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# Organohalogen contamination in breeding glaucous gulls from the Norwegian Arctic: Associations with basal metabolism and circulating thyroid hormones

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*Basal metabolic rate in glaucous gulls was negatively associated with plasma organochlorine concentrations, but not with circulating thyroid hormone levels.*

**Abstract**

Exposure to organohalogens in endotherms has been suggested to impose chemically induced stress by affecting functions related to maintenance energy requirements. Effects on basal metabolic rate (BMR) have been suggested to be, in part, mediated through interactions with the thyroid hormones (THs). We investigated the relationships between plasma concentrations of major organochlorines, PBDEs, hydroxylated (OH)- and methoxylated (MeO)-PBDEs and OH-PCBs, circulating TH levels and BMR in breeding glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic. Negative associations were found between BMR and concentrations of ∑PCB, ∑DDT and particularly ∑chlordane, which combined made up 91% of the total contaminant burden. Levels of THs (thyroxine and triiodothyronine) were not associated significantly with variation of BMR or concentrations of any of the compounds determined. The present study suggests that BMR may be altered in glaucous gulls exposed to high loadings of persistent contaminants in the Norwegian Arctic environment.


*Keywords:* Organohalogen; Basal metabolic rate; Energy expenditure; Thyroid hormone; Norwegian Arctic; Glaucous gull

## 1. Introduction

The concept of energy distribution for maintenance requirements, activity, growth, and reproduction constitutes a well-studied approach that integrates the physiology and ecology of individual animals. Attempts to identify the factors responsible for the variation in the maintenance energy requirements of vertebrates have typically focused on the minimal rate of energy expenditure, or basal metabolic rate (BMR). The BMR is commonly defined as the rate found in a thermoregulating, postabsorptive, adult endotherm resting in its thermoneutral zone, and has been characterized for a variety of wildlife species, including seabirds from a wide geographical range (Ellis, 1984; Ellis and Gabrielsen, 2002). The large variation in BMR within and between seabird species has been attributed to adaptations to specific behavioral traits of the species or in response to environmental conditions. For instance, seabirds living in arctic regions have been distinguished by

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higher BMR compared to species with characteristic southern distributions (Bech et al., 2002; Ellis, 1984; Gabrielsen et al., 1988; Gabrielsen and Mehlum, 1989). Yet, the factors influencing BMR among seabirds and wildlife in general, besides inherent seasonal and circadian rhythms, have rarely been investigated with respect to the physiological costs of exposure to environmental chemical pollution. It has been suggested that sublethal exposure to contaminants may impose a chemically induced stress in vertebrates by affecting respiratory processes and energy budgets via additional metabolic costs incurred by detoxification and excretion (Calow, 1991; Handy and Depledge, 1999).

The few studies that have measured energy expenditure in mammal and bird subjects following long- and short-term dosage regimes with chlorinated compounds are somewhat contradictory. In brief, increased metabolic rate was observed in dichloro-diphenyl-trichloroethane (DDT)-treated short-tailed shrews (*Blarina brevicauda*) (Braham and Neal, 1974) and white-footed mice (*Peromyscus leucopus*) dosed with low levels of polychlorinated biphenyls (PCBs) (Voltura and French, 2000). In contrast, other works have reported a decrease in metabolic rate: mourning doves (*Zenaida macroura* c.) exposed to Aroclor 1254 (Tori and Mayer, 1981) as well as pigeons (*Columbidae* sp.) (Jefferies et al., 1971) and lesser black-backed gulls (*Larus fuscus*) (Jefferies and Parslow, 1972) fed high doses of DDT and PCBs, respectively. Also reported has been unchanged metabolic rate as a function of contaminant dosage relative to control subjects, as for example in PCB-treated white-footed mice (French et al., 2001). Such increased or decreased metabolic rate following toxicant exposure may have partly been mediated through interactions of chemicals with the activities of certain hormones. One likely candidate is the thyroid hormones (THs).

The THs, i.e., thyroxine ($T_4$) and particularly the primary metabolically active triiodothyronine ($T_3$), are considered the prime controllers for the regulation of metabolic functions and thermogenesis in mammals and birds (Danforth and Burger, 1984; McNabb, 2000). Over the last few years, a number of observations have led to the speculation that contamination of certain environmental chemicals is the cause of thyroid function modulation in some avian species. Although somewhat less consistent in birds relative to mammals, a number of studies have reported abnormal TH concentrations and thyroid gland histology in birds exposed to organochlorine compounds under laboratory conditions and in free-ranging populations (Dawson, 2000; McNabb, 2005; Scanes and McNabb, 2003). More recently, in vitro and in vivo studies in non-avian species have reported effects on TH-dependent processes for chemicals of more recent environmental concern such as the polybrominated diphenyl ethers (PBDEs) (Legler and Brouwer, 2003) and their hydroxylated (OH)- and methoxylated (MeO)-PBDE analogues (Hakk and Letcher, 2003; Legler et al., 2002; Meerts et al., 2000), as well as the primary metabolically-derived PCB residues, the OH-PCBs (Letcher et al., 2000).

