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

Follow this and additional works at: https://scholar.uwindsor.ca/biologypub

Part of the Biology Commons

Recommended Citation
Marteinson, S. C.; Drouillard, K. G.; and Verreault, J., "Short-term fasts increase levels of halogenated flame retardants in tissues of a wild incubating bird" (2016). Environmental Research, 146, 73-84.
https://scholar.uwindsor.ca/biologypub/754

This Article is brought to you for free and open access by the Department of Biological Sciences at Scholarship at UWindsor. It has been accepted for inclusion in Biological Sciences Publications by an authorized administrator of Scholarship at UWindsor. For more information, please contact scholarship@uwindsor.ca.
Short-term fasts increase levels of halogenated flame retardants in tissues of a wild incubating bird

Sarah C. Marteinson¹, Ken G. Drouillard² and Jonathan Verreault¹*

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 99 ring-billed gulls breeding near the metropolis of Montreal (QC, Canada) because they have been 100 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 101 alternate between bouts of incubation (and thus fasting) that may last several hours, and foraging 102 trips (Marteinson et al. 2015). Because these gulls do not exhibit prolonged continuous fasts 103 (e.g., several days), we measured their time-activity budget using high-resolution GPS-based 104 tracking during the last 24 hours prior to tissue collection. The relationships between liver and 105 plasma contaminant concentrations and the amount of time spent fasting at the nest site or 106 foraging away from the colony were investigated. To evaluate whether or not birds were in a 107 fasted state, the percent plasma lipids was assessed, and the body condition was examined. We 108 hypothesized first that, *i*) if short term fasting was to elicit lipid mobilization and increased 109 contaminant release from lipid stores that contaminant concentrations would increase in plasma 110 and liver as a function of the duration of time birds spend fasting at the nest site, and *ii*) that this 111 increase would be related to changes in whole body lipid stores during fasting. Alternatively, if 112 contaminant concentrations in plasma were dependent largely on recent dietary intake of 113 contaminated prey, HFR and OC concentrations in blood would be expected to decline with the 114 number of hours spent at the nest site (and thus time since feeding), or increase as the proportion 115 of time spent foraging increased, and be related to time spent foraging in habitats of varying 116 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 ($\pm$ SEM) $205 \pm 32.0$ ng/g wet weight (ww) in liver and $27 \pm 4.05$ 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: $6.06 \pm 1.64$; $syn$-DP: $2.38 \pm 0.67$; (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 ($\pm 0.01$ g) and morphometric measurements (head, culmen, and tarsus length) were recorded using digital callipers ($\pm 1$ 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 $\pm$ SEM: $478 \pm 7$ 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 $^2\text{H}/^1\text{H}$ ratio using a Micromass Isoprime$^{\text{TM}}$ DI gas isotope ratio mass spectrometer (IRMS) coupled to an Aquaprep$^{\text{TM}}$ 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: $N_d = \frac{\text{Mol}_{\text{inj}}}{(E_{\text{mix}} - E_{\text{inj}}) / (E_{\text{wat}} - E_{\text{mix}})}$ where Mol$_{\text{inj}}$ is the moles of $^2\text{H}$ in the injectate, $E_{\text{mix}}$ is the equilibrium enrichment of $^2\text{H}$ in water (ppm of initial enrichment), $E_{\text{wat}}$ is the background level of isotopes from control birds (in ppm) and $E_{\text{inj}}$ 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 through 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 (ant- and 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 13C-BDE-209, 90.0 ± 2.2% for 13C-syn-DP and 90.2 ± 2.2% for 13C-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 13C-syn-DP and 13C-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 (Σ37PBDEs) were used in analyses. The Σ37PBDEs 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 $\Sigma_{38}$PCBs, $p,p\prime$-DDE, $p,p\prime$-DDT, and $\Sigma$ nonachlors (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
temporal variables (capture date, Julian egg laying date), sex, body mass, % plasma lipids, and activity and body condition measures (body condition index, whole body water and lipid percents). Only models with one or two predictor variables as well as the intercept were conducted due to the low sample size. Related variables were not included in the same model. For comparison, the null model was assessed, and only models with AICc values lower than that of the null model were retained. Additionally, models for which predictor variables for which the parameter estimate 95% confidence intervals included zero were not considered. For each model, the ΔAICc was calculated as well as the weight ($w$) (Burnham and Anderson 2002). Simple linear regressions were also conducted with the variables for each model to generate adjusted $R^2$ values. Statistical analyses were conducted using IBM SPSS 20, and a 0.05 significance level was employed where applicable. Means are reported with standard error ranges.

