Changes in prolactin in a highly organohalogen contaminated Arctic top predator seabird, the glaucous gull

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ABSTRACT

The factors influencing prolactin (PRL) variation in birds and in wildlife in general have rarely been investigated with respect to the physiological impacts of exposure to environmental contaminants. We investigated the associations between circulating baseline PRL levels and concentrations of eight persistent organohalogen contaminant (OHC) classes (i.e., major organochlorines and brominated flame retardants, and associated metabolic products) in blood (plasma) of free-ranging glaucous gulls (Larus hyperboreus), a top predator in the Norwegian Arctic, engaged in the process of incubation. We further examined whether plasma OHC concentrations were associated with the variation of PRL in glaucous gulls exposed to a standardized capture/restraint protocol. Plasma OHC concentrations in male glaucous gulls were 2- to 3-fold higher relative to females. Baseline PRL levels tended to be higher in females compared to males, although not significantly ($p = 0.20$). In both males and females, the 30-min capture/restraint protocol led on average to a 26% decrease in PRL levels, which resulted in a rate of PRL decrease of 0.76 ng/mL/min. The baseline PRL levels and the rate of decrease in PRL levels tended to vary negatively with plasma OHC concentrations in males, but not in females, although several of these associations did not adhere with the criterion of significance ($\alpha = 0.05$). Present results suggest that in highly OHC-exposed male glaucous gulls, the control of PRL release may be affected by the direct or indirect modulating actions of OHCs and/or their metabolically derived products. We conclude that potentially OHC-mediated impact on PRL secretion in glaucous gulls (males) may be a contributing factor to the adverse effects observed on the reproductive behavior, development and population size of glaucous gulls breeding in the Norwegian Arctic.

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1. Introduction

In recent years, growing research attention has been given to chemically induced changes on endocrine systems in wildlife species chronically exposed to environmental contaminants. A wide body of evidence now suggests that certain persistent contaminant classes, including various organochlorines and brominated flame retardants, and their metabolically derived products (e.g., hydroxylated (OH)-PCBs), possess structural attributes and mode of action potencies that are similar to endogenous hormones. Hence, these contaminants can perturb endocrine-related mechanisms via signaling pathways, biosynthesis and transport proteins. Alterations of natural hormone levels, beyond the thresholds of homeostatic compensation and variation, may thus lead to detrimental and irreversible changes in the development, reproduction and cognitive and behavioral functions of exposed wildlife, including several avian species (Burger et al., 2002; Dawson, 2000; Giesy et al., 2003). However, the mechanistic linkages between contaminant exposure and endocrine system modulation in birds remain poorly understood.

Hitherto, endocrino-toxicological assessments in free-ranging birds have focused primarily on modulation of the thyroid gland and thyroid hormone levels, and to a lesser extent on reproductive steroid hormones and glucocorticosteroids (Burger et al., 2002; Dawson, 2000; Giesy et al., 2003). Nevertheless, other key hormones involved in the initiation/regulation of physiological and behavioral mechanisms and processes in birds may be affected by chemical interactions during critical phases of their life cycle. One such phase is the breeding period. Prolactin (PRL), an anterior pituitary hormone, is closely associated with reproduction and particularly in parental (Buntin, 1996; Sharp et al., 1988) and

alloparental behaviors (Angelier et al., 2006). Circulating levels of PRL in male and female birds are known to increase at the onset of oviposition and to remain at high levels following clutch completion and throughout the incubation period. PRL levels have also been shown to decline rapidly after hatching in some avian species (Buntin, 1996), or to be maintained at elevated levels in others (Lormée et al., 1999, 2000). The current state of knowledge on the potentiation of PRL secretion in birds identifies several biological and environmental factors that may induce physiological changes in circulating PRL levels. These factors include, among others, photoperiod (i.e., diurnal and annual rhythms), reproductive and nutritional status, maturation (i.e., age and breeding experience), osmosis and stress (Angelier et al., 2006, 2007a; Chastel et al., 2005; Hall et al., 1986; Sharp et al., 1989). For example, it has been reported in long-lived seabird species that an acute stressful event can lead to a marked decrease in plasma PRL levels [e.g., black-legged kittiwakes (*Rissa tridactyla*) (Chastel et al., 2005) and snow petrels (*Pagadroma nivea*) (Angelier et al., 2007b)]. During stressful events such as the presence of a predator, severe weather conditions and sudden decrease in food supply, a decrease in plasma PRL levels can be considered adaptive as it may disrupt the current parental effort (via nest desertion) of an individual and promote its survival and future reproduction (Chastel et al., 2005). Alternatively, an attenuation of the PRL response could be considered as a hormonal tactic permitting the maintenance of parental care behaviors and maximizing current reproductive efforts during temporary stressful conditions (Chastel et al., 2005).

The factors influencing PRL homeostasis in birds and in wildlife in general have rarely been investigated with respect to the physiological impacts of exposure to anthropogenic environmental stressors such as the persistent organic contaminants. In fact, based on the primary role of PRL in avian reproduction, research on the chemically induced, endocrine-disruptive effects on PRL release may be important in understanding life-history tradeoffs in chronically exposed bird populations. The few experimental avian studies carried out *in vivo* with male and laying female mallard ducks (*Anas platyrhynchos*) have shown that the ingestion of petroleum resulted in a marked reduction in circulating PRL levels (Cavanaugh et al., 1983; Harvey et al., 1981). In contrast, in another study of mallard ducks exposed to cadmium, no effect on the plasma PRL levels was observed (Hughes et al., 2003), which may suggest a contaminant-dependent response on PRL control and release in birds. It has also been shown in breeding male American kestrels (*Falco sparverius*) that a PCB-dosed group exhibited a size reduction in brood patch (i.e., a bare area of skin that aids in transferring the body heat of the incubating bird to its eggs) (Fisher et al., 2006), which is in part under PRL control (Buntin, 1996). However, in this particular study the PRL levels were not determined, and thus no link to PRL modulation could be established.