Recently, a study on breeding glaucous gulls (*Larus hyperboreus*) reported significant negative relationships between plasma concentrations of PCBs and selected organochlorine pesticides, and total and unbound plasma $T_4$ and $T_3$ concentrations (Verreault et al., 2004). It was suggested that the glaucous gull, a top-predator species in the Norwegian Arctic marine environment, might be particularly vulnerable to contaminant-mediated alteration in thyroid functions as a result of its high organohalogen burden. In fact, in eggs and plasma of glaucous gulls from the Norwegian Arctic, concentrations of PCBs, organochlorine pesticides (e.g., DDT- and chlordane [CHL]-related compounds), PBDEs, MeO-PBDEs and OH-PCBs/PBDEs were among the highest reported in any arctic seabird species and populations (Verreault et al., 2005a,b). Because a large suite of organohalogens occurring in glaucous gulls was demonstrated to have structure-related affinities with THs, and because THs exert strong control over regulation of metabolic functions, glaucous gulls exposed to high concentrations of these substances may experience altered circulating TH status, basal metabolism and capacity for adaptive thermogenesis. To test this assumption, we investigated the relationships between plasma concentrations of major legacy and emerging organohalogens (PCBs, DDTs, CHLs, PBDEs, MeO-PBDEs and OH-PCBs/PBDEs), circulating TH levels and BMR in breeding glaucous gulls from the Norwegian Arctic. We assumed that glaucous gull males and females during the breeding period would be more responsive to contaminant-induced changes on energetic and thyroid functions due to the particularly high energy expenditure associated with this critical stage of their life-history.

## 2. Materials and methods

### 2.1. Field procedure

BMR measurements and blood samples were obtained from adult male ($n = 11$) and female ($n = 12$) glaucous gulls during the breeding season (May–June) of 2004 at Bear Island (74°22′ N, 19°05′ E) in the Norwegian Arctic. The study period at Bear Island was characterized by continuous daylight, a mean ambient temperature of 2.8 °C (range: -0.8–8.7 °C), and periods of rain, strong winds and even snowfalls. Randomly selected individuals were captured, while incubating, from three major colonies using a nest trap (Verreault et al., 2004). A general qualitative assessment of the birds at the time of sampling revealed they were all in good body condition. 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). A blood sample (for organohalogen and TH measurements) was collected from each individual prior to BMR measurements (see the proceeding sections). A detailed description of blood sampling procedures and sample processing can be found in Verreault et al. (2004, 2005a). The capture and handling methods of glaucous gulls were approved by the Norwegian National Animal Research Authority (P.O. Box 8147 Dep., NO-0033 Oslo, Norway) and the Governor of Svalbard (Box 633, NO-9171 Longyearbyen, Norway).

### 2.2. Organohalogen analyses

The analytical methods (i.e., sample extraction, partitioning and cleanup) for the determination of PCBs, DDTs, CHLs, PBDEs, MeO-PBDEs and OH-PCBs/PBDEs in glaucous gull plasma samples have been described extensively by Verreault et al. (2005a, 2005b). A detailed list on the congeners/compounds determined is provided in footnotes a through g in Table 1. The analysis of PCBs, DDTs and CHLs was performed by automatic injection of

Table 1
Mean (±1 standard error [SE]) and range of plasma lipid percentage and sum (∑) concentrations (ng $g^{-1}$ wet weight) of selected organohalogens in glaucous gull males and females from the Norwegian Arctic

| | Males ($n = 11$) | | Females ($n = 12$) | |
|---|---|---|---|---|
| | Mean ± SE | Range | Mean ± SE | Range |
| Lipid % | 1.54 ± 0.06 | 1.30–1.81 | 1.52 ± 0.06 | 1.12–2.02 |
| ∑PCB[a] | 1183 ± 194 | 457–2548 | 1073 ± 156 | 498–2655 |
| ∑DDT[b] | 494 ± 64.0 | 184–889 | 480 ± 60.0 | 263–903 |
| ∑CHL[c] | 62.6 ± 11.6 | 17.5–134 | 74.0 ± 12.1 | 25.0–155 |
| ∑PBDE[d] | 21.3 ± 5.44 | 8.66–67.5 | 19.3 ± 2.21 | 8.23–35.4 |
| ∑MeO-PBDE[e] | 1.00 ± 0.34 | 0.36–4.30 | 0.67 ± 0.10 | 0.31–1.73 |
| ∑OH-PCB[f,*] | 22.6 ± 4.75 | 7.96–54.6 | 11.9 ± 2.16 | 2.54–25.9 |
| ∑OH-PBDE[g] | 0.44 ± 0.08 | 0.12–0.79 | 0.33 ± 0.07 | 0.06–1.05 |

*Statistically different ($P < 0.05$) between males and females.