**Results**

*Time-activity budget*

The proportions of time ring-billed gulls spent in nest-site attendance and foraging were consistent with those previously reported for a larger set of individuals including the present birds (Marteinson et al. 2015). Birds spent 47-90% of the 24-hour tracking period prior to recapture in nest-site attendance (mean: 70 ± 0.02%). The amount of time that birds had spent in this activity prior to their recapture at the nest ranged from 0.2 to 18.9 hours (mean: 5.4 ± 0.2 h). Birds spent from 0 to 30% of the tracking period in foraging activities (mean: 11 ± 0.02%). The proportions of time ring-billed gulls spent foraging in different sites were consistent with those previously reported for a larger set of individuals, including the present birds (Gentes et al. 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 Σ37PBDE 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 Σ37PBDE 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 Σ38PCB 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 oxychlorodane were not detected in any individuals. Mean concentrations of Σ37PBDEs in liver were similar to Σ38PCBs 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; $-0.31 < r < -0.37$; $0.014 < p < 0.050$), $\Sigma_{37}$PBDEs ($r = -0.33$, $p = 0.031$) (Fig. 3B) as well as $\Sigma$dechloranes ($r = -0.39$, $p = 0.022$). 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; $-0.33 < r < -0.43$; $0.007 < p < 0.046$), $\Sigma_{37}$PBDEs ($r = -0.40$, $p = 0.013$) (Fig. 3A) and $\Sigma$dechloranes ($r = -0.35$, $p = 0.03$) 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 $\Sigma_{37}$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 $\Sigma_{37}$PBDEs in liver and plasma of low plasma lipid birds was $74.4 \pm 19.4$ ng/g ww and $41.0 \pm 6.7$ ng/g ww which were 2.3 and 2.1 times higher, respectively, compared to high lipid plasma birds (mean liver: $33.1 \pm 10.7$ ng/g ww; plasma: $19.4 \pm 4.6$ ng/g ww). The difference between low and high plasma lipid birds was significant for $\Sigma_{37}$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 $\Sigma_{38}$PCB concentration of $161 \pm 51.5$ ng/g ww in liver compared to $134 \pm 32.0$ 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 $\Sigma_{37}$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 $\Sigma_{37}$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). $\Sigma_{37}$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, $\Sigma$dechlorane 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 Σ_{PBDEs} 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 $\Sigma_{37}$PBDEs in models containing plasma lipid % ($w = 0.11-0.05$) and the sex ($w = 0.02$). The variations in $\Sigma_{37}$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 $\Sigma_{37}$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 $\Sigma_{37}$PBDE concentrations. Sex explained only a small proportion of the variations in plasma $\Sigma_{37}$PBDEs ($w = 0.15$). Julian lay date, body mass and measures of body condition did not explain any variation in $\Sigma_{37}$PBDE concentrations in both plasma and liver. Plasma % lipids did not explain any variation in plasma $\Sigma_{37}$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
continuously for four weeks during the incubation, levels of PCB-153, \( p,p' \)-DDE and hexachlorobenzene increased between day five and day 20 in the incubation period (Bustnes et al. 2010). Moreover, in overwintering greater scaup (\textit{Aythya marila}), which experience reduced availability and simultaneous reductions in body fat stores, individuals captured later in the winter had higher wet weight-based levels of \( \Sigma \)PCBs and \( \Sigma \)DDTs (including DDD, DDE) than birds caught early in the winter (Perkins and Barclay 1997). This effect has been confirmed even in species that are not adapted for extreme fasts. For instance, herring gull chicks exposed to a mixture of contaminants (OCs and PBDEs) in the laboratory and subsequently fasted for one week, exhibited contaminant concentrations that were three times higher in liver, plasma, and brain compared to a non-fasted exposed group (Routti et al. 2013). Changes in feeding rates and body mass throughout the breeding season have similarly been associated to contaminant concentrations in some seabirds, both those adapted to long fasts or not, including kittiwakes (\textit{Rissa trydactyla}) (Henriksen et al. 1996), and Adélie penguins (\textit{Pygoscelis adeliae}) and southern fulmars (\textit{Fulmarus galacialoides}) (van den Brink et al. 1998). More specifically, in the penguin and fulmar study, lower body mass during times of fast (egg-laying or incubation) or times of higher physical exertion (chick-provisioning) were associated with higher levels of several OCs in blood and uropygial oil compared to periods where birds were able to replenish fat stores (van den Brink et al. 1998). Similarly for the kittiwakes during breeding, levels of \( \Sigma \)PCBs in the brain were four-fold higher at the end of chick-rearing period during which they lost 20\% of their body mass compared to the pre-breeding period (Henriksen et al. 1996). Collectively, these studies demonstrate that relationships exist between fasting and contaminant 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 rufa*) (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\text{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
20 hours, which can be enough to weight loss in birds that are not adapted for prolonged fasting (e.g. Scollon et al. 2012). However, even though individual ring-billed gulls would have underwent several such relatively short fasting events over their 28 days incubation period, there was no relationship between body condition expressed as whole body lipids and time spent at the nesting site, body condition and number of incubation days nor between body condition measures and contaminant concentrations in liver or plasma. This points to a lack of condition deterioration over time during this phase and suggests that these birds remained within the first metabolic phase of fasting, and were relying more heavily on fatty acids and glycogen reserves compared to stored triglycerides. Loss of fatty acids from adipose tissue would also decrease the partitioning capacity of the tissue, but to a lesser extent than triglycerides (Dulfer and Govers 1995; Dulfer et al. 1996). Ring-billed gulls forage between short-term incubation bouts, and tend to exploit areas of high and predictable food availability (e.g., anthropogenic-related habitats and agricultural fields) and quality (fat and protein content) (Caron-Beaudoin et al. 2013; Patenaude-Monette et al. 2014). This may have allowed them to replenish labile tissue energy stores as they were used up on a daily basis. This is in line with other seabird species exhibiting biparental care that maintain body mass during the incubation period (e.g., Moreno 1989) despite prolonged bouts of intermittent fasting at the nest. Given that whole body lipids in birds of the present study did not change significantly in relation to time devoted to nest-site attendance, fugacity gradients generated as a result of adipose tissue depletion cannot explain the increasing trend of plasma and liver HFR concentrations with time spent at the nest site. This conclusion is reinforced by the fact that OCs showed no evidence of enriched liver concentrations. Mobilization of contaminants from lipid-rich tissues during weight loss would be expected to increase all hydrophobic contaminant concentrations (including OCs and HFRs in present birds) in tissues to
a similar extent. For example, Routti and colleagues (2013) exposed herring gull chicks to a
mixture of environmentally relevant contaminants for 45 days and subsequently fasted (70% food reduction) treatment birds for seven days to compare chemical enrichment in tissues relative to non-fasted individuals (controls). The above study demonstrated that all contaminant classes including \( \Sigma \) PCBs, \( \Sigma \) DDTs, \( \Sigma \) CHLORs and \( \Sigma \) PBDEs increased in liver, brain, and plasma over those of the non-fasted group to a similar extent.

