2016

Short-term fasts increase levels of halogenated flame retardants in tissues of a wild incubating bird

S. C. Marteinson

K. G. Drouillard
University of Windsor

J. Verreault

Short-term fasts increase levels of halogenated flame retardants in tissues of a wild incubating bird

Sarah C. Marteinson[1], Ken G. Drouillard[2] and Jonathan Verreault[1*]

[1] Centre de recherche en toxicologie de l'environnement (TOXEN), Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succursale Centre-ville, Montreal, QC, Canada, H3C 3P8

[2] Great Lakes Institute for Environmental Research (GLIER), University of Windsor, 401 Sunset Avenue, Windsor, ON, Canada, N9B 3P4

[*] Corresponding author. Mailing address: Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, Succursale Centre-ville, Montréal, Québec, H3C 3P8, Canada. Tel. +514-987-3000 ext. 1070; Fax: +514-987-4647; E-mail address: verreault.jonathan@uqam.ca

Abstract

Many species are adapted for fasting during parts of their life cycle. For species undergoing extreme fasts, lipid stores are mobilized and accumulated contaminants can be released to exert toxicological effects. However, it is unknown if short-term fasting events may have a similar effect. The objective of this study was to determine if short successive fasts are related to contaminant levels in liver and plasma of birds. In ring-billed gulls (*Larus delawarensis*), both members of the pair alternate between incubating the nest for several hours (during which they fast) and foraging, making them a useful model for examining this question. Birds were equipped with miniature data loggers recording time and GPS position for two days to determine the proportion and duration of time birds spent in these two activities. Liver and plasma samples were collected, and halogenated flame retardants (HFRs) (PBDEs and dechlorane plus) and organochlorines (OCs) (PCBs, DDTs, and chlordane-related compounds) were determined. Most birds (79%) exhibited plasma lipid content below 1%, indicating a likely fasted state, and plasma lipid percent declined with the number of hours spent at the nest site. The more time birds spent at their nest site, the higher were their plasma and liver concentrations of HFRs. However, body condition indices were unrelated to either the amount of time birds fasted at the nest site or contaminant levels, suggesting that lipid mobilization might not have been severe enough to affect overall body condition of birds and to explain the relationship between fasting and HFR concentrations. A similar relationship between fasting and OC levels was not observed, suggesting that different factors are affecting short-term temporal variations in concentrations of these two classes of contaminants. This study demonstrates that short fasts can be related to increased internal contaminant exposure in birds and that this may be a confounding factor in research and monitoring involving tissue concentrations of HFRs in wild birds.

Keywords: PBDEs; PCBs; Dechlorane Plus; lipid mobilization; Fasting; birds; incubation

Funding sources

Natural Sciences and Engineering Research Council of Canada (NSERC: grant #385787) (J.V.)

Canada Research Chair in Comparative Avian Toxicology (grant #225707 J.V.)

Fonds québécois de la recherche sur la nature et les technologies (to S.C.M.).

Research Approval

All experimental protocols with the ring-billed gull were approved by the Institutional Committee on Animal Care of the Université du Québec à Montréal.

Introduction

For many vertebrate species, chronic and short term fasting occurs regularly as part of their life cycle such as during hibernation, post-weaning, incubation, moulting, and migration, or can be part of regular seasonal restrictions in food availability or foraging frequency. During periods of fasting or starvation, animals mobilize lipid reserves to fuel their daily energy requirements. Depending on the species, body state and size of lipid stores, this metabolic state can last anywhere from several hours to months (Wang et al. 2006). Because many halogenated organic contaminants are predominantly lipid soluble and thus bioaccumulative in lipid-rich tissues, they can be released through lipid mobilization leading to increased circulating levels (Birnbaum 1985). Studies to date on the effects of fasting on contaminant toxicodynamics in free-ranging animals have focused mainly on species that undergo dramatic fasting events where individuals loose a large proportion of their total body lipids such as polar bears (*Ursus maritimus*) during hibernation (Polischuk et al. 2002) and female eider ducks (*Somateria mollissima*) that fast for several weeks during incubation (Bustnes et al. 2010). In these situations, lipid mobilization during fasting can lead to increased circulating levels of contaminants as they are released from fat stores (Birnbaum 1985). Several studies have reported increased enzyme-mediated liver metabolism of contaminants following fasting, further confirming that increased organismal exposure occurs during these natural food deprivation phases (Helgason et al. 2010; Jorgensen et al. 1999; Routti et al. 2013; Vijayan et al. 2006).

The effects of less severe fasting events on contaminant concentrations in birds have received limited attention to date particularly for wildlife species undergoing mild fasting events. Many birds are not adapted for long-term fasting and thus they must forage frequently to cover their high daily energy requirements. In such species, even short-term or milder fasts have the

potential to enhance lipid mobilization, which may as a result impact internal contaminant concentrations (e.g. Routti et al. 2013). However, herring gull chicks (*Larus argentatus*) exposed to a mixture of environmental contaminants and subsequently fed a reduced (by 70%) but not fully restricted diet for one week, demonstrated a 10% loss in body lipids coupled with an increase in contaminants and their metabolites in liver, brain, and plasma (Routti et al. 2013). This study showed that even incomplete fasting can reduce body fat stores, increase lipid mobilization and associated contaminant release in birds that are not adapted for high lipid storage. Conversely, white crowned sparrows (*Zonotrichia leucophrys*) exposed to 1,1,1-trichloro-bis(4-chlorophenyl)ethane (*p,p'*-DDT) in the lab for 5 days and subsequently fasted did not show increases in *p,p'*-DDT or its metabolites, *p,p'*-DDD and *p,p'*-DDE, in various tissues following 20 minute, 4 or 9 hour fasting regimes despite the loss of up to 19% of their body mass (Scollon et al. 2012). Further research on different contaminants, in different species and under varying fasting regimes is clearly needed to resolve this question.

Once fat-soluble contaminants are released from their association with lipids, they enter circulation and may be redistributed into target organs and tissues to exert toxicity (e.g. Bigsby et al. 1997). This means that during periods of fasting, animals may be at an increased risk of adverse effects which may be compounded by other ecological and physiological challenges including or resulting from food shortage (Wingfield 1994), migration, reproduction, or disease (Hall et al. 2008; Keller et al. 2006). Which species are at increased risk of exposure from contaminant release from fat stores, and in what life cycle phases this may occur, are still not well understood nor are the toxicokinetics of different contaminant classes in this situation.

The objective of the present study was to determine if short successive fasts related to incubation bouts were linked to contaminant (chlorinated and brominated) concentrations in liver

and plasma of an omnivorous bird species, the ring-billed gull (*Larus delawarensis*). We used ring-billed gulls breeding near the metropolis of Montreal (QC, Canada) because they have been shown to accumulate elevated levels of halogenated flame retardants (HFRs) (Gentes et al. 2012). Moreover, this species undergoes biparental incubation (Ryder 2012), where both parents alternate between bouts of incubation (and thus fasting) that may last several hours, and foraging trips (Marteinson et al. 2015). Because these gulls do not exhibit prolonged continuous fasts (e.g., several days), we measured their time-activity budget using high-resolution GPS-based tracking during the last 24 hours prior to tissue collection. The relationships between liver and plasma contaminant concentrations and the amount of time spent fasting at the nest site or foraging away from the colony were investigated. To evaluate whether or not birds were in a fasted state, the percent plasma lipids was assessed, and the body condition was examined. We hypothesized first that, *i*) if short term fasting was to elicit lipid mobilization and increased contaminant release from lipid stores that contaminant concentrations would increase in plasma and liver as a function of the duration of time birds spend fasting at the nest site, and *ii*) that this increase would be related to changes in whole body lipid stores during fasting. Alternatively, if contaminant concentrations in plasma were dependent largely on recent dietary intake of contaminated prey, HFR and OC concentrations in blood would be expected to decline with the number of hours spent at the nest site (and thus time since feeding), or increase as the proportion of time spent foraging increased, and be related to time spent foraging in habitats of varying contamination.

Materials and Methods

Model species and fieldwork

During the incubation period (May-June 2011), ring-billed gull males (n = 16) and females (n = 13) were sampled on Deslauriers Island (45°42’45’’N, 73°26’25’’W) located in the St. Lawrence River downstream of Montreal (QC, Canada). Approximately 44,000 ring-billed gull pairs breed on this island annually (P. Brousseau, personal communication). These omnivorous gulls use the surrounding mosaic of agricultural, urban and suburban areas where they feed opportunistically (Patenaude Monette et al., 2014; Caron-Beaudoin et al., 2013). The most prominent HFRs previously determined in these ring-billed gulls were PBDEs, for which the sum of 45 congeners averaged (± SEM) 205 ± 32.0 ng/g wet weight (ww) in liver and 27 ± 4.05 ng/g ww in plasma, which is the highest level recorded in gull tissues to date in Canada (Gentes et al. 2012). Dechlorane plus (DP), a suggested deca-BDE alternative, was also determined in all of the samples at low levels in liver (*anti*-DP: 6.06 ± 1.64; *syn*-DP: 2.38 ± 0.67; (Gentes et al. 2012).

Two-hundred ring-billed gull nests with one egg were georeferenced and monitored daily. Once clutches were completed (i.e., three eggs), males or females were initially captured at random on their nests using a radio-controlled noose trap and the number of incubation days completed was back calculated. Each bird was weighed (± 0.01 g) and morphometric measurements (head, culmen, and tarsus length) were recorded using digital callipers (± 1 mm). A miniature GPS data logger (GiPSy2, TechnoSmArt, Guidonia, Roma, Italy) was then attached on two central tail feathers (rectrices), and was recovered at the second capture two to three days later. GPS units weighed 14-15 g, thus representing 2-4% of the ring-billed gull’s body mass (mean ± SEM: 478 ± 7 g), which was shown not to influence the daily energy expenditure (via field metabolic rate measurement) of ring-billed gulls for the same individuals as utilized in the present study (Marteinson et al. 2015). At the initial and second captures, both occurring during

the incubation phase, blood samples were obtained (3 mL and 8 mL for capture and recapture, respectively) using a heparinized 25-gauge needle and 10 mL-syringe. Because blood collection volume from the initial capture had to be restricted due to the body size of these birds and because their behaviour was monitored thereafter, contaminant analysis in blood was only performed on one blood sample set (the second). Following the second blood collection, birds were euthanized, sexed by gonadal examination, and the liver was collected. Adipose tissue was not harvested because it is not reliably present in large enough amounts in this species during the incubation phase (it is sparsely distributed), thus precluding chemical analysis in all individuals. In the field, blood samples were kept in amber plastic vials in a cooler, and were centrifuged in the laboratory within 10 hours to obtain plasma for chemical analysis (section 2.4). Liver samples were also kept in a cooler in the field, and stored at -20°C until chemical analysis (section 2.4). Approval for all handling and sampling procedures was obtained by the Institutional Committee on Animal Care of the Université du Québec à Montréal, which followed the Canadian Council on Animal Care guidelines.

