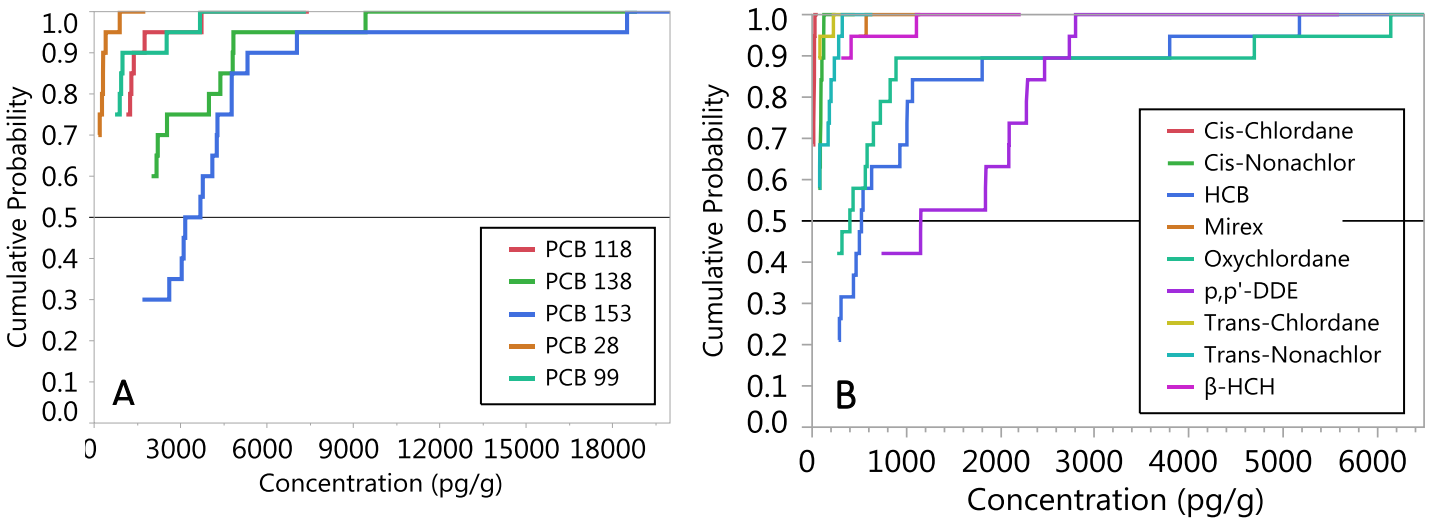

Figure 4. Cumulative probability plots of **(A)** CB -28, -99, -118, -138, and -153 concentrations, and **(B)** organopesticide concentrations. The reference line at 0.5 crosses each concentration at its median. Plot A and B are concentrations in pg/g ww in feathers from 19 kittiwakes from Kongsfjorden.

3.3 Pollutants at Blomstrandhalvøya and Krykkjefjellet

Pollutant profiles are an efficient way to show the contribution of each contaminant to the total pollutant concentration. Figure 5 shows the contribution of each detected pollutant to the total contamination level in plasma for the two colonies. Sexes have been pooled since no significant differences were found between sexes for ΣPCBs, ΣPBDEs, and ΣOCPs in neither Blomstrandhalvøya (p=0.48, p=0.49, and p=0.31 respectively) nor Krykkjefjellet (p=0.38, p=0.23, and p=0.38 respectively). The mean contribution of *cis*-nonachlor, CB -99, -118, and -183 differed significantly (all p<0.05) between the colonies. No significant differences were found for CB -153, -138, -180, and *p,p'*-DDE, which were the major contaminants in plasma for kittiwakes from Blomstrandhalvøya (27.3 %, 22.0 %, 12.8 %, and 6.4 %) and from Krykkjefjellet (25.7 %, 21.6 %, 11.5 %, and 8.0 %) constituting 68.5 % and 66.8 % of the total POP load respectively.

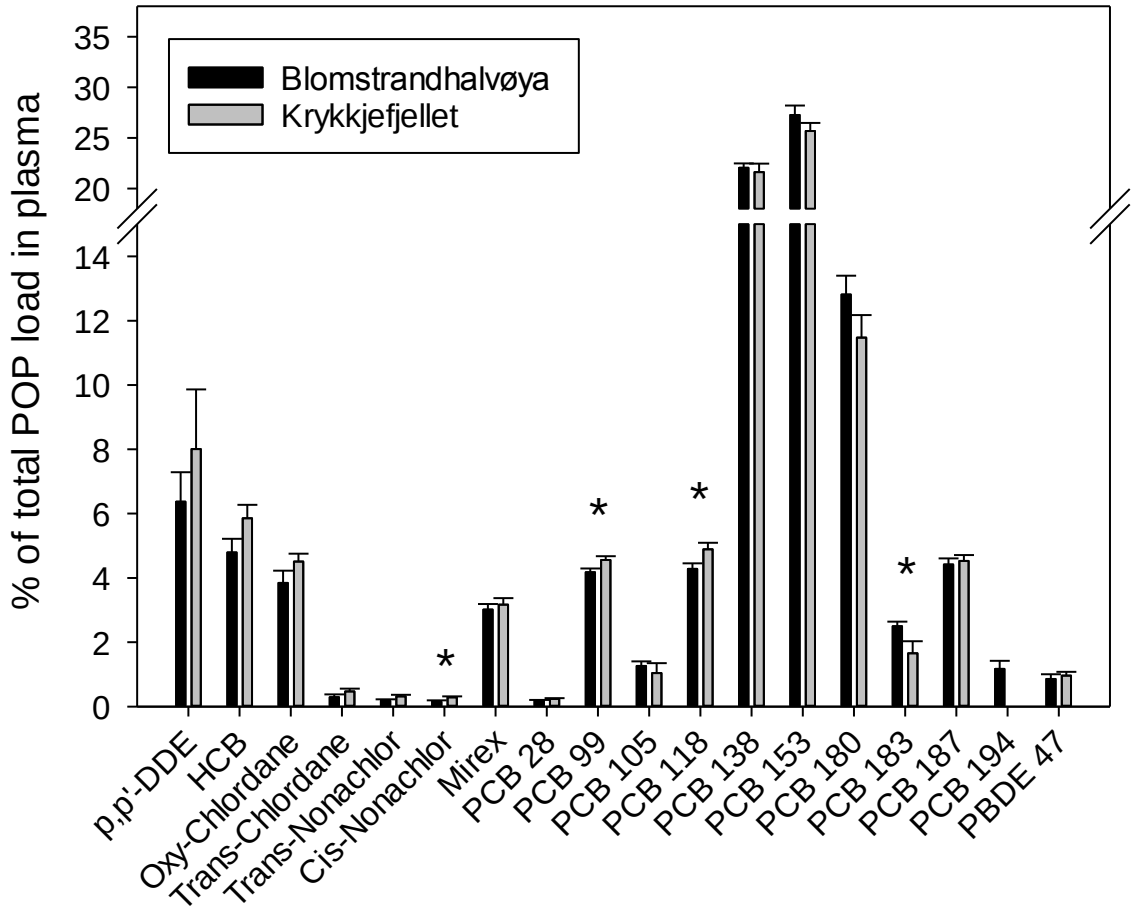

Figure 5. Comparison of POPs expressed as mean percentage (%) of total POP load ± SE in plasma for Blomstrandhalvøya (n=11) and Krykkjefjellet (n=8). *: significant difference between colonies, p<0.05. All compounds were detected in more than 50 % of the samples.

Mean concentration of most pollutants in plasma differed significantly between colonies (fig. 6). Only mean concentration of *p,p'*-DDE, *trans*-chlordane, *cis*- and *trans*-nonachlor, and BDE 47 did not differ significantly. ΣPCBs, ΣOCPs, and ΣPOPs were strongly significantly different between colonies (all $p<0.01$), but ΣPBDEs did not differ ($p=0.95$). Mean ΣPOPs were almost 2.5 times higher for kittiwakes from Blomstrandhalvøya than for kittiwakes from Krykkjefjellet.