One seabird species in the Norwegian Arctic, the glaucous gull (*Larus hyperboreus*), has received particular research interest with regard to organohalogen contaminant (OHC) monitoring and health risk assessments. In fact, comprehensive surveys have demonstrated that the Norwegian Arctic glaucous gull, which occupies an apex position in the marine food web, accumulates some of the highest burdens of chlorinated, brominated and fluorinated contaminants among any Arctic seabirds (Verreault et al., 2005a,b,c). Current understanding of contaminant-induced biological effects in breeding glaucous gulls supports that OHCs, including their metabolically derived products, may compromise endocrine functions. Thus far, field-based studies in glaucous gulls have reported that during the breeding season highly OHC-contaminated individuals, particularly the males, exhibit altered circulating thyroid (thyroxine and triiodothyronine) (Verreault et al., 2004, 2007a) and sex steroid levels (Verreault et al., 2006a). Also documented in breeding glaucous gulls are various toxicological and reproductive effects and

endpoints that were suggested to be mediated through modulation of the thyroid and reproductive steroid hormone systems. These include: a higher rate of wing feather asymmetry (Bustnes et al., 2002), altered basal metabolism (Verreault et al., 2007a), change in reproductive behavior such as lower nest-site attendance (Bustnes et al., 2001, 2003a, 2005; Verboven et al., submitted for publication a), and reduced ability to maintain nest temperature while incubating (Verboven et al., submitted for publication b). Hence, considering the fundamental role PRL plays in avian reproduction and parental behaviors, but also in the initiation and control of molt (Dawson, 2006; Kuenzel, 2003), it could be hypothesized that the adverse effects reported in glaucous gulls on various reproductive parameters and feather growth may, in part, involve a sensitivity of baseline PRL (i.e., PRL levels measured without handling stress) secretion to chemically induced stress. Therefore, we investigated the associations between circulating baseline PRL levels and plasma concentrations of major persistent OHC classes and metabolites in free-ranging glaucous gulls from the Norwegian Arctic engaged in the process of incubation. The OHCs and metabolites investigated are known, or suspected to interfere with endocrine systems in vertebrates based on *in vitro* and *in vivo* studies (Giesy et al., 2003). Hence, we predicted that baseline PRL release should be negatively affected, and thus at lower circulating levels in highly OHC-contaminated individuals. Furthermore, we examined whether OHC concentrations were associated with the variation of PRL levels in glaucous gulls exposed to a stressful event consisting of a standardized capture/restraint protocol, which has been suggested to be a measure of reproductive investment in seabirds (Chastel et al., 2005). Because highly OHC-contaminated glaucous gulls in the Norwegian Arctic were previously reported to exhibit a lower incubation commitment (Bustnes et al., 2001, 2005; Verboven et al., submitted for publication a, submitted for publication b), we also predicted that plasma OHC concentrations and PRL levels in response to stress would be associated.

2. Materials and methods

2.1. Sample collection

Samples of blood were obtained in May and June (2006) from free-ranging adult male ($n = 17$) and female ($n = 25$) glaucous gulls at Bear Island (74° 22′N, 19° 05′E) in the Norwegian Arctic. The study period was characterized by continuous daylight, a mean ambient temperature of 4.4 °C (range: −0.8 to 8.8 °C), and periods of rain, strong winds and even snowfalls. In glaucous gulls, both males and females partake in the care of offspring, which includes incubation, nest defense and chick feeding (Gilchrist, 2001). Therefore, males and females were captured randomly while incubating their eggs in major colonies using a nest trap. The trap consisted of a snare placed on the edge of the nest bowl that was triggered from a distance (~100 m) with a radio transmitter. The trap was triggered approximately ten minutes after the birds had resumed incubating to ensure that these birds and the neighboring nesting pairs in the colony were unstressed.

In order to induce a stress-related response on PRL release in glaucous gulls, a standardized capture/restraint and sampling protocol developed for seabirds was applied (Chastel et al., 2005). The initial blood sample was taken from the wing vein within 3–5 min following capture. It has been shown that based on this sampling regime the initial blood sample reflects the baseline PRL levels (hereafter termed as baseline PRL). Because males and females were captured and sampled for blood at least three days following clutch completion, and throughout the first half of the incubation period (i.e., 28–30 days) at any time during the day or night, the temporal effect (i.e., day in the incubation period and time of the day) on PRL levels was examined. The influence of diurnal rhythm on PRL levels was tested by plotting the time of the initial blood sample and plasma PRL levels. No significant relationship was found for either sex ($p \geqslant 0.43$), which suggests negligible effect of diurnal variation on PRL levels, as also documented in Adélie penguins (*Pygoscelis adeliae*) during the Antarctic summer (Vleck and van Hook, 2002). However, the effect of day of capture in the incubation period on the PRL level variation was consistently controlled for in the statistical models (see Section 2.4). Shortly after initial blood sampling, the birds were placed into individual, opaque cotton bags and left undisturbed. The final blood sample was collected once 30 min had elapsed, and was used to quantify post-handling PRL levels (hereafter termed as handling PRL) (see Section 2.3) as well as OHC concentrations and extractable plasma lipid content (see Section 2.2). Blood samples were collected, processed and stored according

to procedures optimized for hormone and OHC analyses in glaucous gulls (Verreault et al., 2004). Various morphometric measurements were recorded (i.e., head + bill, wing and tarsus length and body mass), and the birds were sexed according to methods described elsewhere (Verreault et al., 2004). All handled birds were observed in their respective nesting colonies shortly after release, and eventually resumed incubating. Capture, handling and sampling methods were approved by the Norwegian Animal Research Authority (Oslo, Norway) and the Governor of Svalbard (Longyearbyen, Norway).

2.2. Chemical analysis

The analytical methods (i.e., sample extraction, partitioning and clean-up, and instrumental quantification) for the determination of chlorinated benzenes (CBzs), dichlorodiphenyldichloroethanes (DDTs), chlordanes (CHLs), PCBs, polybrominated diphenyl ethers (PBDEs), methoxylated (MeO)-PBDEs, OH- and methysulfonyl (MeSO$_2$)-PCBs in glaucous gull plasma samples have been described in detail by Verreault et al. (2005a,b). These methods were applied on the present plasma samples with no modification. A complete list of the congeners/compounds determined in the present study is provided in Table 1.