Time-activity budget determination

The same ring-billed gull individuals were used as those for which details on the activity budget and field metabolic rate have been previously reported (Marteinson et al. 2015). Briefly, the GPS data loggers recorded geographical positions (± 5-10 m), velocity, date, and time at 4-minute intervals for two to three days (Caron-Beaudoin et al. 2013). Using combined information on velocity and position, each position was treated as an instantaneous behavioral sample for which the total time duration could be associated following Marteinson et al. (2015). Positions falling into the two activities of interest to address the present objective were defined as follows: 1) “Nest-site attendance” was assigned to all positions when birds were at the nest

site which included incubation as well as time spent near the nest (e.g., resting, guarding and/or preening), as the two behaviors could not be distinguished due to the spatial precision of the GPS data logger; and 2) "Foraging" was assigned to all positions when birds were away from the colony, but not flying. This coarse definition was used because specific activities such as walking, resting or preening similarly could not be distinguished (Marteinson et al. 2015). Though birds were GPS-tracked for 2-3 days, we assessed only positions from the last 24 hours prior to recapture. As a result, the first portion of each trajectory post-release (1-2 days) was not used in this experiment and any potentially confounding behavioural effects related to the initial capture (contention and handling) did not affect activity assessment. From the trajectories within the 24 hours prior to recapture, the proportions (%) of time spent in each of the two activities were calculated. Because birds were recaptured at the nest site, thus interrupting a bout of nest-site attendance, the number of hours that birds were attending the nest-site prior to recapture could also be determined. The proportion of time birds spent foraging was further categorized into habitat types utilized based on previous research in our laboratory (Caron-Beaudoin et al. 2013; Gentes et al. 2015; Marteinson et al. 2015): 1) agricultural areas, 2) waterways (including the St. Lawrence River and other water bodies), and 3) urban areas (including urban, suburban, landfills, and wastewater treatment plant ponds).

Body condition and nutritional state

The body condition of ring-billed gulls was estimated via two methods. First, the condition index of individuals was determined for each sex separately by plotting the first principal component based on three morphometric measures (head, culmen, and tarsus length) against body mass, from which residuals were obtained and used as body condition index. This measure is useful in estimating body condition which will depend on how both muscle and fat

masses contribute to overall body mass. Second, body composition was estimated to determine the proportion of whole body lipids. To achieve this the determination of total body water percent by measuring the dilution of deuterium-labelled water in blood after a period of 1 hour following injection (described below) was conducted. Total body water percent has been shown to be a reliable non-destructive technique to estimate body condition in birds (McWilliams and Whitman 2013) and is strongly and negatively correlated with total body lipid content in birds and mammals (Farley and Robbins 1994; Mata et al. 2006) including gulls (glaucous gulls, *Larus hyperboreus*: (Shaffer et al. 2006); Verreault et al. 2007; Fig. S1). Briefly, as described in detail in Marteinson et al. (2015), at the initial capture, 0.65 mL of water containing 37% deuterium (371,000 ppm) was injected into the pectoral muscle of the bird and after 1 hour of water equilibration in the body, a 3 mL blood sample was obtained. Duplicate 500 µL aliquot samples of plasma were separated from whole blood and were assessed for $^{2}H/^{1}H$ ratio using a Micromass Isoprime^{TM} DI gas isotope ratio mass spectrometer (IRMS) coupled to a Aquaprep^{TM} system using an equilibration method with the presence of hydrophobic platinum as a catalyst (Horita and Kendall 2004). Baseline levels of deuterium for this field site (colony) were determined from plasma of four control (uninjected) ring-billed gulls. The whole body water percent was calculated by dividing the dilution space for hydrogen (in grams of water) by body mass. The dilution space (N_d) was calculated using the plateau approach as follows: $N_d = Mol_{inj}$ $(E_{mix} - E_{inj}) / (E_{wat} - E_{mix})$ where Mol_{inj} is the moles of ^{2}H in the injectate, E_{mix} is the equilibrium enrichment of ^{2}H in water (ppm of initial enrichment), E_{wat} is the background level of isotopes from control birds (in ppm) and E_{inj} is the enrichment of the injectate (in ppm) (eqn 17.11: Speakman 1997). The percent of whole body lipids was then calculated using the linear equation determined for the relationship between total body water mass and total body lipid mass in

glaucous gulls (y = -0.91x + 69.89) which were inversely correlated (R^2 = 0.72) (data from Shaffer et al. 2006 and Verreault et al. 2007; Fig. S1). The extractable percent lipid content was determined in plasma and liver as part of the chemical analysis (see section 2.4), and was used as a measure of recently acquired lipids though diet and as a proxy of fasting state. Plasma lipid content above 1% were used as a guideline to indicate whether birds had fed recently (K. Drouillard, unpublished data), whereas those below this threshold were likely to be in a fasted state, for example, fasted ring doves were found to have an average of 0.63% lipids in plasma while fasting (Drouillard and Norstrom 2000).

Chemical analysis

Ring-billed gull liver and plasma samples (both from the second capture) were analyzed for 37 PBDE congeners (BDE-1, -2, -3, -7, -10, -15, -17, -28, -49, -66, -71, -77, -85, -99, -100, -119, -126, 138, -139, -140, -153, -154/BB-153, -171, -180, -183/Dec-604, -184, -191, -196, -197/-204, -201, -203, -205, -206, -207, -208, and -209) as well as dechloranes (Dec) (Dec-602, -603, BDE-183/-604, and -604 CB), Chlordene Plus (CP) and Dechlorane Plus (*syn*- and *anti*-DP). Sample extraction and clean-up procedures were performed based on methods described previously in detail (Houde et al. 2014; Gentes et al 2012). Briefly, 1.0 g of liver or plasma was homogenized and ground with diatomaceous earth, and spiked with 200 ppb of an internal standard mixture (BDE-30, BDE-156, ^{13}C-BDE-209, ^{13}C-*syn*-DP, and ^{13}C-*anti*-DP), and extracted with the solvent dichloromethane:*n*-hexane (50:50 volume ratio) using a pressurized liquid extraction system (Fluid Management Systems, Watertown, MA). The extractable lipid percentages in plasma and liver samples were determined gravimetrically. Samples were cleaned-up with a PBDE-free acid-basic-neutral column followed by a PBDE-free neutral alumina column (Fluid Management Systems). The identification and quantification of the

analytes was achieved using a gas chromatograph coupled to a mass spectrometer (GC-MS) (Agilent Technologies 5975C Series, Palo Alto, CA) in the electron capture negative ionization mode (ECNI). Quality assurance procedures included analysis of method blanks and standard reference material (SRM) (NIST 1947 Lake Michigan fish tissue) for each batch of ten samples. Background contamination of method blanks were low, however, blank correction was consistently performed for the following PBDEs: BDE-15, -17, -47, -49, -66, -71, -77, -85, -99, -100, -119, 126, -138, -139, -153, -154/BB-153, -171, -180, -183/Dec-604, -191, -196, -197/-204, -201, -203, and -209. The mean recovery of the internal standards in samples, blanks and SRMs were 85.8 ± 1.7% for BDE-30, 88.0 ± 1.9% for BDE-156, 51.2 ± 1.9% for ^{13}C-BDE-209, 90.0 ± 2.2% for ^{13}C-*syn*-DP and 90.2 ± 2.2% for ^{13}C-*anti*-DP. Concentrations of PBDEs and other HFRs were quantified using an internal standard approach, and thus all analyte concentrations were inherently recovery-corrected. PBDE concentrations (seven congeners) determined in SRM showed less than 22% deviation from certified values.

Levels of OCs were reanalyzed in cleaned-up fractions obtained from liver tissue only (see sample preparation procedure above) because large enough volumes of plasma could not be collected from these mid-size birds. The compounds measured were: PCBs (CB-17, -18, -33, -44, -49, -52, -70, -74, -82, -87, -95, -99, -101, -110, -118, -128, -138, -149, -151, -153, -156, -158, -170, -171, -177, -180, -183, -187, -191, -194, -195, -206, -208, and -209), dichlorodiphenyltrichloroethane (*p,p*'-DDT) and its metabolites (*p,p*'-DDD, *p,p*'-DDE), Mirex, oxychlordane, *cis*- and *trans*-nonachlor, *cis*-chlordane, pentachlorobenzene (QCB), tetrachlorobenzene (1,2,3,4-TCB and 1,2,4,5-TCB), and octachlorostyrene (OCS). The ^{13}C-*syn*-DP and ^{13}C-*anti*-DP internal standards were used to recovery-correct OC concentrations as described above. Chemical analysis was performed using a Hewlett-Packard 5890 GC with 5973

mass selective detector (GC-MSD) operated in the electron impact (EI) mode, and using selected ion monitoring (SIM). For every batch of five samples, a MSD-PCB standard prepared from Aroclor 1242, 1254 and 1260 mixtures (AccuStandards, CT), two OC pesticide standards (Supelco, PA), a method blank and one in-house reference tissue (GLIER Detroit River fish homogenate) were also analyzed. PCB concentrations (32 congeners) determined in the in-house reference tissue showed less than 26% deviation from reference values.

Data analysis

The complete data sets (including contaminant concentrations, time-activity budget, body condition indices, and percent lipids) were obtained for 16 males and 13 females for plasma contaminant concentrations and 14 males and 11 females for liver contaminant concentrations. All statistical analyses were conducted using wet weight (ww)-based concentrations of these lipophilic contaminants did not increase in proportion to tissue lipids (Hebert and Keenleyside 1995). More specifically, for liver samples, HFR concentrations were unrelated to liver lipid percents (Pearson's Correlation Analysis; $0.982 > p > 0.140$), which was consistent with OC concentrations (Spearman's Correlation Analyses; $0.810 > p > 0.071$). Plasma HFR concentrations, were correlated with plasma lipid percents (see results below), although negatively, and thus opposite to what is predicted for lipophilic contaminants according to Hebert and Keenleyside (1995). Regardless, a parallel set of relationships between lipid percents and plasma HFR concentrations based on lipid-normalized data are presented in the Supplementary Information (Fig. S5) which demonstrates similar results to those based on ww concentrations. For HFRs, PBDE congeners that were determined (i.e. above the detection limit) in both liver and plasma of all individuals as well as the sum of all analyzed congeners (Σ_{37}PBDEs) were used in analyses. The Σ_{37}PBDEs were calculated by summing the

concentrations of all congeners above their respective detection limits. The sum of dechloranes was also assessed which, depending on their detection, for plasma included levels of *syn*- and *anti*-DP, and for liver it additionally included Dec-602, -603, and -604 CB as well as CP. For OCs, PCB congeners that were quantified in liver of all individuals were also analyzed as were Σ_{38}PCBs, *p,p*'-DDE, *p,p*'-DDT, and Σnonachlors (sum of *cis*- and *trans*-nonachlor).

Relationships between contaminant concentrations (plasma or liver) and the following variables were assessed using Spearman's Rank Correlation analyses: percent lipids in either liver or plasma, body condition measures (body condition index, whole body water and lipid percents), activity measures (time spent incubating or foraging and number of hours spent at the nest-site prior to recapture); correlation analyses between body condition and activity measures or the number of incubation days completed were also assessed. Spearman's Rank Correlation analyses were used for both sexes combined due to low sample size and to control for the effect of any outliers due to the ranking of the data. The contaminants were listed in order of decreasing *r* value to rank the strength of the relationships because different congeners will have varying halogenation and lipophilicity (expected to show differing behaviour during fasting). Data were additionally split into two groups defined as follows, and compared for contaminant concentrations and condition measures using t-tests: 1) birds in the lower (25%) and upper (75%) quartile groups based on percent plasma lipids and 2) birds in the lower (25%) and upper (75%) quartile groups based on hours spent in nest-site attendance.

To determine and rank which factors influenced the sum concentrations of the contaminant classes, a series of Generalized Linear Models (GLZ) were conducted and ranked using Akaike's Information Criterion corrected for small sample sizes (AICc) (Burnham and Anderson 2002; Burnham et al. 2011). Variables that may affect contaminant levels were assessed including

306 temporal variables (capture date, Julian egg laying date), sex, body mass, % plasma lipids, and
307 activity and body condition measures (body condition index, whole body water and lipid
308 percents). Only models with one or two predictor variables as well as the intercept were
309 conducted due to the low sample size. Related variables were not included in the same model.
310 For comparison, the null model was assessed, and only models with AIC_c values lower than that
311 of the null model were retained. Additionally, models for which predictor variables for which the
312 parameter estimate 95% confidence intervals included zero were not considered. For each model,
313 the ΔAICc was calculated as well as the weight (*w*) (Burnham and Anderson 2002). Simple
314 linear regressions were also conducted with the variables for each model to generate adjusted R^2
315 values. Statistical analyses were conducted using IBM SPSS 20, and a 0.05 significance level
316 was employed where applicable. Means are reported with standard error ranges.

