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Levels and Potential Effects of PCBs, OH-PCBs, OH-PBDEs, and Hg on Plasma Progesterone Levels in Breeding Glaucous Gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard

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Abstract

Arctic life faces the risk of exposure to a magnitude of anthropogenic pollutants. Due to the chemical stability and persistence of many organic pollutants, they are able to undergo long-range transportation from emission sources at southern latitudes. When reaching arctic regions, these pollutants condense and are deposited before accumulating in the marine food web. Due to processes of bioaccumulation and biomagnification, these pollutants may pose a threat to species at higher trophic levels, such as the glaucous gull (*Larus hyperboreus*). Many of the compounds and their metabolic derivatives detected in the glaucous gull have structural similarities to reproductive hormones. They are toxic and can potentially lead to adverse endocrine disrupting effects in glaucous gulls. This study aimed to investigate the potential effects of polychlorinated biphenyls (PCBs), hydroxylated polychlorinated biphenyls (OH-PCBs), hydroxylated polybrominated diphenyl ethers (OH-PBDEs), and mercury on the sex steroid progesterone in incubating glaucous gulls sampled in 2011, 2012, and 2013 in Kongsfjorden, Svalbard. Significant positive relationships were found between progesterone levels and OH-PCBs ($p = 0.057$) and OH-PBDEs ($p = 0.079$ - 0.006) in female glaucous gulls. In male glaucous gulls, a significant positive relationship was found between progesterone levels and PCBs ($p = 0.072$). No relationships were found between mercury and progesterone in either of the sexes. The ultimate effects of altered progesterone levels in glaucous gulls may lead to repercussions in reproductive success, for example through physiological or behavioral manifestations.

Sammendrag

Dyrelivet i Arktis står ovenfor risikoen av å være eksponert til en rekke menneske-skapte miljøgifter. Disse miljøgiftene har en kjemisk stabilitet, som tillater dem å vedvare i miljøet over lang tid. Atmosfærisk transport fører disse persistente miljøgiftene fra sine opphav i sydlige strøk, til nordligere strøk og Arktis. Et kjølig klima i nord fører til at stoffene kondenseres, deponeres i miljøet, og er tilgjengelig for opptak i organismer. Gjennom prosesser som bioakkumulering og biomagnifisering står arter på høyere trofiske nivå, slik som polarmåken (*Larus hyperboreus*) i fare for å bli eksponert for miljøgifter. Tidligere studier har vist at mange miljøgifter og deres metabolitter påvirker hormonsystemet til polarmåker. I dette studiet ble det undersøkt potensielle effekter av polyklorerte bifenyler (PCB), hydroksylerte metabolitter av polyklorerte bifenyler (OH-PCB) og polybromerte difenyletere (OH-PBDE), samt kvikksølv på progesteron-nivåer i rugende polarmåker fra Kongsfjorden, Svalbard. Blodprøver ble tatt sommeren 2011, 2012, og 2013. Hos hunnlige polarmåker ble det observert signifikante positive forhold mellom progesteron-nivåer og OH-PCB ($p = 0.057$) og OH-PBDE ($p = 0.079$ - 0.006). Hos hannfuglene ble det funnet signifikant positive forhold mellom progesteron-nivåer og polyklorerte bifenyler ($p = 0.072$). Det ble ikke funnet noe signifikante forhold mellom progesteron-nivåer og kvikksølv for hverken hunn- eller hannfugler. Dette studiet viser at noen av de undersøkte miljøgiftene påvirker progesteron-nivået hos polarmåker på Svalbard. Endringer i progesteron-nivåer i polarmåker kan medføre endringer i reproduktiv suksess, gjennom utslag i deres adferd eller fysiologisk tilstan

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Abbreviations

Adj R^2	Adjusted R-squared value
AMAP	Arctic Monitoring and Assessment Programme
AICc	Akaike's information criteria for small sample sizes
ANOVA	Analysis of variance
BC	Body condition
BMR	Basal metabolic rate
Bo	Maximum binding
CV	Coefficient of variance
CYP	Cytochrome P450 monooxygenases
ELISA	Enzyme-linked immunosorbent assay
F	F-statistic
FSH	Follicle-stimulating hormone
GC-MS	Gas chromatography mass spectrometry
GnRH	Gonadotropin-releasing hormone
HNO_3	Nitric acid
HPC	Halogenated phenolic compound
HR-ICP-MS	High resolution inductive coupled plasma mass spectrometry
H_2SO_4	Sulfuric acid
i.e.	*id est*
K	Number of parameters
KOH	Potassium hydroxide solution
K_{ow}	Octanol-water partition coefficient
LH	Luteinizing hormone
ln	Natural logarithm
LOQ	Limit of quantificatoin
LOD	Limit of detection
MeHg	Methyl mercury
$MeSO_2$-PCB	Methyl sulfone polychlorinated biphenyl
min	Minute
mg	Milligram
ml	Millimeter

MS	Mass spectrometer
MTBE	Methyl tertiary butyl ether
NILU	Norwegian Institute of Air Research
n	Number of samples
ng	Nanogram
nm	Nanometer
NSB	Non-specific binding
NTNU	Norwegian University of Science and Technology
OH-PBDE	Hydroxylated polybrominated diphenyl ether
OH-PCB	Hydroxylated polychlorinated biphenyl
p	Probability of rejecting null-hypothesis
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
pH	*potentia Hydrogenii*
PL	Phospholipids
pmol	Picomol
POP	Persistent organic pollutant
R^2	R-squared value
r_s	Correlation coefficient spearman
RIA	Radioimmunoassay
SDR	Steroid Displacement Reagent
SE	Standard error of the mean
t	*t*-statistic from the Students t-test
TA	Total activity
TC	Total cholesterol
TG	Tri-glycerides
µL	Microliters
Σ	Sigma symbol denoting the sum of
Δ	Delta symbol denoting difference or change

1. Introduction

1.1. Pollutants in the Arctic

The Arctic is considered one of the least polluted areas on Earth, due to scarce human activity and few local sources of pollutants. Yet a wide range of contaminants can be detected here, including the persistent organic pollutants (POPs) polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), their metabolic derivatives, as well as mercury (Ariya et al., 2004; Hung et al., 2010). The physicochemical properties of POPs allow for long-range atmospheric and ocean current transportation, ultimately leading to their deposition and accumulation in the Arctic (Jæger et al., 2009; Muir and de Wit, 2010). Originating from emission sources at lower latitudes, these compounds are transported to higher colder latitudes, often over the span of multiple seasons, where they finally condense and deposit. The persistence of POPs is attributed to their chemical stability, making them resistant to biological, photolytic, and chemical degradation, with half-lives of up to 9 years in the atmosphere depending on the compound (Hung et al., 2010; Miniero and Iamiceli, 2008). Organisms, therefore, face the risk of long-term exposure to these substances. PCBs and PBDEs are highly lipophilic, increasing their capacity of accumulation in the fatty tissues of organisms, and eventually the biomagnification through trophic levels and food webs (Letcher et al., 2010). Mercury is also detected in arctic marine food webs, most likely originating from atmospheric transport and local geological sources (Atwell et al., 1998). Biomagnification of mercury is seen throughout arctic food webs, and in its methylated form methylmercury (MeHg), is highly bioaccumulative (Campbell et al., 2005; Jæger et al., 2009). In vertebrates, the highest concentrations of mercury are typically found in protein-rich tissues, like liver and muscle tissue, while low levels are found in lipid-rich tissues (Atwell et al., 1998; Campbell et al., 2005). The high levels of POPs and mercury found in upper trophic levels of the Arctic marine food web can lead to numerous health concerns in individuals of top predatory species, such as the polar bear (*Ursus maritimus*) and glaucous gull (*Larus hyperboreus*) (Bytingsvik et al., 2012; Dietz et al., 2013; Verreault et al., 2007c, 2006a, 2005a). Many POPs classes and their metabolically derived products have structural similarities to endogenous reproductive hormones, and can therefore mimic hormones, block them directly, or otherwise disturb their synthesis and metabolism (Damstra et al., 2002).

1.2. The glaucous gull (*Larus hyperboreus*)

The glaucous gull is an opportunistic predator and scavenger, positioned at the top of the Arctic food web. Their feeding ecology spans over many trophic levels, which includes them feeding on fish, molluscs, crustaceans, rodents, birds, eggs, insects, berries, plants, carrion, offal, and even garbage (Anker-Nilssen et al., 2000). Consequently, the glaucous gull is potentially exposed to a wide range of legacy pollutants such as PCBs and mercury, but also many current-use pollutants of recent concern including PBDEs and polyfluorinated alkyl substances (Verreault et al., 2010, 2007c, 2005a). Measured levels of POPs in the glaucous gull are among the highest in comparison to other arctic seabirds (Verreault et al., 2010, 2006a). High levels of PCBs have been measured in glaucous gulls on Bear Island as early as 1972 (Bourne and Bogan, 1972). Over the past decades, the glaucous gull populations in Canada, Iceland, and Svalbard have decreased drastically (Petersen et al., 2015). On Bear Island, the population has declined by 65 % in the past 30 years and is currently categorized as endangered (Erikstad and Strøm, 2012). The prognosis for the Bear Island population indicates that it most likely will go extinct if the factors contributing to the current state of its decline continue (Erikstad and Strøm, 2012). Large burdens of environmental pollutants, along with climate change are some of the major factors contributing to the observed decline (Erikstad and Strøm, 2012; Petersen et al., 2015). Levels of PCBs detected in Svalbard glaucous gulls are very high compared to levels of other POPs and constitute approximately 72 % of the analyzed legacy POPs in a previous study (Verreault et al., 2005b). The effects of such high PCB concentrations in glaucous gulls have been associated with impaired biological functions, including but not limited to disruption of the endocrine systems. Among other adverse effects correlated to PCB burdens are reduced clutch sizes, increased white blood cell counts, asymmetric feather growth, and a generally reduced adult survival (Gabrielsen, 2007; Verreault et al., 2010).

1.3. Physiological traits of importance

Differences in pollutant loads are seen between the two sexes of glaucous gull, with males generally having the greater burden (Bustnes et al., 2000; Verreault et al., 2007b, 2005b). Through egg production, female glaucous gulls have a route of pollutant elimination that males do not have (Verreault et al., 2006b). The mechanisms and rate of deposition of pollutants into eggs during egg synthesis depends on biological factors of the mother and the egg, as well as the properties of the compound itself. Compounds with a low octanol-water partition coefficient (K_{ow}), *i.e.* less lipophilic POPs, have a tendency to be more readily transferred to

eggs from the mother (Verreault et al., 2006b). However, differences seen between sexes can also be partially due to differences in feeding ecology (Bustnes et al., 2000).

The body mass of adult seabirds, including the glaucous gull, fluctuates throughout the year (Gabrielsen, 2009). The entire process of reproduction is very energy demanding, and a large percentage of this energy is derived from body lipids. At the onset of the breeding season, utilization of energy storages leads to a decrease in body mass and fat content, reaching its lowest levels at the end of the chick-rearing period (Sagerup et al., 2009a). Lipophilic compounds stored in fatty tissues, such as POPs, are released into the blood stream when the tissues are utilized. When in the blood, these foreign compounds (xenobiotics) and their potential metabolites may exert toxic effects in the individuals (Sagerup et al., 2009a).

1.4. Biotransformation

An important factor regulating the accumulation of pollutants in organisms is enzyme-mediated biotransformation. The metabolism of POPs is generally catalyzed by biotransforming enzymes, such as cytochrome P450 monooxygenases (CYP), a superfamily of enzymes with broad substrate specificities (Klaassen, 2013). Initial hydrolysis, reduction, and oxidation, often termed phase 1 reactions, are often precursing reactions to the phase 2 reactions of conjugation: glucuronidation, sulfation, and glutathionylation (Klaassen, 2013). These processes of metabolite conversion generally reduce the lipophilicity and toxicity of a compound and ease their rate of excretion (Helgason et al., 2010). Biotransformation of PCBs and PBDEs leads to the formation of different metabolites, including hydroxylated forms of both PCBs (OH-PCBs) and PBDEs (OH-PBDEs). OH-PCBs and their parent PCB congeners have been found at increasingly high concentrations in the glaucous gulls of Svalbard (Kelly et al., 2008; Verreault et al., 2005b). Hydroxylated metabolites are of increasing concern due to their attributes which allow for modes of action as endocrine disrupters in Arctic species, including the glaucous gull (Bytingsvik et al., 2012; Gutleb et al., 2010; Verreault et al., 2008). The transformational mechanisms result in that several precursor PCB compounds that can be metabolized into identical OH-PCBs, which makes it difficult to track parent compound to the metabolites (Helgason et al., 2010; Grimm et al., 2015).

1.5. Progesterone in avian species

Although care must be taken when comparing studies on different classes of animals and between avian orders, chemical structures of steroid hormones remain virtually identical among vertebrates (Nelson, 2005). Progesterone is a sex steroid hormone related to several aspects of the reproductive cycle and is synthesized in both sexes in avian species. The precursor compound for progesterone is pregnenolone, which is synthesized from cholesterol and catalyzed by cytochrome P450 (Nelson and Cox, 2008). In female birds, the main production of progesterone takes place in granulosa and theca cells of ovarian follicles. Circulation of progesterone in the blood is mediated by corticoid-binding globulin, albumin, or other γ-globulins. Progesterone receptors are present in the hypothalamus and pituitary, on the surface of epithelial cells, tubular gland cells, stromal fibroblasts, in the smooth muscle cells of the oviduct, as well as in granulosa, theca, and germinal epithelial cells of follicles (Hahn et al., 2015). Increased progesterone levels in females are induced by ovary stimulation from luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Signaled by a positive feedback mechanism working on the hypothalamus, progesterone increases the release of luteinizing hormone (Hahn et al., 2015). The control of this axis is far more complicated than described here, with numerous factors influencing the balance of both gonadotropins and sex steroids. Progesterone is a prohormone, meaning that it can itself act as a hormone or be converted into a range of other hormones with different endocrine properties. Through steroidogenesis pathways, progesterone is a precursor to glucocorticoids, mineralocorticoids, androgens, and estrogens (Nelson and Cox, 2008). As the precursor to testosterone and estradiol, which also play crucial roles in the reproductive cycle, the importance of normal progesterone levels in relation to successful breeding is further illustrated. For the different sexes, balancing the correct concentrations of each sex hormone through specific steroidogenic enzymes is very important to avoid endocrine abnormalities (Nelson, 2005).

Like many arctic seabirds, the glaucous gull is a seasonal breeder. Seasonal breeding birds have fluctuations in gonadal steroid concentrations throughout their reproductive cycle, which is synchronized with breeding activity (Hahn et al., 2015). Cyclic fluctuations in avian species are seen in both sexes, albeit far greater in females than in males (Bharucha and Padate, 2009). Levels remain at a basal level during the non-breeding phase, elevate significantly in commence with the pre-breeding phase, before a final peak in advance of the actual breeding phase (Bharucha and Padate, 2009). Highest concentrations in the plasma are found 6-4 hours

prior to ovulation (Hahn et al., 2015). Progesterone levels in females then decrease slightly in the post-breeding stage. Both females and males partake in parental duties, a behavior regulated by progesterone and other hormones such as prolactin. Both of these hormones are important for initiating incubation behavior, and since neither of the hormones alone are sufficient to promote nest build behavior or incubation, both are cooperatively essential (Nelson, 2005). Males have basal levels of progesterone throughout the different phases of the reproductive cycle. These levels naturally rise and fall cyclically, affecting parental behavior (Bharucha and Padate, 2009). The synthesis of lipids in female birds in also regulated by progesterone. Avian ovaries have little capacity for synthesis of lipids, therefore, egg yolk lipids are mainly synthesized in the liver, stimulated by progesterone among other hormones (Hahn et al., 2015).