[a] ∑polychlorinated biphenyl (∑PCB): CB28, 31, 42, 44, 52, 60, 64, 66/95, 70, 74, 97, 99, 101, 105, 110, 118, 128, 129/178, 138, 141, 146, 149, 151, 153, 158, 170/190, 171/202/156, 172, 174, 177, 179, 182/187, 180, 183, 194, 195, 200, 201, 203 and 206.

[b] ∑dichloro-diphenyl-trichloroethane (∑DDT): *p,p′*-DDT, *p,p′*-DDD and *p,p′*-DDE.

[c] ∑chlordane (∑CHL): oxychlordane, *cis*-chlordane and *trans*-chlordane.

[d] ∑polybrominated diphenyl ether (∑PBDE): BDE28, 47, 66, 85, 99, 100, 138, 153, 154 (co-elution with brominated biphenyl 153) and 183.

[e] ∑methoxylated (MeO)-PBDE (∑MeO-PBDE): 2′-MeO-BDE28, 4-MeO-BDE42, 6-MeO-BDE47, 3-MeO-BDE47, 4′-MeO-BDE49 and 6-MeO-BDE90/6-MeO-BDE99.

[f] ∑hydroxylated (OH)-PCB (∑OH-PCB): 4-OH-CB146, 3′-OH-CB138, 4-OH-CB163, 4-OH-CB178, 4-OH-CB187, 4′-OH-CB202, 4′-OH-CB201, 4′-OH-CB199, 4,4′-diOH-CB202 and 4′-OH-CB208.

[g] ∑OH-PBDE: 4-OH-BDE42, 6-OH-BDE47, 3-OH-BDE47, 4′-OH-BDE49, 6′-OH-BDE49 and 2′-OH-BDE68.

appropriate fractions isolated from the final sample extracts on a gas chromatograph (GC) (Agilent 6890; Agilent Technologies, Palo Alto, CA, USA) equipped with a splitless injector (Agilent 7673; Agilent Technologies) and a $^{63}$Ni micro-electron capture detection (μECD) detector. Compound separation was completed using a fused silica DB-5 capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA) with helium as the carrier gas and 5% methane/95% argon makeup gas.

For PBDEs, MeO-PBDEs and OH-PCBs/PBDEs the samples were analyzed by GC-quadrupole mass spectrometry (GC-MS) (Agilent 6890; Agilent Technologies). The GC-MS was operated in the negative chemical ionization (NCI) mode using methane as a buffer gas to facilitate electron capture negative ionization [GC-MS(ECNI)], and in the selected ion-monitoring (SIM) mode. The GC parameters were identical to those of the GC-μECD. The GC-MS(ECNI) compound separation was achieved using a fused silica DB-5 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific).

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 PCB, DDT and CHL quantification using CB83 and CB122 as recovery surrogates. An internal standard (IS) approach was used for PBDEs and MeO-PBDEs (IS was BDE71), OH-PCBs (IS was a 12-congener $^{13}$C-OH-PCB mixture) and OH-PBDEs (IS was 2′-OH-BDE28). Quality assurance and quality control procedures included method blanks and duplicate extractions and injections of standard material and cleaned-up glaucous gull plasma extracts for each block of 5–10 samples to monitor for quantitative reproducibility and instrument sensitivity. Mean recoveries (±1 standard error) based on the added ISs and recovery surrogates were 79 ± 4% for CB83 and CB122, 78 ± 5% for BDE71, and 91 ± 4% for the $^{13}$C-OH-PCB mixture and 2′-OH-BDE28. The duplicate extractions and injections demonstrated on average 15% and 5%, respectively, analytical variation of selected compound concentrations. The instrumental limit of detection for individual compounds was determined as three times the signal-to-noise ratio (S/N). The method limit of quantification (MLOQ) was determined as three times the standard deviation of the average blank signal in the case where a compound was present in blanks. When blanks were clean of contamination, three times the standard deviation of the lowest spiked standard was used for the MLOQ calculation.