317 **Results**

318 *Time-activity budget*

319 The proportions of time ring-billed gulls spent in nest-site attendance and foraging were
320 consistent with those previously reported for a larger set of individuals including the present
321 birds (Marteinson et al. 2015). Birds spent 47-90% of the 24-hour tracking period prior to
322 recapture in nest-site attendance (mean: 70 ± 0.02%). The amount of time that birds had spent in
323 this activity prior to their recapture at the nest ranged from 0.2 to 18.9 hours (mean: 5.4 ± 0.2 h).
324 Birds spent from 0 to 30% of the tracking period in foraging activities (mean: 11 ± 0.02%). The
325 proportions of time ring-billed gulls spent foraging in different sites were consistent with those
326 previously reported for a larger set of individuals, including the present birds (Gentes et al.
327 2015). Gulls in the present sub-sample spent 70 ± 0.1% of time foraging in agricultural areas, 29

± 0.06 % in urban areas and 1 ± 0.01% of their time on waterways in the last 24 hours prior to capture. Activities were unrelated to body condition measures (condition index, whole body water or lipid percent).

Contaminant concentrations and profiles in liver and plasma

Concentrations of HFRs (PBDEs and DP isomers) in the present ring-billed gull plasma and liver samples collected in 2011 were comparable to those previously reported for individuals sampled in 2010 (Gentes et al. 2012). The mean Σ_{37}PBDE concentrations in the present ring-billed gulls were 138 ± 22.2 ng/g ww in liver (Fig. 1) and 32.7 ± 4.7 ng/g ww in plasma. Among these, 13 PBDE congeners were quantifiable in all individuals in both liver and plasma including tetra-BDE (BDE-47), penta-BDEs (BDE-99 and -100), hexa-BDEs (BDE-138, -153, and -154/BB-153), hepta-BDE (BDE-183), octa-BDEs (BDE-196, -197, and -201), nona-BDEs (BDE-207 and -208), and deca-BDE (BDE-209). This congener profile was dominated by BDE-209, followed by the major penta-mixture congeners BDE-99, -47, -153, and -100 in descending order in both liver and plasma (Fig. S2). The percent contributions of these five major congeners to Σ_{37}PBDE concentrations in liver samples (with almost identical proportions in plasma) were 26 ± 2%, 24 ± 1%, 18 ± 1%, 7 ± 0.4% and 6 ± 0.4%, respectively. The Σdechlorane concentrations were 6.6 ± 1.3 ng/g ww in liver (Fig. 1) and 0.5 ± 0.1 ng/g ww in plasma.

Several OCs were detected in ring-billed gull liver samples. The mean Σ_{38}PCB concentrations in the gull livers were 138 ± 16.1 ng/g ww (Fig. 1). A total of 19 PCB congeners were detected in the liver of all individuals, which included penta-CBs (CB-99, -118, -105, and -128), hexa-CBs (CB-138, -153, -158, and -156), hepta-CBs (CB-170, -171, -180, -183, and -191), octa-CBs (-194, -195, and -199) and the nona-CB-206. The congeners present in the

highest concentrations were CB-153, -138, -180, -187 and -118 in descending order (Fig. S3). Several other OCs were quantified in liver of all individuals (Fig. 1): *trans*-nonachlor (mean: 10.8 ± 1.7 ng/g ww), *cis*-nonachlor (0.7 ± 0.2 ng/g ww), *p,p*'-DDE (107 ± 12.3 ng/g ww), and *p,p*'-DDT (33.9 ± 4.7 ng/g ww) (Fig. 1). A few other OCs were detected in some of the individuals including QCB (n = 8), *cis*-chlordane (n = 7), *p,p*'-DDD (n = 5), Mirex (n = 16), and *trans*-chlordane (n = 1), whereas 1,2,4,5-TCB, 1,2,3,4-TCB, OCS, and oxychlordane were not detected in any individuals. Mean concentrations of Σ_{37}PBDEs in liver were similar to Σ_{38}PCBs in this tissue (Fig. 1).

Liver, plasma and whole body lipid contents and contaminant concentrations

The mean extractable plasma lipid percent of ring-billed gulls was 0.8 ± 0.04% (range: 0.07 – 1.4%). The majority of birds (79%; n = 23) exhibited plasma lipid content below 1%, with six individuals having percent lipids above that fasting state guideline level (Fig. 2). However, the plasma lipid percent declined significantly with the number of hours birds had spent on their nest site prior to recapture (r = -0.40, p = 0.033), which corresponded to a 0.02% decline in lipid percentage per hour of nest-site attendance (Fig. 2). Based on the patterns observed, plasma lipid contents between 0.5 and 0.8% were the most commonly encountered and no birds had plasma lipids exceeding 0.8% after their nest-site attendance lasted ≥8 hours.

The mean liver lipid percent of ring-billed gulls was 6.0 ± 0.2% (range: 4.1-8.5%). Liver lipid percent was unrelated to the number of hours birds had fasted prior to recapture. Whole body lipid content estimated by the deuterium labelled water method was 23.6 ± 2.4% (range: 17.4-28.3%). Similar to what was observed for liver lipids, whole body lipids did not show any significant relationship with the number of hours birds had fasted prior to recapture.

The plasma lipid percent was negatively associated with the plasma concentrations of several individual PBDEs (in order of decreasing *r*: BDE-154/BB-153, -209, -47, -196, -197, -201, -208, and -207; $-0.31 < r < -0.37$; $0.014 < p < 0.050$), Σ_{37}PBDEs ($r = -0.33$, $p = 0.031$) (Fig. 3B) as well as Σdechloranes ($r = -0.39$, $p = 0.022$). Similarly, liver concentrations of several individual PBDE congeners (in order of decreasing *r*: BDE-183/Dec-604, -153, -154/BB-153, -196, -197/204, -100, -47, -201, -209, and -138; $-0.33 < r < -0.43$; $0.007 < p < 0.046$). Σ_{37}PBDEs ($r = -0.40$, $p = 0.013$) (Fig. 3A) and Σdechloranes ($r = -0.35$, $p = 0.03$) were also negatively associated with plasma lipid content. Despite the variation in bromine content of the various PBDE congeners (from tetra- to deca-brominated) that were related to plasma lipid percent, correlations all showed a very similar *r*, and thus only the relationships between plasma lipid percents and Σ_{37}PBDEs in liver or plasma are displayed graphically for brevity (Fig. 3). Birds with low plasma lipid percents (25% quartile; n = 13) ranged from 0.4-0.7% and those with high plasma lipid percents (75% quartile, n = 11) had mean levels above the 1% threshold (0.93-1.43%). The mean Σ_{37}PBDEs in liver and plasma of low plasma lipid birds was 74.4 ± 19.4 ng/g ww and 41.0 ± 6.7 ng/g ww which were 2.3 and 2.1 times higher, respectively, compared to high lipid plasma birds (mean liver: 33.1 ± 10.7 ng/g ww; plasma: 19.4 ± 4.6 ng/g ww). The difference between low and high plasma lipid birds was significant for Σ_{37}PBDEs in plasma ($t_{22} = 2.55$, $p = 0.018$) with a similar, but non-significant trend for liver concentrations ($t_{18} = 1.86$, $p = 0.079$).

PCB and OC pesticide concentrations in liver were not significantly related to the lipid percents in plasma (Fig 3). For these compounds, birds grouped into low plasma lipid categories showed mean Σ_{38}PCB concentration of 161 ± 51.5 ng/g ww in liver compared to 134 ± 32.0 ng/g

for birds in the high plasma lipid category, representing a 1.2 times decrease which did not differ significantly.

Relationships between contaminant concentrations and time-activity budget

With respect to the HFRs, as the proportion of time that birds spent in nest-site attendance increased, so did their concentrations of Σ_{37}PBDEs in both liver ($r = 0.49$, $p = 0.013$) and plasma ($r = 0.44$, $p = 0.020$). Several of the major congeners in liver (in order of decreasing *r*: BDE-99, -197/204, -201, -209, -153, -47, -154/BB-153, -196, -183/Dec-604, -138, -208, -100, and -207: $0.49 < r < 0.57$; $0.003 < p < 0.033$) and plasma (in order of decreasing *r*: BDE-100, -153, -154/BB-153, -47, -209, -153, and -99: $0.39 < r < 0.43$; $0.022 < p < 0.042$) also significantly increased with the proportion of time birds spent in nest-site attendance. The positive correlation between nest-site attendance and Σ_{37}PBDE concentrations was stronger when the number of hours at the nest site before recapture was considered for both liver ($r = 0.56$, $p = 0.004$; Fig. 4A) and plasma concentrations ($r = 0.57$, $p = 0.002$; Fig. 4B, with a similar figure for lipid-corrected values in plasma: Fig. S5). Σ_{37}PBDE concentrations in this sub-sample of the colony increased on average by 18.0 ng/g ww per hour spent on the nest site prior to capture in liver and 2.5 ng/g ww on average per hour in plasma (Fig. 5). Most of the major congeners in liver (in order of decreasing *r*: BDE-153, -154/BB-153, -138, -99, -100, -197/-204, -209, -201, -183/Dec-604, -196, -207, and -47: $0.44 < r < 0.60$; $0.001 < p < 0.033$) and plasma (in order of decreasing *r*: BDE-209, -154/BB-153, -153, -99, -153, -100, -47, -196, -197, -207, -208, and -201: $0.41 < r < 0.57$; $0.002 < p < 0.032$) also increased with the number of hours spent at the nest site before recapture. Additionally, Σdechlorane concentrations in liver were positively related to the proportion of time spent at the nest-site ($r = 0.40$, $p = 0.050$). Birds with low nest site attendance

(25% quartile, n = 7) were present at the nest site for 1.5 hours or less (mean: 0.8 ± 0.4) and those with high nest-site attendance (75% quartile, n = 7) were present at the nest for 7.3 hours or more (mean: 13.2 ± 1.6 h). Low nest-site-attendance birds exhibited a mean Σ_{37}PBDE concentrations of 14.7 ± 3.0 ng/g ww in plasma and 52.7 ± 13.2 ng/g ww in liver which were 3.8 and 5.2 times lower, respectively, compared to those in the high nest-site attendance group (mean plasma: 56.2 ± 15.4 ng/g ww; mean liver: 275 ± 105 ng/g ww). Concentrations of OCs were unrelated to nest-site attendance measures or the proportion of time spent foraging. Similar to what was reported by Gentes et al. (2015) for a larger set of ring-billed gulls (which included the present individuals), the proportion of time spent foraging in agricultural and urban areas as well as in waterways were not related to HFR concentrations. Similarly, OC concentrations in liver were unrelated to the proportion of time spent in these three different foraging habitats.

Relationships with body condition measures

Body condition index (mean: -0.71 ± 3.74 g), total body water percent (mean: 51 ± 0.42%) and total body lipid percent (mean: 23.61 ± 0.38%) were not related to nest-site attendance measures, proportion of time spent foraging, number of incubation days completed or contaminant concentrations, nor were they related to one another.