1.6. Effects of pollutants on the endocrine system

Disruption of steroid hormone homeostasis is suspected to lead to toxicological effects related to sexual differentiation, growth, development, and behavior (Verreault et al., 2006a, 2010). Three factors dictate the concentrations of steroid hormones in plasma: the rate of biosynthesis, the rate of hormone catabolism, and the affinity to transport proteins (Nelson, 2005). Each of these steps are potential targets for xenobiotics. Due to the crucial nature of steroid hormones in relation to successful reproduction, disruptions of steroidogenesis may cause harmful effects on biochemical to ecological scales (Craig et al., 2011). The timing of progesterone surges is important for the correct physiological response of ovulation, with a sensitivity within a timeframe of hours. High pollutant loads of PCBs have been related to reduced reproductive behavior in Svalbard glaucous gulls (Bustnes et al., 2001). Both the duration of absence and the total amount of time absent from the nest site have been shown to be positively correlated with blood concentrations of PCBs (Bustnes et al., 2005, 2001). In both studies, the reduced reproductive behavior was suggested to result from the effects of pollutant loads on circulating hormone levels, including gonadal steroid hormones. In addition, MeHg has been found to impact vertebrate reproduction as well, disrupting endocrine systems (Tartu et al., 2013). From studies on black-legged kittiwakes, mercury was suggested to be acting as an endocrine disrupter. MeHg is thought to mainly influence the hypothalamus disrupting gonadotropin-releasing hormone (GnRH) synthesis and secretion, thus altering LH and FSH levels and ultimately progesterone levels (Tartu et al., 2013).

2. Aim of the study

The aim of the study is to quantify levels of PCBs, the hydroxylated metabolites OH-PCBs and OH-PBDEs, as well as Hg in plasma of breeding glaucous gulls from Kongsfjorden, Svalbard, and investigate a possible relationship with plasma levels of progesterone. In the present study, it is hypothesized that the endocrine disruptive properties of some of these pollutants may result in correlations between pollutants and the reproductive hormone, progesterone. The link between pollutant exposure and observed endocrine disruption in birds is poorly understood, and this study aims to contribute to the understanding of the relationship between POPs and mercury with the sex steroid hormone progesterone.

3. Methods

3.1. Sampling and data collection

Blood samples were collected from 50 incubating glaucous gulls (male n = 17, female n = 33) during the breeding seasons of 2011, 2012, and 2013 in varying locations within Kongsfjorden, on the western coast of Spitsbergen in the Norwegian Arctic. Blood sampling of glaucous gulls was done by researchers from the Norwegian Polar Institute. Samples were taken from the wing vein, during the period between May and July at varying times of the day. Blood samples were centrifuged immediately after returning from the field to separate plasma and red blood cells. Plasma and red blood cells were transferred to separate cryovials, frozen down at -20°C and kept frozen until analyses were performed. Biometric measurements were recorded for each individual bird at the time of capture, including the length of the head, bill, wing, and gonys depth. Captured birds that were not already ringed, were ringed with numbered steel rings from the Norwegian Ringing Center at Stavanger Museum. Individuals had their sex determined prior to this study, either by morphological or molecular sexing as described by (Løseth, 2014).

3.2. Chemical analyses

The analysis of the hydroxylated PCBs and PBDEs in the plasma samples was performed by gas chromatography-mass spectrometry at the Norwegian Institute for Air Research (NILU) in Tromsø. The analysis of mercury was performed by high-resolution inductive coupled plasma - mass spectrometry (HR-ICP-MS) conducted by an Element 2 HR-ICP-MS at the Institute of Chemistry at the Norwegian University of Science and Technology (NTNU), by Syverin Lierhagen.

3.3. Extraction and clean-up of OH-PCBs and OH-PBDEs

Three parallels of glaucous gull plasma samples, one parallel from each year of sampling, were each processed for extraction separately. Plasma samples were thawed and vortexed to ensure homogeneity, 500-1000 µL was transferred to 15 mL glass tubes, and the exact weight of each sample was noted. Internal standards for halogenated phenolic compounds (HPC), $MeSO_2$-PCBs, PBDEs, and POPs (details in Appendix A) were added to all samples. OH-PCBs are weak acids, and extraction requires an acidic pH. Hydrochloric acid (0.5 mL, 6M), isopropanol (4 mL), and saturated ammonium sulfate aqueous solution (1 mL) were added to

each sample, followed by mixing and vortexing. Acidifying the plasma leads to protonation, increasing the polarity of the compounds. Ammonium sulfate ionizes the water phase, forcing the hydroxylated compounds towards the organic phase. Organic extraction was conducted twice with 5 mL of methyl tertiary butyl ether(MTBE)/n-hexane (50/50), and extracts were pooled together. The extracted phase was transferred to a new 15 mL glass tube and concentrated to 0.5 mL using the RapidVap™ (Labconco™ Vacuum Evaporation System). n-hexane was added to all samples (5 mL). The organic phase was extracted twice, first with 4 ml of an aqueous potassium hydroxide solution (14 g KOH in 250 mL ethanol and water (30/70)), secondly with 2 mL of KOH solution. The KOH solution deprotonates the hydroxylated compounds and forces them towards the aqueous phase. Hydroxylated compounds were then re-extracted, by acidifying with 10-15 drops of sulfuric acid (H_2SO_4) giving a pH < 3, and adding 6 mL MTBE/n-hexane (50/50). The organic phase was extracted to new 15 mL glass tubes and concentrated to 0.5 mL using the RapidVap.

The concentrated organic phase consisting of hydroxylated compounds was derivatized using diazomethane, before being cleaned with acidic silica (25 %) columns. Silica was burned at 600 °C for 8 hours, and acidic silica (25 %) was prepared by adding H_2SO_4 that was shaken to ensure a homogenous mixture. Plastic columns and frits were washed with dichloromethane and air dried. Columns were packed with one frit, 0.7 g acidic silica, 0.2 g silica, and a final frit to seal the column. The fraction containing hydroxylated PCBs and packed silica columns were loaded and run in the RapidTrace Automated SPE Workstation (Biotage Inc). After completion, 200 µL of isooctane was added to each sample, before they were concentrated to 0.2 mL in the RapidVap. Samples were transferred to vials and ^{13}C PCB-159 was added as a recovery standard. Due to a power outage in the final stages before transferring the samples to GC-MS vials, the recovery percentage of the 2012 samples were sub-optimal (2 – 52 %). Internal standard recovery above 60 % was considered optimal, and samples with a recovery below 20 % were to be used in analysis with caution. The samples were then ready for gas chromatography-mass spectrometry (GC-MS), which was conducted at NILU. Quantification of PCBs in the same blood samples was performed previously (Løseth, 2014).

3.4. Instrumental analysis

Analysis of the hydroxylated compounds was performed on an Agilent 7890A GC with a 5975C mass spectrometer (MS) (Waldorf, Germany) operated in single ion monitoring mode (SIM). For each sample, 1 µL was injected splitless in a split/splitless inlet at 250 °C set at a constant flow of 1.0 mL/min using a DB5-MS column with the dimensions of 30 m x 0.25 mmID, 0.25 µm stationary phase. The temperature program began at 70 °C where it was held for 2 minutes, and raised by 30 °C/min to 250 °C, and finally, 5 °C/min until it reached the final temperature of 300 °C, which was held stable for 5 minutes.

3.5. Quantification

Quantification of compounds was done by the internal standard method, with internal standards and recovery standards provided by NILU. Samples with unknown concentrations of compounds were measured along with the added standards containing known concentrations of both ^{12}C and the equivalent labeled ^{13}C. After completion of GC-MS, the peak areas of the standards added were used to create a standard curve, which allowed the calculation of sample extract concentrations with Equation 1.

$$C_{sample} = Rf(C_{std} \times Area_{sample}) / Area_{std} \qquad \text{Equation 1}$$

In Equation 1, C_{sample} is the concentration of the unknown sample, Rf the response factor as calculated from the areas and concentrations of ^{12}C and ^{13}C from the GC-MS, C_{std} is the concentration of the known standard, $Area_{sample}$ is the area found from the GC-MS of the sample, and $Area_{std}$ is the known area of the internal standard from the GC-MS.

3.6. Quality assurance

For every tenth sample of glaucous gull plasma analyzed, one human blood plasma sample (standard reference material from NIST SRM 1958) was included as a reference as well as a water blank. The human plasma samples were spiked with the internal standard for halogenated phenolic compounds (details in Appendix A). Human plasma and water blanks were treated the same as the glaucous gull samples.

3.7. Mercury detection in red blood cells

Designated UltraClave 15 mL vials were washed with HNO_3 and two times with distilled water. Glaucous gull red blood cells (300-600 mg) were transferred to the vials, exact weights noted, and 12 ml 50 % HNO_3 was added to each vial. Vials were loaded into the Milestone UltraClave and digested. Samples were then diluted to 61ml (± 0.5 g) in a Teflon coated bottle and transferred to 15 ml vials. High resolution inductive coupled plasma – mass spectrometry (HR-ICP-MS) was conducted on the prepared samples by an Element 2 HR-ICP-MS at the Institute of Chemistry at the Norwegian University of Science and Technology (NTNU), by Syverin Lierhagen.

3.8. Progesterone analysis

Progesterone analysis was conducted at the Department of Biology at NTNU prior to this study (Løseth, 2014), and was performed as follows. Levels of progesterone in the blood plasma were quantified using enzyme-linked immunosorbent assay (Progesterone ELISA kit, ADI-900-011, Enzo Life Sciences), as described in the kit manual. The Progesterone ELISA kit is a competitive immunoassay and utilizes a polyclonal antibody to bind to progesterone in a competitive manner. Initially, after a simultaneous incubation at room temperature, the excess reagents were washed away and the substrate was added. Following a short incubation, the enzyme reaction was stopped and the absorbance of the yellow color generated was read on a microplate reader at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of progesterone found in the plasma samples. The measured optical density for the samples was used to calculate the concentration of progesterone in the sample.

Analysis of each sample was done in duplicate, where both duplicates were used to calculate the mean concentrations of progesterone per sample. Each 96-well plate analyzed included three wells dedicated to blanks, which were treated equally as the samples. Two wells were dedicated for the calculation of total activity (TA) of the reagents, which only contained conjugate, substrate, and stop solution. Three wells were dedicated for non-specific binding (NSB), which contained assay buffer, conjugate, substrate, and stop solution. And three wells were dedicated for maximum binding (Bo) activity, which contained assay buffer, conjugate, antibody, substrate, and stop solution. In two of the plates, an excess 50 µL of assay buffer was accidently added to the Bo wells. Therefore, values from the Bo wells on the third plate, which contained the correct amount, were used in later calculations. All standard and sample wells

contained either diluted standard solution or diluted samples, as well as a conjugate, antibody, substrate, and stop solution. Due to the sensitivity of the kit used, samples were diluted 5 times (100 µL plasma). The dilution factor was determined by testing plasma with varying dilutions, quality controls, and several replicates. The analyses were conducted with and without the kit Steroid Displacement Reagent (SDR, 1 µL SDR per 10 µL plasma) in order to assess bound and free progesterone in the plasma samples.

To ensure that the results from each kit were comparable, a selection of samples were analyzed on more than one plate, and a coefficient of variance (CV) was calculated from these. If the calculated CV was below 20 %, this ensured the desired inter-assay precision. The inter-assay CV values ranged from 1-18 %, which meant that the progesterone levels measured between plates were comparable. The intra-assay CV values ranged from 0-213 %, but excluding two major outliers resulted in a range of 0-31 %, with a median of 4.75 %. The female individual with a CV of 213% was not included in further analysis. Measurements with CV values between 20-31 % were still included in the statistics because these progesterone levels were well within the range of the dataset.

3.9. Lipid analyses

Lipid analyses were conducted at Unilab Analyse AS, Tromsø, Norway, prior to this study (Løseth, 2014). Briefly put, lipid quantification was performed on plasma samples using a Roche Diagnostics kit and a Cobacs c 11 analyzer (F. Hoffmann-La Roche Ltd., Rotkreuz, Switzerland). Lipids analyzed were tri-glycerides (TG), phospholipids (PL), free cholesterol (FC), and total cholesterol (TC), and a total lipid level was calculated using Equation 2.

$$\text{Total lipid} = 1.677 \times (TC - FC) + FC + TG + PL \qquad \text{Equation 2}$$

3.10. Statistical analysis

All statistics were done using R (v3.2.3: R 2016), and additional packages used are presented in Appendix E. The limit of detection (LOD) and limit of quantification (LOQ) for all the compounds detected in the glaucous gull plasma samples are presented in Appendix B. Compounds with more than 50 % of the values above the limit of quantification (LOQ), were included in the statistical analysis. Of the included compounds that had values below the limit of detection (LOD), these values were assigned a random value between zero and the LOD of the compound (as generated by the statistical software R 3.2.3). Values that were below the LOQ but above the LOD were used directly. These criteria resulted in the following compounds being excluded from statistical analysis: 4-OH-PCB-130, 5-OH-BDE-47, 4-OH-BDE-49, 2-OH-BDE-68, 5-OH-BDE-100, 4-OH-BDE-101, 4-OH-BDE-103.

Correlation tests were performed using Spearman rank correlation. The Spearman rank correlation is fit for relationships that aren't perfectly linear, which was the case between some parent compounds and metabolites. Linear models require response variables to be normally distributed. Therefore, a normality test of the response variable, which was progesterone, was conducted. Normality was found after ln-transforming, and progesterone was therefore ln-transformed in the statistical analysis. Body condition was defined by obtaining the residuals when regressing body mass against head size (Schulte-Hostedde et al., 2005). This was initially conducted for each sex separately, but no difference was found (t = 1.86e-16, p = 1.00) in the resulting female and male body conditions. Therefore, the sexes were pooled for the regression, and the residuals from this were used directly as a measure of body condition.

All compounds were calculated to pmol/mL, to better fit the biological relationship between one unit of pollutant to one unit of progesterone.

3.10.1. Multivariate statistics

Relationships and correlations between variables were investigated by performing principal component analysis. This was performed separately for each sex as the pollutant load differed between them. None of the explanatory variables were ln-transformed, but instead centered and scaled to unit variance. The variables included were progesterone, lipid content, body condition, twelve PCBs, eight OH-PCBs, OH-HpCS, and Hg.

Linear models were used to test how progesterone levels were related to PCBs, OH-PCBs, OH-PBDEs, Hg, body condition, year of capture, and capture period in the breeding season, as well as interaction effects between variables with suspected interactions. Due to the high correlation of the PCBs, OH-PCBs, and OH-PBDEs, they were summed separately into the groups: ΣPCBs, ΣOH-PCBs, and ΣOH-PBDEs. Many variations of models can be made from these variables, so a model selection approach using Akaike's information criteria for small sample sizes (AICc) was employed to find the most parsimonious model. Models with low ΔAICc values and ΔAICc between 0-2, show substantial support and should be taken into regard (Burnham and Anderson, 2004). Standard linear models require independence of data. This was not the case for 22 of the samples, as they were collected from 11 birds sampled in separate years. To control for this, mixed linear models were employed to correct for nested data. This allowed all of the samples to be included in the analysis. However, when creating models for each sex separately there were not enough male samples for mixed models to be employed. So for males, the two of the samples that were from recaptured birds were excluded from the dataset and standard linear models were used. Variables were mean-centered and scaled to unit variance in order to standardize the range of the data. Models were diagnosed to check the fit of the model, by investigating residual normality of the models and checking for potential outliers.

4. Results

4.1. Pollutant levels

The molar concentrations of PCBs, OH-PCBs, OH-PBDEs, and Hg are presented in Table 1, and concentrations in ng/mL are presented in Appendix C. For both sexes, the PCB congeners of highest prevalence were CB-153 > CB-138 > CB-180 > CB-118, and OH-PCB congeners of highest prevalence were 4-OH-CB-187 > 4-OH-CB-146 > 4-OH-CB-107.