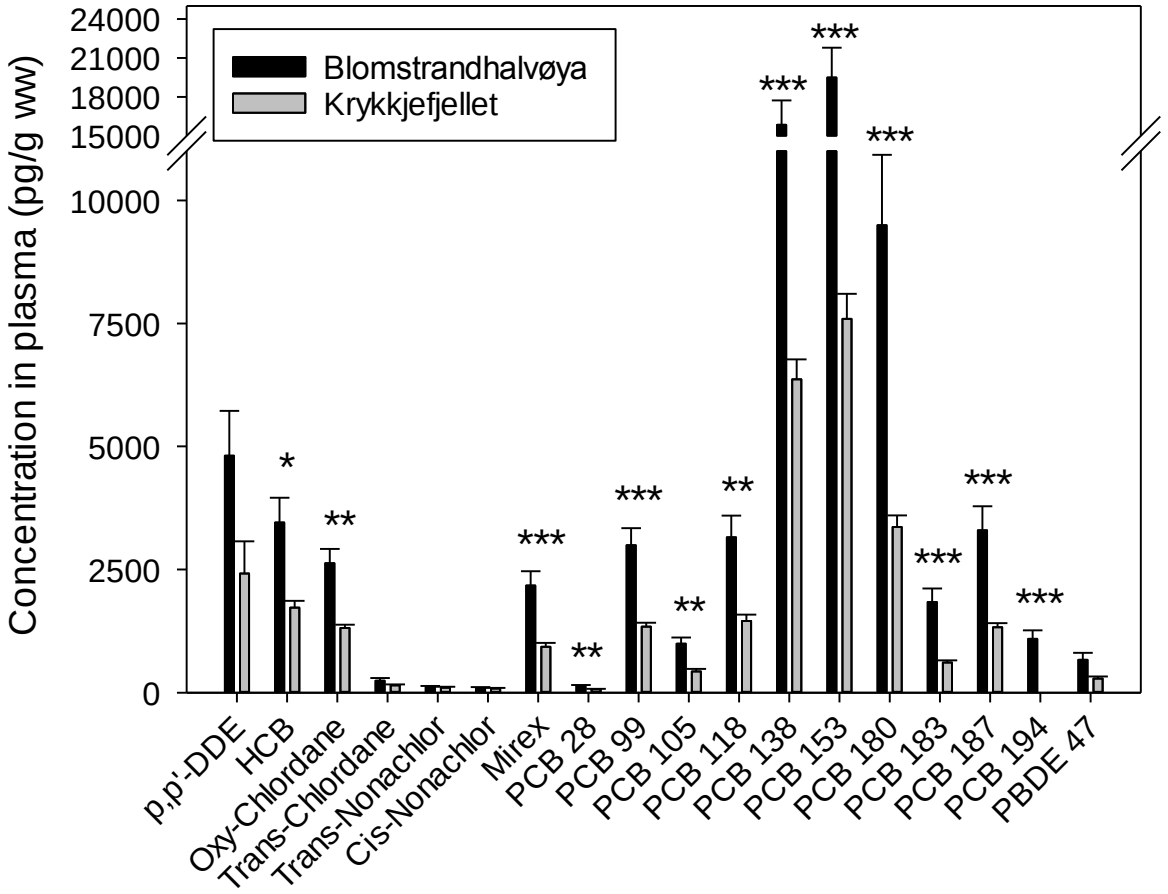

Figure 6. Comparison of mean concentration of POPs in pg/g ww in plasma ± SE between Blomstrandhalvøya (n=11) and Krykkjefjellet (n=8). *: significant difference between the two colonies, $p < 0.05$. **: significant difference between the colonies, $p < 0.01$. ***: significant difference between the colonies, $p < 0.001$.

Most of the investigated compounds were detected in less than 50 % of the samples for feathers, and therefore the pollutant profiles have been constructed with the sum of different pollutant groups (fig. 7). Pollutants in feathers did not differ significantly between colonies for ΣPCBs, ΣPBDEs, ΣOCPs, expressed as mean percentage of total POP load. A significant difference were found in feather concentrations between sexes for ΣPOPs and ΣPCBs, where males had the highest levels (p=0.03 and p=0.02 respectively). Kittiwakes from Krykkjefjellet had no detected values of PBDEs in feathers. CB 153, *p,p'*-DDE, HCB, and CB 138 were the major contaminants in feathers for kittiwakes (33.0 %, 23.1 %, 10.7 %, and 10.7 % respectively) constituting 77.5 % of the total POP load.

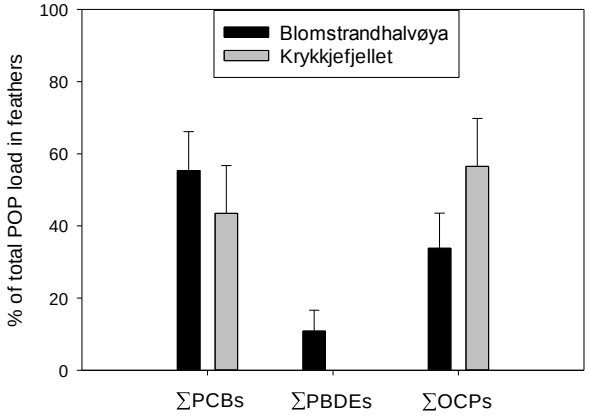

Figure 7. Sum (Σ) of PCBs, PBDEs, and OCPs expressed as mean percentage (%) ± SE of total POP load in feathers from kittiwakes from Blomstrandhalvøya (n = 11) and Krykkjefjellet (n = 8).

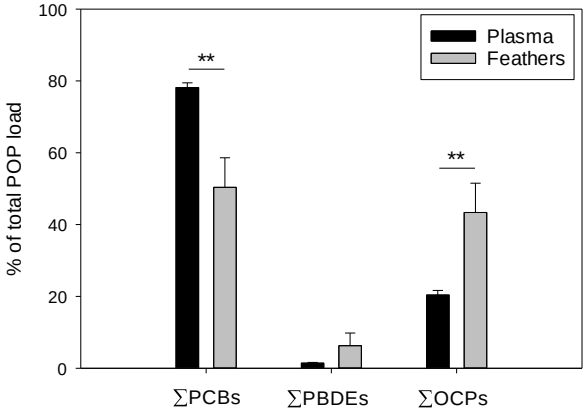

Figure 8. Sum (Σ) of PCBs, PBDEs, and OCPs expressed as mean percentage (%) of total POP load ± SE in plasma and feathers for 19 kittiwakes from Svalbard. **: significant difference between plasma and feather samples, $p < 0.001$.

3.4 Pollutants in feathers and plasma

As seen on figure 8, the mean contribution of ΣPCBs and ΣOCPs differed significantly between plasma and feather samples (p=0.002 and p=0.009 respectively). Statistical comparison between ΣPBDEs was not possible since PBDEs were detected in less than 50 % of the feather samples.

Figure 9, shows the different compounds expressed as mean percentage of the total contaminant load in plasma and feathers. Compounds detected in less than 50 % of the samples have been included in the graph, and the number of detections is noted as the number above the column. If nothing is noted, the compound was detected in all 19 samples. Statistical comparison of mean contributions have only been carried out if the compound was detected in more than 50 % of the samples in both plasma and feathers. *p,p'*-DDE, HCB, and *trans*-chlordane had significantly different

mean contributions to mean level of POPs, where *p,p'*-DDE was the most striking with 7.1 % in plasma versus 23.1 % in feathers.

Pearson correlations were carried out between log transformed plasma and feather concentrations in order to identify any associations. No significant correlations were found, except for a spurious negative relationship for *oxy*-chlordane (r=-0.58, p=0.008). Due to high differences in contaminant load between colonies, correlations were also investigated for each colony separately. The only significant relationship between feather and plasma concentrations was for CB 153 in Krykkjefjellet (r=0.81, p=0.02) as seen on figure 10. This relationship is only significant when including the three non-detects which have been given a value between LOD and LOQ as explained in 2.7.

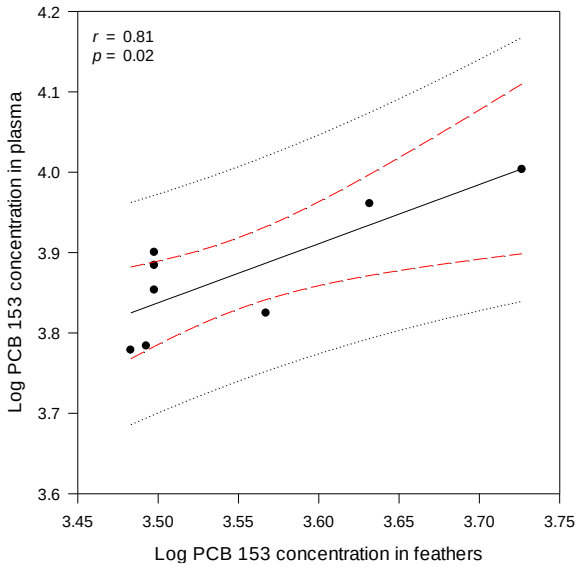

Figure 10. Correlation plot between log PCB 153 concentrations in feather and plasma for 8 kittiwakes from Krykkjefjellet, Kongsfjorden. The *p*- and *r*-value are displayed in the upper right corner. The unbroken line is the regression, the red dashed line is the 95 % confidence interval for the regression, and the dotted line is the 95 % confidence interval for the samples.