Chlorinated and brominated compound quantification was performed using a gas chromatograph–mass spectrometer (GC–MS) (Agilent 6890; Agilent Technologies, Palo Alto, CA, USA) operating in the electron impact (EI) (for CBzs, DDTs, CHLs and PCBs) or electron capture negative ionization (ECNI) mode (for PBDEs, MeO–PBDEs, OH–PCBs and MeSO$_2$–PCBs). The GC–MS(EI) separation was completed using a fused silica DB-5 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA), whereas a fused silica DB-5 HT capillary column (15 m, 0.25 mm i.d., 0.10 μm film thickness) (J&W Scientific) was used for GC–MS(ECNI) separation. The GC–MS(ECNI) utilized methane as a buffer gas. The GC–MS(EI) and GC–MS(ECNI) analyte determinations were accomplished in the selected ion-monitoring (SIM) mode. The analytes were identified on the basis of their retention times on the DB-5 columns, and verified by matching retention times with authentic standard mixtures. An external standard method was used for CBz, DDT, CHL and PCB quantification using a spiked recovery surrogate mixture comprised of six ^{13}C-labeled PCB congeners and three tetra- through hexa-CBzs. An internal standard (IS) approach was used for quantification of PBDEs and MeO–PBDEs (IS was BDE71), OH–PCBs (IS was a 4-congener ^{13}C-OH–PCB mixture) and MeSO$_2$-PCBs (IS was 3-MeSO$_2$-2-CH$_3$-2′,3′,4′,5,5′-pentaCB).

Quality assurance and quality control procedures included a standard reference material (polar bear plasma pool #01-2004) from the National Wildlife Research Centre, method blanks, duplicate extractions and injections of authentic standards and cleaned-up glaucous gull plasma extracts for each block of six samples to monitor for quantitative reproducibility and instrument sensitivity. Mean (±1 standard error) recoveries based on the added recovery surrogates and ISs were: 86 ± 1% for ^{13}C-PCBs, 63 ± 2% for ^{13}C-CBzs, 95 ± 3% for BDE71, 70 ± 3% for ^{13}C-OH–PCBs and 103 ± 4% for 3-MeSO$_2$-CH$_3$-2,3′,4′,5,5′-pentaCB. The CBzs, DDTs, CHLs and PCBs were adjusted for recoveries, while the PBDEs, MeO–PBDEs, OH–PCBs and

MeSO$_2$–PCBs, which were determined using an IS quantification approach, were inherently recovery-corrected. Blank samples showed negligible background contamination for all analyte classes, and thus no background correction was necessary. The duplicate extractions and injections demonstrated on average 10% and 5%, respectively, analytical variation of selected compound concentrations. The analyte-specific method limits of quantification (MLOQs) were set as a signal being 10 times the standard deviation of the noise.

2.3. Prolactin assay

Plasma levels of PRL were determined by a heterologous radioimmunoassay (RIA) based on the methods described by Cherel et al. (1994) and validated for several seabirds (e.g., Angelier et al., 2006, 2007a; Lormée et al., 2000), also including a few Charadriiforms species such as the black-legged kittiwake (Chastel et al., 2005) and common guillemot (Uria aalge) (Tremblay et al., 2003). Pooled plasma samples of glaucous gulls produced a dose–response curve that paralleled a chicken PRL standard curve (AFP 4444B; obtained from Dr. Parlow, NHPP, Harbor-UCLA Medical Center, Torrance, CA, USA, Fig. 1). Parallelism between the glaucous gull and chicken curve indicates that the concentration-dependent binding dynamics of the glaucous gull PRL with the antibody is similar to that of the chicken PRL (Fig. 1). Hence, this RIA was concluded to be an appropriate assay to assess relative levels of plasma PRL in incubating glaucous gulls. Only one assay was performed, the intra-assay coefficient of variation being 8.4% (n = 6 duplicates). The rate of decrease in PRL levels in glaucous gulls following the capture/restraint protocol was calculated as the decrease in PRL level (ng/mL) per handling time unit (min).

2.4. Data treatment

The OHCs determined in the present study were categorized and summed ($\sum$) in eight classes (i.e., $\sum_4$Cbz, $\sum_3$DDT, $\sum_6$CHL, $\sum_{58}$PCB, $\sum_{38}$PBDE, $\sum_{15}$MeO–PBDE, $\sum_{28}$OH–PCB and $\sum_{15}$MeSO$_2$–PCB) composed of closely related congeners/compounds with respect to their chemical structures if they were detected in 60% or more of the plasma samples. For these OHCs, the samples with concentrations of a given congener or compound below the method limit of quantification (MLOQ) were arbitrarily assigned a value, randomly selected from a normal distribution, between zero and the compound-specific MLOQ. In males and females, the OHC classes were positively inter-correlated ($r \geqslant 0.58$; $p \leqslant 0.0001$). Hence, in order to reduce the number of predictor variables and to improve the statistical power of the analyses, principal components (PCs) were extracted based on the wet weight concentrations (log$_{10}$-transformed) of these eight OHC classes. Because the first extracted PC (hereafter referred to as PC 1 OHC) accounted for 80.8% of the total variance, and because it was strongly and positively correlated with concentrations of each of the eight OHC classes ($r \geqslant 0.83$; $p \leqslant 0.0001$), analyses were performed using PC 1 OHC only. The correlations between the eight OHC classes and the selected dependent and independent variables also were tested. However, because strong inter-correlations were found

Table 1
Mean (±1 standard error (SE)) and data range of percentage extractable plasma lipid content and organohalogen contaminant (OHC) class concentrations (ng/g wet wt.) in plasma of incubating glaucous gull males and females from the Norwegian Arctic. The levels of significance (p-level) for the differences in the eight OHC class concentrations between males and females are identified

		Males (n = 17)		Females (n = 25)	
	p-Level	Mean ± SE	Data range	Mean ± SE	Data range
Plasma lipid %	0.15	0.77 ± 0.04	0.44–1.0	0.86 ± 0.04	0.40–1.26
$\sum_4$CBz[a]	0.002	24.2 ± 3.6	7.1–65.7	13.0 ± 1.6	4.3–34.5
$\sum_6$CHL[b]	0.003	44.0 ± 7.0	11.9–119	22.7 ± 2.4	8.3–49.1
$\sum_3$DDT[c]	0.0001	274 ± 32.9	102–625	137 ± 16.5	38.4–324
$\sum_{58}$PCB[d]	0.001	647 ± 113	90.3–1611	263 ± 35.7	54.2–814
$\sum_{28}$OH–PCB[e]	0.0001	19.6 ± 3.7	0.9–59.7	5.8 ± 0.9	0.8–16.3
$\sum_{15}$MeSO$_2$–PCB[f]	0.003	0.60 ± 0.08	0.12–1.14	0.30 ± 0.06	0.02–1.26
$\sum_{38}$PBDE[g]	0.001	19.6 ± 3.4	4.7–54.5	8.5 ± 1.1	2.5–24.9
$\sum_{15}$MeO–PBDE[h]	0.08	2.18 ± 0.44	0.05–5.66	0.92 ± 0.13	0.19–3.22

[a] Sum of 1,2,4,5-TeCBz, 1,2,3,4-TeCBz, PnCBz and HxCBz.