Table 1. Concentrations of PCBs, OH-PCBs, and OH-PBDEs congeners (with LOQ > 50 %) and Hg in pmol/mL, with range, median, mean and standard error of the mean (SE) in plasma samples of incubating female (n = 33) and male (n = 17) glaucous gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard, sampled in 2011, 2012, and 2013. The star (*) beside a compound name indicates a significant difference of the levels between sexes (ANOVA test). If the minimum value was below LOD, it is indicated by a < symbol. The actual range may in these cases be lower than shown in the table. Concentrations of all compounds in ng/mL are in Appendix C.

	Females (n = 33)			Males (n = 17)		
	Range	Median	Mean ± SE	Range	Median	Mean ± SE
CB-28*	0.21 - 1.28	0.49	0.52 ± 0.05	0.36 - 1.61	0.90	0.89 ± 0.10
CB-52	<0.002 - 2.58	0.41	0.63 ± 0.12	<0.01 - 1.70	0.41	0.46 ± 0.11
CB-99*	1.57 - 34.00	6.37	9.22 ± 1.25	7.35 - 42.58	16.94	21.51 ± 3.16
CB-101	<0.01 - 6.46	0.66	1.08 ± 0.26	<0.02 - 3.89	0.07	1.08 ± 0.32
CB-105*	1.06 - 13.97	3.43	4.46 ± 0.54	0.94 - 18.84	6.89	8.60 ± 1.35
CB-118*	4.07 - 52.69	12.44	17.24 ± 2.20	12.68 - 92.52	31.25	40.02 ± 6.11
CB-138*	7.95 - 102.8	22.89	33.22 ± 4.18	26.82 - 184.0	65.95	81.74 ± 12.88
CB-153*	17.62 - 241.0	46.83	68.28 ± 9.56	55.7 - 448.9	115.6	176.6 ± 31.26
CB-180*	6.53 - 133.6	18.90	31.00 ± 5.41	22.08 - 208.4	50.09	76.04 ± 14.54
CB-183*	1.09 - 16.67	2.78	4.46 ± 0.67	3.57 - 25.17	8.04	10.89 ± 1.81
CB-187*	2.00 - 24.59	4.17	5.72 ± 0.78	5.67 - 23.65	10.55	13.22 ± 1.52
CB-194*	0.75 - 17.61	2.51	3.95 ± 0.74	2.24 - 28.39	6.75	9.66 ± 1.87
ΣPCBs*	44.4 - 602.9	118.1	179.0 ± 24.3	143.9 - 1039	332.5	440.7 ± 73.45
4-OH-HpCS*	<0.0003 - 0.14	0.02	0.03 ± 0.004	0.01 - 0.11	0.05	0.05 ± 0.01
4-OH-CB-107	0.06 - 2.19	0.38	0.58 ± 0.08	0.03 - 1.63	0.53	0.72 ± 0.10
3-OH-CB-138*	<0.002 - 0.29	0.02	0.06 ± 0.01	<0.01 - 0.30	0.07	0.10 ± 0.02
4-OH-CB-146*	0.12 - 6.09	0.50	0.79 ± 0.17	0.60 - 3.88	1.47	1.69 ± 0.22
3-OH-CB-153*	<0.001 - 0.43	0.04	0.09 ± 0.02	<0.02 - 0.80	0.09	0.17 ± 0.05
4-OH-CB-163*	<0.002 - 0.28	0.02	0.03 ± 0.01	0.02 - 0.18	0.06	0.07 ± 0.01
4-OH-CB-172*	<0.0002 - 0.54	0.03	0.05 ± 0.02	0.03 - 0.24	0.10	0.11 ± 0.02
4-OH-CB-187*	0.32 - 22.05	1.40	2.47 ± 0.66	2.62 - 18.48	5.00	7.00 ± 1.05
4'-OH-CB-193*	<0.001 - 0.16	0.01	0.02 ± 0.01	<0.02 - 0.12	0.05	0.06 ± 0.01
ΣOH-PCBs*	<0.64 - 32.17	2.87	4.11 ± 0.95	3.76 - 24.74	8.00	9.97 ± 1.41
5-OH-BDE-99	<0.0002 - 0.21	0.02	0.04 ± 0.01	<0.0001 - 0.18	0.03	0.04 ± 0.01
6-OH-BDE-47/75	<0.0007 - 1.10	0.06	0.10 ± 0.02	<0.02 - 0.68	0.19	0.14 ± 0.03
ΣOH-PBDEs	<0.003 - 1.12	0.09	0.14 ± 0.02	0.06 - 0.68	0.11	0.18 ± 0.04
Hg	221.5 - 4307	2258	2191 ± 196.2	264.8 - 5319	2956	2912 ± 361.8

There is a significant difference in pollutant load between the sexes for sum of PCBs (F = 15.26, p = 0.0004) and sum of OH-PCBs (F = 22.56, p = <0.0001) with males displaying the higher concentrations. No difference in OH-PBDE (F = 0.76, p = 0.39) or Hg (F = 0.95, p = 0.34) concentrations was found between the sexes. A noteworthy compound is 6-OH-BDE-47/75, which is the sum of 6-OH-BDE-47 and 6-OH-BDE-75, as the GC-MS in unable to distinguish between the two congeners. They both have the same molecular weight, so this should not affect any calculations to mol.

4.2. Relationship between PCBs and hydroxylated metabolites

As Table 1 shows, OH-PCBs levels in both male and female glaucous gulls are generally lower than PCBs levels. Table 2 shows suggested parental PCB compounds for the three most prevalent hydroxylated metabolites (Grimm et al., 2015), as well as the correlation coefficient between the two. Spearman correlation tests were performed for the most prevalent OH-PCBs: 4-OH-CB-187, -146, and -107. Relatively high and significant correlations were found between the parent compounds and their suggested metabolites.

Table 2. Spearman correlations between OH-PCB metabolites and their suggested parent compounds, either via epoxide or direct insertion, or via a 1,2-shift (Grimm et al., 2015). Correlation values are from 0 to 1, where 1 indicates a 100 % correlation.

Metabolite	Parent compound			
	Epoxide or direct insertion	1,2-shift	r_s	p-value
4-OH-CB-187	CB-187		0.73	<0.0001
4-OH-CB-187		CB-183	0.69	<0.0001
4-OH-CB-146		CB-138	0.74	<0.0001
4-OH-CB-146		CB-153	0.75	<0.0001
4-OH-CB-107		CB-105	0.59	<0.0001
4-OH-CB-107		CB-118	0.65	<0.0001

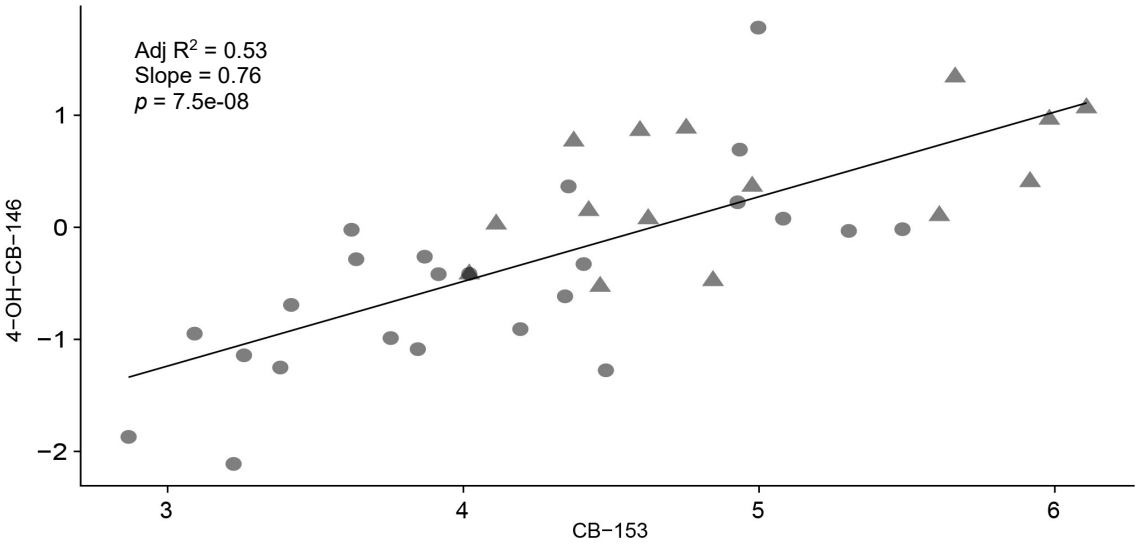

Figure 2. Plot showing the parent compound to metabolite relationship with the highest correlation coefficient, CB-153 to OH-CB-146 from a 1,2-shift. Both sexes are pooled together excluding recaptured birds, where circle points are females (n = 24) and triangle points males (n = 15). Adjusted R^2, slope, and *p*-value are shown in the upper left corner. Both variables are ln-transformed in the current figure for the sake of presentation.

Further, the ratio of the metabolites to the parent compound was calculated, with the mean ratio of the ΣOH-PCBs to ΣPCBs being 2.7 % in males, and 2.5 % in females.

Table 3. Ratios are calculated between the metabolite and parent compound for each sex separately, then compared in a Student t.test. Metabolite and parent compound values are ln-transformed, to better fit the t.test requirements. The significance is set to 95 %, where significant variables are in bold and 90 % significance is underlined.

Compounds			Ratio mean		Difference between sexes	
Metabolite		Parent compound	Males	Females	*t*	*p*-value
ΣOH-PCBs	/	ΣPCBs	0.027	0.025	0.64	0.53
4-OH-CB-187	/	CB-187	0.603	0.429	-1.68	<u>0.10</u>
4-OH-CB-187	/	CB-183	0.780	0.637	-1.44	0.16
4-OH-CB-146	/	CB-138	0.024	0.024	-0.06	0.95
4-OH-CB-146	/	CB-153	0.012	0.012	-0.09	0.93
4-OH-CB-107	/	CB-105	0.139	0.143	-1.44	0.17
4-OH-CB-107	/	CB-118	0.019	0.04	-3.03	**0.006**

The only 95 % significant difference of parent compound to metabolite ratio between sexes, is for CB-118 to 4-OH-CB-107 (t = -3.03, p = 0.006), where females have a higher mean ratio of 4 % compared to 1.9 % in males (Table 3).

4.3. Progesterone levels

Levels of progesterone are presented in Table 4. No significant difference in progesterone levels was found between the two sexes ($F = 0.94$, $p = 0.34$). Body condition was found to significantly affect progesterone ($F = 8.90$, $p = 0.01$). There is no significant difference in the measurements done with and without steroid displacement reagent (SDR) ($t = 2.49$, $p = 0.01$). Therefore, measurements done without SDR will be used in further statistics.

Table 4. Levels of progesterone (pmol/mL) measured with and without SDR, with range, median, mean and standard error of the mean (SE) in plasma samples of incubating female (n = 33) and male (n = 17) glaucous gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard, sampled in 2011, 2012, and 2013. Levels of progesterone in ng/mL are presented in Appendix D.

	Females, n = 33			Males, n = 17		
Progesterone	Range	Median	Mean ± SE	Range	Median	Mean ± SE
SDR	0.48 - 3.61	1.09	1.26 ± 0.13	0.32 - 3.83	0.81	1.25 ± 0.24
Without SDR	0.46 - 3.50	1.01	1.19 ± 0.12	0.31 - 2.85	0.78	1.14 ± 0.19

4.4. Relationships between progesterone and PCBs, OH-PCBs, OH-PBDEs, and Hg

The principal component analysis was performed separately for each sex, resulting in two plots (a scores and a loadings plot) for each sex (Figure 2). The PCAs include progesterone, twelve PCBs, eight OH-PCBs, two OH-PBDEs, OH-HpCS, Hg, lipid content, and body condition. Recaptured birds were not included in the PCAs, as the PCAs are only intended for descriptive statistics. Females are presented in the F plots, where the two first components (PC1 = 49.41 and PC2 = 18.55) account for 67.96 % of the variance. Males are presented in the M plots, where the two first components (PC1 = 53.70 and PC2 = 14.26) account for 67.96 % of the variance. For both sexes, the loadings of the PCA indicated a clustering of most PCBs and OH-PCBs in two separate clusters and a slight clustering of the two OH-PBDEs. The mean of factor 1 loadings (F1) for PCBs in females F1 = 0.74, and in males F1 = 0.79. The mean of F1 loadings for OH-PCBs and OH-HpCS in females F1 = 0.82, and males F1 = 0.74. This shows that a great portion of the variance in PCBs and OH-PCBs in explained along PC1. Notably, 4-OH-CB-107 behaves differently between the two sexes, having a greater association with PCBs for male birds. The loadings mean for the two OH-PBDEs in females F1 = -11, and in males F1 = -0.25. For females, Hg was slightly correlated with the PCBs and OH-PCBs with an F1 = 0.21, and to a larger degree for males where F1 = 0.51.

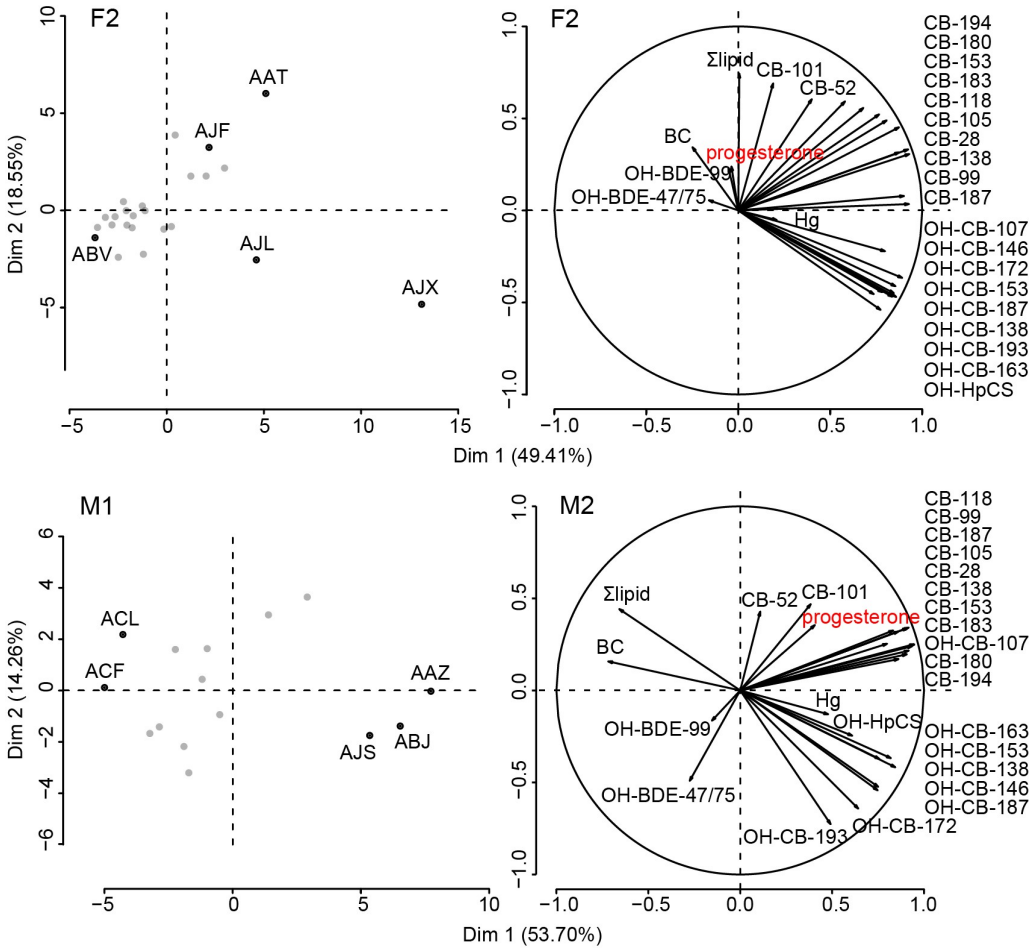

Figure 2. Principal component analysis scores and loading plots of incubating female (F plots, n = 24) and male (M plots, n = 15) glaucous gulls from Kongsfjorden, Svalbard, excluding recaptured birds. The PCA includes progesterone (emphasized in red), 12 PCBs, 8 OH-PCBs, 2 OH-PBDEs, OH-HpCS, Hg, lipid content (Σlipid), and body condition (BC). Females plots: PC1 = 49.41 and PC2 = 18.55, Male plots: PC1 = 53.70 and PC2 = 14.26. In the scores plot, the five individuals that contributed to the most variation are labeled with their ring code. The PCA score plot shows the relationship between the individuals sampled while the PCA loadings plot shows the relationship between the variables. A complete PCA summary is presented in Appendix F.