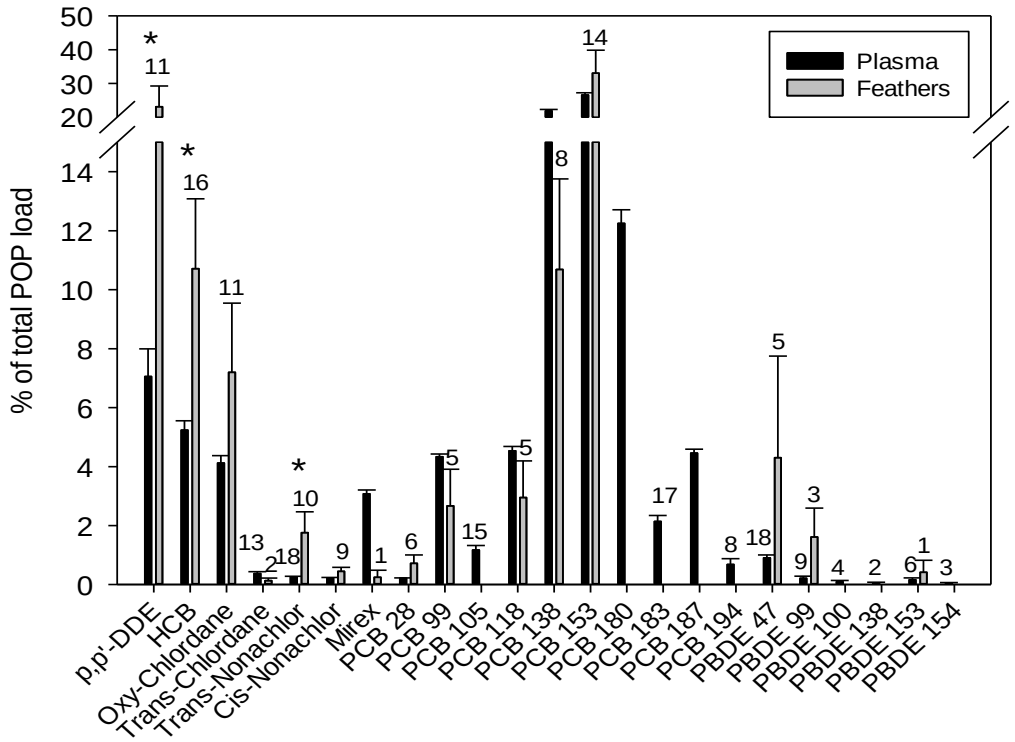

Figure 9. Different POP concentrations expressed as mean percentage (%) of total POP load ± SE in plasma (black bars) and feather (grey bars). The number above the SE denotes the number of detections above LOD, if not detected in all samples (n = 19). *: significant difference between the two colonies, $p < 0.05$.

3.5 Thyroid levels

Levels of fT3 and TT3 have been investigated in the present study (fig. 11), and fT3 levels differed significantly between sexes (p=0.007) with a range from 2.45 to 6.11 pg/mL for males and 1.25 to 3.48 pg/mL for females. No significant differences were found between colonies. TT3 levels ranged from 1.68 to 5.12 ng/mL for males and from 0.70 to 3.52 ng/mL for females and no significant differences were found between sexes or colonies. The ratio between fT3 and TT3 ranged from 0.47 to 1.78 for males and from 0.56 to 1.53 for females and did not significantly differ between neither colonies nor sexes.

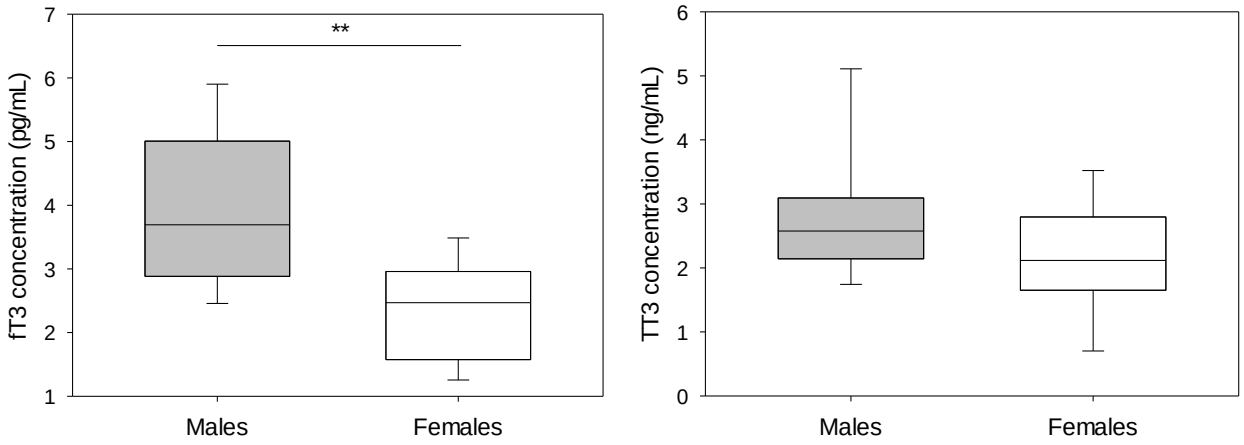

Figure 11. fT3 (left) and TT3 (right) levels in 11 male and 8 female kittiwakes from Kongsfjorden, Svalbard. Concentrations are presented as boxplots, where first and third quartiles form bottom and top of the box, and whiskers are minimum and maximum values. Median is given as the horizontal line inside the box. **: significant difference between the sexes, $p < 0.01$.

3.6 Associations between contaminants, thyroid hormones, and physiological parameters

Principal component analysis (PCA) has been performed to visualize the patterns of pollutant groups in relation to thyroid hormones and BCI. The scores and loadings plot for all samples are presented in figure 12. A clear division between BCI, thyroid hormones and pollutants on the loadings plot is obvious. BCI and all pollutants are negatively correlated among the first principal component (PC1) explaining almost 59 % of the total variance, whereas the thyroid hormones are orthogonal to both BCI and the pollutants on the second principal component (PC2), explaining 19 % of the total variance. Grouping of colonies is clear on the scores plot on PC1 (Kruskall-Wallis test, p=0.006), where kittiwakes from Krykkjefjellet are more positively related to BCI, and kittiwakes from Blomstrandhalvøya are more positively related to all pollutant groups. This reflects the higher levels of pollutants found in kittiwakes from Blomstrandhalvøya. The opposite positions of pollutants and BCI moreover suggest an inverse association between pollutants and BCI. By inspecting Pearson correlations this was found to be significant for all compounds detected in plasma (all r>-0.53 and p<0.05), except for *trans*-chlordane, *cis*- and *trans*-nonachlor, BDE 47, and ΣPBDEs. Further, males tend to score higher on PC2 than females do (Kruskall-Wallis test, p=0.08), which is in accordance with the higher levels of fT3 found in males. According to the 95 % prediction ellipse one female kittiwake from Krykkjefjellet is reported as an outlier, but this sample is included in the PCA to show the individual variation.