Model selection for ΣPBDEs in liver and plasma

AIC analysis was conducted exclusively for PBDEs because only this contaminant class showed relationships with predictor variables as described above (Table 1). The variation in liver concentrations of Σ_{37}PBDEs were best explained by the model including the number of hours spent at the nest-site prior to recapture in combination with the capture date (w = 0.43),

which explained 38% of the variation. The remaining models that showed significant effects all had considerably lower AIC weights in comparison to the top models (w = 0.12-0.02) demonstrating their minimal effect on liver Σ_{37}PBDEs in models containing plasma lipid % (w = 0.11-0.05) and the sex (w = 0.02). The variations in Σ_{37}PBDEs concentrations in plasma were best explained by the proportion of time birds had spent in nest-site attendance (w = 0.29) which accounted for 18% of the variation. The second best model closely followed and was the same as the top model for Σ_{37}PBDEs in liver- i.e., the number of hours spent at the nest-site in combination with capture date (w = 0.22). After these two top models, AIC weights ranged from 0.15 to 0.07, thus comparatively having a minimal effect on plasma Σ_{37}PBDE concentrations. Sex explained only a small proportion of the variations in plasma Σ_{37}PBDEs (w = 0.15). Julian lay date, body mass and measures of body condition did not explain any variation in Σ_{37}PBDE concentrations in both plasma and liver. Plasma % lipids did not explain any variation in plasma Σ_{37}PBDEs.

Discussion

This study demonstrates that time-activity budgets of wild birds can influence their contaminant concentrations in liver and plasma. More specifically, the percentage of time spent in nest-site attendance, rather than the time engaged in foraging activities, was significantly related to increased plasma and liver concentrations of PBDEs and DP isomers in ring-billed gulls during the incubation period. This suggests that short nest-site attendance bouts in birds have a greater and previously underestimated influence on within-tissue concentration variations compared to foraging, at least with respect to HFRs. However, unexpectedly, a similar pattern of enrichment of OCs in liver as a function of time spent in nest-site attendance did not occur

despite the fact that log K_{ow} values of the contaminant classes are largely overlapping: e.g., 4.6-6.9 for PCBs (Han et al. 2006), 5.75 for DDT (Veith et al. 1979), and 5.7-8.3 for PBDEs (Braekevelt et al. 2003; Han et al. 2006). This may imply different causal factors contributing to short-term temporal variations in tissue-specific contaminant levels between present suite of OCs and HFRs. Plasma lipids decreased with time spent fasting as would be predicted, and HFR concentrations increased with time spent fasting which agrees in part with our first hypothesis. However, this did not occur concurrently with the required changes in body condition during fasting in these gulls, that is, there was no relationship between whole body lipids and time spent fasting (incubating) or HFR concentrations. Therefore, we must reject our first hypothesis that short-term fasting caused whole body lipid mobilization and contaminant release in these incubating gulls. The lack of relationship between time spent foraging or in different habitats, which should have varying contamination (e.g. urban vs. agricultural), with plasma or liver HFR or OC concentrations implies that we must also reject our second hypothesis predicting that recent intake of a contaminated diet may be at the basis for the relationships between HFRs and time spent fasting in these birds. These results suggest that other underlying mechanisms may explain the relationships between HFR concentrations and time spent fasting (incubating) in ring-billed gulls. These other possible mechanisms may be related to the effect of time of capture during the incubation phase or differing depuration rates related to metabolic biotransformation of individual PBDE congeners in the gulls. Further study on these avenues and others is warranted and larger sample sizes are needed.

Few studies have been conducted on the effects of fasting on contaminant concentrations in birds. A few examples demonstrate that concentrations of contaminants increase during prolonged periods of food restriction. For example, in common eiders, where the female fasts

484 continuously for four weeks during the incubation, levels of PCB-153, *p,p*'-DDE and
485 hexachlorobenzene increased between day five and day 20 in the incubation period (Bustnes et
486 al. 2010). Moreover, in overwintering greater scaup (*Aythya marila*), which experience reduced
487 availability and simultaneous reductions in body fat stores, individuals captured later in the
488 winter had higher wet weight-based levels of ΣPCBs and ΣDDTs (including DDD, DDE) than
489 birds caught early in the winter (Perkins and Barclay 1997). This effect has been confirmed even
490 in species that are not adapted for extreme fasts. For instance, herring gull chicks exposed to a
491 mixture of contaminants (OCs and PBDEs) in the laboratory and subsequently fasted for one
492 week, exhibited contaminant concentrations that were three times higher in liver, plasma, and
493 brain compared to a non-fasted exposed group (Routti et al. 2013). Changes in feeding rates and
494 body mass throughout the breeding season have similarly been associated to contaminant
495 concentrations in some seabirds, both those adapted to long fasts or not, including kittiwakes
496 (*Rissa trydactyla*) (Henriksen et al. 1996), and Adélie penguins (*Pygoscelis adeliae*) and
497 southern fulmars (*Fulmarus galacialoides*) (van den Brink et al. 1998). More specifically, in the
498 penguin and fulmar study, lower body mass during times of fast (egg-laying or incubation) or
499 times of higher physical exertion (chick-provisioning) were associated with higher levels of
500 several OCs in blood and uropygial oil compared to periods where birds were able to replenish
501 fat stores (van den Brink et al. 1998). Similarly for the kittiwakes during breeding, levels of
502 ΣPCBs in the brain were four-fold higher at the end of chick-rearing period during which they
503 lost 20% of their body mass compared to the pre-breeding period (Henriksen et al. 1996).
504 Collectively, these studies demonstrate that relationships exist between fasting and contaminant
505 concentrations, but the processes behind these remain difficult to explain.

Contaminant mobilization during tissue lipid metabolism is commonly invoked as a mechanism describing the increase in tissue contaminant concentrations post-fasting (Daley et al. 2014). When animals cannot obtain energy from food, they must draw upon stored energy reserves. The loss of lipids due to metabolism of triglycerides stored in adipose tissue, which reflects the main proximate component contributing to the partitioning capacity of organisms for hydrophobic contaminants, results in a fugacity gradient between adipose tissue and blood. This favors net diffusion of chemicals from adipose tissue until a new inter-tissue equilibrium is achieved. The process of lipid mobilization during fasting typically follows a defined sequence of events. In the first metabolic phase of fasting, stored liver glycogen and fatty acids from fat stores are used to supply glucose to tissues (Wang et al. 2006). However, these sources of energy are rapidly depleted (from hours to days) after which the organism will enter the second phase of food deprivation in which glycerol is released from adipose tissue and used for the production of glucose (Wang et al. 2006). This second phase is longer and, depending on the species and size of lipid stores, can last weeks to months (Wang et al. 2006). Fasting birds typically enter phase two after a few days of fasting - two days in red-legged partridges (*Alectoris rufai*) (Rodriguez et al. 2005) which are not well-adapted to fasting condition, four days in gentoo penguins (*Pygoscelis papua*) which are adapted to short-term fasts, and seven days in King penguins (*Aptenodytes patagonica*) which are adapted to several months of fasting (Cherel et al. 1993). Gulls (Larids) fall into the middle of this spectrum, entering phase two after approximately four to five days, as documented for example in herring (Totzke et al. 1999) and yellow-legged gulls (*Larus cachinnans)* (Alonso-Alvarez and Ferrer 2001).

During lipid loss, contaminant levels become concentrated in adipose tissue as lipids are depleted leading to an elevation in chemical fugacity. This creates a fugacity gradient between

adipose and blood that favors chemical diffusion and mobilization from the major storage compartment (fat) to other tissues in the body (Daley et al. 2014). For most bioaccumulative contaminants, inter-tissue distribution kinetics are considered rapid compared to whole body elimination. Therefore, the kinetics associated with tissue-to-tissue transfer are commonly ignored when formulating simple, non-physiologically based bioaccumulation models (Selck et al. 2013). For example, in the ring dove (*Streptopelia risoria*), rapidly perfused organs such as brain, liver and gonads achieved 90% steady state with blood PCB-153 in less than three days, while fat and carcass required ten days to achieve steady state with blood (Daley et al. 2013). In contrast, whole body elimination rates for PCB-153 in the same species was reported to be on the order of 2.9 to 3.7 years (Drouillard and Norstrom 2003). The rate at which contaminants are released from fat stores during fasting and how this differs between different chemicals remains largely unknown. Some evidence indicates that not all contaminant classes follow the same dynamics of release during lipolysis. For example, in fasting polar bears, some OCs increased in concentrations in adipose tissue during fasting including Σchlordanes and PCBs, suggesting that they are less readily released from this compartment, whereas others declined such as ΣDDTs and Σ hexachlorocyclohexanes, suggesting that they were released more easily into circulation and/or were potentially eliminated from the body at a higher rate through metabolic biotransformation (Polischuk et al. 2002). There is some evidence to suggest that contaminants with lower log K_{ow} (i.e., are less lipophilic) may be released more efficiently from blubber in grey seals (*Halichoerus grypus*) (Vanden Berghe et al. 2012).

However, in the present study, there is no evidence to support mobilization of lipid stores as the mechanism behind the relationships between HFR concentrations and time spent fasting. For these birds, the maximum time ring-billed gulls spent on their nesting site was in the order of

552 20 hours, which can be enough to weight loss in birds that are not adapted for prolonged fasting
553 (e.g. Scollon et al. 2012). However, even though individual ring-billed gulls would have
554 underwent several such relatively short fasting events over their 28 days incubation period, there
555 was no relationship between body condition expressed as whole body lipids and time spent at the
556 nesting site, body condition and number of incubation days nor between body condition
557 measures and contaminant concentrations in liver or plasma. This points to a lack of condition
558 deterioration over time during this phase and suggests that these birds remained within the first
559 metabolic phase of fasting, and were relying more heavily on fatty acids and glycogen reserves
560 compared to stored triglycerides. Loss of fatty acids from adipose tissue would also decrease the
561 partitioning capacity of the tissue, but to a lesser extent than triglycerides (Dulfer and Govers
562 1995; Dulfer et al. 1996). Ring-billed gulls forage between short-term incubation bouts, and tend
563 to exploit areas of high and predictable food availability (e.g., anthropogenic-related habitats and
564 agricultural fields) and quality (fat and protein content) (Caron-Beaudoin et al. 2013; Patenaude-
565 Monette et al. 2014). This may have allowed them to replenish labile tissue energy stores as they
566 were used up on a daily basis. This is in line with other seabird species exhibiting biparental care
567 that maintain body mass during the incubation period (e.g., Moreno 1989) despite prolonged
568 bouts of intermittent fasting at the nest. Given that whole body lipids in birds of the present study
569 did not change significantly in relation to time devoted to nest-site attendance, fugacity gradients
570 generated as a result of adipose tissue depletion cannot explain the increasing trend of plasma
571 and liver HFR concentrations with time spent at the nest site. This conclusion is reinforced by
572 the fact that OCs showed no evidence of enriched liver concentrations. Mobilization of
573 contaminants from lipid-rich tissues during weight loss would be expected to increase all
574 hydrophobic contaminant concentrations (including OCs and HFRs in present birds) in tissues to

575 a similar extent. For example, Routti and colleagues (2013) exposed herring gull chicks to a
576 mixture of environmentally relevant contaminants for 45 days and subsequently fasted (70%
577 food reduction) treatment birds for seven days to compare chemical enrichment in tissues
578 relative to non-fasted individuals (controls). The above study demonstrated that all contaminant
579 classes including ΣPCBs, ΣDDTs, ΣCHLORs and ΣPBDEs increased in liver, brain, and plasma
580 over those of the non-fasted group to a similar extent.