### 2.3. Basal metabolic rate measurements

BMR, measured as the volume of $O_2$ ($VO_2$) consumed per unit time (ml $O_2$ $min^{-1}$), was determined using open-circuit respirometry. Because glaucous gull males and females alternate between nest attendance and feeding bouts, and thus have an unpredictable rest phase in their circadian cycle, BMR measurements were performed at any time during the day (or night). Nevertheless, BMR measurements were carried out exclusively within the thermoneutral zone of the glaucous gull, i.e., at ambient air temperatures of 2 °C or more (Gabrielsen and Mehlum, 1989). Briefly, birds were placed into an outdoor opaque metabolic chamber (volume of approximately 42 L) in which fresh dry air was pumped at a flow rate maintained at 2.5 −3.5 L $min^{-1}$. The exact flow rate was measured using a pre-calibrated mass flowmeter (Bronkhorst Hi-Tech, Ruurlo, The Netherlands). The $CO_2$-content in the dry effluent air was quantified using a $CO_2$ analyzer (Serwomex 4400, Crowborough, UK). Both influent and effluent air was dried with drierite (Krugersdorp, South Africa). Voltage outputs from the flowmeter and $CO_2$ analyzer were recorded by a data logger (Grant Squirrel, Cambridge, UK) at 1-min intervals. Birds were kept undisturbed in the metabolic chamber until a minimum and constant $CO_2$ outflow was achieved, i.e., typically after 3–5 h. The lowest mean $CO_2$-output was calculated (assuming a $CO_2$-content of the incurrent air of 0.03%) for a 10-min period and was used to represent the BMR of an individual. Since the birds were assumed to be postabsorptive at the time of BMR determination, a respiratory quotient of 0.71 was used when converting values of $CO_2$-output to rates of $O_2$-consumption ($VO_2$) (Gabrielsen and Mehlum, 1989). The birds were weighed and then released into the colony upon completion of BMR measurements.

### 2.4. Thyroid hormone analyses

Concentrations of total (T) and free (F) $T_4$ and $T_3$ in glaucous gull plasma were determined using solid-phase radioimmunoassay (RIA) human kits (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA, USA). The analytical procedures of the RIA kits were applied without modification.

### 2.5. Statistical analyses

The selected organohalogens were analyzed as sums (∑) of closely-related congeners/compounds, with respect to chemical structures, if they were detected in 60% or more of the samples. For these compounds, the samples with concentrations below the MLOQ were assigned a randomly generated value between zero and the compound-specific MLOQ. Concentrations of all organohalogen classes, BMR and TH levels were $\log_{10}$-transformed to meet the criterion of normal distribution.

The explanatory effects of organohalogen concentrations and environmental and biological variables (covariables) on the BMR and TH variation were investigated using principal component analysis (PCA) on the correlation matrix and general linear models (GLMs) using the statistical package Statistica® (StatSoft, Tulsa, OK, USA). The strength and significance of the associations between the selected variables and the principal components (PCs) extracted was assessed using the correlation coefficients (i.e., PC loadings). The covariables selected for the analyses, owing to their potential regulatory roles and/or indirect influence on BMR and TH oscillations, were: day of BMR measurement during the incubation period (day 1 through day 28), time of day (or night) at the moment of BMR measurement, mean ambient temperature and body condition. Body condition of a bird was defined as the residual obtained when the observed body mass was regressed against a body size index extracted using PCA from four morphological measurements: wing length, bill height, tarsus length and total head and bill length. Body condition was generated separately for males and females, as the glaucous gull is sexually dimorphic (Løvenskiold, 1964). In order to control for the variation in extractable lipid content among plasma samples, lipid percentage was included as covariate in the GLMs (Type III sums of squares) if it was significant predictor of

wet weight-based organohalogen concentrations. Furthermore, because sex-specific differences in circulating TH levels (Verreault et al., 2004) and patterns/concentrations of legacy and emerging organohalogens were previously reported in glaucous gull plasma (Verreault et al., 2005a,b), the effect of sex was controlled for in the GLMs. Correlations between two variables were expressed using partial $r$, i.e., the correlation controlled for the effects of one or more predictors (i.e., sex and/or lipid percentage).

## 3. Results

### 3.1. Organohalogens

The selected chlorinated and brominated compound classes (i.e., PCBs, DDTs, CHLs, PBDEs, MeO-PBDEs and OH-PCBs/PBDEs) monitored in glaucous gull plasma were detected at mean sum concentrations ranging from 0.33 to 1183 ng $g^{-1}$ wet weight (Table 1). The congener/compound concentrations and profiles of these organohalogens in glaucous gull plasma have been comprehensively reviewed elsewhere, and thus will not be further described here as they showed great consistency with those related studies (Verreault et al., 2004, 2005a,b). However, the only contradictory result with these studies was the non-significant difference in ∑PCB, ∑DDT and ∑CHL concentrations between males and females. Concentrations of organohalogens in glaucous gull plasma were not influenced statistically by any of the selected biological and environmental covariables (i.e., day of BMR determination, time of day, mean ambient temperature and body condition).