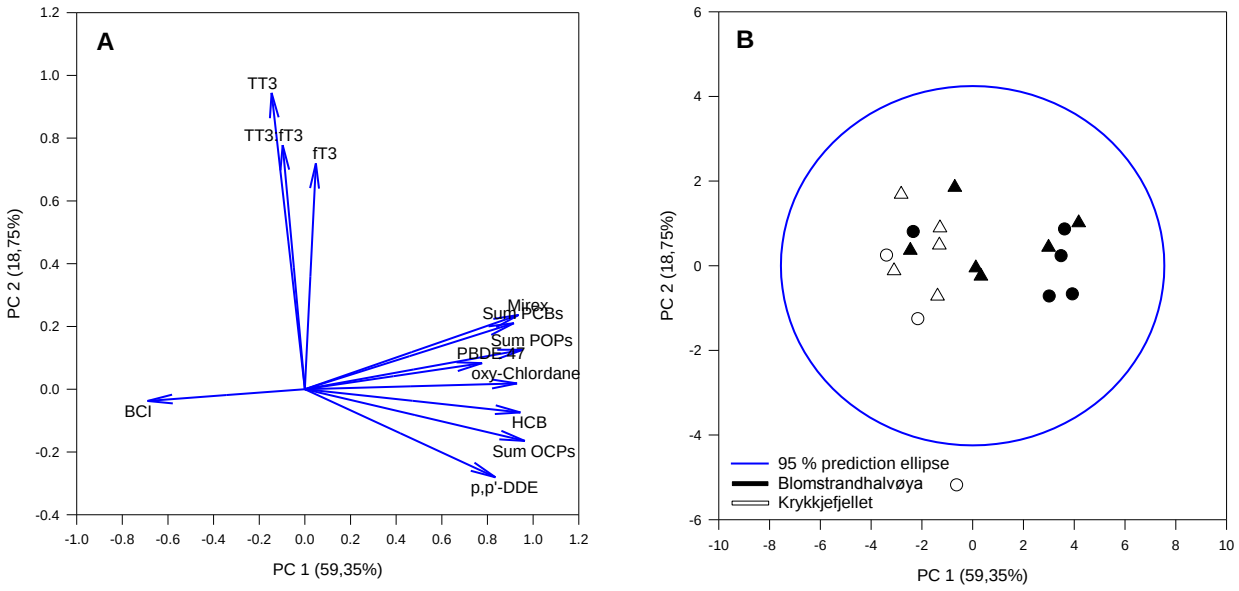

Figure 12. PCA loadings **(A)** and scores **(B)** plot of POPs, BCI, and thyroid hormones in plasma from 19 kittiwakes from Kongsfjorden, Svalbard. The first and second principal component (PC1 and PC2) explain 78.1 % of the total variation in the data. Triangles represent males and circles represent females.

CB -28, -138, and -187, BDE 47, and the ΣPBDEs (mainly consisting of BDE 47) were negatively correlated with TT3:fT3 ratio (all $r \geq -0.60$ and all $p \leq 0.05$) for kittiwakes from Blomstrandhalvøya, but not for Krykkjefjellet. When combining the colonies no correlations were found between any contaminants and thyroid hormones. Figure 13 shows the significant correlations between the aforementioned compounds and the TT3:fT3 ratio.

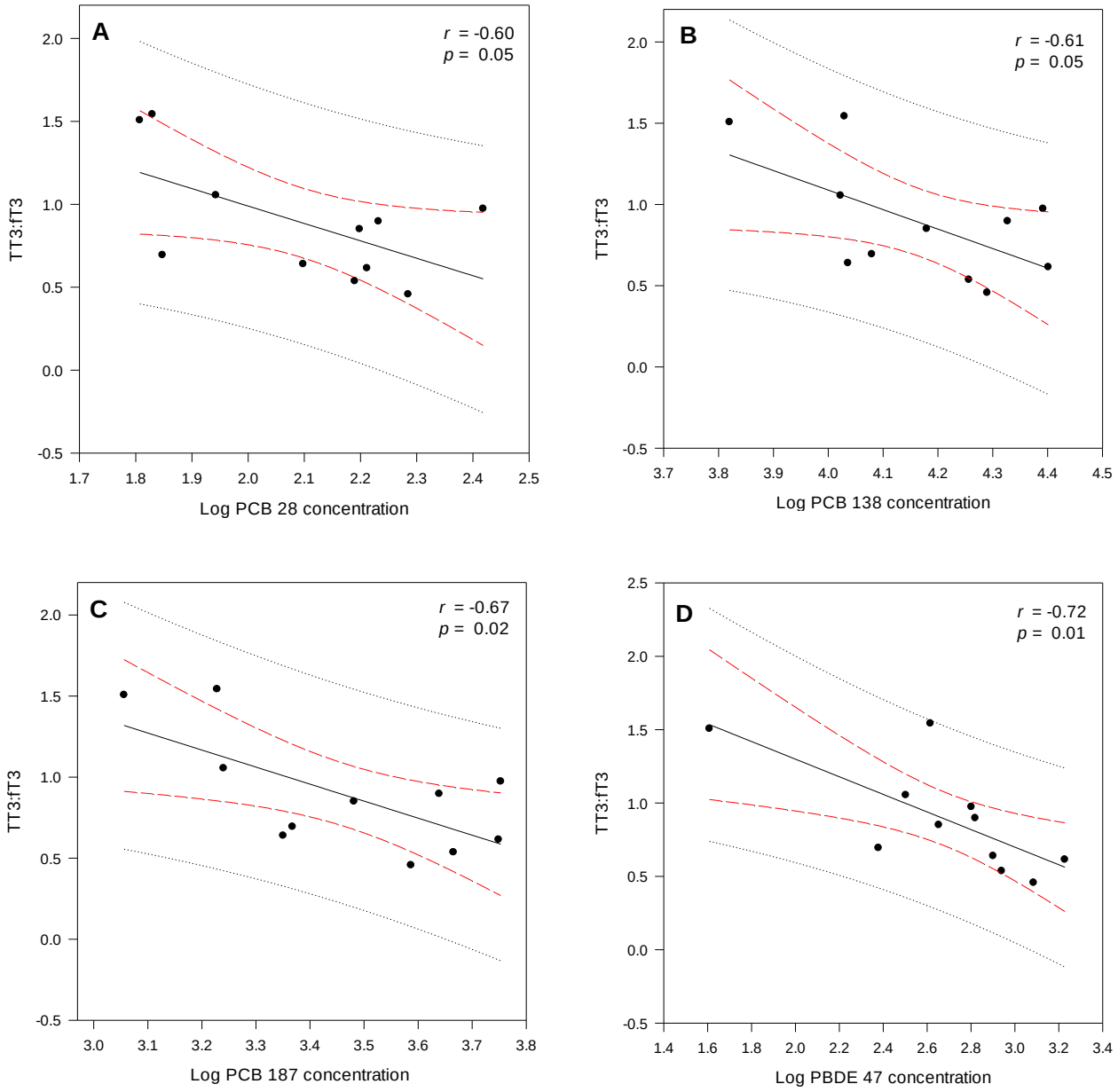

Figure 13. Correlation plots between TT3:fT3 ratio and the log concentration of **(A)** PCB 28, **(B)** PCB 138, **(C)** PCB 187, and **(D)** PBDE 47 in 11 kittiwakes from Blomstrandhalvøya, Kongsfjorden. The *p*- and *r*-values are displayed in the upper right corner. The unbroken line is the regression, the red dashed line is the 95 % confidence interval for the regression, and the dotted line is the 95 % confidence interval for the samples.

4. Discussion

4.1 Levels of pollutants in kittiwakes from Svalbard

Levels of different legacy pollutants in kittiwakes have mainly been studied in liver (Borgå et al., 2001; Tomy et al., 2004; Borgå et al., 2005; Solheim, 2010), in eggs (Braune, 2007; Helgason et al., 2008), and in plasma (Nordstad et al., 2012; Nøst et al., 2012; Blévin et al., 2014; Tartu et al., 2014). PCBs, OCPs, PBDEs, and perfluorinated acids (PFAs) have been investigated and quantified previously, but to the knowledge of the author, this is the first study to detect and quantify PFRs in feathers and plasma from Kittiwakes from Svalbard. High levels of PFRs were found in blank samples (0.03 to 4.47 ng/g ww and 0.02 to 26.5 ng/g feather for plasma and feather respectively) indicating possible external contamination in the lab, since most PFR levels in the samples were below PFR levels in the blank samples. Therefore, the PFR results should be interpreted with caution. Nevertheless, the detection of PFRs in the Arctic wildlife increases the evidence for their long-range atmospheric transport, and since levels of PFRs in the Arctic atmospheric are exceeding both contemporary and historical levels of PBDEs (Salamova et al., 2014), levels of PFRs in Arctic wildlife will presumably increase.

Female birds can transfer halogenated pollutants to their offspring through the egg yolk (Gabrielsen et al., 1995), which mainly consists of lipids (Royle et al., 1999), and therefore female birds may possess the ability to reduce the total contaminant load. Female birds had significantly lower levels of ΣPOPs and ΣPCBs in feathers than males, but no differences were found in plasma. As pollutant concentrations in plasma act as a snapshot in time, the maternal transfer of pollutants can be masked since plasma levels are highly variable and are dependent on whether the bird has just eaten, or is fasting and remobilizing lipids. Levels of pollutants could have been higher in females before egg formation, but since most feathers are grown at wintering areas for kittiwakes and therefore reflect pollutant levels at the wintering areas, the higher levels of pollutants in females before breeding seem unlikely, as the feather levels for males were significantly higher. The lack of difference in pollutant load between sexes has been reported before in liver from glaucous gull (Sagerup et al., 2009), but sex-related differences have on the other hand been found in plasma from glaucous gull (Verreault et al., 2004).