581 It is more likely that the relationships between HFR concentrations and time spent fasting
582 in ring-billed gulls at the nest site was related to recent contaminant exposure which may have
583 changed as the spring season progressed. Three alternative explanations would be that ring-
584 billed gulls had been: i) exposed to a "diluted meal" during foraging activities rather than the
585 contaminated diet we predicted from the habitats they use, ii) exposed to a non-dietary source of
586 HFRs at the nesting site itself or iii) exposure to PBDEs may have altered the incubation
587 behavior of these gulls. In the diluted meal hypothesis, birds returning from foraging and which
588 have fed on a relatively clean meal (low HFR concentrations) would be expected to experience
589 dilution of plasma contamination due to an influx of clean dietary lipids (e.g., portomicrons)
590 and/or production of *de novo* lipids generated from assimilated nutrients (Drouillard and
591 Norstrom 2000; Drouillard and Norstrom 2001). In this scenario, differences in the behavior of
592 different contaminant classes might be explained by the fact that the diluted meal was less
593 contaminated with HFRs, but not OCs. Based on Fig. 2, peak plasma lipid contents were
594 observed in birds had been incubating for less than a few hours prior to capture, followed by
595 depletion of plasma lipids to apparent fasting levels after eight to ten hours. This profile is very
596 similar to what was observed in ring doves after achieving C_{max} of plasma lipids following a
597 controlled feeding study that demonstrated an exponential return of plasma lipids approaching

598 fasting levels after approximately seven hours (Drouillard and Norstrom 2000). This would be
599 surprising because these birds do not rely heavily on aquatic habitats for feeding which act as
600 sinks for PCBs (reviewed in: Beyer and Biziuk 2009), but they do frequently utilize
601 anthropogenic-related habitats to forage (e.g., Caron-Beaudoin et al., 2013) which can be highly
602 contaminated with HFRs (Venier and Hites 2008). As further evidence, recent (last 24 hours)
603 use of landfills and wastewater treatment plant ponds has been linked to increased concentrations
604 of BDE-209 in these gulls nesting in the same colony as utilized in the present study (Gentes et
605 al. 2015).
606 However, the contaminant patterns in liver of ring-billed gulls do not support this
607 hypothesis. Dilution of liver lipids post-feeding is also possible given that liver is capable of
608 generating lipids from absorbed carbohydrates and proteins. Indeed, lipid-normalized
609 egg/maternal tissue concentration ratios in herring gulls and other bird species have been
610 hypothesized to occur as a result of dilution of the liver lipid pool during yolk production
611 resulting from *de novo* lipid production in the liver (Braune and Norstrom 1989; Drouillard and
612 Norstrom 2001; Norstrom et al. 1986). In the case of herring gulls, a mean lipid-normalized
613 egg/maternal tissue ratio of 0.7 was apparent, suggesting a 1.4-fold dilution factor of liver OC
614 levels during yolk production (Braune and Norstrom 1989). A much higher egg/maternal tissue
615 dilution factor of 2.9-fold was observed in ring doves which approaches the 5.2-fold differences
616 in liver HFR residues observed in ring-billed gulls from the present study. However, the period
617 of yolk formation is considered an extreme case where *de novo* lipid production in the liver is
618 expected to be maximized and does not likely reflect the situation present for incubating ring-
619 billed gulls. Second, there was no evidence for a change in liver lipid content with time at the
620 nesting site, which would be expected to be observed under a situation of lipid dilution in liver.

621 Finally, *de novo* lipid production by the liver would dilute all hydrophobic chemicals to the same
622 extent, but only HFRs and not OCs showed changes in liver concentration with time on the
623 nesting activity in ring-billed gulls. Thus, the "diluted meal" hypothesis could be considered
624 consistent with the observed trends in plasma concentrations of HFRs and lack of trends for OCs,
625 although not consistent with the patterns observed for liver.
626 The second hypothesis implies that ring-billed gulls were exposed to elevated HFR and
627 baseline OC concentrations while at the nesting site. The exposure source was apparently not
628 related to dietary exposure at the nesting site because the change in plasma lipid content with
629 time at the nest is consistent with birds reverting to a fasting state during this activity.
630 Alternative exposure routes could be as a result of inhaled HFR-contaminated dusts/particles or
631 air at the local nesting site. Assimilated material from the respiratory tract would be expected to
632 show up rapidly in perfused tissues including blood and liver, followed by slower redistribution
633 to adipose tissues (Daley et al. 2013). HFRs can be present in the atmosphere in their volatilized
634 form which is more common for lower-brominated congeners, or in association with
635 dust/particles which is more common for higher-brominated congeners including BDE-209
636 (reviewed in: Hale et al. 2006). Several HFRs including PBDEs have been measured in outdoor
637 air globally (reviewed in: Hale et al. 2006) with the highest concentrations recorded outside
638 point-source areas in urbanized environments (e.g., Venier and Hites 2008). Recently, PBDE
639 concentrations in outdoor air have begun to exceed concentrations of PCBs in several reports
640 (reviewed in: Hale et al. 2006). This is consistent with the finding that the present ring-billed
641 gulls demonstrated similar mean ΣPBDEs concentrations to ΣPCBs, the latter of which have
642 previously dominated in bird tissue. Few studies have measured HFRs in Canadian city air
643 though, recently in Toronto, air contained 38 pg/m^3 ΣPBDEs in combined gas and particle

644 phases, as well as a number of emerging HFRs including *syn*- and *anti*-DP (Shoeib et al. 2015).
645 Concentrations of contaminants in the atmosphere can be affected by temperature and weather as
646 well as other atmospheric variables (e.g. (Melymuk et al. 2012) and references therein) and since
647 these change throughout the spring season when incubation takes place in the present location,
648 this may play a role in the identified effect of capture date on PBDE concentrations in these
649 gulls. However, the diet of these birds also changes as the spring progresses (Patenaude-Monette
650 et al. 2014), and further research is required to determine how the capture date may be related to
651 contaminant exposure in these birds. The contribution of atmospheric exposure to HFR
652 contamination in wildlife is largely unknown. However, it has been confirmed as an important
653 source of exposure to humans via indoor air and house dust (reviewed in: (Hale et al. 2006)), and
654 thus wildlife living in urban areas may be receiving significant (and underestimated) exposure to
655 HFRs via air and associated particles and via ingestion of these contaminated particles during
656 preening. Further research on this avenue of exposure to HFRs in ring-billed gulls is currently
657 ongoing in our laboratory to determine how much this route of exposure may be contributing to
658 their body burdens.

659 Finally, the possibility that exposure to PBDEs may have altered the incubation or nest-
660 site attentiveness of these gulls should be explored as they have been shown to disrupt related
661 endpoints. In laboratory studies, exposure to PBDEs has caused reduced nest-related behavior
662 during courtship and brood rearing in American kestrels (*Falco sparverius*) (Fernie et al. 2008,
663 Marteinson et al. 2010), as well as reduced nest temperatures during incubation (Sullivan et al.
664 2013). Additionally, in wild male glaucous gulls, greater plasma sum PBDE concentrations were
665 associated with reductions in the hormone prolactin which regulates incubation behavior
666 (Verreault et al., 2008, and references therein). As such to date, exposure to PBDEs appears to

667 have a negative impact on incubation and prolactin levels in birds, suggesting that this may not
668 explain the positive correlation between PBDEs and time spent at the nest in the present ring-
669 billed gulls, however further research on this avenue may be warranted.
670
671 **Conclusions**
672 This study demonstrates increased HFR concentrations in ring-billed gull plasma and
673 liver tissues during the incubation period while birds were present at the nesting site. More
674 specifically, bouts of nest-site attendance in ring-billed gulls lasting only a few hours were
675 associated with significant increase in levels of PBDEs and DP isomers (liver and plasma), but
676 not OCs (liver). Conversely, the amount of time that birds were engaged in foraging activities,
677 body condition and percent body fat were unrelated to contaminant concentrations. The changes
678 in plasma HFR concentrations may be consistent with the hypothesis that birds were exposed to
679 a diluted meal with respect to HFR levels, although this would not apply for OCs. However, the
680 patterns in liver do not support this explanation because all hydrophobic organic contaminants
681 should increase in liver as fasting duration increases. Alternatively, these gulls may have been
682 significantly exposed to non-dietary sources of HFRs at the nest site (i.e., via inhalation of air
683 and HFR-laden particles or ingestion via preening of particles adsorbed to their plumage).
684 Ultimately, further study is needed to understand the increased liver and plasma HFR
685 concentrations in these birds while they spend time fasting at the nest site. However, regardless
686 of the underlying mechanisms and exposure routes, the results presented herein highlight the fact
687 that short-term fasting events related to, for example nest-site attendance, may significantly
688 influence levels of HFRs in birds, and hence the potential toxicity associated with these
689 compounds. As such, the amount of time spent in this activity just prior to capture may become

690 a confounding factor in monitoring and research on contaminants, including biological effect
691 studies in wild birds. This may also apply to a wide variety of other situations where short-term
692 fasting occurs including other reproductive phases, migration, or even daily overnight fasting.
693 Further research on how contaminant concentrations relate to these life cycle stages is warranted.
694
695 **Acknowledgements**
696 Funding for this study was provided by the Natural Sciences and Engineering Research Council
697 of Canada (NSERC: grant #385787) (J.V.), the Canada Research Chair in Comparative Avian
698 Toxicology (grant #225707 J.V.) and *Fonds québécois de la recherche sur la nature et les*
699 *technologies* (to S.C.M.). For field assistance the authors thank V. Aponte, S. Pellerin Plourde,
700 M. Patenaude-Monette, M.-L. Gentes, F. St-Pierre, and M. Tremblay. For assistance with stable
701 isotope analysis, the authors thank J.-F. Hélie and A. Adamowicz, and for ArcGIS analysis the
702 authors thank M. Patenaude-Monette and M.-L. Gentes.
703
704 **References**

705 Alonso-Alvarez C, Ferrer M. 2001. A biochemical study of fasting, subfeeding, and recovery
706 processes in yellow-legged gulls. *Physiol Biochem Zool* 74:703-713.
707 Beyer A, Biziuk M. 2009. Environmental fate and global distrbution of polychlorinated
708 biphenyls. In: Reviews of Environmental Contamination and Toxicology, Vol.
709 201:Springer, 137-154.
710 Bigsby RM, Caperell Grant A, Madhukar BV. 1997. Xenobiotics released from fat during fasting
711 produce estrogenic effects in ovariectomized mice. *Cancer Res* 57:865-869.

712 Birnbaum LS. 1985. The role of structure in the disposition of halogenated aromatic xenobiotics.
713 *Environ Health Persp* 61:11-20.
714 Braekevelt E, Tittlemier SA, Tomy GT. 2003. Direct measurement of octanol-water partition
715 coefficients of some environmentally relevant brominated diphenyl ether congeners.
716 *Chemosphere* 51:563-567.
717 Braune BM, Norstrom RJ. 1989. Dynamics of organohclorine compounds in herring gulls: Iii.
718 Tissue distribution and bioaccumulation in lake ontario gulls. *Env Toxicol Chem* 8:957-
719 968.
720 Burnham KP, Anderson DR. 2002. Model selection and multimodel inference - a practical
721 information-theoretic approach. U.S.A.:Springer.
722 Burnham KP, Anderson DR, Huyvaert KP. 2011. AIC model selection and multimodel inference
723 in behavioral ecology: Some background, observations, and comparisons. *Behav Ecol*
724 *Sociobiol* 65:23-35.
725 Bustnes JO, Moe B, Herzke D, Hanssen SA, Nordstad T, Sagerup K, et al. 2010. Strongly
726 increasing blood concentrations of lipid-soluble organochlorines in high arctic common
727 eiders during incubation fast. Chemosphere 79:320-325.
728 Caron-Beaudoin E, Gentes ML, Patenaude-Monette M, Hélie J-F, Giroux JF, Verreault J. 2013.
729 Combined usage of stable isotopes and gps-based telemetry to understand the feeding
730 ecology of an omnivorous bird in a heterogeneous landscape. *Can J Zool* 91:689-697.
731 Cherel Y, Freby F, Gilles J, Robin JP. 1993. Comparative fuel metabolism in gentoo and king
732 penguins - adaptation to brief versus prolonged fasting. *Polar Biol* 13:263-269.