### 3.2. Basal metabolic rate

The mean and range of body mass and BMR values of glaucous gull males and females are listed in Table 2. BMR (ml $O_2$ $min^{-1}$) was also calculated on a mass-specific basis (ml $O_2$ $g^{-1}$ $h^{-1}$). Because males were heavier than females ($F_{1,21} = 115.5$; $p < 0.001$), and because body mass had a positive association with non-mass-specific BMR variation ($F_{1,21} = 9.76$; $p = 0.005$), males exhibited significantly higher non-mass-specific BMR than females ($F_{1,21} = 6.45$; $p = 0.02$). Mass-specific BMR, however, did not differ between sexes of glaucous gulls, although it showed a weak tendency to be higher in females ($F_{1,21} = 2.33$; $p = 0.14$). Nevertheless, in order to control for the effect of body mass in BMR comparisons, and thus to minimize mass-related differences between and among sexes, mass-specific BMR (hereafter referred to as BMR only) was used in further statistical treatments. Neither day of BMR measurement, time of day, mean ambient temperature, nor body condition were significant predictors of BMR oscillations in the investigated birds. Measurements of BMR in the present glaucous gulls (Table 2) were within the upper range previously reported for this species breeding in the Norwegian Arctic (mean: $0.88 \pm 0.07$ ml $O_2$ $g^{-1}$ $h^{-1}$, $n = 9$; Gabrielsen and Mehlum, 1989).

Principal component analysis of the variables BMR, TH levels and the selected organohalogen classes yielded three principal components (PCs) with eigenvalues greater than 1.5. The graphic representation of the first two PCs, PC I and PC II, which accounted for 52.8% of the total variation, is shown in Fig. 1. The scatterplot of PC I vs. PC II illustrated that in the present glaucous gulls BMR was negatively, although weakly, associated with concentrations of the persistent chlorinated compound classes ∑PCB, ∑DDT and ∑CHL. The combined concentrations of ∑PCB, ∑DDT and ∑CHL made up on average 91% (range: 89–95%) of the total sum of organohalogens selectively monitored in plasma. In contrast, the brominated substances ∑PBDE and ∑MeO-PBDE, as well as the more polar ∑OH-PCBs, were clustered along the PC I axis and showed no significant relationship with BMR.

Table 2
Mean (±1 SE) and range of body mass, basal metabolic rate (BMR) and plasma levels of total and free thyroxine ($T_4$) and triiodothyronine ($T_3$) in glaucous gull males and females from the Norwegian Arctic

| | Males ($n = 11$) | | Females ($n = 12$) | |
|---|---|---|---|---|
| | Mean ± SE | Range | Mean ± SE | Range |
| Body mass (g)* | 1738 ± 34.0 | 1570–1920 | 1363 ± 15.0 | 1280–1460 |
| BMR (ml $O_2$ $min^{-1}$)* | 35.2 ± 1.66 | 27.5–45.4 | 30.2 ± 1.11 | 25.2–38.2 |
| BMR (ml $O_2$ $g^{-1}$ $h^{-1}$) | 1.21 ± 0.05 | 1.01–1.50 | 1.33 ± 0.05 | 1.08–1.64 |
| Total $T_4$ (nmol $L^{-1}$) | 22.4 ± 1.67 | 15.1–36.6 | 22.7 ± 1.37 | 13.9–31.0 |
| Free $T_4$ (pmol $L^{-1}$) | 24.3 ± 2.16 | 16.0–44.2 | 25.3 ± 1.55 | 16.7–34.3 |
| Total $T_3$ (nmol $L^{-1}$)* | 4.02 ± 0.41 | 1.80–6.60 | 2.69 ± 0.31 | 0.90–5.10 |
| Free $T_3$ (pmol $L^{-1}$)* | 4.71 ± 0.57 | 2.30–7.90 | 2.96 ± 0.42 | 0.80–6.70 |

*Statistically different ($P < 0.05$) between males and females.