4.2 Differences between the colonies

The level of ΣPCBs in plasma found in this study for kittiwakes from Krykkjefjellet is similar to median levels of ΣPCBs in kittiwakes reported by Nordstad et al. (2012), but levels for kittiwakes from Blomstrandhalvøya in the current study were more than twice as high. The same was evident for *p,p'*-DDE, HCB, and *oxy*-chlordane, where kittiwakes from Blomstrandhalvøya had higher mean levels than previously reported for kittiwakes in Kongsfjorden. In Johnsen (2011) levels of CB 153, *p,p'*-DDE, HCB, and *oxy*-chlordane were similar to levels found in the current study in kittiwakes from Krykkjefjellet, but again, mean levels in kittiwakes from Blomstrandhalvøya in the current study were more than twice as high. Both Johnsen (2011) and Nordstad et al. (2012) used Krykkjefjellet as study colony. The higher levels of almost all halogenated pollutants found at Blomstrandhalvøya may be caused by several factors, as individual variations in breeding status, body size, sex, feeding ecology, and area may all affect the trophic transfer of pollutants (Henriksen et al., 1996; Borgå et al., 2004). However, only few differences were found between POP profiles for the two colonies, suggesting that feeding ecology was most likely the same. As kittiwakes often forage in upwelling zones in front of glaciers (Strøm, 2006; Coulson, 2011), birds from Blomstrandhalvøya presumably forage in front of Blomstrandbreen, whereas kittiwakes from Krykkjefjellet presumably forage in front of Kongsbreen or Kronebreen. Feeding ecology will probably be the same in front of these glaciers as all calve into Kongsfjorden, but the abundance of food may differ. If so, birds foraging in upwelling zones with low abundance of food could possibly have lower body mass, or spend more time searching for food. Females from Blomstrandhalvøya showed a tendency to have lower BCI than the rest of the birds from both colonies, suggesting that the higher redistribution of stored lipids and thereby release of pollutants, may have caused the higher levels of pollutants. Body mass and BCI did not differ significantly between the colonies for male kittiwakes, but males from Blomstrandhalvøya still had significantly higher levels of POPs than males from Krykkjefjellet. Nevertheless, median levels of male body mass for Blomstrandhalvøya were 20 g lower than for males from Krykkjefjellet (although not significant), which partly could explain the higher levels of pollutants found in male kittiwakes at Blomstrandhalvøya.

The levels of pollutants are very dependent on the time of sampling (Henriksen et al., 1996) and even a small reduction in the median body mass (1.5 %) between the pre-breeding and incubation period for male kittiwakes can almost triple levels of ΣPCBs (calculated from Nordstad et al. (2012)). In the

present study, breeding males had higher levels of pollutants than non-breeders, but this is based on only two breeding males from Blomstrandhalvøya. Body condition of incubating birds is thought to be better than birds in early chick rearing period (Moe et al., 2002) and better of non-breeding than breeding birds (Tartu et al., 2014), but no difference were found in body condition between breeding and non-breeding kittiwakes in the present study. Breeding status can be difficult to assess in the field, and thus mask any effects of breeding. No significant differences in POP levels between sexes were found within the colonies when taking breeding status into account, but a tendency was found for females at Blomstrandhalvøya to have higher levels of pollutants than males, independent of their breeding status. Higher biota-sediment accumulation factor (BSAF) of some polycyclic aromatic hydrocarbons (PAHs) in a zoo plankton species have been found at Blomstrandhalvøya compared to Ny-Ålesund (Szczybelski et al., unpublished), but whether the higher BSAF at Blomstrandhalvøya is causing higher levels of some PAHs in kittiwakes from Blomstrandhalvøya is not known. Further investigation is needed to fully comprehend this interesting difference between the locations, and it should be evaluated whether it is a reoccurring event, as this study has only compared pollutant levels between colonies for the breeding season of 2014.

4.3 Feathers as biomarkers of exposure

Levels of POPs in kittiwake feathers quantified in this study were lower than previously reported levels. In Solheim (2010) levels of ΣPCBs, *p,p'*-DDE, and ΣPOPs found in kittiwake feathers from Kongsfjorden were up to ten times higher than reported levels in this study. Solheim (2010) investigated levels of pollutants on the feathers to compare the pollutant profiles to preen oil, whereas this study investigated POP levels in feathers. As lipid content in feathers mainly is due to the application of preen oil on the surface of feathers and not the actual lipid content of the feather, the POP levels in feathers are often lower than internal levels. The striking differences between reported levels of pollutants in feathers cannot be explained.

4.3.1 Detection of POPs, PBDEs and PFRs in feathers

In this study, 14 out of 28 POPs, 3 out of 8 PBDEs, and 6 out of 13 PFRs were detected in levels above LOD in feathers. Since feathers contain less lipids than plasma, levels of POPs in feathers compared to plasma are expected to be lower, which in this study was true for most compounds, but not for PFRs. As explained earlier, pollutants in feathers may arise from the 3-6 weeks of growing, where

feathers are connected to the bloodstream and its circulating contaminants (Burger, 1993; Jaspers et al., 2004; van den Steen et al., 2007). Preening of feathers with lipid rich preen oil (Jaspers et al., 2008), and deposition of airborne particles containing pollutants, may also give rise to high levels of pollutants in feathers (Burger, 1993). Although high levels of PFRs in the Arctic atmosphere have been reported (Salamova et al., 2014), external contamination by deposition of airborne particles is less important as kittiwakes are continuously exposed to seawater, where external contamination is washed off. Furthermore, the study by (Jaspers et al., 2007a) found no significant effect of external contamination onto feathers for PCBs and PBDEs. Preen oil has been found to be the source of external contamination onto feathers in a terrestrial bird species (Jaspers et al., 2008), and the pollutant profile in preen oil has been reported similar to pollutant profile in feathers for kittiwakes (Solheim, 2010). The higher levels of PFRs in feathers may partly origin from preen oil, but feathers could also work as an excretion route for PFRs as seen for lead and cadmium (Burger, 1993), causing the levels of PFRs in feathers to exceed levels in plasma. High levels of PFRs in blank samples are mainly caused by contamination in the lab, as PFRs can be found in plastic materials, analysis instruments, dust and solvents (Mikael Harju, personal communication). All precautions have been followed during cleanup and extraction, but it is unfortunately not possible to completely avoid external contamination in the lab.

4.3.2 Lack of correlation between feathers and plasma

Kittiwakes from Blomstrandhalvøya had significantly higher levels of almost all compounds in plasma compared to kittiwakes from Krykkjefjellet, but no differences between colonies were found for feather levels. The pollutant profile for plasma and feathers had small similarities like high contributions of both CB -138 and -153, but general comparisons were difficult to carry out, due to low pollutant detections in feathers. However, by inspecting the mean contribution of each compound group to the total contaminant load (fig. 8), both ΣPCBs and ΣOCPs strongly differed. Levels of ΣPCBs contributed significantly more to the total contaminant load in plasma, whereas the mean contribution of ΣOCP in feathers were more than twice as high compared to plasma (41.4 % vs 20.4 % respectively). This discrepancy between pollutant profiles in feather and plasma explains why no significant correlations were found between pollutants in feathers and plasma. In general, none or low correlations between feathers and internal levels have been reported for aquatic birds (Jaspers et al., 2007b), and correlations between feathers and preen oil have mostly been absent (Solheim,

2010). As the sampled primary feathers in kittiwakes are grown from September to May (Baird, 1994; González-Solís et al., 2011), they will mainly reflect the wintering areas since most of the kittiwakes do not arrive at Kongsfjorden before April (Strøm, 2006). The kittiwake is not a true migrant, but wintering areas differ from breeding grounds (Strøm, 2006; Coulson, 2011), and during winter kittiwakes can be found in most of the North Atlantic (Barrett and Bakken, 1997; Coulson, 2011; González-Solís et al., 2011). The plasma levels of pollutants reflect the pollution at the breeding area, and any correlation between feathers and plasma levels should therefore not be expected.