It is more likely that the relationships between HFR concentrations and time spent fasting in ring-billed gulls at the nest site was related to recent contaminant exposure which may have changed as the spring season progressed. Three alternative explanations would be that ring-billed gulls had been: i) exposed to a “diluted meal” during foraging activities rather than the contaminated diet we predicted from the habitats they use, ii) exposed to a non-dietary source of HFRs at the nesting site itself or iii) exposure to PBDEs may have altered the incubation behavior of these gulls. In the diluted meal hypothesis, birds returning from foraging and which have fed on a relatively clean meal (low HFR concentrations) would be expected to experience dilution of plasma contamination due to an influx of clean dietary lipids (e.g., portomicrons) and/or production of de novo lipids generated from assimilated nutrients (Drouillard and Norstrom 2000; Drouillard and Norstrom 2001). In this scenario, differences in the behavior of different contaminant classes might be explained by the fact that the diluted meal was less contaminated with HFRs, but not OCs. Based on Fig. 2, peak plasma lipid contents were observed in birds had been incubating for less than a few hours prior to capture, followed by depletion of plasma lipids to apparent fasting levels after eight to ten hours. This profile is very similar to what was observed in ring doves after achieving \( C_{\text{max}} \) of plasma lipids following a controlled feeding study that demonstrated an exponential return of plasma lipids approaching
fasting levels after approximately seven hours (Drouillard and Norstrom 2000). This would be surprising because these birds do not rely heavily on aquatic habitats for feeding which act as sinks for PCBs (reviewed in: Beyer and Biziuk 2009), but they do frequently utilize anthropogenic-related habitats to forage (e.g., Caron-Beaudoin et al., 2013) which can be highly contaminated with HFRs (Venier and Hites 2008). As further evidence, recent (last 24 hours) use of landfills and wastewater treatment plant ponds has been linked to increased concentrations of BDE-209 in these gulls nesting in the same colony as utilized in the present study (Gentes et al. 2015).