[b] Sum of heptachlor epoxide, oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor.

[c] Sum of p,p′-DDT, p,p′-DDE and p,p′-DDD.

[d] Sum of CB22, 28/31, 20/33, 41/64, 42, 44, 47/48, 49, 52, 56/60, 66, 70/76, 74, 85, 87, 90/101, 92, 95, 97, 99, 105, 110, 114, 118, 128, 130, 137, 138, 141, 146, 149, 151, 153, 156, 157, 158, 167, 170/190, 171, 172, 174, 176, 177, 178, 179, 180, 183, 187, 189, 194, 195, 196/203, 199, 200, 202, 206, 207 and 208.

[e] Sum of 4′-OH–CB79, 4-OH–CB97, 4-OH–CB107/4′-OH–CB108, 2′-OH–CB114, 4′-OH–CB120, 4′-OH–CB127, 4′-OH–CB130, 4-OH–CB134, 4-OH–CB146, 4′-OH–CB159, 4-OH–CB162, 4-OH–CB163, 4′-OH–CB177, 4-OH–CB178, 3′-OH–CB180, 3′-OH–CB182, 3′-OH–CB183, 3′-OH–CB184, 4-OH–CB187, 4-OH–CB193, 4′-OH–CB198, 4′-OH–CB199, 4′-OH–CB200, 4′-OH–CB201, 4′-OH–CB202, 4,4′-diOH–CB202, 3′-OH–CB203 and 4′-OH–CB208.

[f] Sum of 3-MeSO$_2$-CB49, 4-MeSO$_2$-CB49, 3-MeSO$_2$-CB52, 4-MeSO$_2$-CB52, 4-MeSO$_2$-CB64, 3-MeSO$_2$-CB70, 4-MeSO$_2$-CB70, 4-MeSO$_2$-CB87, 3-MeSO$_2$-CB101, 4-MeSO$_2$-CB101, 3-MeSO$_2$-CB110, 4-MeSO$_2$-CB110, 3-MeSO$_2$-CB132, 4-MeSO$_2$-CB132 and 3-MeSO$_2$-CB149.

[g] Sum of BDE17, 25, 28, 47, 49, 54, 66, 75, 77, 85, 99, 100, 116, 119, 138, 139, 140, 153, 154/BB153, 155, 156, 171, 180, 181, 183, 184, 190, 191, 196, 197, 201, 202, 203, 205, 206, 207, 208 and 209.

[h] Sum of 6′-MeO–BDE17, 4′-MeO–BDE17, 2′-MeO–BDE28, 4-MeO–BDE42, 5-MeO–BDE47, 6-MeO–BDE47, 3-MeO–BDE47, 4′-MeO–BDE49, 6′-MeO–BDE49, 2′-MeO–BDE68, 6-MeO–BDE85, 6-MeO–BDE90, 6-MeO–BDE99, 2-MeO–BDE123 and 6-MeO–BDE137.
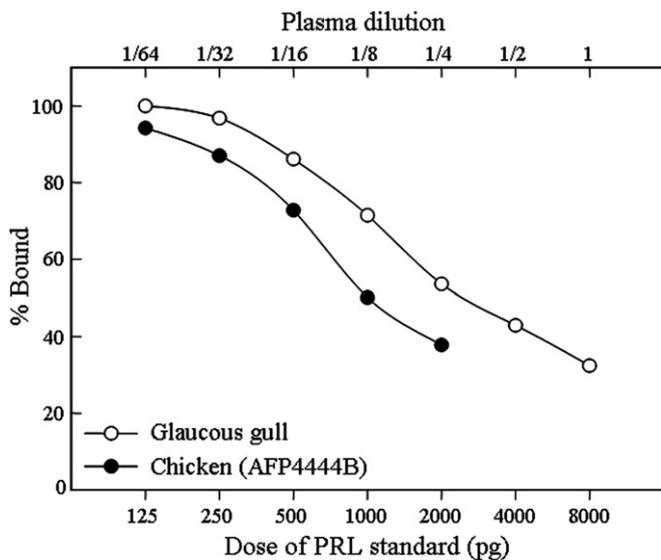
Plasma dilution

Fig. 1. Dose–response curve of prolactin in chicken (AFP 4444B) and glaucous gull from the Norwegian Arctic (Bear Island). The dose of prolactin standard is expressed in pg/tube.

between the congeners and compounds constituting those eight classes, analyses were not performed on single congeners/compounds. In order to detect potential sex-specific variation patterns in the associations between PRL levels and OHC concentrations, PC 1 OHC factor scores were tested based on a quartile categorization (i.e., groups I–IV). The quartiles generated were comprised of 4 to 5, or 6 to 7 individuals for males and females, respectively, and were significantly different from each other for both males ($p \leqslant 0.03$) and females ($p \leqslant 0.003$).

The structures in the relationships between baseline and handling PRL levels ($\log_{10}$-transformed), rate of decrease in PRL levels, PC 1 OHC as well as potentially confounding environmental and biological variables (see next paragraph) were investigated using PC analysis. The results from this multivariate exploratory technique allowed identifying the potential predictor variables to the variation of PRL levels and rate of decrease in PRL levels, which were further analyzed using general linear models (GLMs) (e.g., ANOVA). Differences between the categorical predictors (i.e., sex and PC 1 OHC quartiles) and their possible interactions, for any continuous variables, were further examined using the Fisher *post hoc* test. The statistical package utilized was Statistica® (StatSoft, Tulsa, OK, USA) and α was set at 0.05.

The environmental and biological variables selected for the analyses were: day of capture in the study period (i.e., calendar dates: June 1st–June 23rd), extractable plasma lipid percentage and body condition. Body condition of an individual was defined as the body size index extracted using PC analysis from three morphological measurements (i.e., wing length, tarsus length and head + bill length), and corrected for body mass in the GLMs (Garcia-Berthou, 2001). Because PC 1 explained 76.3% and 65.1% of the variation in body size for male and females, respectively, only PC 1 was used. The body size index and body condition were generated separately for males and females as the glaucous gull is sexually dimorphic. It has previously been shown in glaucous gulls at a post-egg laying stage, for an identical suite of OHCs analyzed as in the present study (Table 1), that significant differences in plasma concentrations are found between males and females (Verreault et al., 2005a,b, 2006a, 2007a). Furthermore, in toxicological assessments on various endocrine parameters (Verreault et al., 2004, 2006a, 2007a) and reproductive and developmental endpoints (Bustnes et al., 2001, 2002, 2003a, 2005; Verboven et al., submitted for publication a, submitted for publication b) in breeding glaucous gulls, notable sex-specific disparities have been reported. As a consequence, males and females were investigated as distinct groups rather than controlling for the effect of sex in the statistical models where the variation of baseline and handling PRL levels and rate of decrease in PRL levels was examined relative to PC 1 OHC (and PC 1 OHC quartiles).