High correlations between feathers and internal levels have been reported for several bird species such as great tit (Dauwe et al., 2005), herring gull, common moorhen, common buzzard, barn owl, and sparrowhawk (Jaspers et al., 2007b). However, these birds are resident, and are exposed to the same pollutant profile winter and summer. In such cases, the feathers serve as a good biomonitoring tool for pollutants, since feathers will reflect the same pollutant profile as found in internal tissues. The significantly higher mean contribution of OCPs to the total contaminant load in feathers compared to plasma may therefore best be explained by the growing of primary feathers at the overwintering areas at southern latitudes, which are closer to source regions of pesticides (Bailey et al., 2000; Burkow and Kallenborn, 2000).

Feathers have proven to be good biomarkers for pollution in terrestrial and resident bird species. This study adds further evidence for their applicability in detecting and quantifying different pollutant groups as PBDEs, PCBs, OCPs, and PFRs. However, as kittiwakes are not resident, primary feathers may not be a good biomarker for pollution at Svalbard. Sampling of feathers grown at the breeding ground, such as nestling feathers, will presumably act as better biomarkers for pollution at Svalbard. As feathers are a common trait in birds, the use of feathers in non-destructive biomonitoring of pollution is important for conservation of endangered species, but just as well because sampling procedures require no special skills or training.

4.4 Thyroid hormones and pollution

Plasma levels of TT3 reported in this study were within previously reported TT3 levels in kittiwakes ranging from 2.4 to 4.2 ng/mL (Rønning et al., 2008; Johnsen, 2011). Mean fT3 levels (3.9 pg/mL for males and 2.4 pg/mL for females) ranged around 54 % lower than reported levels for both male and female kittiwakes (Welcker et al., 2013). Male kittiwakes had significantly higher levels of fT3 than

females in the current study, and similar results for kittiwakes were reported in Welcker et al. (2013) although not significant, and for glaucous gull (Verreault et al., 2004). The latter study further found negative correlations showing decreasing levels of T4 and T4:T3 ratio with increasing pollutant load, but only for male gulls, indicating a sex-specific thyrotoxicity.

The present study reported significant negative correlations between decreasing TT3:fT3 levels and increasing levels of CB -28, -138, and -187, BDE 47, and ΣPBDEs, but only for kittiwakes from Blomstrandhalvøya. No sex differences were found, but kittiwakes from Blomstrandhalvøya had significantly higher levels of pollutants than birds from Krykkjefjellet, and the higher levels of circulating contaminants may have caused the decreasing levels of TT3:fT3. As fT3 is the active hormone and tightly controlled due to its wide array of effects during neural development, molt, and thermogenesis (Dawson, 2000), any pollutant interaction with fT3 hormones might be masked by the negative feedback mechanism with the HPT axis. This could lead to increased deiodinase activity and thereby reduced levels of T4, or release of T3 from its carrier proteins (McNabb, 2007). Any interaction between pollutants and THs could only be studied by the measured TT3:fT3 ratio in this study, as levels of T4 could not be investigated, due to low sample amount available. Since the TT3:fT3 ratio was decreasing with increasing pollutant load, either TT3 levels decreased or fT3 levels increased. As most of the pollutants had positive, but not significant correlations with fT3 levels (Appendix III), increased levels of fT3 might be a possible explanation. Positive correlations between fT3 levels and pollutants have previously been reported in glaucous gull (Verreault et al., 2004) and polar bears (Braathen et al., 2004), but negative correlations have been reported as well (Braathen et al., 2004). If thyrotoxicity is sex-specific as suggested in Verreault et al. (2004), the positive correlations between fT3 and pollutant levels, although not significant, might partly explain the higher levels of fT3 found in males from both colonies, since males seem more susceptible to thyrotoxicity.

Although several mechanisms have been proposed as possible interaction pathways, most are based on laboratory studies on rodents and mammals (Dawson, 2000). Verreault et al. (2004) suggested that the negative associations between TH and pollutants were caused by interference with TH plasma carrier proteins, as some OH-PCBs have structural resemblance with TH. As avian transthyretin (TTR) has higher affinity for T3 than T4, most TTR will be saturated with T3. The displacement of T3 from TTR could facilitate excretion of T3 via urine, thereby reducing levels of TT3

in plasma and cause the TT3:fT3 ratio to decrease with increasing levels of pollutants. This is another possible explanation of the decreasing TT3:fT3 ration with increasing pollutant levels.

The correlations reported in the current study suggest contaminant mediated thyrotoxicity, as high levels of circulating contaminants affected the TT3:fT3 ratio. However, food availability and fasting during the breeding period may also cause a decrease in especially T3 levels in birds (McNabb, 2007), although no significant correlations were found between body mass and THs.

For future investigation of correlations between THs and pollutants, T4 levels should be measured as well to investigate specifically whether levels of fT3 go up, whether TT3 levels go down, or both.

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5. Conclusion

This study provided new insights in the reliability of using feathers as biomarkers of exposure for emerging and legacy pollutants. All investigated compound classes including OCPs, PBDEs, PCBs, and PFRs could be detected and quantified in feathers from kittiwakes, but none of the investigated compounds correlated with plasma levels. These results suggest that kittiwake feathers reflect contamination at the overwintering area where they were grown, whereas plasma levels reflect the breeding grounds at Svalbard. Therefore, only nestling feathers or feathers grown at Svalbard should be used for biomonitoring pollutants at Svalbard. This study was the first to report detection and quantification of PFRs in kittiwake feathers from Svalbard (despite high blank levels) and confirm their occurrence in Arctic wildlife.

A strong significant difference was found in plasma levels of organic pollutants between two kittiwake colonies in Kongsfjorden, as kittiwakes from Blomstrandhalvøya had almost 2.5 times higher levels of POPs than kittiwakes from Krykkjefjellet. The heavily polluted birds from Blomstrandhalvøya also showed significant negative correlations between the TT3:fT3 ratio and BDE 47, CB -28, -138, and -187, whereas no such relationship was found for birds from Krykkjefjellet. Higher levels of fT3 in male kittiwakes and positive, but not significant, correlations with increasing pollutant load suggest sex-specific thyrotoxicity.

Future research should include further investigation of the unexpected difference in pollutant load between kittiwakes from Blomstrandhalvøya and Krykkjefjellet, as only suggestions of causation explaining this discrepancy were made in this study. By studying an equal amount of breeding and non-breeding male and female kittiwakes from both colonies in the beginning and at the end of the breeding season, this difference could be investigated more thoroughly. In addition, measurements of stable isotopes could give insight into differences in feeding behavior, when comparing the different colonies. By including measurements of T4 levels in kittiwakes in future studies as well, light might be shed on the interesting negative correlation between the decreasing TT3:fT3 ratio and the increasing pollutant load.

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7. Appendices

Appendix I

List of IUPAC abbreviations and names of detected compounds.

Compound	IUPAC
OCPs	
p,p'-DDE	*p,p'*-dichlorodiphenyltrichloroethane
HCB	Hexachlorobenzene
β-HCH	β-1,2,3,4,5,6-Hexachlorocyclohexane
Oxy-chlordane	1-exo-2-endo-4,5,6,7,8,8a-octochloro-2,3-exo-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindane
Trans-chlordane	Trans-1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane
Trans-nonachlor	Trans-1,2,3,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane
Cis-nonachlor	Cis-1,2,3,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane
Mirex	1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1H-1,3,4-(methanetriyl)cyclobuta[cd]pentalene
PCBs	
PCB 28	2,4,4'-trichlorobiphenyl
PCB 52	2,2',5,5'-tetrachlorobiphenyl
PCB 99	2,2',4,4',5-pentachlorobiphenyl
PCB 101	2,2',4,5,5'-pentachlorobiphenyl
PCB 105	2,3,3',4,4'-pentachlorobiphenyl
PCB 118	2,3',4,4',5-pentachlorobiphenyl
PCB 138	2,2',3,4,4',5'-hexachlorobiphenyl
PCB 153	2,2',4,4',5,5'-hexachlorobiphenyl
PCB 180	2,2',3,4,4',5,5'-heptachlorobiphenyl
PCB 183	2,2',3,4,4',5',6-heptachlorobiphenyl
PCB 187	2,2',3,4',5,5',6-heptachlorobiphenyl
PCB 194	2,2',3,3',4,4',5,5'-octachlorobiphenyl
PBDEs	
PBDE 47	2,2',4,4'-tetrabromodiphenyl ether
PBDE 99	2,2',4,4',5-pentabromodiphenyl ether
PBDE 100	2,2',4,4',6-pentabromodiphenyl ether
PBDE 138	2,2',3,4,4',5'-hexabromobiphenyl ether
PBDE 153	2,2',4,4',5,5'-hexabromodiphenyl ether
PBDE 154	2,2',4,4',5,6'-hexabromodiphenyl ether

Appendix I - continued

List of IUPAC abbreviations and names of detected compounds.