Body condition was negatively correlated with the clusters of PCBs and OH-PCBs in both sexes, where females and males had F1 = -0.25 and F1 = -0.73 respectively. Lipid content had a visible difference between the two sexes, having a greater correlation with body condition in males F1 = -0.66, than what is seen in females F1 = 0.005. There was a correlation along PC2 between progesterone and OH-PBDEs in females. There was no apparent correlation between progesterone, PCBs, OH-PCBs, Hg, or body condition in females. However, the plot did suggest a negative relationship between OH-PCBs and progesterone in females. In males,

progesterone was positively correlated with the PCB cluster and negatively correlated with both OH-PBDEs.

Based on the PCA findings, OH-PBDEs were expected to affect progesterone concentrations differently for the two sexes. PCBs and OH-PCBs were negatively correlated with body condition and lipid content. This led to the suspicion that there may be an interaction effect between body condition and PCBs and or OH-PCBs when explaining the variation in progesterone. Lipid content was expected to be statistically linked to progesterone in females, as progesterone stimulates lipid synthesis in female ovaries. Therefore, lipid content was not included as an explanatory variable. The associations observed in the PCAs are further investigated below using linear models based on Aikaike's information criteria.

Table 5.
Model selection table including the five best models for each sex, explaining the variation of progesterone levels in incubating glaucous gulls in relation to PCBs, OH-PCBs, OH-PBDEs, Hg, and BC (body condition). Progesterone was ln-transformed to ensure normality, while PCB, OH-PCB, Hg, and BC are scaled (mean centered). The * symbol indicates interaction effects between two variables, also including each variable separately. K is the number of parameters in the model. AICc (Akaike's information criteria for small sample sizes) shows the ranking for each model, and ΔAICc depicts the difference between models in relation to the best model. AIC weight indicates the level of support among candidate models. Females were modeled using mixed linear models to account for recaptured birds, where each bird's unique ring number was set as a random effect. Males were modeled using standard linear models without recaptured birds.

Models	Model ID	K	AICc	ΔAICc	AIC Weight
Females					
progesterone ~ ΣPCBs*BC + ΣOH-PCBs + ΣOH-PBDEs	1	8	42.86	0.00	0.38
progesterone ~ ΣPCBs*BC + ΣOH-PBDEs	2	7	44.00	1.14	0.21
progesterone ~ ΣPCBs*BC + ΣOH-PCBs + ΣOH-PBDEs + Hg	3	9	44.63	1.77	0.16
progesterone ~ ΣPBDEs*BC	4	6	44.68	1.82	0.15
progesterone ~ ΣPCBs*BC + ΣOH-PCBs	5	7	45.60	2.74	0.10
Males					
progesterone ~ ΣPCBs*BC	1	5	41.08	0.00	0.65
progesterone ~ ΣOH-PCBs*BC	2	5	43.80	2.72	0.17
progesterone ~ ΣOH-PBDEs*BC	3	5	44.47	3.38	0.12
progesterone ~ ΣPCBs*BC + ΣOH-PCBs	4	6	46.12	5.04	0.05
progesterone ~ ΣPCBs*BC + ΣOH-PCBs*BC	5	7	51.60	10.52	0.00

The five best-ranked models explaining the variation in progesterone levels in relation to the detected compounds are presented in Table 5. Body condition was found to be a variable of importance besides the compounds themselves. Year and capture period showed no importance in explaining progesterone levels for either of the sexes. Of the included compounds, ΣPCBs, ΣOH-PCBs and ΣOH-PBDEs showed variable importance, while no significant effect of Hg was found in any of the best models. Four of the female models and one male model had ΔAICc values between 0-2. ΔAICc values between 0-2 indicate that the model likely explain the data equally well, and any models within that range should all be considered. ΣOH-PBDEs, ΣPCBs : body condition, and ΣOH-PBDEs : body condition were the significant variables explaining progesterone variation in females depending on the model (Table 4), while ΣPCBs were found to be the significant variable explaining progesterone variation in males (Table 5). Body condition seemed to be of importance in both sexes, as it was included in all five of the best female and male models.

Table 6.
Progesterone levels in incubating female glaucous gulls (n = 32) as affected by ΣPCBs, ΣOH-PCB, ΣOH-PBDEs, Hg, body condition, and the interaction effects ΣPCBs : body condition and ΣOH-PBDEs : Body condition. Mixed linear model summary tables including estimates, standard errors (SE) and significance levels (*p*-value) are shown for female models 1 - 4. Progesterone was ln-transformed to ensure normality, while ΣPCBs, ΣOH-PCB, ΣOH-PBDEs, Hg, and body condition are scaled (mean centered). The estimate value of the intercept represents the y-axis intercept, and the sum of the estimates represents the slope. The significance is set to 95 %, where significant variables are in bold and 90 % significance is underlined.

	Model 1		Model 2		Model 3		Model 4	
Variable	estimate	*p*	estimate	*p*	estimate	*p*	estimate	*p*
Intercept	0.055	0.621	0.05	0.678	0.06	0.567	0.09	0.428
ΣPCBs	-0.09	0.467	0.07	0.472	-0.07	0.643		
ΣOH-PCBs	0.21	0.121			0.16	0.260		
ΣOH-PBDEs	0.18	<u>0.079</u>	0.22	**0.036**	0.12	0.281	0.34	**0.006**
Hg					-0.05	0.222		
Body condition	-0.006	0.193	-0.07	0.114	-0.06	0.317	-0.09	<u>0.091</u>
ΣPCBs : Body condition	0.21	**0.017**	0.16	**0.029**	0.22	**0.054**		
ΣOH-PBDEs : Body condition							0.21	**0.045**

The female model summaries in Table 6 show that in models 1 to 4 the accumulative effect of ΣPCBs, ΣOH-PCBs, ΣOH-PBDEs, Hg, body condition, ΣPCBs : body condition, and ΣOH-PBDEs : body condition may all lead to an increase of progesterone in incubating female glaucous gulls. The summed values of the effects were 0.56, 0.43, 0.38, and 0.55 for models 1, 2, 3, and 4 respectively.

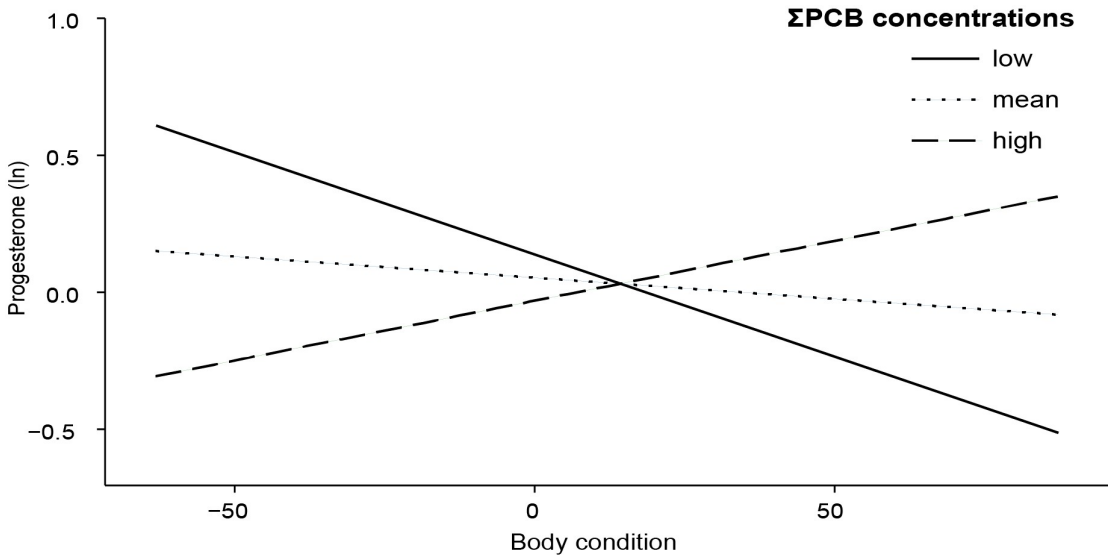

Figure 3. Plot of the interaction effect between ΣPCBs and body condition from female model 1, with progesterone levels as a function of body condition. The three different lines solid, dotted, and dashed, indicate low, mean, and high levels of ΣPCBs respectively. Their slopes show the differing effects low, mean, and high levels of ΣPCBs have on the function of progesterone and body condition.

Figure 3 shows the interaction effects of ΣPCBs and body condition on progesterone in female glaucous gulls. The effect of ΣPCBs on progesterone levels in individuals with low to mean levels of PCBs (solid and dotted lines), is less in individuals with increasing body condition. In contrast, the effect of ΣPCBs on progesterone levels in individuals with high levels of PCBs (dashed line), is predicted to be higher in individuals with a good body condition as shown by the trend line. Since trend lines predict how the interactions are expected to work based on observed data, the data was further examined. The distribution of ΣPCBs in female glaucous gulls is heavily skewed towards individuals with lower PCB burdens, with 64 % of individuals having ΣPCB levels below the mean. Also, the body condition of the female glaucous gulls was normally distributed. Most individuals, therefore, fall within the category low to mean levels of ΣPCBs, and at mid-ranged body conditions. The solid and dotted trend lines are thus based on a larger sample size.

Table 7.
Progesterone levels in incubating male glaucous gulls (n = 15) as affected by ΣPCBs, body condition, and the interaction between ΣPCBs and body condition. Linear model summary table including estimates, standard errors (SE) and significance levels (*p*-value) is shown for male model 1. Progesterone was ln-transformed to ensure normality while ΣPCBs and body condition are scaled (mean centered). The estimate value of the intercept represents the y-axis intercept, and the sum of the estimates for ΣPCBs, body condition, and ΣPCBs:body condition represents the slope. The significance is set to 95 %, where significant variables are in bold and 90 % significance is underlined.

	Model 1		
Variable	estimate	SE	*p*
Intercept	0.19	0.22	0.934
ΣPCBs	0.56	0.28	<u>0.072</u>
Body condition	0.28	0.26	0.303
ΣPCBs : Body condition	0.28	0.26	0.320

The summary of male model 1 in Table 7 shows that ΣPCBs, body condition, and the interaction between ΣPCBs and body condition may all lead to an increase in progesterone levels in male incubating glaucous gulls. The summed value of the effects was 1.31.

ΣOH-PBDEs was only constituted of the two compounds 5-OH-BDE-99 and 6-OH-BDE-47/75. In regard to the importance of ΣOH-PBDEs in explaining progesterone variation in females, the fact that 5-OH-BDE-99 was detected in fewer females (45 % < LOD) compared to males (35 % < LOD) was worrying. Because of this discrepancy and the lack of enough data for OH-PBDEs, new female models were made excluding ΣOH-PBDEs to investigate the relationship with the other compound groups.

Table 8.
Model selection table including the five best female models excluding OH-PBDEs, explaining the variation of progesterone levels in incubating glaucous gulls in relation to PCBs, OH-PCBs, Hg, and BC (body condition). Progesterone was ln-transformed to ensure normality, while PCBs, OH-PCBs, Hg, and BC are scaled (mean centered). The * symbol indicates interaction effects between two variables. K is the number of parameters in the model. Mixed linear models were used to account for recaptured birds, where each bird's unique ring number was set as a random effect.

Models	Model ID	K	AICc	ΔAICc	AIC Wt
progesterone ~ ΣPCBs*BC + ΣOH-PCBs + Hg	6	8	43.60	0.00	0.56
progesterone ~ ΣPCBs*BC + ΣOH-PCBs	7	7	45.60	2.00	0.21
progesterone ~ ΣPCBs*BC + ΣOH-PCBs*BC	8	8	47.09	3.49	0.10
progesterone ~ ΣPCBs*BC + ΣOH-PCBs*BC + Hg	9	9	47.32	3.72	0.09
progesterone ~ ΣOH-PCBs*BC	10	6	48.46	4.86	0.05

Upon excluding the OH-PBDEs, the models showed an importance of PCBs, OH-PCBs, and Hg in explaining progesterone variation. Two models fell within the ΔAICc range of 0-2, and both were considered.

Table 9.
Progesterone levels in incubating female glaucous gulls (n = 33) as affected by ΣPCBs, ΣOH-PCBs, Hg, body condition, and the interaction effect ΣPCB : body condition. Mixed linear model summary tables including estimates, standard errors (SE) and significance levels (*p*-value) are shown for female model 6 and 7. Progesterone was ln-transformed to ensure normality, while ΣPCBs, ΣOH-PCBs, Hg, and body condition are scaled (mean centered). The estimate value of the intercept represents the y-axis intercept, and the sum of the estimates for ΣPCBs, ΣOH-PCBs, Hg, body condition, and ΣPCBs : body condition represents the slope. The significance is set to 95 %, where significant variables are in bold.

	Model 6			Model 7		
Variable	estimate	SE	*p*	estimate	SE	*p*
Intercept	0.07	0.11	0.528	0.06	0.11	0.577
ΣPCBs	-0.09	0.12	0.537	-0.12	0.12	0.364
ΣOH-PCBs	0.18	0.11	0.177	0.27	0.11	**0.057**
Hg	-0.05	0.02	0.118			
Body condition	-0.02	0.03	0.515	-0.02	0.03	0.618
ΣPCBs : Body condition	0.25	0.05	**0.014**	0.27	0.06	**0.006**

The summaries from the two best female models excluding OH-PBDEs are shown in Table 9, where OH-PCBs and the interaction effect between ΣPCBs and body condition are the significant variables. It is worth noting that Hg here is almost significant but still falls outside of the 90 % significance. The accumulative effects of these variables may lead to an increase in progesterone levels in both models, with an effect of 0.34 and 0.46 for model 6 and 7 respectively.

5. Discussion

Studies have been conducted on the glaucous gull since the 1970s, resulting in large amounts of information on the levels, patterns, and temporal trends of their environmental pollutant burdens, as well as the effects these compounds have on a physiological, behavioral, and ecological scale (Verreault et al., 2010). In this study the effects that PCBs, OH-PCBs, OH-PBDEs, and Hg have on progesterone levels in breeding glaucous gulls was investigated, to further contribute to the current understanding of how anthropogenic pollutants exert their effects on arctic seabirds.

5.1. Levels of pollutants

5.1.1. PCBs

The pollutant loads of glaucous gulls have shown to be dominated by PCBs, constituting up to 70 % of their legacy pollutant load (Verreault et al., 2005b). In the current study 12 PCB congeners were quantifiable in the glaucous gull plasma samples (Table 1), and their concentrations were found to be lower than what is seen in previous studies. Compared to concentrations detected in two populations of glaucous gulls sampled on Bear Island in 1997 (Bustnes et al., 2003), the concentrations found in the current study were approximately 4.5 times lower in females and 4.3 times lower in males, based on 8 congeners. And in comparison to a study with samples from 2001 by Bustnes et al. (2004), PCB concentrations in the current study had a median 5 times lower in females and 3.3 lower in males based on 6 congeners. Specifically, two of the congeners (CB-118 and -138) were both 11 times lower in females in the current study. Fortunately, the levels of PCBs have slowly decreased following the regulations of PCBs under the Stockholm Convention. Temporal trends of PCBs have been found to significantly decrease by 1.9 % annually in a wide range of arctic biota (Rigét et al., 2010). This holds true for the glaucous gull as well, with a decrease in blood levels of PCBs in the Svalbard glaucous gull population between 1995 – 2004 (Rigét et al., 2010). Thus, the lower PCB levels found in the current study are to be expected based on the reducing temporal trends. Congeners of highest prevalence in the current study were CB-153 > CB-138 > CB-180, following the same pattern as previous studies on glaucous gulls (Bustnes et al., 2004, 2003). When comparing with other arctic bird species, CB-153 is also found to be the most prevalent congener, as seen in a study investigating the little auk (*Alle alle*), Brünnich's guillemot (*Uria lomvia*), black guillemot (*Cepphus grylle*), and black-legged kittiwake (*Rissa*

tridactyla) (Borgå et al., 2005). The persistence of these specific compounds is partly due to PCB congeners containing five or more chlorine and para-chlorine atoms have greater metabolic resistance (Letcher et al., 2000). This is reflected in the most prevalent congeners found in this study: CB-153 (hexachloride), CB-138 (hexachloride), CB-180 (heptachloride), and CB-118 (pentachloride).