3. Results

3.1. OHC concentrations

The sum concentrations of closely related compounds/congeners (i.e., $\sum_4 CBz$, $\sum_3 DDT$, $\sum_6 CHL$, $\sum_{58} PCB$, $\sum_{38} PBDE$, $\sum_{15} MeO–PBDE$, $\sum_{28} OH–PCB$ and $\sum_{15} MeSO_2–PCB$) determined in plasma of glaucous gull males and females can be found in Table 1. The

OHC compositional patterns and concentrations in glaucous gull plasma were comparable to those previously reported for this species during the incubation stage in which an analogous suite of OHCs was analyzed (Verreault et al., 2006a,b, 2007a). Moreover, consistent with previous glaucous gull investigations, males retained approximately 2- to 3-fold higher OHC concentrations in plasma than females, with more pronounced disparities for the $\sum_{28} OH–PCB$ and $\sum_{58} PCB$ (Table 1). A comprehensive description and discussion on the sex-specific factors of OHC accumulation, toxicokinetics and fate of chlorinated and brominated contaminants and metabolites in glaucous gull plasma can be found in Verreault et al. (2005a,b, 2006b, 2007b).

3.2. PRL and stress induction

The mean baseline PRL levels in incubating glaucous gull plasma samples tended to be higher in females relative to males, although not significantly ($p = 0.20$). This gender-dependent peculiarity was in accordance with several seabird species exhibiting shared parental duties for which slightly elevated baseline PRL levels were found in females during most of the breeding period (Angelier et al., 2006, 2007a; Cherel et al., 1994). The 30-min capture/restraint protocol applied on glaucous gulls led to a significant decrease in circulating PRL levels in both males and females, which was consistent with studies of several long-lived seabird species exposed to a similar treatment (Angelier et al., 2007b; Chastel et al., 2005). Following capture/restraint, the baseline PRL levels decreased on average by 26% (range: 3–45%) in males and females, which resulted in a rate of PRL decrease averaging 0.76 ng/mL/min (range: 0.08–1.61 ng/mL/min). The rate of decrease in PRL levels in glaucous gulls was identical between sexes ($p = 0.73$). Baseline and handling PRL levels co-varied positively in both sexes ($r \geqslant 0.64$; $p \leqslant 0.005$).

3.3. Associations among variables

The structures in the relationships between baseline and handling PRL levels, rate of decrease in PRL levels, PC 1 OHC as well as the potential confounding variables extractable plasma lipid percentages, body condition and capture day in the study period, were investigated separately for each sex using PC analysis (Fig. 2A and B). In males and females, the cumulated variance of PC 1 and PC 2 accounted for 57.5% and 54.0%, respectively, of the total variance. Because the contribution from PC 3 in the total variance was substantially less than PC 2 in both sexes, and because this PC did not provide noteworthy additional information on the variable association patterns, PC 3 was left out from further analyses and discussions.

In males, the spatial orientation of the variables based on PC 1 and PC 2 pointed to an inverse association between baseline PRL levels and the rate of decrease in PRL levels, and the PC 1 OHC factor scores (Fig. 2A). Using GLMs, the baseline PRL levels and rate of decrease in PRL levels in males tended to vary negatively with PC 1 OHC, although these associations did not comply with the criterion of significance (Table 2). Moreover, negative relationships were found in males between baseline PRL levels and rate of decrease in PRL levels, and the eight OHC classes tested individually, although only the $\sum_{38} PBDE$ concentrations ($\log_{10}$-transformed) yielded a statistically significant correlation with the baseline PRL levels (Table 2). Baseline and handling PRL levels in males, as well as the rate of decrease in PRL levels, varied irrespective of the extractable plasma lipid percentages ($p \geqslant 0.28$) body condition ($p \geqslant 0.13$) and capture day in the study period ($p \geqslant 0.44$). In contrast, in females, no significant predictive effect of PC 1 OHC was found on baseline and handling PRL level and rate of decrease in PRL level variation (Fig. 2B). In fact, PC 1 OHC scores in females
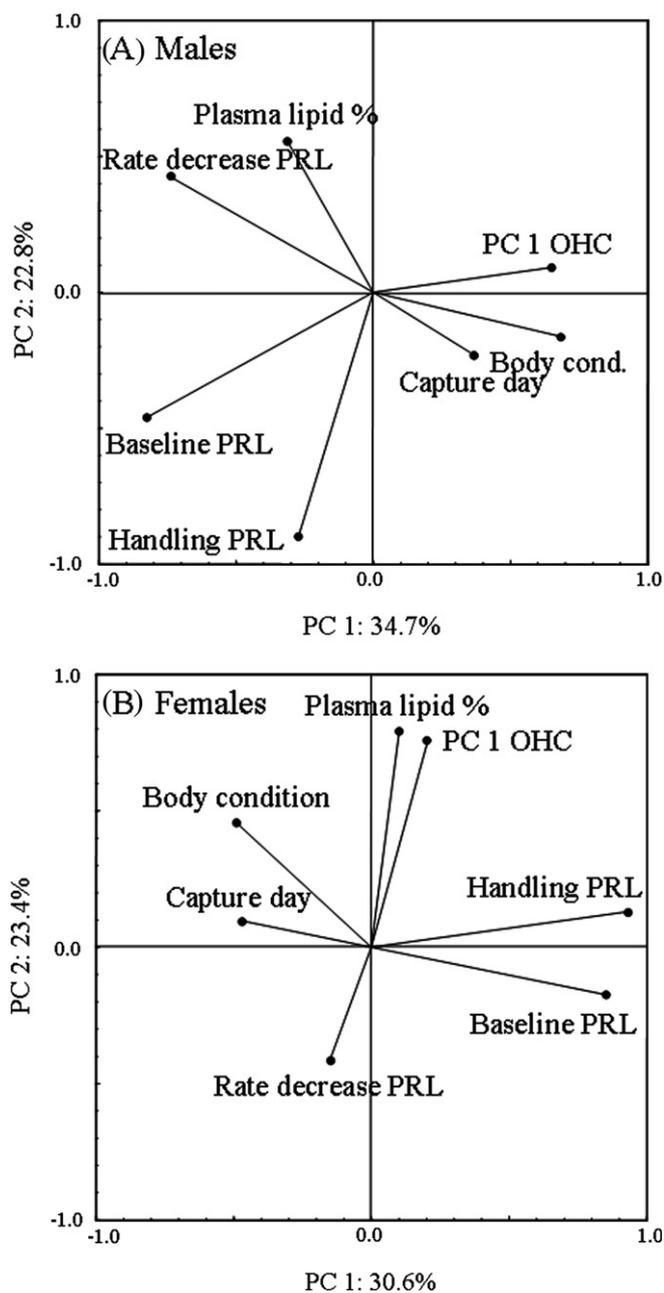
Fig. 2. Structures in the relationships between baseline and handling prolactin (PRL) levels (log$_{10}$-transformed), rate of decrease in PRL levels, organohalogen contaminant (OHC) concentrations (PC 1 OHC), as well extractable plasma lipid percentages, body condition and capture day in the study period for incubating male (A) and female (B) glaucous gulls. The relative percentages of the total variance explained by each of the two first principal components (PCs), PC 1 and PC 2, are provided.