Compound	IUPAC
PFRs	
BdPhP	2,4-bis(diphenylphosphino)pentane
DBPhP	dibutyl phenyl phosphate
EHDP	ethane-1-hydroxy-1,1-diphosphonate
sumTCP	sum of tricresyl phosphate
TBEP	tris(2-butoxyethyl)phosphate
TCEP	tris(2-chloroethyl) phosphate
TCPP	tri(3-chloropropyl) phosphate
TDCPP	tris(1,3-dichloro-2-propyl)phosphate
TEHP	tris-2-ethyl hexyl phosphate
TiBP	tris isobutyl phosphate
TnBP	tri-n-butyl phosphate
TPP	triphenyl phosphate
TPrP	tripropyl phosphate

Appendix II

Individual biometrics and levels of TT3 and fT3 of the 19 studied kittiwakes from Kongsfjorden, Svalbard.

ID number	Sex	Colony	Body Mass (g)	Centered BCI	Skull length (mm)	Tarsus (mm)	Wing length (mm)	TT3 (ng/mL)	fT3 (pg/mL)
KBK14-15	Male	Krykkjefjellet	425	0.55	95.06	36.66	326	3.09	6.11
KBK14-16	Male	Krykkjefjellet	432.5	0.32	96.45	36.78	326	5.06	4.65
KBK14-17	Female	Krykkjefjellet	365	0.05	90.30	35.41	312	0.70	1.25
KBK14-18	Female	Krykkjefjellet	360	0.84	87.64	32.31	308	1.71	1.41
KBK14-19	Male	Krykkjefjellet	410	1.41	91.19	35.52	318	2.14	3.69
KBK14-20	Male	Krykkjefjellet	420	0.31	95.18	34.70	320	2.65	3.92
KBK14-21	Female	Krykkjefjellet	395	1.00	90.78	34.88	304	2.53	3.49
KBK14-22	Male	Krykkjefjellet	405	-0.85	96.83	37.12	326	1.68	2.48
KBK14-24	Male	Blomstrandhalvøya	390	0.99	90.30	36.35	325	1.99	2.92
KBK14-25	Male	Blomstrandhalvøya	400	0.89	91.59	35.00	316	2.58	2.45
KBK14-26	Female	Blomstrandhalvøya	315	-2.79	92.94	33.62	311	2.39	2.63
KBK14-27	Female	Blomstrandhalvøya	315	-0.63	87.08	33.10	310	3.52	2.31
KBK14-28	Male	Blomstrandhalvøya	430	1.22	93.73	34.76	325	2.33	3.38
KBK14-31	Female	Blomstrandhalvøya	300	-0.90	86.27	33.62	320	1.63	2.92
KBK14-32	Female	Blomstrandhalvøya	310	-0.28	85.60	33.62	306	1.85	2.06
KBK14-33	Male	Blomstrandhalvøya	380	-0.92	94.46	34.32	314	2.69	5.01
KBK14-34	Male	Blomstrandhalvøya	430	0.14	96.66	34.64	314	5.12	2.88
KBK14-35	Male	Blomstrandhalvøya	400	-0.81	96.20	35.13	316	2.39	5.07
KBK14-36	Female	Blomstrandhalvøya	300	-0.54	85.31	32.62	321	2.89	2.97

Appendix **III**

Pearson product-moment correlation coefficients (*r*) between log concentrations of contaminants in plasma (pg/g ww) and fT3 levels (pg/mL plasma) for both colonies. The result is reported as not detected (ND) if less than 50 % of the samples had concentrations above LOD.

	Blomstrandhalvøya			Krykkjefjellet		
Compound	*r*-value	*p*-value	Samples above LOD in plasma	*r*-value	*p*-value	Samples above LOD in plasma
p,p'-DDE	0.29	0.38	11/11	-0.57	0.14	8/8
HCB	-0.01	0.98	11/11	-0.35	0.40	8/8
Oxy-Chlordane	0.06	0.86	11/11	-0.45	0.26	0/8
Trans-Chlordane	-0.08	0.82	6/11	-0.29	0.48	7/8
Trans-Nonachlor	0.63	0.04	10/11	-0.47	0.24	8/8
Cis-Nonachlor	0.48	0.14	11/11	-0.02	0.95	8/8
Mirex	0.26	0.45	11/11	0.52	0.19	8/8
ΣOCPs	0.21	0.54	11/11	-0.60	0.12	8/8
PCB 28	0.22	0.51	11/11	-0.16	0.70	8/8
PCB 99	0.31	0.35	11/11	0.10	0.81	8/8
PCB 105	0.29	0.39	10/11	-0.47	0.24	5/8
PCB 118	0.22	0.52	11/11	-0.17	0.70	8/8
PCB 138	0.48	0.13	11/11	-0.06	0.90	8/8
PCB 153	0.45	0.17	11/11	0.14	0.75	8/8
PCB 183	0.43	0.19	11/11	0.11	0.79	6/8
PCB 187	0.55	0.08	11/11	0.23	0.58	8/8
PCB 194	0.31	0.36	8/11	ND	ND	0/8
ΣPCBs	0.45	0.17	11/11	0.09	0.83	8/8
PBDE 47	0.54	0.09	10/11	0.33	0.43	8/8
ΣPBDEs	0.40	0.22	10/11	0.03	0.94	7/8
ΣPOPs	0.42	0.20	11/11	-0.19	0.66	8/8

Appendix IV

Individual plasma concentrations of all detected organic pollutants in kittiwakes from Krykkjefjellet (n=8) and Blomstrandhalvøya (n=11). All concentrations are in pg/g ww.

	Krykkjefjellet								Blomstrandhalvøya										
Compound	KBK14-15	KBK14-16	KBK14-17	KBK14-18	KBK14-19	KBK14-20	KBK14-21	KBK14-22	KBK14-24	KBK14-25	KBK14-26	KBK14-27	KBK14-28	KBK14-31	KBK14-32	KBK14-33	KBK14-34	KBK14-35	KBK14-36
OCPs																			
p,p'-DDE	2556	1514	6561	3313	1482	1905	946	1075	1946	4622	6147	1334	874	6606	7131	8015	2263	9996	3993
HCB	2110	1578	2150	2016	1008	1789	1368	1778	2733	2431	4393	1634	1401	6207	5167	3715	1672	3139	5475
ß-HCH	0	0	0	0	0	0	0	0	0	0	0	0	0	724	1109	0	0	0	0
Oxy-chlordane	1470	1058	1424	1438	1185	1187	1168	1585	2563	2071	2614	1847	1005	3790	3423	3298	1516	2715	4072
Trans-chlordane	203	70	201	193	135	164	139	0	360	282	0	0	0	451	403	445	0	0	453
Trans-nonachlor	59	45	138	95	73	180	33	166	157	123	0	32	115	101	40	130	218	250	43
Cis-nonachlor	72	69	49	114	71	133	45	115	246	73	38	34	72	104	65	126	95	110	59
Mirex	968	877	789	606	940	1299	807	1175	1618	1588	2387	1045	834	2908	2801	3854	1416	2221	3250
ΣOCPs	7439	5210	11312	7775	4894	6657	4505	5894	9623	11191	15580	5924	4301	20891	20140	19583	7182	18431	17346
PCBs																			
PCB 28	86	64	68	92	51	84	36	77	125	88	171	64	70	193	158	163	68	155	262
PCB 99	1366	1136	1337	1165	1229	1790	1203	1505	1826	2135	4318	1235	2106	4263	3465	3982	2040	3333	4233
PCB 105	515	0	644	493	0	527	0	492	816	779	1497	0	504	1047	1326	1615	567	1103	1294
PCB 118	1559	1166	2044	1200	1238	1975	1208	1249	1963	2305	4730	1259	1675	4065	3891	4877	1719	2928	5289
PCB 138	5724	5297	6594	5274	5514	8347	6554	7637	10863	10527	21237	6605	12006	19490	15120	25189	10698	18045	24644
PCB 153	7951	6079	7659	6008	6679	10082	7134	9143	12037	11816	28140	8497	16735	20782	19808	30271	14909	21035	30506
PCB 180	3539	3558	2984	2332	3252	4402	2843	4006	5166	4276	14076	4257	6263	9496	9101	15452	6997	11245	18132
PCB 183	595	629	704	0	0	742	683	679	896	874	3157	994	1114	1780	1894	3005	1138	2528	2870
PCB 187	1274	1202	1327	1100	1327	1843	1184	1376	2240	1738	4355	1138	2331	3862	3030	5607	1690	4628	5659
PCB 194	0	0	0	0	0	0	0	0	0	0	1762	0	931	974	1037	1400	1075	1062	2354
ΣPCBs	22610	19130	23361	17665	19290	29791	20846	26164	35932	34538	83442	24050	43735	65953	58829	91562	40901	66060	95241

Appendix IV - continued

Individual plasma concentrations of all detected organic pollutants in kittiwakes from Krykkjefjellet (n=8) and Blomstrandhalvøya (n=11). All concentrations are in pg/g ww.