5.1.2. OH-PCBs

Of the hydroxylated compounds, nine OH-PCBs and eight OH-PBDE congeners, as well as OH-HpCS, were detected in the glaucous gull plasma samples. Of these compounds, eight OH-PCBs and two OH-PBDE congeners were at sufficient levels to be quantified (Table 1). In comparison to a former study, OH-PCB levels in the current study were found to be lower by a factor of approximately 2.5 in females and 1.9 in males, based on three congeners (4-OH-CB-187, 4-OH-CB-146, and 4-OH-CB-107) (Verreault et al., 2005b). The most prevalent hydroxylated PCB congeners in the current study were 4-OH-CB-187, 4-OH-CB-146, and 4-OH-CB-107. Two other arctic seabird species, the northern fulmar (*Fulmarus glacialis*) and the black-legged kittiwake, both displayed 4-OH-CB-187 and 4-OH-CB-146 as the most prevalent congeners (Helgason et al., 2010). Avian species in general though seem to produce and or retain fewer hydroxylated metabolites as opposed to arctic marine mammals such as the polar bear (*Ursus maritimus*), where a study detected 16 OH-PCB congeners (Sandala et al., 2004). However, this may only reflect the higher PCB burden many mammals obtain, resulting in more metabolites being detectable. Further, patterns and levels of hydroxylated metabolites vary among species due to variation in enzymatic capacity, specifically CYP enzymes. But also the intra-specific variations in diet, body condition, age, sex, biotransformation capacity, and reproductive status all impact toxicokinetics (Letcher et al., 2000). The polar nature of hydroxylated compounds theoretically means they are less bioaccumulative. Therefore, metabolic formation within the body could more likely be the source as opposed to uptake from dietary sources. OH-PCBs are produced via phase 1 biotransformation but are further liable to phase 2 biotransformation through conjugation with glucuronic acid or sulfate, which increases their ultimate water solubility and rate of excretion. This is reflected in that OH-PCBs are predominantly reported in blood and to a lesser degree in tissues, as opposed to the PCB metabolites $MeSO_2$-PCBs, which are persistent in fatty tissue and the liver and are resistant to further metabolism (Letcher et al., 2000). Yet retention of hydroxylated compounds may be happening, as they are detectable at

high concentrations in organisms. One of the mechanisms that result in retention of hydroxylated PCBs and PBDEs is through the competitive binding of these compounds with natural ligands for protein binding sites. The structural similarities of OH-PCBs and OH-PBDEs to the natural ligand thyroxine results in their high binding affinities to the thyroxine carrier protein transthyretin. This has been reported in *in vitro* studies on glaucous gull liver tissue, in polar bears of Svalbard, and in human *in vitro* studies (Gutleb et al., 2010; Meerts et al., 2000; Ucán-Marin et al., 2010). OH-PCBs/-PBDEs also show a higher affinity for transthyretin than its intended endogenous hormone thyroxine, actually leading to thyroxine being outcompeted by OH-PCBs/-PBDEs (Chauhan et al., 2000; Meerts et al., 2000; Ucán-Marin et al., 2010). This indicates that the presence of these hydroxylated compounds in blood is not necessarily linked to their lipophilic properties or the lipid content of the blood, but rather from protein binding. Not only does the competitive binding of OH-PCBs/-PBDEs disrupt hormonal balance, the binding of the OH-group shelters it from further metabolism and excretion by denying enzyme-mediated conjugative processes (Letcher et al., 2000).

5.1.3. OH-PCB to PCB relationship

When exploring the relationship between parent compounds and metabolites, levels in glaucous gulls from the current study showed significantly high correlations between a selection of proposed metabolites and parent compounds. Relationships were investigated between the three most prevalent metabolites 4-OH-CB-187, 4-OH-CB-146, and 4-OH-CB-107, and two proposed parent compounds for each metabolite (Table 2). These relationships had a correlation range of 0.59 - 0.75, indicating their origin as metabolites from the given parent compounds. Ratios were also calculated between the same set of metabolites to parent compounds, to investigate the extent of metabolism (Table 3). These ratios varied a lot, ranging from 0.012 to 0.78. The greatest ratios were found between 4-OH-CB-187 and CB-187 (males = 0.60, females = 0.43) and between 4-OH-CB-187 and CB-183 (males = 0.78, females = 0.64). These results indicate that CB-187 and CB-183 are metabolized to a large degree in both female and male glaucous gulls, both instances forming the same metabolite, namely 4-OH-CB-187. T-tests were used to check whether females and males had varying ratios between metabolites and parent compounds, which would indicate a difference in toxicokinetics between the two sexes. Only one relationship had a significant difference between males and females, which was 4-OH-CB-107 from CB-118 (t = -3.03, p = 0.006), where females had the higher ratio value meaning a higher degree of

metabolism from CB-118. 4-OH-CB-107 behaved notably different from the other hydroxylated PCBs in the PCA as well (Figure 2), displaying a greater association with PCBs in males compared to females. In humans, a difference in OH-PCB patterns was established based on the consumption of fatty fish, where 4-OH-CB-107 was particularly influenced based on diet (Hagmar et al., 2001). Interestingly, 4-OH-CB-107 formation was the only metabolite to parent compound ratio with a significant difference between the sexes in the current study. Along with the previously discussed significance of how diet based differences lead to varying congener profiles, this may indicate that 4-OH-CB-107 is especially prone to intra-specific variations, which are possibly due to dietary and enzymatic differences between sexes. Naturally, care must be taken when extrapolating results from mammals to birds. A study on herring gulls (*Larus argentatus*) showed that reduced food intake led to an increase in biotransforming enzymes and consequent OH-PCBs (Routti et al., 2013). This further depicts the impact diet has, as well as body condition on congener patterns. Ratios between OH-PCBs and PCBs have been calculated in previous studies on glaucous gulls. These studies have used the ratio between ΣOH-PCBs and ΣPCBs, making comparisons to the current study difficult as the number of OH-PCBs and PCBs included in the sums vary. Yet, the mean ratio for both females and males was found to be 0.02 between Σ_5PCB (118, 138, 153, 187, 105) and Σ_4 OH-PCB (107, 120, 146, 187) in a previous study (Verreault et al., 2005b). In the current study, mean ratio values are skewed from the relationship between 4-OH-CB-187 and its parent compounds being particularly high. Calculation of the median of OH-PCBs to PCBs ratios results in females = 0.04 and males = 0.03, which is very comparable to the (Verreault et al., 2005b). ΣOH-PCBs to ΣPCBs ratios in blood have widely varying relationships based on species and range from approximately 10 % in humans, 90 % in specific albatross samples (although the mean of samples was 57 %), to 459 % (± 358 %) in female polar bears and 830 % (± 556 %) in male polar bears (Klasson-Wehler et al., 1998; Letcher et al., 2000; Sandala et al., 2004). In northern fulmar chicks, ΣOH-PCBs to ΣPCBs ratios were found to be 4.2 (±1.0) (420 % ± 100 %) (Helgason et al., 2010), which is higher than what was found in the current study. There is a seemingly huge difference in ΣOH-PCBs to ΣPCBs ratios not only between mammals and birds but also within arctic seabirds and birds of differing age. Therefore, comparing ratios with other species than the glaucous gull was not emphasized.

5.1.4. Mercury

In the current study levels of mercury were measured in red blood cells, as opposed to other seabird studies where mercury is generally measured in tissues such as feathers, liver, or muscle. Comparing levels detected in the current study with previous studies cannot be done directly, but can still give indications of the pollutant load. Total mercury levels in the liver have been found to range from 0.30 - 0.74 μg/g, and up to 1.50 μg/g in livers of glaucous gulls found dead on Bear Island (Jæger et al., 2009; Sagerup et al., 2009a, 2009b). The current study detected red blood cell concentrations lower than in the mentioned studies, with levels at 0.49 μg/ml (±0.27). According to the Arctic Monitoring and Assessment Programme (AMAP) from 2002, these levels are all well below the threshold for toxic effects of mercury in tissues of terrestrial birds and marine bird eggs (Demore et al., 2005). Although comparing seabirds to terrestrial birds should be done carefully, marine seabirds are thought to be much more tolerant to mercury, due to constant environmental exposure to mercury over an evolutionary time-span (Thompson and Furness, 1989). The tolerance birds have to mercury may be reflected in the fact that although the proportion of total mercury found in glaucous gulls consists of 62 % organic MeHg, nearly 100 % of mercury found in bird feathers is generally MeHg (Jæger et al., 2009; Thompson and Furness, 1989). This indicates a very effective route of elimination for MeHg.

5.1.5. Sexual differences in pollutant burden

Significant differences in organic pollutant concentrations between sexes have been found in this and other studies on glaucous gulls, with males consistently having the higher body burdens of PCBs, OH-PCBs, and OH-PBDEs (Bustnes et al., 2004; Verreault et al., 2006a). Multiple factors can contribute to these observed differences. Females have a selective transfer of their organic pollutant load to eggs. A study investigating the maternal transfer of POPs to eggs in glaucous gulls, concludes that the characteristic congener pattern found in female plasma and in eggs suggests a highly selective retention of organic compounds in glaucous gulls (Verreault et al., 2006b). Mercury, on the other hand, was not found at significantly different concentrations between females and males in the current study, which is supported by other studies on glaucous gulls and black-legged kittiwakes (Burger et al., 2009; Sagerup et al., 2009a; Tartu et al., 2013). Elimination of mercury through feather molting is the most important route for birds (Thompson and Furness, 1989). Due to the differences in the

elimination of organic pollutants and mercury, the resulting body burden patterns are different for the two types of compounds.

5.2. Progesterone levels

No differences in progesterone levels were found between the two sexes (Table 4). Certain individuals of both sexes were found to be major outliers (three females, two males), with either very high or low concentrations, but nothing from the laboratory results indicated that these results should be excluded. A previous study investigating progesterone in incubating glaucous gulls showed significant differences in concentrations between females and males (Verreault et al., 2006a). In contrast, a study on incubating yellow-eyed penguins showed no significant differences in progesterone levels between the two sexes, although results from a study on the same species are better to compare. When comparing the progesterone levels with the aforementioned study by Verreault et al. (2006a), the current study detected lower mean levels by a factor of 3.4 for males, and 4 for females. The Verreault et al. (2006a) study used radioimmunoassay when analyzing progesterone while the current study employed enzyme-linked immunosorbent assay. When using the ELISA kit, four parallels were used, two with steroid displacement reagent, and two without. There was no significant difference between the parallels with steroid displacement reagent and the parallels without (Table 4), where the use of SDR should have resulted in higher levels. Why there was no difference is uncertain, but this may have been a weakness in the ELISA method employed, where the RIA method Verreault and colleagues (2006a) used may have detected a more accurate concentration of the progesterone plasma levels.

5.3. Effects of pollutants on progesterone

Significant positive relationships between the detected organic pollutants and progesterone in incubating glaucous gulls were found in the current study. In males, a significant relationship between progesterone and ΣPCBs (Table 7) was shown, leading to an ultimate increase in progesterone levels. Females, on the other hand, seemed more prone to be affected by hydroxylated compounds, where both OH-PBDEs (Table 6) and OH-PCBs (Table 9) showed significant positive relationships. The accumulative effects of the variables led to a greater increase in progesterone in males than what was seen for females. This may be tied to their greater blood burden of most pollutants. The previously detected the positive relationship between PCBs and progesterone in glaucous gulls, showed that only males were significantly

affected (Verreault et al., 2006a). The findings from the Verreault et al. (2006a) study support the findings in the current study, giving more data on the relationship between PCBs and progesterone. The relationship between PCBs and progesterone has also been shown in mammalian species, as exemplified in a study on polar bears of Svalbard, where lactating female polar bears with blood burdens of PCBs had altered progesterone levels (Haave et al., 2003). This relationship was non-existent in single polar bears, suggesting reproductive status may be an important factor when investigating sex steroid hormones. A study on female mink (*Mustela vison*) showed the same relationship between PCBs and progesterone as the current study, with an increase in progesterone levels when exposed to PCBs (Patnode and Curtis, 1994). Verreault et al. (2006a) also discovered a significant relationship between PBDEs and progesterone, where PBDEs actually explained the greater part of the variation (24 %) in progesterone despite being found at concentrations 36-fold times lower than the PCBs. The current study found that OH-PBDEs and OH-PCBs were important variables in describing female progesterone variations, and PCBs were important variables in males. It would be interesting to explore if there was a similar sexual difference between OH-PBDEs and PBDEs as was seen for OH-PCBs and PCBs. Perhaps PCBs and PBDEs are more impactful pollutants in males, while OH-PCBs and OH-PBDEs are more impactful in females.

Differences in which compounds significantly affected males and females in the current study indicated potential influences of many factors. Differences in composition of congener profile, mechanistic modes of action, and variations in physiological status between the sexes, could all be linked to the observed differences. But likely, the differences result from a combination of these factors. The endocrine disrupting effects of PCBs and OH-PCBs can affect steroid hormone systems like estrogens, progesterone, androgens, and adrenal steroids through a variety of actions (Robertson and Hansen, 2001). Effects of PCBs on progesterone potentially involve alterations in progesterone levels directly, or in the expression of the progesterone receptor. Studies on mammals show that estrogen functions as a major regulator of the progesterone receptor in reproductive organs (Robertson and Hansen, 2001). Ultimately, PCBs that act estrogenically can through estrogen receptor signaling alter progesterone receptor expression, which has been shown in a study on mink where PCBs lead to an increase in progesterone receptor expression (Patnode and Curtis, 1994). Hydroxylated PCB metabolites also have relatively high affinity for estrogen receptor β, and through binding to and action with this receptor can alter progesterone receptor expression (Connor et al., 1997). Specific actions of PCBs in females can be related to the effects PCBs have in ovarian follicles, where

PCBs disrupt steroidogenesis in mammalian cell cultures (Wójtowicz et al., 2005). In the *in vitro* study mentioned above disruption in cholesterol mobilization occurs in small ovarian follicles exposed to PCBs, leading to insufficient substrate availability for hormone synthesis. PCBs also affected larger ovarian follicles, altering cytochrome P450 aromatase activity, which is an important enzyme in steroidogenesis. Normal progesterone levels depend on properly regulated processes of enzymes involved in both synthesis of progesterone from precursor steroids, metabolism, and conversion of progesterone to other steroids.

Mercury was not found to be significant in explaining progesterone variation in either female or male glaucous gulls in the current study, although it was included in three female models (Table 6 and 9). A study on breeding black-legged kittiwakes investigated the relationship between mercury and whether or not birds bred that season (Tartu et al., 2013). The authors showed that non-breeding birds had higher levels of mercury than breeding birds, in which non-breeding birds showed that gonadotropin-releasing hormone (GnRH) -induced luteinizing hormone levels were affected. Breeding birds, on the other hand, had no significant mercury-related variation in luteinizing hormone. Mean mercury levels measured in breeding kittiwakes were 1.8 μg/g, which was believed to be below the threshold for affecting breeding success. In relation to the current study, assuming that levels and effects in black-legged kittiwakes can be extrapolated to glaucous gulls, it seems that breeding birds do not have high enough body burdens of mercury to significantly affect reproductive mechanisms. The current study only captured incubating glaucous gulls. The fact that they are breeding may imply that they have mercury levels below the required levels for any effects to be seen, as indicated by (Tartu et al., 2013). Also, as mentioned before, levels detected in the current study had a mean of 0.49 μg/ml (±0.27), which is below threshold levels thought to result in effects in birds. Future studies interested in similar relationships as the current study should consider sampling non-breeding birds as well.