only varied significantly and positively as a function of the extractable plasma lipid percentages ($r = 0.41$; $p = 0.04$). Moreover, female baseline PRL levels were associated negatively with body condition ($r = -0.47$; $p = 0.03$).

The quartiles (groups I–IV) generated based on the PC 1 OHC factor score distribution were used in order to detect potential sex-specific variation (interactive or non-interactive) patterns in baseline and handling PRL levels (and rate of decrease in PRL levels) as a function of the OHC concentrations (Fig. 3A and B). In males, baseline PRL levels were significantly lower ($p = 0.05$) in the top 25% quartile (IV) relative to the lowest (I). No such difference in baseline PRL levels was found for females. However, in response to the capture/re-

straint-related stress, handling PRL levels in both males and females varied following an unpredictable pattern with respect to the PC 1 OHC quartiles. No significant difference in handling PRL levels was found between any of the PC 1 OHC quartiles for both males and females. By comparison, following a similar quartile-based approach, the variation in the rate of decrease in PRL levels highly differed between the sexes (Fig. 4A and B). In males, the rate of decrease in PRL levels was significantly lower ($p = 0.03$) in quartile IV relative to quartile I. In females, the rate of decrease in PRL levels fluctuated irrespective of the PC 1 OHC quartiles, although it tended to be highest in the mid-quartiles relative to the quartiles I and IV.

4. Discussion

4.1. PRL variation and OHCs

In the present field-based study of Arctic-breeding glaucous gulls exposed to a complex cocktail of OHCs in the marine ecosystem, negative associations between circulating baseline PRL levels and plasma OHC concentrations were found in males, but not in females. Furthermore, in response to a 30-min stressful event induced through a capture/restraint protocol, baseline PRL levels in males decreased at a rate that tended to be negatively associated with plasma OHC burdens. Hence, highly OHC-contaminated glaucous gull males appeared to exhibit an attenuated stress-related PRL responsiveness, which would suggest that they maintained higher PRL levels compared to lower-contaminated individuals. Interestingly, these findings were contrasting with our original predictions suggesting that the rate of decrease in PRL levels following a handling stress would be lowest in the low OHC-exposed birds. However, the reasons for this (weak) association are uncertain, and thus further studies would be required. Nonetheless, the present results suggest that in chronically OHC-exposed male glaucous gulls, the control of PRL release may be affected by the direct or indirect modulating actions, depending on the level of exposure, of OHCs and/or their metabolically derived products. In contrast, in females, the associations between PRL levels and the rate of decrease in PRL levels and plasma OHC concentrations were notably absent. This may indicate a sex-specific physiological sensitivity of PRL control mechanisms in glaucous gulls exposed to OHCs and acute stress. Alternatively, because female glaucous gulls tended to have higher baseline PRL levels than males (but not significant) and can excrete a substantial portion of their OHC burden via oviposition, they may not have reached the threshold concentrations of OHCs that are necessary to overwhelm and impair the homeostatic mechanisms of PRL release. However, based on the lack of statistical significance of most of these PRL–OHC associations in males at the established α-value, which may be the consequence of a low sample size and/or a large amount of individual variation, these trends should be interpreted with great caution. In fact, inherent factors controlling PRL secretion in birds, other than body condition and time of the year (season) or day, may not have been factored out in the present field-based investigation of glaucous gulls. For instance, it has been shown in several long-lived seabirds that baseline PRL levels increase with the breeding experience and age (Angelier et al., 2006, 2007a). Moreover, PRL responsiveness to an experimental stress has been reported to be influenced by age in some seabird species where older breeders have been shown to maintain higher stress-induced PRL levels than younger individuals (Angelier et al., 2007b). While the present glaucous gull sub-population was comprised exclusively of adult and breeding individuals, and thus older than 5 years of age (Gilchrist, 2001), the exact age of the individuals was unknown. However, it has previously been established in adult breeding glaucous gulls from the Norwegian Arctic that based on recovered individuals that were ring-marked as chicks, the effect of age on the variation of OHC concentrations in blood is negligible (Bustnes et al., 2003b).

Table 2
Relationships between concentrations of eight organohalogen contaminant (OHC) classes ($\log_{10}$-transformed ng/g wet wt.) and the first principal component extracted from OHC concentrations (PC 1 OHC) and baseline prolactin (PRL) levels ($\log_{10}$-transformed ng/mL) and rate of decrease in PRL levels in plasma of incubating male glaucous gulls ($n = 17$)