However, the contaminant patterns in liver of ring-billed gulls do not support this hypothesis. Dilution of liver lipids post-feeding is also possible given that liver is capable of generating lipids from absorbed carbohydrates and proteins. Indeed, lipid-normalized egg/maternal tissue concentration ratios in herring gulls and other bird species have been hypothesized to occur as a result of dilution of the liver lipid pool during yolk production resulting from de novo lipid production in the liver (Braune and Norstrom 1989; Drouillard and Norstrom 2001; Norstrom et al. 1986). In the case of herring gulls, a mean lipid-normalized egg/maternal tissue ratio of 0.7 was apparent, suggesting a 1.4-fold dilution factor of liver OC levels during yolk production (Braune and Norstrom 1989). A much higher egg/maternal tissue dilution factor of 2.9-fold was observed in ring doves which approaches the 5.2-fold differences in liver HFR residues observed in ring-billed gulls from the present study. However, the period of yolk formation is considered an extreme case where de novo lipid production in the liver is expected to be maximized and does not likely reflect the situation present for incubating ring-billed gulls. Second, there was no evidence for a change in liver lipid content with time at the nesting site, which would be expected to be observed under a situation of lipid dilution in liver.
Finally, *de novo* lipid production by the liver would dilute all hydrophobic chemicals to the same extent, but only HFRs and not OCs showed changes in liver concentration with time on the nesting activity in ring-billed gulls. Thus, the “diluted meal” hypothesis could be considered consistent with the observed trends in plasma concentrations of HFRs and lack of trends for OCs, although not consistent with the patterns observed for liver.

The second hypothesis implies that ring-billed gulls were exposed to elevated HFR and baseline OC concentrations while at the nesting site. The exposure source was apparently not related to dietary exposure at the nesting site because the change in plasma lipid content with time at the nest is consistent with birds reverting to a fasting state during this activity. Alternative exposure routes could be as a result of inhaled HFR-contaminated dusts/particles or air at the local nesting site. Assimilated material from the respiratory tract would be expected to show up rapidly in perfused tissues including blood and liver, followed by slower redistribution to adipose tissues (Daley et al. 2013). HFRs can be present in the atmosphere in their volatilized form which is more common for lower-brominated congeners, or in association with dust/particles which is more common for higher-brominated congeners including BDE-209 (reviewed in: Hale et al. 2006). Several HFRs including PBDEs have been measured in outdoor air globally (reviewed in: Hale et al. 2006) with the highest concentrations recorded outside point-source areas in urbanized environments (e.g., Venier and Hites 2008). Recently, PBDE concentrations in outdoor air have begun to exceed concentrations of PCBs in several reports (reviewed in: Hale et al. 2006). This is consistent with the finding that the present ring-billed gulls demonstrated similar mean ΣPBDEs concentrations to ΣPCBs, the latter of which have previously dominated in bird tissue. Few studies have measured HFRs in Canadian city air though, recently in Toronto, air contained 38 pg/m³ ΣPBDEs in combined gas and particle
phases, as well as a number of emerging HFRs including syn- and anti-DP (Shoeib et al. 2015).