733 Daley JM, Norstrom RJ, Drouillard KG. 2013. Tissue distribution kinetics of 2,2',4,4',5,5'-
734 hexachlorobiphenyl in ringdoves after oral dosing. *Bull Environ Contam Toxicol* 91:367-
735 371.
736 Daley JM, Paterson G, Druillard KG. 2014. Bioamplification as a non-steady state
737 bioaccumulation mechanism for persistent organic pollutants (pops) in wildlife. Rev
738 *Environ Contam Toxicol* 227:107-155.
739 Drouillard KG, Norstrom RJ. 2000. Dietary aborption efficiencies and toxicokinetics of
740 polychlorinated biphenyls in ring doves following exposure to arochlor mixtures. *Environ*
741 *Toxicol Chem* 19:2707-2714.
742 Drouillard KG, Norstrom RJ. 2001. Quantifying maternal and dietary sources of 2,2',4,4',5,5'-
743 hexachlorobiphenyl deposited in eggs of the ring dove (*Streptopelia risoria*). *Environ*
744 *Toxicol Chem* 20:561-567.
745 Dulfer WJ, Govers HA. 1995. Solubility and micelle-water partitioning of polychlorinated
746 biphenyls in solutions of bile salt micelles. *Chemosphere* 30:293-306.
747 Dulfer WJ, Groten JP, Govers HA. 1996. Effect of fatty acids and the aqueous diffusion barrier
748 on the uptake and transport of polychlorinated biphenyls in caco-2 cells. *J Lipid Res*
749 37:950-961.
750 Farley SD, Robbins CT. 1994. Development of 2 methods to estimate body-composition of
751 bears. *Can J Zool* 72:220-226.
752 Fernie, KJ, Shutt, JL, Letcher, RJ, Ritchie JI, Sullivan, K, Bird, DM. 2008. Changes in
753 reproductive courtship behaviors of adult American kestrels (*Falco sparverius*) exposed to
754 environmentally relevent levels of the polybrominated diphenyl ether mixture, DE-71.
755 *Toxicol Sci*, 102: 171-178.

Gentes ML, Letcher RJ, Caron-Beaudoin E, Verreault J. 2012. Novel flame retardants in urban-feeding ring-billed gulls from the St. Lawrence River, Canada. *Environ Sci Technol* 46:9735-9744.

Gentes ML, Mazerolle MJ, Giroux J-F, Patenaude-Monette M, Verreault J. 2015. Tracking the sources of polybrominated diphenyl ethers in birds: Foraging in waste management facilities results in higher decaBDE exposure in males. *Environ Res* 138:361-371.

Hale RC, La Guardia MJ, Harvey E, Gaylor MO, Mainor TM. 2006. Brominated flame retardant concentrations and trends in abiotic media. *Chemosphere* 64:181-186.

Hall AJ, Gulland FMD, Ylitalo GM, Greig DJ, Lowenstine L. 2008. Changes in blubber contaminant concentrations in california sea lions (*Zalophus californianus*) associated with weight loss and gain during rehabilitation. *Environ Sci Technol* 42:4181-4187.

Han X, Wang Z, Zhai Z, Wang L. 2006. Estimation of *n*-octanol/water partition coefficients (kow) of all PCB congeners by ab initio and a cl substitution position method. *QSAR Combin Sci* 25:333-341.

Hebert CE, Keenleyside WJ. 1995. To normalize or not to normalize? Fat is the question. *Envion Toxicol Chem* 14:801-807.

Helgason LB, Verreault J, Braune BM, Borga K, Primicerio R, Jenssen BM, et al. 2010. Relationship between persistent halogenated organic contaminants and tcdd-toxic equivalents on erod activity and retinoid and thyroid hormone status in northern fulmars. *Sci Total Environ* 408:6117-6123.

Henriksen EO, Gabrielsen GW, Skaare JU. 1996. Levels and congener pattern of polychlorinated biphenyls in kittiwakes (*Rissa tridactyla*), in relation to mobilization of body-lipids associated with reproduction. *Environ Pollut* 92:27-37.

779 Horita J, Kendall C. 2004. Stable isotope analysis of water and aqueous solutions by
780 conventional dual-inlet mass spectrometry. In: Handbook of stable isotope analytical
781 techniques, Vol. 1, (De Groot P, ed). New York:Elsevier, 1-37.
782 Houde M, Berryman D, de Lafontaine Y, Verreault J. 2014. Novel brominated flame retardants
783 and dechloranes in three fish species from the St. Lawrence River, Canada. *Sci Total*
784 *Environ* 479-480:48-56.
785 Jorgensen EH, Bye BE, Jobling M. 1999. Influence of nutritional status on biomarker responses
786 to PCB in the arctic charr (*Salvelinus alpinus*). *Aquat Toxicol* 44:233-244.
787 Keller JM, Kucklick JR, Harms CA, Segars AL, Cluse W, Godfrey M, et al. 2006. Organic
788 contaminant concentrations are higher in debilitated loggerhead turtles compared to
789 apparently healthy ones. 26th Annual Symposium on Sea Turtle Biology and Conservation
790 - Book of Abstracts - International Sea Turtle Society: 63.
791 Marteinson SC, Fernie KJ, Shutt JL, Letcher RJ, Ritchie JI, Bird DM. 2010. Embryonic exposure
792 to male American kestrels (*Falco sparverius*) to DE-71 flame retardant via maternal
793 transfer reduces reproductive success and decreases courtship and brood rearing behaviors.
794 *Environ Toxicol CHem* 29:1740-1747.
795 Marteinson SC, Giroux J-F, Hélie J-F, Gentes ML, Verreault J. 2015. Field metabolic rate is
796 dependent on time-activity budget in ring-billed gulls (*Larus delawarensis*) breeding in an
797 anthropogenic environment PLoS1. DOI: 10.1371/journal.pone.0126964
798 Mata AJ, Caloin M, Robin JP, Le Maho Y. 2006. Reliability in estimates of body composition of
799 birds: Oxygen-18 versus deuterium dilution. *Physiol Biochem Zool* 79:202-209.
800 McWilliams SR, Whitman M. 2013. Non-destructive techniques to assess body composition of
801 birds: A review and validation study. *J Ornithol* 154:597-618.

802 Melymuk L, Robson M, Helm PA, Diamond ML. 2012. PCBs, PBDEs, and PAHs in Toronto
803 air: Spatial and seasonal trends and implications for contaminant transport. *Sci Total*
804 *Environ* 429:272-280.
805 Moreno J. 1989. Strategies of mass change in breeding birds. Biol J Linn Soc 37:297-310.
806 Norstrom RJ, Clark TP, Jeffery DA, Won HT, Gilman AP. 1986. Dynamics of organochlorine
807 compounds in herring gulls: I. Distribution and clearance of [14c]-DDE in free living gulls
808 (*Laurus argentatus*). *Environ Toxicol Chem* 5:41-48.
809 Patenaude-Monette M, Bélisle M, Giroux J-F. 2014. Balancing energy budget in a central-place
810 forager: Which habitat to select in a heterogeneous environment? PLos1. DOI:
811 10.1371/journal.pone.0102162
812 Perkins CR, Barclay JS. 1997. Accumulation and mobilization of organochlorine contaminants
813 in wintering greater scaup. *J Wildlife Manage* 61:444-449.
814 Polischuk SC, Norstrom RJ, Ramsay MA. 2002. Body burdens and tissue concentrations of
815 organochlorines in polar bears (*Ursus maritimus*) vary during seasonal fasts. *Environ*
816 *Pollut* 118:29-39.
817 Rodriguez P, Tortosa FS, Villafuerte R. 2005. The effects of fasting and refeeding on
818 biochemical parameters in the red-legged partridge (*Alectoris rufa*). *Comp Biochem Phys A*
819 140:157-164.
820 Routti H, Helgason LB, Arukwe A, Wolkers H, Heimstad ES, Harju M, et al. 2013. Effect of
821 reduced food intake on toxicokinetics of halogenated organic contaminants in herring gull
822 (*Larus argentatus*) chicks. *Environ Toxicol Chem* 32:156-164.
823 Ryder JP. 2012. Ring-billed gull. In: Birds of North America, Part 2012:Cornell Lab of
824 Ornithology.

825 Scollon EJ, Carr JA, Rintoul DA, McMurry ST, Cobb GP. 2012. Metabolism and distribution of
826 *p,p'*-DDT during flight of the white-crowned sparrow, *Zonotrichia leucophrys*. *Environ*
827 *Toxicol Chem* 31:336-346.
828 Shaffer SA, Gabrielsen GW, Verreault J, Costa DP. 2006. Validation of water flux and body
829 composition in glaucous gulls (*Larus hyperboreus*). *Physiol Biochem Zool* 79:836-845.
830 Shoeib M, Ahrens L, Jantunen L, Harner T. 2015. Concentrations in air of organobromine and
831 organophosphate flame retardants in Toronto Canada. *Atmos Environ* 99:140-147.
832 Sullivan KM, Marteinson SC, Letcher, RJ, Bird DM, Ritchie IJ, Shutt, JL, Fernie KJ. 2013.
833 Changes in the incubation by American kestrels (*Falco sparverius*) during exposure to the
834 polybrominated diphenyl ether (PBDE) mixture DE-71. *J Toxicol Environ Health A* 76:
835 978-989.
836 Totzke U, Fenske M, Huppop O, Raabe H, Schach N. 1999. The influence of fasting on blood
837 and plasma composition of herring gulls (*Larus argentatus*). *Physiol Biochem Zool* 72:426-
838 437.
839 van den Brink NW, van Franeker JA, Ruiter-Dijkman EM. 1998. Fluctuating concentrations of
840 organochlorine pollutants during a breeding season in two antarctic seabirds: Adélie
841 penguin and southern fulmar. *Environ Toxicol Chem* 17:702-709.
842 Vanden Berghe M, Weijs L, Habran S, Das K, Bugli C, Rees JF, et al. 2012. Selective transfer of
843 persistent organic pollutants and their metabolites in grey seals during lactation. *Environ*
844 *Int* 46:6-15.
845 Veith GD, DeFoe DL, Bergstedt BV. 1979. Measuring and estimating the bioconcentration
846 factor of chemicals in fish. Journal of the Fisheries Research Board of Canada 36:1040-
847 1048.

Venier M, Hites RA. 2008. Flame retardants in the atmosphere near the Great Lakes. *Environ Sci Technol* 42:4745-4751.

Verreault J, Shahmiri S, Gabrielsen GW, Letcher RJ. 2007. Organohalogen and metabolically-derived contaminants and associations with whole body constituents in Norwegian Arctic glaucous gulls. *Environ Int* 33:823-830.

Verreault J, Verboven N, Gabrielsen GW, Letcher RJ, Chastel O. 2008. Changes in prolactin in a highly organohalogen contaminated Arctic top predator seabird, the glaucoud gull. *Gen Comp Endocrinol* 156:569-576.

Vijayan MM, Aluru N, Maule AG, Jorgensen EH. 2006. Fasting augments PCB impact on liver metabolism in anadromous arctic char. *Toxicol Sci* 91:431-439.

Wang T, Hung CCY, Randall DJ. 2006. The comparative physiology of food deprivation: From feast to famine. *An Rev Physiol* 68:223-251.

Wingfield JC. 1994. Modulation of the adrenocortical response to stress in birds. In: Perspectives in comparative endocrinology, (Davey KG, Peter RE, eds). Ottawa, Canada:National Research Council of Canada, 520-528.

Table 1: Generalized linear models ranked by Akaike's Information Criterion for small sample sizes (AIC_c) and linear regression adjusted R^2 for variables that explain Σ_{37}PBDEs determined in plasma and liver of ring-billed gulls (*Larus delawarensis*) breeding near Montreal (QC, Canada).