|	Baseline PRL levels				Rate of decrease in PRL levels			
	$r(x,y)$	r^2	t	p-Level	$r(x,y)$	r^2	t	p-Level
$\sum_4$CBz	−0.42	0.17	−1.78	0.09	−0.16	0.02	−0.61	0.54
$\sum_6$CHL	−0.38	0.14	−1.58	0.13	−0.39	0.15	−1.62	0.12
$\sum_3$DDT	−0.33	0.11	−1.35	0.19	−0.42	0.18	−1.79	0.09
$\sum_{58}$PCB	−0.37	0.14	−1.56	0.13	−0.34	0.12	−1.41	0.17
$\sum_{28}$OH–PCB	−0.33	0.11	−1.33	0.20	−0.35	0.12	−1.43	0.17
$\sum_{15}$MeSO$_2$–PCB	−0.35	0.12	−1.44	0.16	−0.24	0.06	−0.98	0.34
$\sum_{38}$PBDE	−0.50	0.25	−2.24	0.04	−0.42	0.18	−1.82	0.08
$\sum_{15}$MeO–PBDE	−0.41	0.17	−1.75	0.10	−0.31	0.09	−1.24	0.23
PC 1 OHC	−0.43	0.18	−1.84	0.08	−0.36	0.13	−1.51	0.15
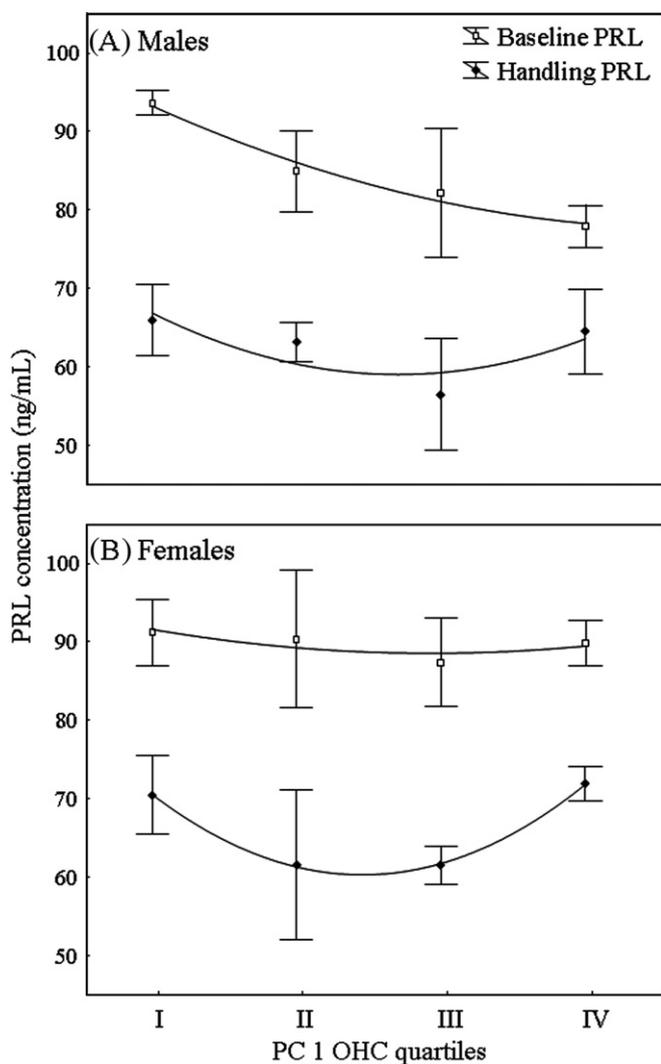
Fig. 3. Variation of baseline and handling prolactin (PRL) levels in incubating male (A) and female (B) glaucous gulls. Mean PRL levels (±1 standard error) are categorized based on quartiles (i.e., groups I–IV) generated from a first principal component (PC) extracted from organohalogen contaminant (OHC) concentrations (PC 1 OHC).

Fig. 4. Rate of decrease in prolactin (PRL) levels following a capture/restraint-related stress in incubating male (A) and female (B) glaucous gulls. Mean rates of decrease in PRL levels (±1 standard error) are categorized based on quartiles (i.e., groups I–IV) generated from a first principal component (PC) extracted from organohalogen contaminant (OHC) concentrations (PC 1 OHC).

There are three *in vivo* studies that have addressed the potential impacts of petroleum and cadmium ingestion on PRL levels in a captive bird; the mallard duck (Cavanaugh et al., 1983; Harvey et al., 1981; Hughes et al., 2003). These studies are to our knowledge the only studies where anthropogenic chemical stressors were investigated in relation to PRL perturbation. In line with find-
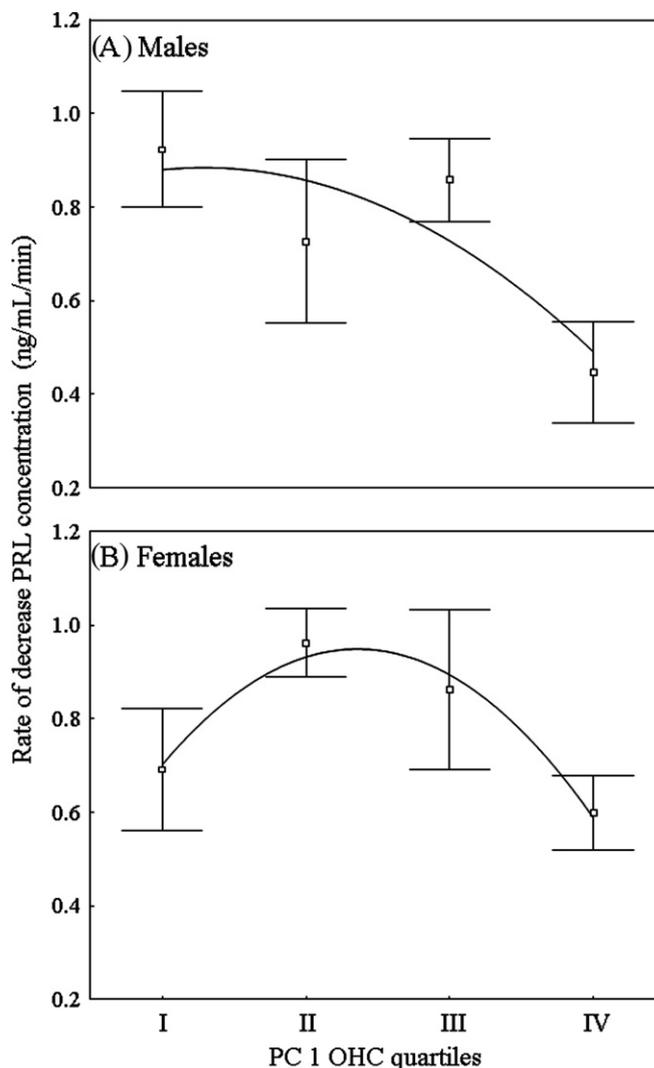
ings in the present study, mallard ducks exposed to petroleum showed significant reductions in circulating PRL levels compared to unexposed, control specimens (Cavanaugh et al., 1983; Harvey et al., 1981). However, the laboratory conditions employed in these controlled captive studies may poorly reflect the environmental conditions experienced by free-ranging birds, which include vari-

ous stressors such as changing weather conditions and food availability, and risk of predation. In addition, avian wildlife, in contrast to most laboratory specimens, is exposed to a complex contaminant mixture that has accumulated in tissues and body compartments over time, and the multiple chemical interactions that can exist among those contaminants (e.g., additive, synergic and blocking effects). Nonetheless, investigations in captive rodent species under controlled laboratory conditions also have provided substantial evidence suggesting that *in vivo* exposure to a variety of chemicals (e.g., methyl *tert*-butyl ether, atrazine, chlordecone and PCBs) may elicit alterations in PRL secretion, including a suppressing response (de Krey et al., 1994; Rosecrans et al., 1984; Stoker et al., 1999; Williams et al., 2000).