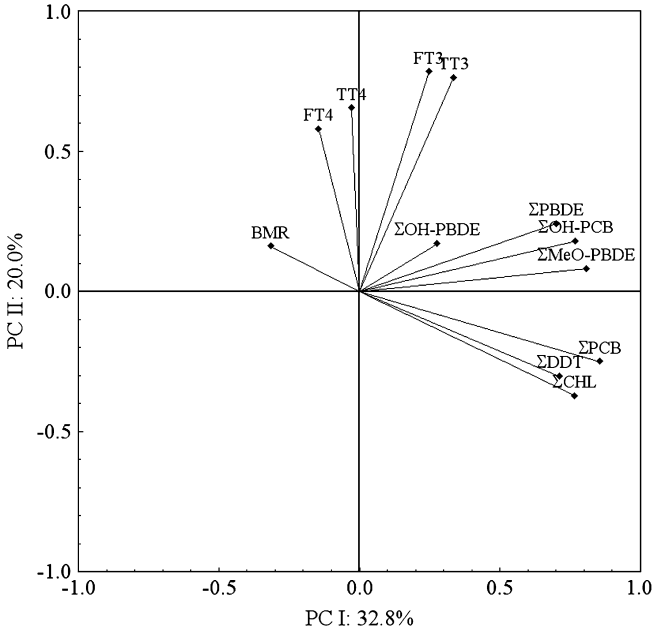


Fig. 1. Ordination diagram based on principal component analysis on the correlation matrix of basal metabolic rate (BMR), total (T) and free (F) thyroxine ($T_4$) and triiodothyronine ($T_3$) levels, as well as sum (∑) concentrations of polychlorinated biphenyl (∑PCB), dichloro-diphenyl-trichloroethane (∑DDT), chlordane (∑CHL), polybrominated diphenyl ether (∑PBDE), methoxylated-PBDE (∑MeO-PBDE) and hydroxylated-PCB and -PBDE (∑OH-PCB and ∑OH-PBDE) for male and female glaucous gulls. The relative percentage of the total variance explained by each of the principal components (PCs) is given. See Table 1 for congener/compound composition of organohalogen sums.

∑OH-PBDE concentrations were not distinguished by significant loadings on any of PC I, PC II or PC III.

Regression analyses in GLMs showed the concentrations of ∑CHL in a model comprising ∑PCB, ∑DDT and the covariate sex was significant contributor to the variation of BMR (Table 3). The overall fit of the current model, which was the best significant whole model tested, showed 45% ($F_{4,18} = 3.61$; $p = 0.02$) of the variability around the BMR mean was explained by these variables. No significant interaction was found between the predictor variables tested. ∑CHL concentrations were negatively correlated with BMR (partial $r = -0.56$, $p = 0.01$) (Fig. 2). The composition of individual CHL components making up ∑CHL did not vary consistently among birds, and was not related to variation in BMR values.

### 3.3. Thyroid hormones

Levels of circulating $TT_3$ and $FT_3$ were 49% and 59%, respectively, higher in male glaucous gulls in comparison to females ($F_{1,21} \geq 5.54$; $p \leq 0.03$) (Table 2). No such sex-specific difference was observed for $FT_4$ and $TT_4$ concentrations (Table 2). The covariables day of BMR measurement, time of day, mean ambient temperature, and body condition did not contribute significantly to TH variation in bird plasma. Present levels of circulating total and free $T_4$ and $T_3$ were within a range similar to that previously reported for incubating glaucous gulls from the Norwegian Arctic (Verreault et al., 2004).

The PCA plot did not show significant associations between levels of free and total $T_4$ and $T_3$ and BMR (Fig. 1). Moreover, $T_4$ or $T_3$ levels of any description were not associated with concentrations of the determined classes of organohalogens. However, regression analyses showed the concentrations of ∑DDT corrected for sex and lipid percentage were negatively related, although not significantly, with the $TT_4$ to $TT_3$ concentration ratios ($TT_4/TT_3$) (partial $r = -0.39$, $p = 0.07$). Similar negative, but non-significant, trends were found between $TT_4/TT_3$ ratios and ∑CHL and ∑PCB concentrations.

## 4. Discussion

The results from the present study have shown that BMR, as a general proxy for the energy requirement for physiological maintenance, may be altered in glaucous gulls exposed to high loadings of persistent and bioaccumulative organohalogens, with possible enhanced contributions from CHL, PCB and DDT loadings. Current reduction of BMR in glaucous gulls was indeed supported by outcomes of experimental designs in which avian species were exposed to organochlorine compounds. In fact, a few research groups have reported reduced metabolic rate in birds fed high doses of PCBs and DDTs; for example, in mourning doves (Tori and Mayer, 1981), pigeons (Jefferies et al., 1971) and lesser black-backed gulls (Jefferies and Parslow, 1972). These results were corroborated by two human studies in which the decrease in metabolic rate observed in subjects after weight loss was associated statistically with an increase in plasma concentrations of organochlorines (e.g., PCBs, DDTs, CHLs and hexachlorobenzene [HCB]) (Pelletier et al., 2002; Tremblay et al., 2004). In contrast, other experimental studies in mammalian species have reported conflicting observations that indicated either increased (Braham and Neal, 1974; Voltura and French, 2000) or unchanged (French et al., 2001) metabolic rate following short- and/or long-term exposure to PCBs and DDTs. Despite the scarcity of studies available and dissimilarities in methodological designs, it cannot be completely ruled out that the effect of organohalogen exposure on bioenergetics of endotherms are likely species-specific.