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

Finally, the possibility that exposure to PBDEs may have altered the incubation or nest-site attentiveness of these gulls should be explored as they have been shown to disrupt related endpoints. In laboratory studies, exposure to PBDEs has caused reduced nest-related behavior during courtship and brood rearing in American kestrels (Falco sparverius) (Fernie et al. 2008, Marteinson et al. 2010), as well as reduced nest temperatures during incubation (Sullivan et al. 2013). Additionally, in wild male glaucous gulls, greater plasma sum PBDE concentrations were associated with reductions in the hormone prolactin which regulates incubation behavior (Verreault et al., 2008, and references therein). As such to date, exposure to PBDEs appears to
have a negative impact on incubation and prolactin levels in birds, suggesting that this may not explain the positive correlation between PBDEs and time spent at the nest in the present ring-billed gulls, however further research on this avenue may be warranted.

**Conclusions**

This study demonstrates increased HFR concentrations in ring-billed gull plasma and liver tissues during the incubation period while birds were present at the nesting site. More specifically, bouts of nest-site attendance in ring-billed gulls lasting only a few hours were associated with significant increase in levels of PBDEs and DP isomers (liver and plasma), but not OCs (liver). Conversely, the amount of time that birds were engaged in foraging activities, body condition and percent body fat were unrelated to contaminant concentrations. The changes in plasma HFR concentrations may be consistent with the hypothesis that birds were exposed to a diluted meal with respect to HFR levels, although this would not apply for OCs. However, the patterns in liver do not support this explanation because all hydrophobic organic contaminants should increase in liver as fasting duration increases. Alternatively, these gulls may have been significantly exposed to non-dietary sources of HFRs at the nest site (i.e., via inhalation of air and HFR-laden particles or ingestion via preening of particles adsorbed to their plumage).

Ultimately, further study is needed to understand the increased liver and plasma HFR concentrations in these birds while they spend time fasting at the nest site. However, regardless of the underlying mechanisms and exposure routes, the results presented herein highlight the fact that short-term fasting events related to, for example nest-site attendance, may significantly influence levels of HFRs in birds, and hence the potential toxicity associated with these compounds. As such, the amount of time spent in this activity just prior to capture may become
a confounding factor in monitoring and research on contaminants, including biological effect studies in wild birds. This may also apply to a wide variety of other situations where short-term fasting occurs including other reproductive phases, migration, or even daily overnight fasting. Further research on how contaminant concentrations relate to these life cycle stages is warranted.

Acknowledgements

Funding for this study was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC: grant #385787) (J.V.), the Canada Research Chair in Comparative Avian Toxicology (grant #225707 J.V.) and Fonds québécois de la recherche sur la nature et les technologies (to S.C.M.). For field assistance the authors thank V. Aponte, S. Pellerin Plourde, M. Patenaude-Monette, M.-L. Gentes, F. St-Pierre, and M. Tremblay. For assistance with stable isotope analysis, the authors thank J.-F. Hélie and A. Adamowicz, and for ArcGIS analysis the authors thank M. Patenaude-Monette and M.-L. Gentes.

References


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.

sources of polybrominated diphenyl ethers in birds: Foraging in waste management

Hale RC, La Guardia MJ, Harvey E, Gaylor MO, Mainor TM. 2006. Brominated flame retardant

contaminant concentrations in california sea lions (*Zalophus californianus*) associated with

(kow) of all PCB congeners by ab initio and a cl substitution position method. *QSAR

Hebert CE, Keenleyside WJ. 1995. To normalize or not to normalize? Fat is the question. *Environ

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


Scollon EJ, Carr JA, Rintoul DA, McMurry ST, Cobb GP. 2012. Metabolism and distribution of 
*p,p*-DDT during flight of the white-crowned sparrow, *Zonotrichia leucophrys*. *Environ 

Shaffer SA, Gabrielsen GW, Verreault J, Costa DP. 2006. Validation of water flux and body 

Shoeib M, Ahrens L, Jantunen L, Harner T. 2015. Concentrations in air of organobromine and 