However, what are the effects of altered progesterone levels? As an important part of a complex mix of steroid hormones, all requiring their own unique timing and concentrations in order to secure optimal body function, progesterone ultimately effects reproduction. The birds sampled in this study were incubating birds, already indicating success to a certain degree. Perhaps more extensive alterations in steroid hormone balance are seen in non-breeding birds? Even though the current sampled birds were incubating, progesterone influences behavioral aspects of reproduction, which can still affect the success of the entire breeding season. A study on PCBs

in incubating glaucous gulls on Bear Island showed a strong significant relationship between PCBs and nest site attentiveness, where both male and female birds with high blood burdens of PCBs were more frequently absent from their nest site, and were missing for longer periods of time (Bustnes et al., 2001). Parental behavior in these birds was affected in one the most critical phases of reproduction, as both parents and offspring are vulnerable to predation when not attending the nest. The authors of the study concluded that the observed behavior was likely due to neurological effects or through endocrine disruption. Verboven et al. (2009) conducted a similar study and found no relationship between POP exposure and incubation behavior. What the study did find though, was a relationship between POP levels and altered thermal conditions inside the nest, which could negatively affect the offspring. Another study on breeding glaucous gulls linked high organic pollutant levels with earlier egg laying (Bustnes et al., 2003). Progesterone may be a factor involved in these process, as it generally is an important sex steroid in reproduction, and specifically in relation to behavior and the timing of ovulation.

5.4. Body condition

The best models for both sexes showed the importance of body condition in explaining the variation of progesterone, although the variable in itself was not significant in any of the models. The interaction effect between ΣPCBs and body condition was significant in all five of the female models where it was included. The interaction effect between ΣPCB and body condition was also present in the best model for male glaucous gulls, although not significant. The interaction effect of ΣPCBs and body condition in female glaucous gulls presented in Figure 3, is likely rooted in the complexity of body condition, energy allocation, and mobilization of pollutants within an individual. Low, average, and good body condition values may all imply and reflect different physiological statuses within an individual. Notably, it is not necessarily the fluctuation in body condition from an average state that is indicated, merely the current relative size in relation to the population sampled. An individual with a poor body condition could in fact be emaciated, and may have mobilized lipid storages increasing the effects of PCBs. At the same time, an individual with a good body condition may have increased effects of PCBs from a high body burden of dietary PCBs. (Verreault et al., 2007a) showed that PCBs and other POPs may impair the basal metabolic rate (BMR) of glaucous gulls during the breeding season. BMR generally mediates physiological maintenance, which in turn is moderated by numerous mechanisms and hormones, including thyroid hormones. A

study on house sparrows (*Passer domesticus*) linked plasma thyroid hormone levels to BMR variation in birds (Chastel et al., 2003). As previously discussed, hydroxylated compounds are potent disrupters of the thyroid hormone system (Ucán-Marin et al., 2010), which may in turn affect BMR and body condition of glaucous gulls. Verreault et al. (2004) found a negative correlation between plasma levels of thyroid hormones and POPs in male glaucous gulls. With all these factors considered, the methods in which POPs potentially affect body condition are many. These varying scenarios could all potentially result in the differing slopes of the interaction presented in Figure 3, making interpretation difficult. The skewed distribution of ΣPCBs towards low to mean values is also important to consider when interpreting the interaction effect. Possibly making the low to mean level trend lines more credible than the high level trend line. One thing is clear though, and that is the evident importance of this relationship, as this interaction effect was significant in all the female models ranked best in the AICc table (Table 5). As mentioned, the same interaction effect was present in male models as well, albeit not significant. This may reflect the physiological differences between the two sexes.

5.5. Lipid content

Correlations between progesterone and plasma lipid content were only found in female birds in the current study. This can be explained by the specific metabolism of plasma lipoproteins in females, as it has been shown to differ significantly between female laying hens and juvenile and male chickens. Avian ovaries have little capacity for synthesis of lipids, and therefore, all egg yolk lipids are mainly synthesized in the livers of female hens, which is a process stimulated by progesterone, estrogens, and other steroid hormones (Hahn et al., 2015). The observed correlation if therefore expected, but increased lipid content is a result from progesterone levels, not the other way around. Due to this relationship between lipid content and progesterone, lipids were not included as an explanatory variable in any of the models.

5.6. Further research

The current study would have benefitted from including parent PBDE compounds, as to fully investigate the relationship between parent PBDEs and hydroxylated metabolites. Also, the PCB metabolites $MeSO_2$-PCBs would be interesting to include as they are also linked to endocrine disruption, and may be correlated with hydroxylated PCBs (Letcher et al., 2000). Studying mercury levels in non-breeding individuals would also be interesting as they may show higher levels than breeding individuals, perhaps above threshold levels that could possibly lead to effects on progesterone. The low sample size of both sexes, but particularly for males, reduces the overall statistical power of the current study. A larger sample size could clarify the current results.

5.7. Closing remarks

Seabirds, in general, can provide knowledge to researchers on the health status of aquatic ecosystems, whether it is through monitoring pollution, investigating aquatic productivity or climate change (Mallory et al., 2010). Continuous research and addition of information on the well-being of seabird populations is, therefore, an important task in understanding how smaller pieces affect the bigger puzzle of pollution and aquatic ecosystems. Nature is highly complex and the relationships between biological systems are even more intricate and vulnerable to drastic change and altering external factors. It is our job, if not responsibility, to continue assessing and monitoring these systems, as humankind has an ever-growing impact on nature. Be it one biological mechanism, species, or ecosystem at a time.

6. Conclusion

The current study revealed that PCBs, OH-PCBs, and OH-PBDEs affect the two sexes of incubating glaucous gulls. Significant positive relationships were found between progesterone levels and hydroxylated polychlorinated biphenyls and polybrominated diphenyl ethers in female glaucous gulls. In male glaucous gulls, a significant positive relationship was found between progesterone levels and polychlorinated biphenyls. Mercury was not found to be an important factor in describing variation in progesterone for either of the sexes. Considering the plasma levels of mercury detected in comparison to reported threshold levels for effects in terrestrial avian species, effects are not expected at current mercury levels in breeding gulls. The mechanisms through which the organic compounds exert endocrine disrupting effects could be through direct manipulation of progesterone synthesis, metabolism, and transport, or indirectly through estrogenic activation or inhibition of progesterone receptors. The resulting alterations in progesterone levels and or activity of progesterone could ultimately affect reproductive success and fitness in glaucous gulls.

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Appendices

Appendix A

Internal standards

Internal standards as provided by the Norwegian Institute of Air Research in Tromsø.

"POP I", 1:10 diluted in isooctane	100 µL
"PBDE I", 1:10 diluted in isooctane	100 µL
"Meso PCBs", ca. 200 pg/µL	50 µL
"HPC I (OH-PCBs)", ca. 50 pg/µL	100 µL
Human serum reference spike:	
"12 HPC" 50 pg/µL	50 µL

Appendix B

Detected compounds

Table B. Limit of detection (LOD) and limit of quantification (LOQ) for each compound detected in the glaucous gull plasma samples of the current study, along with the percentage of the samples detected above LOQ. In the current study, compounds were included if their percentage above LOQ was at least 50 %. Compounds that did not meet this requirement were excluded from statistical analysis, and are highlighted in grey.

Compound	LOD	LOQ	% > LOQ
CB-28	2.6	7.9	100
CB-52	8.3	25.0	76
CB-99	18.8	56.4	100
CB-101	21.4	64.3	57
CB-105	20.5	61.6	100
CB-118	17.7	53.2	100
CB-138	595	1785	100
CB-153	455	1364	100
CB-180	19.4	58.3	100
CB-183	14.7	44.0	100
CB-187	17.5	52.4	100
CB-194	183	550	90
PCP	59.7	124	2
4-OH-HpCS	0.5	1.4	96
4-OH-CB-107	8.4	25.1	98
3-OH-CB-153	4.1	13.7	63
4-OH-CB-146	2.2	6.6	100
3-OH-CB-138	2.4	8.0	69
4-OH-CB-130	4.9	14.7	6
4-OH-CB-163	2.3	6.9	69
4-OH-CB-187	0.3	0.6	100
4-OH-CB-172	1.8	4.8	88
4'-OH-CB-193	1.4	2.8	88
2-OH-BDE-68	3.8	11.3	0
6-OH-BDE-47/75	2.7	8.2	92
5-OH-BDE-47	3.9	11.8	0
4-OH-BDE-49	4.2	12.5	4
5-OH-BDE-100	4.0	11.9	0
4-OH-BDE-103	4.0	11.9	0
5-OH-BDE-99	4.0	11.9	53
4-OH-BDE-101	4.3	12.8	0

Appendix C

Pollutant loads

Table C1. Concentrations of PCBs, OH-PCBs, and OH-PBDEs congeners (with LOQ > 50 %) and Hg in ng/mL, with range, median, mean and standard error of the mean (SE) in plasma samples of incubating female (n = 33) and male (n = 17) glaucous gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard, sampled in 2011, 2012, and 2013. The stars (*) beside a compound name indicate a significant difference of the levels between sexes (ANOVA test). If the minimum value was below LOD, it is indicated by a < symbol. The actual range may in these cases be lower than shown in the table.

	Females, n = 33			Males, n = 17		
ng/mL	Range	Median	Mean ± SE	Range	Median	Mean + SE
CB-28*	0.05 - 0.33	0.13	0.13 ± 0.01	0.09 - 0.42	0.23	0.23 ± 0.10
CB-52	<0.001 - 0.75	0.12	0.18 ± 0.04	<0.002 - 0.50	0.12	0.13 ± 0.03
CB-99*	0.51 - 11.10	2.08	3.01 ± 0.41	2.40 - 13.9	5.53	7.02 ± 1.03
CB-101	<0.002 - 2.11	0.22	0.35 ± 0.09	<0.01 - 1.27	0.02	0.35 ± 0.10
CB-105*	0.35 - 4.56	1.12	1.46 ± 0.18	0.31 - 6.15	2.25	2.81 ± 0.44
CB-118*	1.33 - 17.20	4.06	5.63 ± 0.73	4.14 - 30.2	10.2	13.06 ± 1.99
CB-138*	2.87 - 37.10	8.26	11.99 ± 1.53	9.68 - 66.4	23.8	29.5 ± 4.64
CB-153*	6.36 - 87.00	16.9	24.64 ± 3.50	20.10 - 162	41.7	63.72 ± 11.28
CB-180*	2.58 - 12.48	7.47	12.26 ± 2.17	8.73 - 82.4	19.8	30.06 ± 5.74
CB-183*	0.43 - 6.59	1.10	1.76 ± 0.27	1.41 - 9.95	3.18	4.31 ± 0.72
CB-187*	0.79 - 9.72	1.65	2.26 ± 0.31	2.24 - 9.35	4.17	5.22 ± 0.60
CB-194*	0.32 - 7.57	1.08	1.7 ± 0.32	0.96 - 12.2	2.9	4.15 ± 0.80
ΣPCBs*	16.16 - 220.9	42.94	65.38 ± 9.03	52.50 - 379.1	120.7	160.6 ± 26.86
4-OH-HpCS*	<0.0001 - 0.05	0.01	0.01 ± 0.001	0.004 - 0.04	0.02	0.02 ± 0.002
4-OH-CB-107	0.02 - 0.75	0.13	0.20 ± 0.03	0.01 - 0.56	0.18	0.25 ± 0.03
3-OH-CB-138*	<0.0009 - 0.11	0.01	0.02 ± 0.0009	<0.004 - 0.12	0.03	0.04 ± 0.008
4-OH-CB-146*	0.04 - 2.30	0.19	0.30 ± 0.07	0.23 - 1.46	0.55	0.64 ± 0.08
3-OH-CB-153*	<0.0004 - 0.16	0.01	0.03 ± 0.007	<0.01 - 0.30	0.03	0.06 ± 0.02
4-OH-CB-163*	<0.0006 - 0.11	0.01	0.01 ± 0.003	0.01 - 0.07	0.02	0.03 ± 0.004
4-OH-CB-172*	<0.0001 - 0.22	0.01	0.02 ± 0.006	0.01 - 0.10	0.04	0.05 ± 0.01
4-OH-CB-187*	0.13 - 9.07	0.59	1.02 ± 0.28	1.08 - 7.60	2.06	2.88 ± 0.44
4'-OH-CB-193*	<0.0002 - 0.07	0.005	0.01 ± 0.0002	<0.01 - 0.05	0.02	0.02 ± 0.003
ΣOH-PCBs*	<0.25 - 12.83	1.14	1.62 ± 0.39	1.49 - 9.93	3.16	3.98 ± 0.58
5-OH-BDE-99	<0.0001 - 0.11	0.01	0.02 ± 0.005	<0.0001 - 0.10	0.02	0.02 ± 0.007
6-OH-BDE-47/75	<0.0003 - 0.54	0.03	0.05 ± 0.01	<0.01 - 0.33	0.03	0.07 ± 0.02
ΣOH-PBDEs	0.001 - 0.55	0.05	0.07 ± 0.02	0.01 - 0.33	0.06	0.09 ± 0.02
Hg	44.44 - 863.9	453.0	439.5 ± 40.99	53.11 - 1067	592.9	584.3 ± 74.38

Individual levels of pollutants

Table C2. Levels of PCBs (ng/ml) for each individual female glaucous gull from Kongsfjorden, Svalbard, sampled in 2011, 2012, and 2013. Code is the unique ring code for each bird. Recaptured birds are included in the table, and the sample excluded from analysis is indicated with a * symbol.