Hitherto, the experiments performed in birds and mammals that have investigated a dose-dependent response of basal metabolism following contaminant exposure have been limited to chlorinated contaminants. Therefore, structure-activity relationships have yet to be defined for organohalogenated environmental contaminants with respect to physicochemical properties and type of halogenation (e.g., chlorinated vs. brominated) or the presence or absence of more polar, phenyl-substituted moieties (e.g., methoxyl and hydroxyl). Nonetheless, our data suggested that the BMR variation in glaucous gulls exposed to a complex cocktail of chlorinated and brominated organohalogens in the arctic marine food

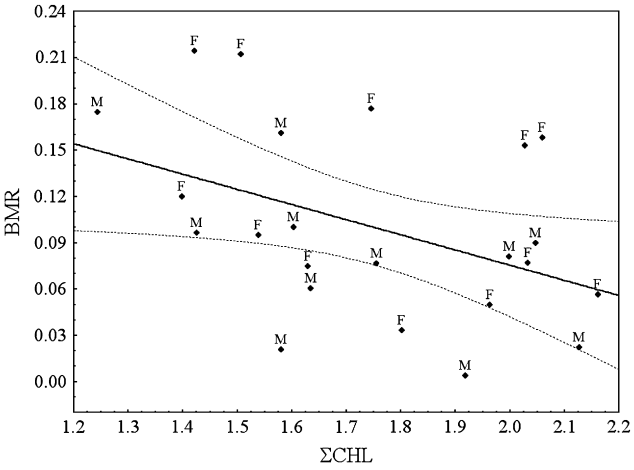


Fig. 2. Raw plot of basal metabolic rate (BMR) (ml $O_2$ $g^{-1}$ $h^{-1}$) as a function of sum (∑) plasma concentrations of chlordane (∑CHL) (ng $g^{-1}$ wet weight) compounds in glaucous gull males (M) and females (F). Data are $log_{10}$-transformed.

Table 3
Results from general linear models (Type III sums of squares) showing the explanatory effects of $log_{10}$-transformed ∑polychlorinated biphenyl (∑PCB), ∑dichloro-diphenyl-trichloroethane (∑DDT) and ∑chlordane (∑CHL) concentrations on the variation of basal metabolic rate (BMR) in glaucous gull males and females from the Norwegian Arctic

| | Degrees of freedom | Mean square | *F* Statistic | *p* Value |
|---|---|---|---|---|
| Intercept | 1 | 0.001 | 0.32 | 0.58 |
| ∑PCB | 1 | 0.003 | 1.04 | 0.32 |
| ∑DDT | 1 | 0.005 | 1.98 | 0.18 |
| ∑CHL | 1 | 0.02 | 8.19 | 0.01 |
| Sex | 1 | 0.02 | 6.49 | 0.02 |
| Error | 18 | 0.003 | | |

web may be particularly sensitive to higher concentrations of PCBs, DDTs and particularly CHLs. Alternatively, because the combined concentrations of these three distinct organochlorine classes made up on average 91% of the total, currently known organohalogen burden in glaucous gulls, the negative associations with BMR may have arisen as a result of the overall high levels of contamination. Nonetheless, other organohalogen residues and retained degradation products that have not been accounted for in the present study also may have modulation potential, via similar or differing mechanisms, on the regulating functions of BMR. However, present field study did not demonstrate causality. Hence, it was not possible to expand on whether the observed decline in BMR of glaucous gulls may have occurred via direct induction of the determined organohalogen compounds on metabolic systems or on their primary controllers, the THs (Danforth and Burger, 1984; McNabb, 2000). In fact, BMR variation in glaucous gulls was not predicted statistically by total or unbound circulating levels of $T_4$ or $T_3$. Few other studies have attempted to explain the variation in metabolic rate as a function of circulating TH levels in birds, although they have showed somewhat contradictory results. For example, Burger and Denver (2002) found no correlation between plasma TH levels and BMR in northern cardinals (*Cardinalis cardinalis*). Concurrently, Chastel et al. (2003) showed significant positive relationships between plasma $T_3$ levels and BMR in free-ranging house sparrows (*Passer domesticus*). However, since BMR in the present study was established approximately 3–5 h following sampling of blood utilized for TH quantification (see Section 2), plasma TH status of glaucous gulls at the time of BMR determination might have been slightly different. It has been documented that in birds, $T_4$ and $T_3$ half-lives are equivalent and relatively short (3–9 h) (McNabb, 2000). Moreover, the present lack of association between TH levels and BMR also may be a consequence of relative low number of birds investigated.