4.2. Postulated mechanisms of action

It has been established that PRL secretion in birds can both be stimulated (Hall et al., 1986) and inhibited (Reddy et al., 2002) by feedback mechanisms of the gonadal steroid hormones (i.e., progesterone, 17β-estradiol and testosterone). For example, in pre-incubated anterior pituitary glands of broiler fowls, exposure to progesterone resulted in a marked reduction in baseline PRL release in a dose-related manner (Hall et al., 1984). Furthermore, in white leghorn chickens and girirani birds (a hybrid chicken breed) treated with the anti-PRL agent bromocriptine, levels of PRL were lower compared to the untreated groups, and were negatively correlated with those of progesterone and 17β-estradiol in both species (Reddy et al., 2002, 2006). The current state of knowledge on the toxicological actions of OHCs in vertebrates also suggests that certain OHCs, all identified and quantified in glaucous gull blood and tissues, may interfere with enzymes involved in steroidogenesis [e.g., hydroxysteroid dehydrogenase (HSD) and cytochrome P450 (CYP) enzymes], or less directly through modulation of the feedback mechanisms in the hypothalamus–pituitary–gonadal axis (Sanderson and van den Berg, 2003). For example, it has been suggested that OHCs may exert their effects at the anterior pituitary gland level by directly modulating PRL release (positively and negatively), as shown in *in vivo* and *in vitro* studies for various chemicals including bromocriptine in birds (Youngren et al., 1998) and estrogen-like OHCs in mammalian cell lines (Abraham and Frawley, 1997; Rousseau et al., 2002; Steinmetz et al., 1997). Other postulated mechanisms were suggested to be mediated through a chemically induced deregulation of neurotransmitters (e.g., dopamine) involved in the modulation of PRL secretion, as for instance demonstrated in rats administered orally with the pesticide methoxychlor (Lafuente et al., 2000).

In a recent study of incubating glaucous gulls also from Bear Island, plasma progesterone levels were found to be positively correlated with concentrations of PCBs, DDTs, CHLs and PBDEs in male glaucous gulls, but not in females (Verreault et al., 2006a). In this study, in which no relationship was found between OHC and testosterone and 17β-estradiol (not detected), it was suggested that OHCs may have the potential to interfere with certain steroidogenenic enzymes, and thus impinge on circulating progesterone homeostasis in highly OHC-exposed males. Hence, it could be hypothesized that the negative relationships between baseline PRL levels and plasma OHC concentrations in the present male glaucous gulls may, in fact, be the indirect result of an OHC-mediated disturbance on gonadal progesterone synthesis and/or metabolism, leading to abnormally high progesterone levels in the circulatory system. This would in turn create a negative feedback response on PRL secretion. However, the reverse mechanism could also occur where a direct OHC-induced inhibition of PRL secretion (e.g., in the anterior pituitary gland) would influence the circulating progesterone status. Alternatively, levels of progesterone precursors (e.g., cholesterol and pregnenolone) in the steroidogenic

cascade of glaucous gull males might have been impacted through the actions of specific OHCs on HSD and/or aryl hydrocarbon receptor-responsive CYP enzymes (Sanderson and van den Berg, 2003).

It has also been suggested that in birds, levels of thyroid hormones (thyroxine and triiodothyronine) may influence the status of circulating PRL (Hall et al., 1986). However, this endocrine interactive pathway has as yet to be confirmed. Nevertheless, in glaucous gull males from the Norwegian Arctic it has been reported that plasma thyroxine levels were negatively related to blood concentrations of major organochlorines (i.e., CBzs, PCBs, DDTs and oxychlordane) (Verreault et al., 2004). In this study, adverse effects of thyroid hormone homeostasis were found in males exclusively, which again suggests possibly enhanced sensitivity of endocrine functions in breeding male glaucous gulls relative to females.

4.3. Modulation of PRL and biological implications

It has been reported in Norwegian Arctic glaucous gulls, including the presently investigated colonial population from Bear Island, that behaviors related to nest-site attentiveness and incubation were negatively related to blood concentrations of major, currently determined OHCs (Bustnes et al., 2001, 2005; Verboven et al., submitted for publication a). Furthermore, in the study by Verboven et al. (submitted for publication a), it was observed that OHC-contaminated glaucous gulls were less able to maintain a constant nest temperature. Hence, as nest-site attendance and incubation are strongly influenced by hormonal fluctuations in birds, this may suggest that the concomitant effects on circulating baseline PRL (present study), sex steroid (Verreault et al., 2006a) and thyroid hormone homeostasis (Verreault et al., 2004, 2007a) are explaining, in part, the impaired reproductive behaviors observed in glaucous gulls (males). Ultimately, this may also be a possible explanation as to the poor reproductive performance documented in glaucous gulls from these same breeding colonies (Bustnes et al., 2003a, 2005; Verboven et al., submitted for publication b). However, the lower incubation commitment in male glaucous gulls apparently can not be explained based on their PRL response to a stressful event (i.e., capture/restraint-related) as the highly OHC-contaminated individuals tended to maintain higher PRL levels compared to those that had accumulated lower OHC concentrations. Moreover, feather growth is one other endocrine-dependent physiological mechanism that has been suggested to be OHC-sensitive in glaucous gulls (Bustnes et al., 2002). Because PRL, in orchestration with other hormones (e.g., thyroid and growth hormones), has been demonstrated to play a role in the control of molt (Dawson, 2006; Kuenzel, 2003), it could be speculated that the higher rate of wing feather asymmetry reported in glaucous gull males (Bustnes et al., 2002) could be partly linked to the alterations on PRL release. Taken together, these possibly OHC-associated effects on the reproductive behaviors and development of glaucous gulls from the Norwegian Arctic may contribute to adverse changes on their population size status (Bustnes et al., 2003a, 2005). In fact, it has recently been documented that since 1986 and up until the most recent census in 2006, the breeding population of glaucous gulls from these Norwegian Arctic colonies has decreased by nearly 65% (Strøm, 2006).

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