Code	Year	CB-28	CB-52	CB-99	CB-101	CB-105	CB-118	CB-138	CB-153	CB-180	CB-183	CB-187	CB-194
AAD	2011	0.13	0.01	2.04	0.01	1.12	3.88	7.74	18.10	7.55	1.10	1.25	1.10
AAD*	2013	0.12	0.10	2.41	0.02	1.18	4.39	9.73	18.00	8.62	1.29	1.55	1.16
AAT	2013	0.30	0.75	7.65	1.32	4.03	17.20	31.00	87.00	52.80	6.59	4.91	7.32
ABA	2012	0.08	0.003	1.11	0.002	0.59	1.76	3.78	7.94	4.22	0.43	0.79	0.35
ABA	2013	0.14	0.01	2.97	0.01	1.43	4.79	11.00	12.70	5.43	1.02	2.18	0.65
ABC*	2011	0.12	0.14	2.68	0.60	1.39	6.36	11.50	35.40	20.40	2.44	1.74	3.19
ABC	2012	0.14	0.09	4.14	0.63	2.20	9.38	19.40	49.70	30.70	3.75	2.60	5.11
ABD	2011	0.27	0.12	7.51	0.02	3.67	14.70	26.90	58.10	28.00	3.75	3.68	3.94
ABV*	2011	0.05	0.002	0.89	0.005	0.35	1.39	2.87	6.53	2.63	0.49	0.99	0.42
ABV	2012	0.06	0.01	0.78	0.02	0.35	1.33	2.93	6.36	2.58	0.47	0.90	0.37
ABX	2011	0.08	0.15	1.15	0.22	0.58	2.05	5.06	9.37	4.73	0.68	0.82	0.70
ABX*	2012	0.07	0.12	1.24	0.22	0.61	2.38	5.32	11.20	5.91	0.80	0.97	0.90
ACB*	2011	0.13	0.17	2.27	0.21	1.17	4.44	7.10	12.30	6.23	1.12	1.52	0.72
ACB	2012	0.11	0.09	1.99	0.09	1.08	3.78	6.21	13.50	5.48	0.90	1.23	0.73
ACC	2011	0.11	0.24	2.77	0.55	1.34	5.65	10.10	27.80	15.20	1.95	1.82	2.34
ACH	2011	0.12	0.25	2.05	0.34	0.98	3.79	8.26	15.40	6.33	1.02	1.45	0.80
ACJ	2011	0.14	0.004	2.00	0.01	1.05	3.65	7.26	17.30	7.47	1.12	1.79	1.15
ADD	2012	0.07	0.08	1.20	0.02	0.60	2.23	3.50	9.05	2.63	0.44	0.85	0.32
ADD*	2013	0.08	0.003	1.85	0.01	0.87	3.23	5.43	13.50	4.22	0.77	1.31	0.51
ADF	2012	0.14	0.01	3.68	0.02	1.61	6.03	18.30	23.90	8.82	1.61	3.33	0.96
ADH	2012	0.13	0.001	4.29	0.01	1.12	4.59	16.70	29.60	10.70	1.91	3.11	1.25
ADJ	2012	0.13	0.19	2.18	0.43	1.09	4.06	8.07	16.90	7.79	1.10	1.73	1.13
ADL	2012	0.07	0.16	1.49	0.25	0.72	2.48	5.86	10.60	4.91	0.77	1.41	0.76
ADL*	2013	0.09	0.09	1.69	0.07	0.88	2.92	7.15	8.43	4.17	0.71	1.23	0.71
ADS	2012	0.09	0.24	1.72	0.47	0.87	3.00	6.08	11.00	5.37	0.83	1.16	0.83
AFB	2013	0.22	0.74	5.19	2.11	2.37	8.69	20.80	31.90	11.60	2.11	4.65	1.42
AFC*	2012	0.10	0.22	1.76	0.48	0.93	3.05	6.31	13.70	4.34	0.80	1.65	0.43
AFC	2013	0.24	0.71	6.05	1.63	2.10	9.89	19.20	28.10	11.80	2.32	5.25	1.21
AJB	2013	0.14	0.30	2.08	0.51	1.05	3.49	8.43	13.70	6.89	1.05	1.47	1.08
AJC	2013	0.14	0.38	2.48	0.70	1.26	4.51	8.59	20.10	9.13	1.44	2.03	1.14
AJF	2013	0.15	0.25	0.51	0.46	2.65	12.10	24.90	72.50	48.70	5.54	2.13	7.57
AJL	2013	0.17	0.03	6.44	0.01	2.31	8.78	23.00	50.10	22.00	3.07	3.45	2.80
AJX	2013	0.33	0.42	11.10	0.24	4.56	15.70	37.10	53.40	27.10	4.79	9.72	2.98

Table C3. Levels of PCBs (ng/ml) for each individual male glaucous gull sampled in Kongsfjorden, Svalbard in 2011, 2012, and 2013. Code is the unique ring code for each bird. Recaptured birds are included in the table, and the sample excluded from analysis is indicated with a * symbol.

Code	Year	CB-28	CB-52	CB-99	CB-101	CB-105	CB-118	CB-138	CB-153	CB-180	CB-183	CB-187	CB-194
#	2012	0.24	0.21	12.60	0.80	5.24	24.80	59.40	134.00	66.70	9.29	6.88	8.97
AAJ	2013	0.15	0.00	3.43	0.02	1.62	6.66	16.50	28.60	16.10	2.42	2.50	2.63
AAV	2011	0.23	0.50	5.53	0.93	2.36	9.85	23.80	52.30	21.80	3.69	6.32	2.90
AAZ	2011	0.39	0.20	13.90	0.86	6.15	30.20	63.60	162.00	75.50	9.95	6.75	9.63
ABJ	2011	0.30	0.01	13.40	0.02	4.73	23.60	66.40	143.00	82.40	9.59	9.35	12.20
ACA	2011	0.18	0.20	5.80	0.53	2.25	10.20	25.60	45.80	19.80	3.18	4.62	2.56
ACF	2011	0.09	0.12	2.40	0.02	0.94	4.14	9.68	20.10	9.78	1.41	2.24	1.57
ACL	2011	0.17	0.19	3.85	0.60	1.76	7.19	14.70	31.30	8.73	1.54	3.91	0.96
ACL*	2012	0.29	0.23	8.49	1.27	3.92	17.10	33.20	70.60	35.90	5.68	8.68	4.38
ADN	2012	0.12	0.14	2.67	0.10	1.17	5.34	11.20	30.10	19.20	2.65	3.75	3.52
ADN*	2013	0.14	0.05	2.93	0.02	1.41	5.79	10.50	26.80	14.00	2.05	2.84	2.00
ADT	2012	0.09	0.01	3.85	0.02	1.17	5.25	17.50	35.80	13.50	2.11	2.80	1.60
AJD	2013	0.26	0.04	6.55	0.02	0.31	11.00	22.80	36.70	12.90	2.16	4.17	1.28
AJH	2013	0.42	0.04	13.60	0.02	5.43	23.10	43.30	98.40	43.00	6.08	8.58	6.46
AJN	2013	0.20	0.04	3.93	0.01	2.10	7.97	13.80	22.00	9.81	1.62	2.89	1.17
AJS	2013	0.40	0.27	11.30	0.75	4.58	19.30	45.60	104.00	35.70	6.28	8.62	4.22
AJV	2013	0.25	0.05	5.15	0.01	2.60	10.60	23.90	41.70	26.20	3.49	3.92	4.49

Table C4. Levels of OH-PCBs (ng/ml) for each individual female glaucous gull sampled in Kongsfjorden, Svalbard in 2011, 2012, and 2013. Code is the unique ring code for each bird. Recaptured birds are included in the table, and the sample excluded from analysis is indicated with a * symbol.

Code	Year	OH-HpCS	OH-CB 107	OH-CB 153	OH-CB 146	OH-CB 138	OH-CB 163	OH-CB 187	OH-CB 172	OH-CB 193
AAD	2011	0.009	0.152	0.043	0.254	0.029	0.005	0.502	0.007	0.005
AAD*	2013	0.016	0.238	0.121	0.296	0.038	0.011	0.521	0.020	0.008
AAT	2013	0.007	0.370	0.030	0.380	0.029	0.011	0.797	0.019	0.003
ABA	2012	0.014	0.265	0.031	0.150	0.026	0.010	0.777	0.016	0.005
ABA*	2013	0.011	0.164	0.029	0.209	0.027	0.008	0.207	0.007	0.004
ABC*	2011	0.007	0.092	0.019	0.186	0.008	0.004	0.341	0.016	0.006
ABC	2012	0.009	0.305	0.045	0.481	0.005	0.010	1.000	0.028	0.007
ABD	2011	0.005	0.120	0.056	0.416	0.040	0.011	0.871	0.022	0.008
ABV*	2011	0.002	0.065	0.011	0.071	0.009	0.001	0.336	0.011	0.004
ABV	2012	0.000	0.060	0.007	0.059	0.005	0.002	0.273	0.0001	0.003
ABX	2011	0.004	0.079	0.010	0.123	0.006	0.005	0.492	0.010	0.004
ABX*	2012	0.000	0.021	0.001	0.044	0.001	0.001	0.210	0.004	0.002
ACB*	2011	0.011	0.094	0.001	0.170	0.006	0.005	0.217	0.002	0.004
ACB	2012	0.015	0.157	0.009	0.378	0.007	0.006	0.654	0.010	0.004
ACC	2011	0.012	0.108	0.0004	0.208	0.004	0.004	0.515	0.011	0.005
ACH	2011	0.004	0.011	0.009	0.144	0.007	0.008	0.610	0.014	0.007
ACJ	2011	0.013	0.161	0.044	0.297	0.030	0.014	1.241	0.027	0.009
ADD	2012	0.005	0.052	0.006	0.047	0.007	0.001	0.131	0.000	0.0002
ADD*	2013	0.004	0.059	0.020	0.127	0.017	0.004	0.262	0.003	0.002
ADF	2012	0.011	0.583	0.077	0.155	0.051	0.010	0.455	0.011	0.008
ADH	2012	0.007	0.134	0.075	0.278	0.054	0.015	1.199	0.012	0.006
ADJ	2012	0.005	0.112	0.001	0.130	0.004	0.004	0.498	0.002	0.002
ADL	2012	0.011	0.105	0.001	0.110	0.008	0.003	0.590	0.008	0.001
ADL*	2013	0.009	0.196	0.012	0.153	0.013	0.007	0.532	0.012	0.005
ADS	2012	0.009	0.097	0.009	0.193	0.004	0.005	0.809	0.009	0.004
AFB	2013	0.004	0.106	0.002	0.108	0.004	0.005	0.627	0.013	0.004
AFC*	2012	0.016	0.134	0.005	0.147	0.002	0.007	0.890	0.011	0.003
AFC	2013	0.011	0.298	0.014	0.555	0.009	0.025	2.960	0.038	0.015
AJB	2013	0.007	0.192	0.009	0.290	0.011	0.008	0.802	0.011	0.006
AJC	2013	0.008	0.355	0.018	0.255	0.009	0.008	0.557	0.010	0.002
AJF	2013	0.010	0.319	0.034	0.374	0.016	0.007	0.645	0.019	0.005
AJL	2013	0.024	0.537	0.148	0.773	0.111	0.054	3.977	0.051	0.021
AJX	2013	0.051	0.751	0.163	2.295	0.103	0.108	9.072	0.221	0.066

Table C5. Levels of OH-PCBs (ng/ml) for each individual male glaucous gull sampled in Kongsfjorden, Svalbard in 2011, 2012, and 2013. Code is the unique ring code for each bird. Recaptured birds are included in the table, and the sample excluded from analysis is indicated with a * symbol.

Code	Year	OH-HpCS	OH-CB 107	OH-CB 153	OH-CB 146	OH-CB 138	OH-CB 163	OH-CB 187	OH-CB 172	OH-CB 193
#	2012	0.010	0.448	0.018	0.576	0.011	0.022	2.025	0.032	0.016
AAJ	2013	0.016	0.141	0.046	0.828	0.032	0.020	3.921	0.076	0.036
AAV	2011	0.017	0.183	0.028	0.553	0.019	0.022	2.058	0.031	0.016
AAZ	2011	0.030	0.558	0.198	1.113	0.109	0.057	5.432	0.094	0.038
ABJ	2011	0.022	0.425	0.305	1.006	0.116	0.040	5.080	0.075	0.051
ACA	2011	0.032	0.161	0.013	0.238	0.004	0.012	1.448	0.014	0.014
ACF	2011	0.004	0.139	0.009	0.252	0.010	0.008	1.144	0.031	0.019
ACL	2011	0.006	0.138	0.012	0.226	0.005	0.010	1.080	0.011	0.008
ACL*	2012	0.018	0.318	0.017	0.542	0.014	0.019	2.666	0.022	0.015
ADN	2012	0.010	0.083	0.028	0.443	0.025	0.015	2.556	0.046	0.022
ADN*	2013	0.009	0.107	0.043	0.554	0.033	0.011	1.418	0.041	0.011
ADT	2012	0.017	0.177	0.076	0.907	0.039	0.034	4.231	0.046	0.029
AJD	2013	0.021	0.318	0.069	0.412	0.050	0.027	1.446	0.022	0.012
AJH	2013	0.012	0.302	0.015	0.427	0.028	0.022	2.060	0.020	0.006
AJN	2013	0.009	0.010	0.034	0.394	0.025	0.021	1.479	0.061	0.045
AJS	2013	0.042	0.426	0.093	1.463	0.087	0.071	7.604	0.101	0.044
AJV	2013	0.024	0.240	0.070	0.925	0.041	0.026	3.330	0.048	0.023

Table C6 Levels of OH-PBDEs and Hg (ng/ml) for each individual female glaucous gull sampled in Kongsfjorden, Svalbard in 2011, 2012, and 2013. Code is the unique ring code for each bird. Recaptured birds are included in the table, and the sample excluded from analysis is indicated with a * symbol.

Code	Year	OH-BDE99	OH-BDE47/75	Hg
AAD	2011	0.089	0.064	44.4
AAD*	2013	0.030	0.082	864.0
AAT	2013	0.082	0.034	494.4
ABA	2012	0.002	0.003	130.5
ABA*	2013	0.003	0.016	513.8
ABC*	2011	0.000	0.045	342.2
ABC	2012	0.0006	0.123	462.6
ABD	2011	0.003	0.028	749.9
ABV*	2011	0.069	0.026	92.4
ABV	2012	0.017	0.002	631.7
ABX	2011	0.033	0.033	339.8
ABX*	2012	0.002	0.022	443.4
ACB*	2011	0.002	0.023	758.0
ACB	2012	0.003	0.047	554.1
ACC	2011	0.001	0.040	150.7
ACH	2011	0.028	0.036	421.3
ACJ	2011	0.002	0.062	295.8
ADD	2012	0.016	0.016	736.8
ADD*	2013	0.0002	0.044	274.2
ADF	2012	0.030	0.021	683.3
ADH	2012	0.013	0.026	795.5
ADJ	2012	0.010	0.011	486.6
ADL	2012	0.002	0.051	490.1
ADL*	2013	0.021	0.019	217.6
ADS	2012	0.009	0.537	346.7
AFB	2013	0.004	0.029	266.6
AFC*	2012	0.002	0.019	NA
AFC	2013	0.012	0.101	123.5
AJB	2013	0.114	0.026	197.1
AJC	2013	0.027	0.022	711.2
AJF	2013	0.022	0.029	495.6
AJL	2013	0.016	0.021	199.4
AJX	2013	0.001	0.0003	752.9

Table C7. Levels of OH-PBDEs and Hg (ng/ml) for each individual male glaucous gull sampled in Kongsfjorden, Svalbard in 2011, 2012, and 2013. Code is the unique ring code for each bird. Recaptured birds are included in the table, and the sample excluded from analysis is indicated with a * symbol.

Code	Year	OH-BDE99	OH-BDE47/75	Hg
#	2012	0.020	0.024	593.0
AAJ	2013	0.013	0.026	329.4
AAV	2011	0.022	0.045	786.3
AAZ	2011	0.051	0.043	1067.1
ABJ	2011	0.019	0.038	517.1
ACA	2011	0.001	0.016	710.7
ACF	2011	0.083	0.029	54.9
ACL	2011	0.003	0.056	53.1
ACL*	2012	0.0001	0.044	754.8
ADN	2012	0.001	0.329	541.6
ADN*	2013	0.030	0.030	406.4
ADT	2012	0.028	0.293	909.2
AJD	2013	0.001	0.012	1037.4
AJH	2013	0.021	0.008	506.5
AJN	2013	0.099	0.095	633.5
AJS	2013	0.001	0.022	825.4
AJV	2013	0.014	0.030	206.0

Appendix D

Individual levels of progesterone

Table D1. Levels of progesterone (ng/mL) measured with and without SDR, with range, median, mean and standard error of the mean (SE) in plasma samples of breeding females (n = 33) and male (n = 17) glaucous gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard, sampled in 2011, 2012, and 2013.

	Females, n = 33			Males, n = 17		
Progesterone	Range	Median	Mean ± SE	Range	Median	Mean ± SE
SDR	0.15 - 1.13	0.34	0.40 ± 0.04	0.10 - 1.20	0.25	0.39 ± 0.08
Without SDR	0.15 - 1.10	0.32	0.37 ± 0.04	0.10 - 0.90	0.24	0.36 ± 0.06

Table D2. Levels of progesterone (ng/ml) with and without steroid displacement reagent for each individual female glaucous gull sampled in Kongsfjorden, Svalbard in 2011, 2012, and 2013. Code is the unique ring code for each bird. Recaptured birds are included in the table, and the sample excluded from analysis is indicated with a * symbol.