Recently, growing evidence has suggested that avian species exposed to chemicals of environmental concern may be susceptible to alteration in TH homeostasis in the circulatory system and thyroid gland (Dawson, 2000; McNabb, 2005; Scanes and McNabb, 2003). Yet, the present glaucous gull investigation failed to support this assumption as no significant association was found between plasma total and free $T_4$ and $T_3$ levels, and concentrations of organohalogens and metabolites known to have a higher potential to modulate circulating TH levels. Interestingly, current results were in partial disagreement with previous findings from our own glaucous gull research group that indicated marked and significant reduction in total and free $T_4$ levels, associated with a slight increase in $T_3$ levels, with increasing concentrations of specific organochlorines (Verreault et al., 2004). Such a discrepancy between the present results and those in the Verreault et al. (2004) study, in which a larger number of samples ($n = 66$) were obtained from breeding individuals in analogous colonies at Bear Island, could be a consequence of unequal sample sizes. Alternatively, given that the extrinsic conditions were constant between the breeding seasons, it cannot be completely disregarded that biological factors such as population differences in diet (e.g., iodine content), nutritional status, age, activity level, and perhaps also inherited sensitivity of the thyroid system to organohalogens, may have had influence on the results. On the other hand, somewhat consistent findings between our present and former assessments of glaucous gulls emerged through examinations of circulating TH concentration ratios. In fact, concentrations of specific organochlorines classes (e.g., DDTs) in glaucous gulls were previously shown to have significant negative effect on $TT_4/TT_3$ concentration ratios (Verreault et al., 2004). The same trend was confirmed in the present dataset although it did not comply with the criterion of significance.

The general understanding of thyroidotoxicity in vertebrates suggests that structural resemblance of certain organohalogens with THs may distort circulating TH levels through competitive interaction with binding sites on their carrier proteins (Brouwer et al., 1998). Competitive binding affinity of a suite of organochlorine compounds, as well as PBDEs and OH-PCBs/PBDEs, with the $T_4$ transport protein transthyretin (TTR) has been reported in a number of in vivo and in vitro studies (Hakk and Letcher, 2003; Legler and Brouwer, 2003; Legler et al., 2002; Letcher et al., 2000; Meerts et al., 2000). It could be suggested that the displacement of $T_4$ from TTR binding sites facilitates the excretion of the unbound $T_4$ fraction in urine or bile, thereby decreasing the proportion of circulating $T_4$ relative to $T_3$, thus leading to a decline in the $TT_4/TT_3$ ratio. Alternatively, declining $TT_4/TT_3$ ratios with increasing plasma organohalogen concentrations could be in part explained by disruption of peripheral conversion of $T_4$ to $T_3$ mediated via mono-deiodinase enzymes (Brouwer et al., 1998).

Organohalogen-mediated impairment of BMR in the glaucous gull during the breeding season, which is a critical energy-exigent phase in the annual cycle of a bird, may have particular consequences on its total energy budget and thermoregulation. Any reduction in BMR that is not compensated by additional energy mobilization would ultimately impinge on the ability of a glaucous gull to respond to severe temperature changes by maintaining energetic homeostasis. This would add to the behaviors of high energetic cost of glaucous gull pairs associated with protection of the nest from predators and conspecifics. Hence, depressed basal metabolism in situations of repeated, stringent environmental conditions and stressful events (nest defense) could result in insufficient energy allocated by parent birds for production. This includes components of feeding rate (fat storage), heat transfer to eggs during incubation as well as heating of the chick and brooding. Overall, the likely endpoints of altered energetic budget in glaucous gulls may be poor reproductive success and adult survival. From a long-term perspective, this would have critical impact on the population recruitment and status of this arctic top-predator seabird species. It has recently been reported that in glaucous gulls, blood residues of PCBs, HCB and the *cis*- and *trans*-chlordane metabolite oxychlordane were related to reduced nest site attentiveness, reproductive performance and adult survival (Bustnes, 2006; Bustnes et al., 2005). Coincidentally, annual monitoring of

Bear Island glaucous gull colonies have revealed increasing number of dead or dying adults toward the end of the incubation and beginning of chick-rearing period (Strøm, H., personal communication).

## 5. Conclusions

The results from the present investigation suggest that variation in energy balance, measured as BMR, could be perceived as a valuable biomarker in health risk assessments of glaucous gull populations from the Norwegian arctic marine environment. We conclude that modification of adaptive thermogenic capacity in breeding glaucous gulls, as a potential result of exposure to enhanced environmental organohalogen contamination, may pose physiological constraints on vulnerable individuals in the context of a predicted changing arctic climate. Additional research is needed to determine whether organohalogen contaminants and their degradation products have impacts on the daily energy expenditure, or field metabolic rate, of glaucous gulls, i.e., the energetic costs associated with routine activities (e.g., swimming, brooding, foraging and flight) in individuals throughout a day.

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