Response variable	**Model**	**Parameter estimate** B (CI)	**ΔAIC_c**	***w***	***R^2***
Σ_{37}PBDEs liver (*n* = 25)	# hours at nest-site + capture date	1.0(0.3 – 1.7); -8.8 (-14 – -2.7)	0	0.43	0.38
	% nest-site attendance + capture date	499.5 (33.0 – 966.0); -7.5 (-14.3 – -0.7)	2.49	0.12	0.36
	plasma lipid % + date	-7.7 (-14.5 – -0.9); -200.4 (-395.5 – -5.3)	2.79	0.11	0.35
	plasma lipid % + % nest-site attendance	-201.3 (-397.9 – -4.64)513.5 (42.3 – 984.8)	3.07	0.09	0.35
	capture date	-10.1 (-17.0 – -3.2)	3.69	0.07	0.25
	% nest-site attendance	682.4 (237.8 – 1158.1)	3.95	0.06	0.24
	# hours at nest-site	1.2 (0.4 – 2.0)	3.96	0.06	0.24
	% plasma lipids	-2.8 (-476.6 – -76.0)	4.41	0.05	0.23
	sex	-147.3 (-270.0 – -24.5)	5.82	0.02	0.18
	null	n/a	8.22	0.01	n/a
Σ_{37}PBDEs plasma (*n* = 29)	% nest-site attendance	117.0 (21.7 – 212.2)	0	0.29	0.18
	# hours at nest-site + date	0.1 (0.0 – 0.3); -1.5 (-2.8 – -0.1)	0.51	0.22	0.24
	sex	-24.5 (-47.6 – -1.4)	1.24	0.15	0.14
	date	-1.5 (-3.0 – - 0.4)	1.48	0.14	0.13
	# h nest-site	0.2 (0.0 – -0.1)	1.7	0.12	0.12
	null	n/a	2.70	0.07	n/a

B = parameter estimates. CI = 95% confidence intervals. ΔAIC_c = the difference in Akaikes Information Criterion (corrected for small sample sizes value) between a model and the top model. *w* = model weight. The R^2 refers to the adjusted coefficient of determination generated from linear regression analysis. Variables with AICc values above the null model (thus explaining no variation in the response variable) included body mass, condition index, Julian lay date, proportion of time spent foraging, proportion

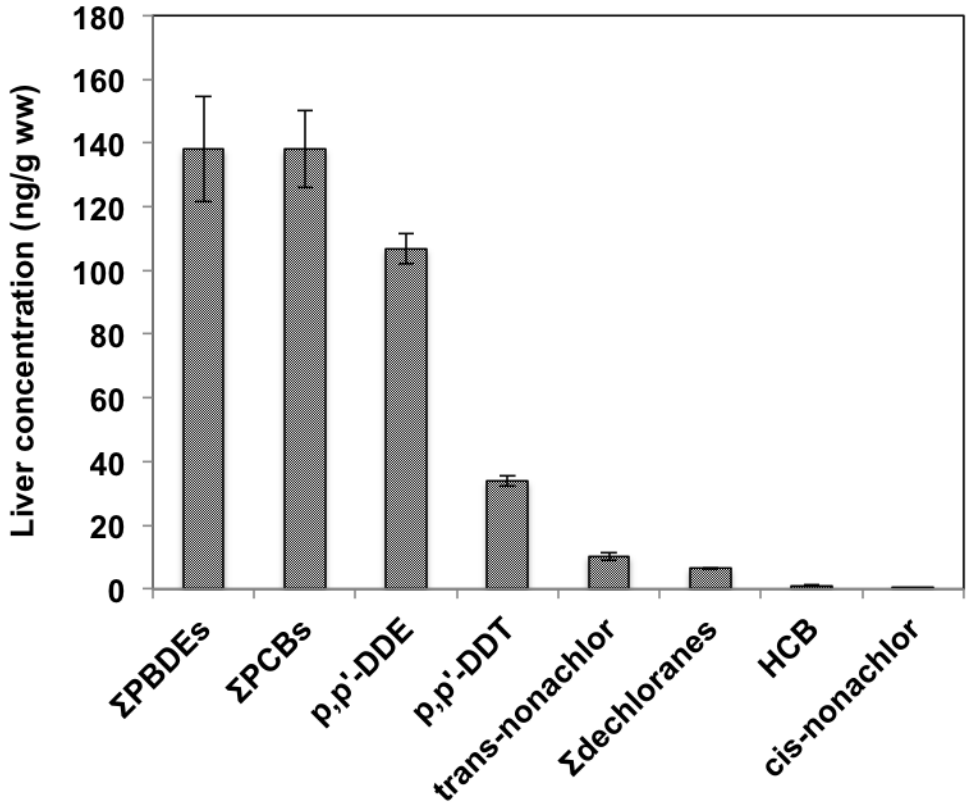
871 of time spent in different habitat types (urban, agricultural, waterway), percent body water or fat and for plasma Σ_{37}PBDEs the %

872 plasma lipids.

873

874

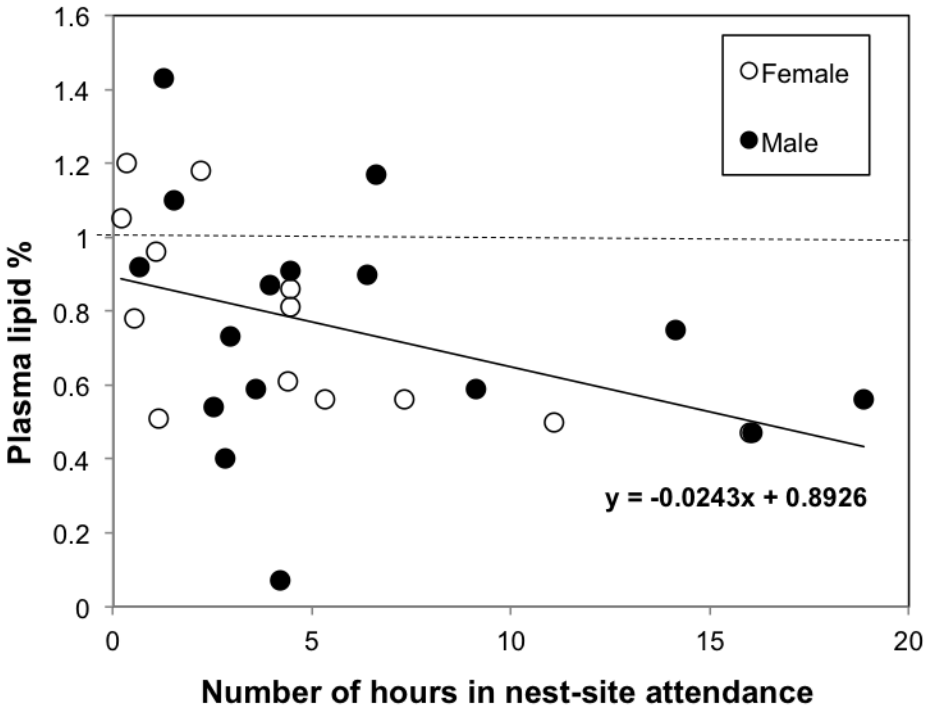
875 **Figure 1**: Mean concentrations (± SEM) of organochlorines and halogenated flame retardants in
876 liver of ring-billed gulls (*Larus delawarensis*) collected during the incubation period near
877 Montreal (QC, Canada).



878

879

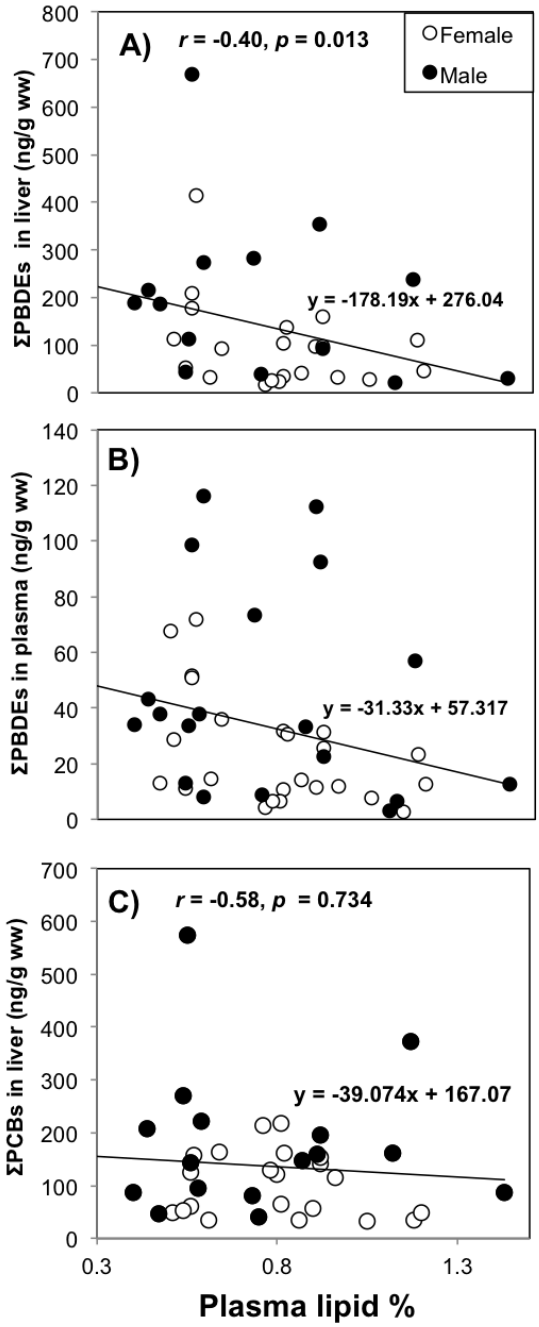
880 **Figure 2**: Relationship (Spearman's Rank Correlation) between plasma lipid percent and
881 number of hours spent at the nest site in the last 24 hours prior to recapture in incubating ring-
882 billed gulls (*Larus delawarensis*) near Montreal (QC, Canada). Birds with less than 1% plasma
883 lipids (dashed line) were assumed to be in a fasted state (K. Drouillard, unpublished data).



884

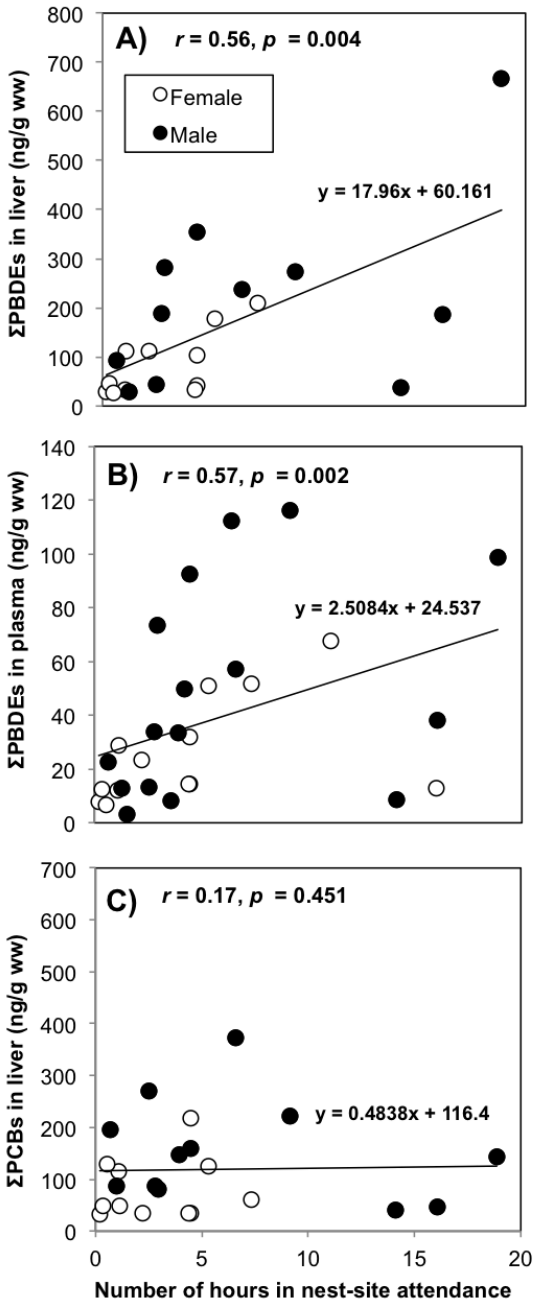
885

886 **Figure 3**: Relationships (Spearman's Rank Correlation) between the concentrations of Σ_{37}PBDE
887 in liver (A) and plasma (B) and (C) Σ_{38}PCBs in liver and the percent lipids determined in plasma
888 of incubating ring-billed gulls (*Larus delawarensis*) near Montreal (QC, Canada).