	Krykkjefjellet								Blomstrandhalvøya										
Compound	KBK14-15	KBK14-16	KBK14-17	KBK14-18	KBK14-19	KBK14-20	KBK14-21	KBK14-22	KBK14-24	KBK14-25	KBK14-26	KBK14-27	KBK14-28	KBK14-31	KBK14-32	KBK14-33	KBK14-34	KBK14-35	KBK14-36
PBDEs																			
PBDE 47	509	217	237	250	264	386	135	291	796	318	660	0	239	1218	450	1690	412	871	634
PBDE 99	211	0	0	199	144	303	0	0	0	0	402	0	0	0	270	503	0	0	0
PBDE 100	0	0	0	0	185	0	0	0	208	0	182	0	0	0	0	323	0	0	0
PBDE 138	0	0	101	0	0	0	0	0	0	0	0	0	0	0	0	598	0	0	0
PBDE 153	0	0	0	140	0	156	0	305	0	0	324	0	0	0	0	0	267	0	238
PBDE 154	0	0	0	0	0	114	0	0	0	0	121	0	0	301	0	0	0	0	0
ΣPBDEs	721	217	338	589	594	960	135	595	1004	318	1687	0	239	1519	721	3115	679	871	871
ΣPOPs	30770	24558	35011	26028	24778	37409	25486	32653	46559	46047	100709	29975	48275	88362	79690	114260	48761	85362	113457

Appendix V

Individual feather concentrations of all detected organic pollutants in kittiwakes from Krykkjefjellet (n=8) and Blomstrandhalvøya (n=11). All concentrations are in pg/g ww.

	Krykkjefjellet								Blomstrandhalvøya										
Compound	KBK14-15	KBK14-16	KBK14-17	KBK14-18	KBK14-19	KBK14-20	KBK14-21	KBK14-22	KBK14-24	KBK14-25	KBK14-26	KBK14-27	KBK14-28	KBK14-31	KBK14-32	KBK14-33	KBK14-34	KBK14-35	KBK14-36
OCPs																			
p, p'-DDE	1155	0	2093	2276	2289	2089	0	1841	2732	2469	1847	1152	0	0	0	0	0	2798	0
HCB	0	0	307	524	267	467	1012	1007	5176	932	3798	290	1066	632	541	439	502	1806	0
β-HCH	0	0	0	0	1108	0	0	0	0	0	0	0	0	414	0	0	0	0	0
Oxy-chlordane	317	435	0	401	565	891	829	584	6146	651	4697	0	725	0	0	0	0	0	0
Trans-chlordane	0	0	0	0	0	226	0	82.6	0	0	0	0	0	0	0	0	0	0	0
Cis-chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0	24.8	20.3	30.3	16.7	24.2	25.4
Trans-nonachlor	82.4	79.3	320	183	64.4	170	282	78.4	0	0	0	0	0	0	0	201	0	236	0
Cis-nonachlor	0	0	0	0	104	125	87.3	0	123	81.5	94.5	0	0	0	0	92.6	0	91.7	26.9
Mirex	0	0	0	0	0	0	0	0	0	574	0	0	0	0	0	0	0	0	0
ΣOCPs	1554	514	2719	3383	4396	3969	2210	3591	14176	4708	10436	1441	1790	1070	561	762	519	4956	52
PCBs																			
PCB 28	0	0	0	0	195	273	0	401	308	0	0	0	889	0	0	0	0	315	0
PCB 99	0	0	0	0	0	0	0	938	0	0	890	0	3677	0	0	0	2519	985	0
PCB 118	0	0	0	1753	1249	1378	0	0	0	0	0	0	3729	0	0	0	0	1293	0
PCB 138	0	2168	0	2531	0	3987	0	0	4813	2210	0	0	9426	0	0	0	4384	4837	0
PCB 153	0	3109	0	3041	3691	5327	0	4283	3164	4105	2609	0	18508	4777	0	7043	4780	4260	3774
ΣPCBs	0	5277	0	7325	5134	10965	0	5621	8285	6314	3499	0	36229	4777	0	7043	11683	11689	3774
PBDEs																			
PBDE 47	0	0	0	0	0	0	0	0	0	606	619	0	1408	0	1049	0	0	624	0
PBDE 99	0	0	0	0	0	0	0	0	0	938	0	0	2828	0	0	1588	0	0	0
PBDE 153	0	0	0	0	0	0	0	0	0	0	0	0	3569	0	0	0	0	0	0
ΣPBDEs	0	0	0	0	0	0	0	0	0	1544	619	0	7805	0	1049	1588	0	624	0
ΣPOPs	1554	5792	2719	10709	9531	14935	2210	9213	22462	12567	14554	1442	45825	5848	1610	9394	12202	17269	3826

Appendix VI

Individual feather and plasma levels of PFRs in the four studied kittiwakes from Krykkjefjellet. Feather concentrations are ng/g feather weight, and plasma concentrations are in ng/g ww. The mean of four blank samples are included to show the high levels of PFRs in the blanks.

	Feathers					Plasma				
Compound	Blank value	KBK14-17	KBK14-18	KBK14-19	KBK14-20	Blank value	KBK14-17	KBK14-18	KBK14-19	KBK14-20
TCEP	0.30	0	0	0	0.35	0.75	0	0	0	0
TPrP	0.05	0	0	0	0	0.13	0	0	0	0
TCPP	26.55	0	0	0	0	4.39	0.048	0	0	0
TiBP	6.08	0	0	0	0	5.83	0	0	0	0
TnBP	3.19	0	0.50	0	0	3.77	0	0	0	0
BdPhP	0.02	0	0	0	0	0.03	0	0	0	0
TPP	4.78	0	4.04	0	0.68	0.04	0	0	0	0
DBPhP	0.02	0	0	0	0	0.10	0	0	0	0
TDCPP	1.31	0	0	0	0	2.00	0	0	0	0
TBEP	18.51	0	2.72	0	0	3.48	0	0	0	0
sumTCP	0.17	0	0.07	0	0	0.08	0	0	0	0
EHDP	7.12	0	7.56	0	0	0.38	0	0	0	0
TEHP	1.55	0	0.77	0	0	0.09	0	0	0	0
Sum PFR		0	15.65	0	1.03		0.048	0	0	0

Appendix VII

Limit of detection (LOD) for all detected compounds in feather and plasma samples. LOD for OCPs, PCBs, and PBDEs in feather and plasma are in pg/g ww. LOD for PFRs for in plasma are in ng/g ww, and ng/g feather weight for feathers.

	LOD	
Compound	Plasma	Feather
OCPs		
p,p'-DDE	88	737
HCB	714	281
β-HCH	705	310
Oxy-chlordane	58	266
Trans-chlordane	32	67
Trans-nonachlor	24	13
Cis-nonachlor	12	78
Mirex	194	66
PCBs		
PCB 28	12	146
PCB 99	85	727
PCB 105	135	1492
PCB 118	106	1119
PCB 138	184	1995
PCB 153	148	1677
PCB 180	224	2376
PCB 183	189	1982
PCB 187	222	2324
PCB 194	217	2322
PBDEs		
PBDE 47	74	440
PBDE 99	112	1068
PBDE 100	89	723
PBDE 138	99	662
PBDE 153	78	992
PBDE 154	77	678
PFRs		
TCEP	0.25	0.25
TCPP	0.5	0.5
TnBP	1.0	1.0
TPP	0.01	0.01
TBEP	0.01	0.01
sumTCP	0.03	0.03
EHDP	0.02	0.02
TEHP	0.01	0.01