Code	Year	Progesterone SDR	Progesterone
AAD	2011	0.21	0.20
AAD*	2013	0.21	0.19
AAT	2013	0.51	0.49
ABA	2012	0.35	0.29
ABA*	2013	0.43	0.41
ABC*	2011	0.16	0.15
ABC	2012	0.20	0.24
ABD	2011	0.28	0.27
ABV*	2011	0.26	0.24
ABV	2012	0.17	0.17
ABX	2011	0.34	0.36
ABX*	2012	0.44	0.40
ACB*	2011	0.23	0.20
ACB	2012	0.22	0.22
ACC	2011	0.40	0.35
ACH	2011	0.74	0.76
ACJ	2011	0.25	0.22
ADD	2012	0.38	0.39
ADD*	2013	0.97	1.10
ADF	2012	0.23	0.25
ADH	2012	0.50	0.43
ADJ	2012	0.15	0.15
ADL	2012	0.38	0.38
ADL*	2013	0.43	0.44
ADS	2012	0.96	0.90
AFB	2013	1.13	0.74
AFC	2012	0.27	0.24
AFC	2013	0.34	0.36
AJB	2013	0.32	0.30
AJC	2013	0.47	0.51
AJF	2013	0.24	0.21
AJL	2013	0.52	0.43
AJX	2013	0.37	0.32

Table D3. Levels of progesterone (ng/ml) with and without steroid displacement reagent for each individual male glaucous gull sampled in Kongsfjorden, Svalbard in 2011, 2012, and 2013. Code is the unique ring code for each bird. Recaptured birds are included in the table, and the sample excluded from analysis is indicated with a * symbol.

Code	Year	Progesterone SDR	Progesterone
#	2012	0.66	0.61
AAJ	2013	0.27	0.28
AAV	2011	0.27	0.29
AAZ	2011	1.06	0.90
ABJ	2011	0.25	0.24
ACA	2011	0.10	0.10
ACF	2011	0.10	0.12
ACL	2011	0.36	0.37
ACL*	2012	0.71	0.74
ADN	2012	0.25	0.21
ADN*	2013	0.25	0.23
ADT	2012	0.18	0.16
AJD	2013	1.20	0.86
AJH	2013	0.39	0.37
AJN	2013	0.23	0.22
AJS	2013	0.21	0.14
AJV	2013	0.18	0.23

Appendix E

R-packages

Table E. Additional packages used in R (3.2.3) during the statistical analysis.

Package name	Usage
nlme	Mixed linear models
lme4	Mixed linear models
ggplo2	Plotting graphs and models
AICcmodavg	AICc tables
FactoMineR	PCA
sjPlot	Plotting interaction effects

Appendix F

Principal component analysis summaries

Table E1. Summary of the principal component analysis (PCA) for male glaucous gulls, excluding recaptured birds. The first section shows the amount of variance each dimension of the PCA explains, the percentage of total variance the dimension explains, and the cumulative percentage of variance all the dimensions explain. The second section includes the following for each individual and the three first dimensions of the PCA: each individuals loading value for the given dimension (Dim), contribution each individual has towards the variance of the given dimension (ctr), and the value indicating the quality of the projection along the given dimension (cos^2, a value from 0 to 1). The third section includes Dim, ctr, and cos^2 for each variable included in the PCA, for the three first dimension.

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10
Variance	14.499	3.851	2.376	1.546	1.359	1.194	0.621	0.492	0.358	0.244
% of var	53.699	14.262	8.802	5.725	5.033	4.421	2.299	1.823	1.326	0.903
Cumulative % of var	53.699	67.962	76.764	82.488	87.521	91.942	94.241	96.064	97.39	98.293

Individuals	Dim.1	ctr	cos^2	Dim.2	ctr	cos^2	Dim.3	ctr	cos^2
1-2011	-2.236	2.298	0.331	1.6	4.431	0.170	1.709	8.197	0.194
6-2011	-4.998	11.486	0.77	0.118	0.024	0.000	-1.742	8.509	0.094
9-2011	7.73	27.472	0.893	-0.026	0.001	0.000	-0.326	0.298	0.002
10-2011	6.532	19.621	0.756	-1.382	3.308	0.034	-2.544	18.154	0.115
11-2011	-0.994	0.454	0.067	1.632	4.614	0.18	2.206	13.65	0.329
16-2011	-4.281	8.429	0.676	2.183	8.249	0.176	0.379	0.404	0.005
2-2012	-1.711	1.346	0.156	-3.202	17.752	0.547	0.49	0.675	0.013
3-2012	2.907	3.885	0.316	3.641	22.945	0.495	-0.591	0.981	0.013
10-2012	-2.859	3.759	0.427	-1.414	3.462	0.104	0.38	0.406	0.008
4-2013	-1.191	0.652	0.084	0.439	0.334	0.011	0.546	0.838	0.018
9-2013	1.395	0.895	0.099	2.946	15.028	0.443	-1.567	6.887	0.125
11-2013	-1.901	1.662	0.291	-2.177	8.206	0.381	-0.45	0.569	0.016
13-2013	-3.23	4.798	0.425	-1.671	4.833	0.114	-1.78	8.885	0.129
17-2013	5.343	13.125	0.592	-1.748	5.29	0.063	3.353	31.537	0.233
18-2013	-0.505	0.117	0.044	-0.938	1.523	0.153	-0.065	0.012	0.001

Variables	Dim.1	ctr	cos^2	Dim.2	ctr	cos^2	Dim.3	ctr	cos^2
progesterone	0.406	1.135	0.164	0.355	3.270	0.126	-0.095	0.377	0.009
SUMlipid	-0.658	2.986	0.433	0.442	5.069	0.195	0.265	2.949	0.070
CB.28	0.799	4.400	0.638	0.253	1.668	0.064	0.073	0.221	0.005
CB.52	0.109	0.082	0.012	0.428	4.763	0.183	0.642	17.318	0.412
CB.99	0.907	5.678	0.823	0.338	2.971	0.114	-0.127	0.678	0.016
CB.101	0.383	1.014	0.147	0.469	5.702	0.22	0.577	13.986	0.332
CB.105	0.860	5.100	0.739	0.320	2.656	0.102	-0.136	0.774	0.018
CB.118	0.916	5.79	0.839	0.341	3.024	0.116	-0.151	0.958	0.023
CB.138	0.945	6.159	0.893	0.251	1.636	0.063	-0.143	0.866	0.021
CB.153	0.947	6.184	0.897	0.249	1.609	0.062	-0.126	0.664	0.016
CB.180	0.906	5.662	0.821	0.196	1.000	0.039	-0.285	3.409	0.081
CB.183	0.934	6.021	0.873	0.24	1.497	0.058	-0.158	1.057	0.025
CB.187	0.832	4.772	0.692	0.325	2.751	0.106	0.062	0.162	0.004
CB.194	0.861	5.113	0.741	0.170	0.753	0.029	-0.356	5.335	0.127

Table F1 continuation. Summary of the principal component analysis (PCA) for male glaucous gulls, excluding recaptured birds. The first section shows the amount of variance each dimension of the PCA explains, the percentage of total variance the dimension explains, and the cumulative percentage of variance all the dimensions explain. The second section includes the following for each individual and the three first dimensions of the PCA: each individuals loading value for the given dimension (Dim), contribution each individual has towards the variance of the given dimension (ctr), and the value indicating the quality of the projection along the given dimension (cos2, a value from 0 to 1). The third section includes Dim, ctr, and cos2 for each variable included in the PCA, for the three first dimension.

OH-HpCS	0.609	2.56	0.371	-0.246	1.569	0.06	0.574	13.846	0.329
OH-CB-107	0.916	5.793	0.84	0.216	1.212	0.047	0.03	0.038	0.001
OH-CB-138	0.844	4.909	0.712	-0.417	4.524	0.174	-0.078	0.257	0.006
OH-CB-146	0.750	3.884	0.563	-0.524	7.118	0.274	0.238	2.377	0.056
OH-CB-153	0.756	3.945	0.572	-0.372	3.591	0.138	-0.269	3.034	0.072
OH-CB-163	0.819	4.63	0.671	-0.368	3.514	0.135	0.315	4.167	0.099
OH-CB-172	0.642	2.844	0.412	-0.642	10.705	0.412	0.024	0.024	0.001
OH-CB-187	0.747	3.853	0.559	-0.542	7.642	0.294	0.259	2.817	0.067
OH-CB-193	0.492	1.667	0.242	-0.726	13.706	0.528	-0.177	1.324	0.031
Hg	0.478	1.578	0.229	-0.129	0.435	0.017	0.385	6.242	0.148
OH-BDE-99	-0.154	0.164	0.024	-0.164	0.701	0.027	-0.549	12.66	0.301
OH-BDE-47/75	-0.275	0.521	0.076	-0.491	6.268	0.241	0.057	0.135	0.003
BC	-0.718	3.557	0.516	0.158	0.645	0.025	-0.321	4.325	0.103

Table F2. Summary of the principal component analysis (PCA) for female glaucous gulls, excluding recaptured birds. The first section shows the amount of variance each dimension of the PCA explains, the percentage of total variance the dimension explains, and the cumulative percentage of variance all the dimensions explain. The second section includes the following for each individual and the three first dimensions of the PCA: each individuals loading value for the given dimension (Dim), contribution each individual has towards the variance of the given dimension (ctr), and the value indicating the quality of the projection along the given dimension (cos^2, a value from 0 to 1). The third section includes Dim, ctr, and cos^2 for each variable included in the PCA, for the three first dimension.

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10
Variance	13.341	5.007	2.491	1.488	1.247	0.852	0.712	0.646	0.389	0.314
% of var	49.412	18.546	9.225	5.512	4.617	3.156	2.637	2.393	1.441	1.165
Cumulative % of var	49.412	67.957	77.182	82.694	87.31	90.467	93.104	95.497	96.938	98.103

Individuals	Dist	Dim.1	ctr	cos^2	Dim.2	ctr	cos^2	Dim.3	ctr	cos^2
3-2011	2.559	-1.254	0.491	0.240	0.228	0.043	0.008	0.475	0.378	0.035
5-2011	5.079	2.977	2.769	0.344	2.175	3.937	0.183	-1.838	5.652	0.131
7-2011	3.364	-2.226	1.547	0.438	0.45	0.168	0.018	1.303	2.839	0.150
13-2011	3.957	-1.778	0.987	0.202	-0.894	0.665	0.051	-0.219	0.08	0.003
14-2011	3.496	-3.155	3.109	0.814	-0.359	0.107	0.011	-0.031	0.002	0.000
15-2011	3.145	-1.200	0.449	0.145	-2.254	4.228	0.514	-0.538	0.483	0.029
17-2011	4.008	-2.489	1.935	0.386	-2.412	4.839	0.362	-0.425	0.302	0.011
1-2012	3.784	0.241	0.018	0.004	-0.832	0.575	0.048	-1.928	6.221	0.26
5-2012	2.921	-2.046	1.307	0.491	-0.758	0.478	0.067	-0.455	0.346	0.024
6-2012	6.140	-2.656	2.203	0.187	-0.323	0.087	0.003	3.318	18.415	0.292
7-2012	4.396	-3.561	3.960	0.656	-0.878	0.641	0.040	-0.931	1.450	0.045
8-2012	2.788	-0.147	0.007	0.003	-0.966	0.777	0.120	-1.113	2.071	0.159
9-2012	2.981	-2.082	1.354	0.488	-0.014	0.000	0.000	-0.053	0.005	0.000
11-2012	3.448	1.242	0.482	0.130	1.762	2.582	0.261	-1.536	3.946	0.198
12-2012	4.359	-3.689	4.249	0.716	-1.404	1.641	0.104	-0.895	1.340	0.042
14-2012	3.110	-2.816	2.476	0.819	-0.747	0.465	0.058	0.113	0.021	0.001
1-2013	4.178	-1.721	0.925	0.170	-0.276	0.063	0.004	0.808	1.091	0.037
2-2013	2.614	-1.116	0.389	0.182	-0.017	0.000	0.000	0.435	0.317	0.028
5-2013	8.215	5.101	8.125	0.386	6.014	30.10	0.536	-0.838	1.175	0.010
6-2013	5.718	0.436	0.059	0.006	3.874	12.49	0.459	3.522	20.75	0.379
8-2013	5.466	2.178	1.482	0.159	3.239	8.729	0.351	-2.833	13.43	0.269
10-2013	6.124	4.614	6.648	0.567	-2.545	5.388	0.173	-0.788	1.039	0.017
16-2013	4.647	2.029	1.286	0.191	1.766	2.594	0.144	3.017	15.22	0.421
19-2013	14.14	13.12	53.74	0.861	-4.829	19.41	0.117	1.430	3.422	0.010

Table F2 continuation. Summary of the principal component analysis (PCA) for female glaucous gulls, excluding recaptured birds. The first section shows the amount of variance each dimension of the PCA explains, the percentage of total variance the dimension explains, and the cumulative percentage of variance all the dimensions explain. The second section includes the following for each individual and the three first dimensions of the PCA: each individuals loading value for the given dimension (Dim), contribution each individual has towards the variance of the given dimension (ctr), and the value indicating the quality of the projection along the given dimension (cos^2, a value from 0 to 1). The third section includes Dim, ctr, and cos^2 for each variable included in the PCA, for the three first dimension.

Variables	Dim.1	ctr	cos^2	Dim.2	ctr	cos^2	Dim.3	ctr	cos^2
progesterone	-0.037	0.010	0.001	0.239	1.139	0.057	0.656	17.29	0.431
SUMlipid	0.005	0.000	0.000	0.743	11.04	0.553	0.172	1.195	0.030
CB.28	0.874	5.727	0.764	0.316	1.990	0.100	0.148	0.879	0.022
CB.52	0.399	1.196	0.160	0.604	7.297	0.365	0.593	14.14	0.352
CB.99	0.901	6.082	0.811	0.078	0.123	0.006	0.153	0.943	0.023
CB.101	0.189	0.269	0.036	0.690	9.516	0.477	0.592	14.07	0.350
CB.105	0.923	6.391	0.853	0.332	2.198	0.110	-0.051	0.104	0.003
CB.118	0.873	5.715	0.762	0.452	4.076	0.204	-0.099	0.397	0.010
CB.138	0.929	6.476	0.864	0.306	1.874	0.094	-0.092	0.341	0.008
CB.153	0.765	4.389	0.586	0.522	5.436	0.272	-0.311	3.883	0.097
CB.180	0.680	3.462	0.462	0.557	6.196	0.310	-0.358	5.154	0.128
CB.183	0.806	4.875	0.650	0.489	4.783	0.240	-0.254	2.589	0.064
CB.187	0.928	6.451	0.861	0.035	0.024	0.001	0.235	2.215	0.055
CB.194	0.580	2.520	0.336	0.592	7.009	0.351	-0.407	6.637	0.165
OH-HpCS	0.773	4.483	0.598	-0.540	5.829	0.292	0.107	0.455	0.011
OH-CB-107	0.797	4.766	0.636	-0.222	0.980	0.049	-0.131	0.685	0.017
OH-CB-138	0.736	4.062	0.542	-0.455	4.131	0.207	-0.197	1.563	0.039
OH-CB-146	0.891	5.949	0.794	-0.366	2.679	0.134	0.118	0.560	0.014
OH-CB-153	0.782	4.585	0.612	-0.444	3.937	0.197	-0.229	2.102	0.052
OH-CB-163	0.858	5.517	0.736	-0.470	4.411	0.221	0.155	0.963	0.024
OH-CB-172	0.854	5.467	0.729	-0.412	3.385	0.169	0.177	1.264	0.031
OH-CB-187	0.845	5.354	0.714	-0.449	4.034	0.202	0.231	2.150	0.054
OH-CB-193	0.837	5.248	0.700	-0.469	4.385	0.220	0.188	1.412	0.035
Hg	0.208	0.325	0.043	-0.052	0.054	0.003	-0.391	6.147	0.153
OH-BDE-99	-0.053	0.021	0.003	0.230	1.054	0.053	-0.048	0.094	0.002
OH-BDE-47/75	-0.160	0.193	0.026	0.055	0.061	0.003	0.448	8.060	0.201
BC	-0.249	0.466	0.062	0.344	2.359	0.118	-0.342	4.710	0.117