889

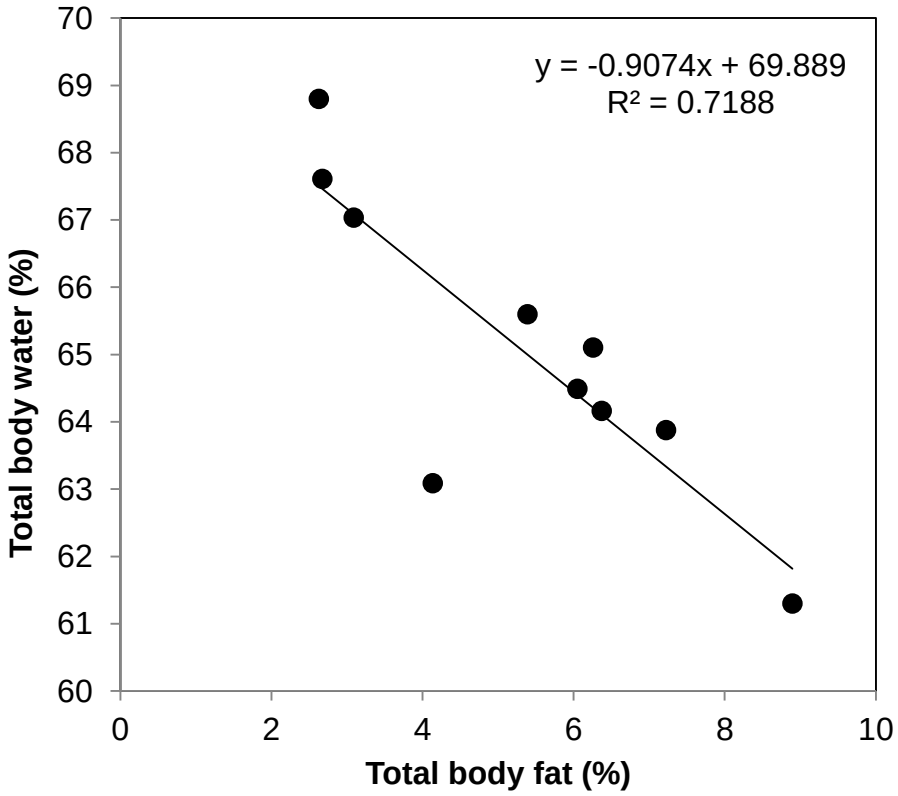
Figure 4: Relationships (Spearman's Rank Correlation) between the number of hours birds spent attending the nest-site in the last 24 hours prior to recapture and Σ_{37}PBDE concentrations in A) plasma and B) liver, and C) Σ_{38}PCBs in liver of incubating ring-billed gulls (*Larus delawarensis*) near Montreal (QC, Canada).



Supplementary information

Methods

Figure S1: Relationship between total body water and total body fat of glaucous Gulls (*Larus hyperboreus*) from Svalbard, Norway. Data from Shaffer et al. (2006) and Verreault et al. (2007).



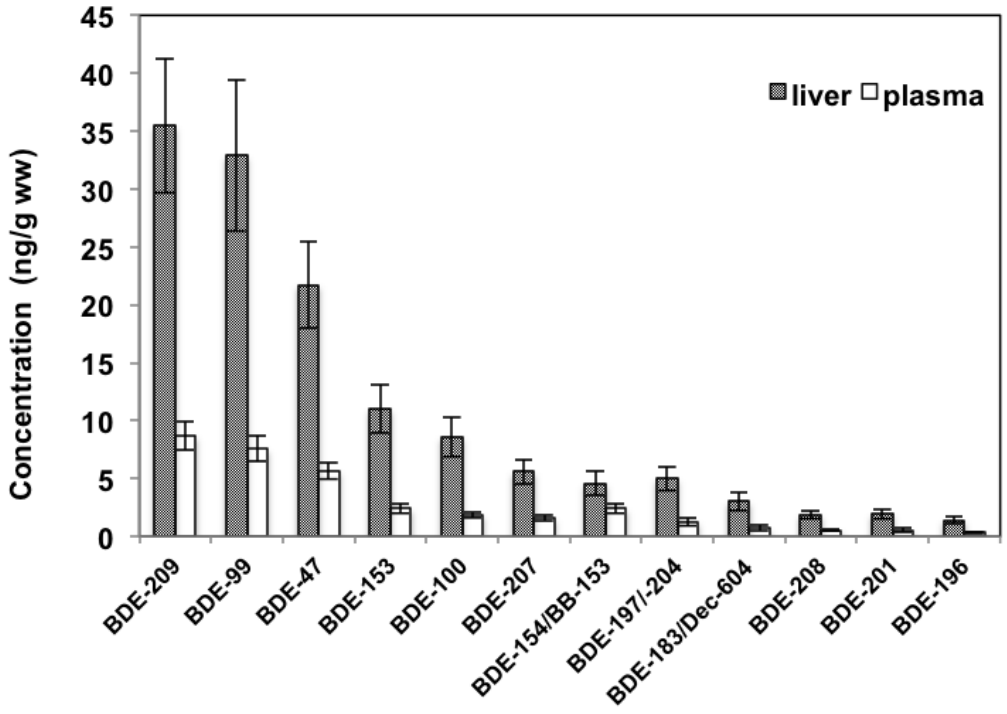
905

906 **Results**

907 **Figure S2**: Concentrations of major PBDE congeners determined in liver and plasma of ring-

908 billed gulls (*Larus delawarensis*) during the incubation phase near Montreal (QC, Canada).

909



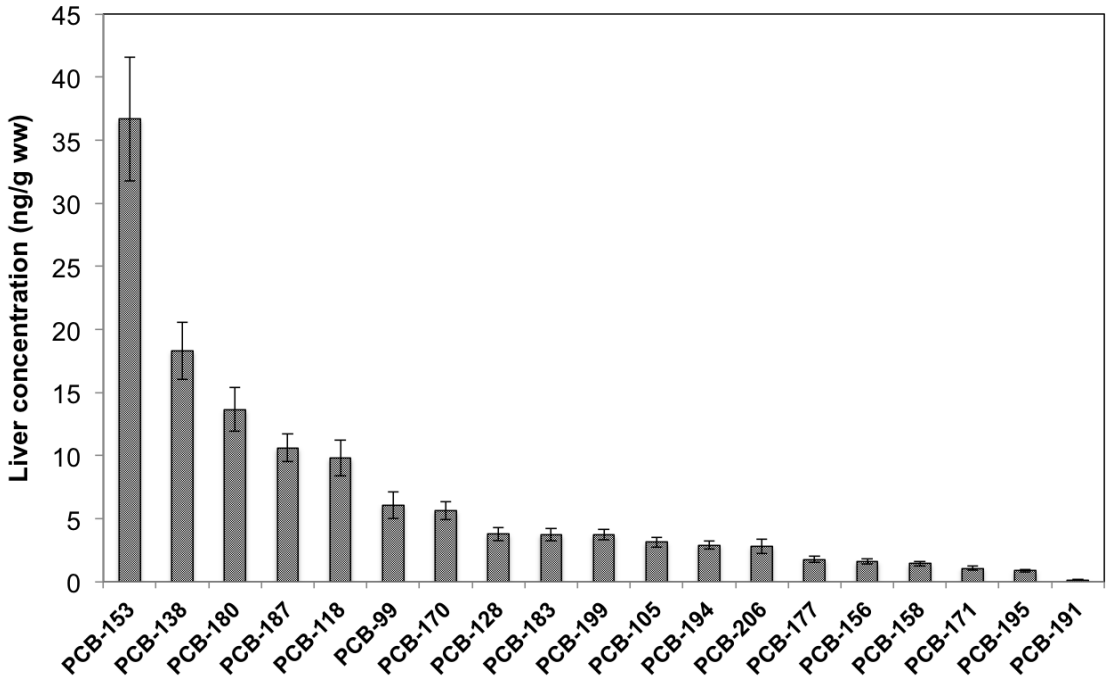
910

911

912

913 **Figure S3**: Concentrations of major PCB congeners determined in liver of ring-billed gulls

914 (*Larus delawarensis*) during the incubation phase near Montreal (QC, Canada).

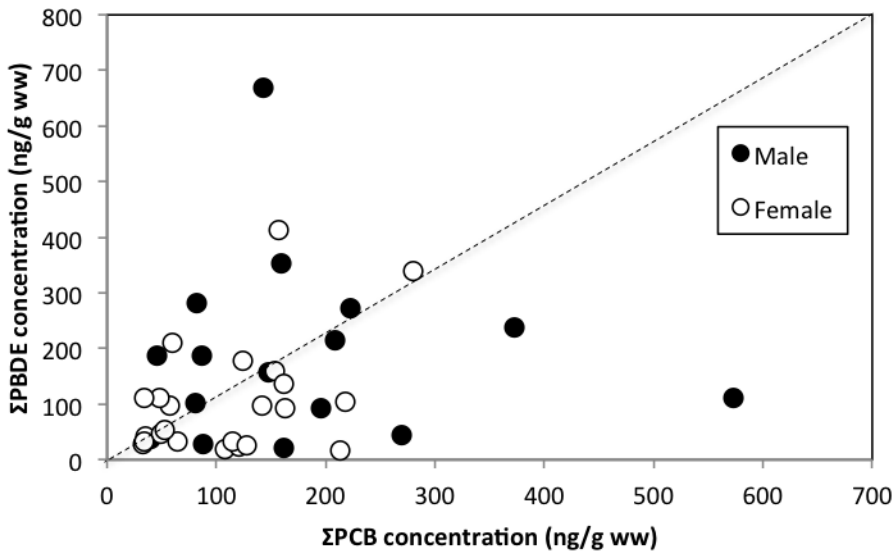


915

916

917

Figure S4: Concentrations of Σ_{37}PBDE vs. Σ_{38}PCBs in liver of ring-billed gulls (*Larus delawarensis*) nesting near Montreal (QC, Canada). Points above the dashed line represent birds for which ΣPBDE exceed ΣPCB concentrations and vice versa for points below this line.



Relationships between lipid-corrected plasma HFR concentrations and time-activity budget

As the proportion of time that birds spent in nest-site attendance increased, so did their lipid-corrected concentrations of Σ_{37}PBDEs plasma ($r = 0.46$, $p = 0.012$) as well as several of the individual PBDE congeners (in order of decreasing r: BDE-154/BB-153, -47, -99, -100, -153, -201, -197, -183: $0.39 < r < 0.43$, $0.007 < p < 0.003$). The positive relationship between nest-site attendance and PBDEs was stronger when the number of hours at the nest site before recapture was considered; all lipid-corrected congener concentrations were positively related to this activity measure ($0.046 > p > 0.001$) as were Σ_{37}PBDEs ($r = 0.61$, $p = 0.001$; Fig. S5) and Σdechloranes ($r = 0.39$, $p = 0.043$).

Figure S5: Relationship (Spearman's Rank Correlation) between the number of hours birds were attending the nest-site in the last 24 hours prior to recapture and lipid corrected Σ_{37}PBDE concentrations in plasma of incubating ring-billed gulls (*Larus delawarensis*) near Montreal (QC, Canada).