Changes in the incubation by American kestrels (*Falco sparverius*) during exposure to the 
polybrominated diphenyl ether (PBDE) mixture DE-71. *J Toxicol Environ Health A* 76: 
978-989.

and plasma composition of herring gulls (*Larus argentatus*). *Physiol Biochem Zool* 72:426- 
437.

van den Brink NW, van Franeker JA, Ruiter-Dijkman EM. 1998. Fluctuating concentrations of 
organochlorine pollutants during a breeding season in two antarctic seabirds: Adélie 

persistent organic pollutants and their metabolites in grey seals during lactation. *Environ 

Veith GD, DeFoe DL, Bergstedt BV. 1979. Measuring and estimating the bioconcentration 
factor of chemicals in fish. Journal of the Fisheries Research Board of Canada 36:1040- 
1048.


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 $\Sigma_{37}$PBDEs determined in plasma and liver of ring-billed gulls ($Larus delawarensis$) breeding near Montreal (QC, Canada).

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Model</th>
<th>Parameter estimate</th>
<th>$\Delta AIC_c$</th>
<th>$w$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Sigma_{37}$PBDEs</td>
<td># hours at nest-site + capture date</td>
<td>1.0 (0.3 − 1.7); -8.8 (-14 − 2.7)</td>
<td>0</td>
<td>0.43</td>
<td>0.38</td>
</tr>
<tr>
<td>liver</td>
<td>% nest-site attendance + capture date</td>
<td>499.5 (33.0 − 966.0); -7.5 (-14.3 − -0.7)</td>
<td>2.49</td>
<td>0.12</td>
<td>0.36</td>
</tr>
<tr>
<td>(n = 25)</td>
<td>plasma lipid % + date</td>
<td>-7.7 (-14.5 − -0.9); -200.4 (-395.5 − -5.3)</td>
<td>2.79</td>
<td>0.11</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>plasma lipid % + % nest-site attendance capture date</td>
<td>-201.3 (-397.9 − -4.64)</td>
<td>3.07</td>
<td>0.09</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>% nest-site attendance</td>
<td>682.4 (237.8 − 1158.1)</td>
<td>3.95</td>
<td>0.06</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td># hours at nest-site</td>
<td>1.2 (0.4 − 2.0)</td>
<td>3.96</td>
<td>0.06</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>% plasma lipids</td>
<td>-2.8 (-476.6 − -76.0)</td>
<td>4.41</td>
<td>0.05</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>sex</td>
<td>-147.3 (-270.0 − -24.5)</td>
<td>5.82</td>
<td>0.02</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>null</td>
<td>n/a</td>
<td>8.22</td>
<td>0.01</td>
<td>n/a</td>
</tr>
</tbody>
</table>

| $\Sigma_{37}$PBDEs | % nest-site attendance | 117.0 (21.7 − 212.2) | 0              | 0.29 | 0.18  |
| plasma             | # hours at nest-site + date | 0.1 (0.0 − 0.3); -1.5 (-2.8 − -0.1) | 0.51          | 0.22 | 0.24  |
| (n = 29)           | 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. $\Delta AIC_c =$ the difference in Akaike’s 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
of time spent in different habitat types (urban, agricultural, waterway), percent body water or fat and for plasma Σ_{37}PBDEs the % plasma lipids.
Figure 1: Mean concentrations (± SEM) of organochlorines and halogenated flame retardants in liver of ring-billed gulls (*Larus delawarensis*) collected during the incubation period near Montreal (QC, Canada).
Figure 2: Relationship (Spearman’s Rank Correlation) between plasma lipid percent and number of hours spent at the nest site in the last 24 hours prior to recapture in incubating ring-billed gulls (*Larus delawarensis*) near Montreal (QC, Canada). Birds with less than 1% plasma lipids (dashed line) were assumed to be in a fasted state (K. Drouillard, unpublished data).
Figure 3: Relationships (Spearman’s Rank Correlation) between the concentrations of $\Sigma_{37}$PBDE in liver (A) and plasma (B) and (C) $\Sigma_{38}$PCBs in liver and the percent lipids determined in plasma of incubating ring-billed gulls (Larus delawarensis) near Montreal (QC, Canada).
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).

\[ y = -0.9074x + 69.889 \]

\[ R^2 = 0.7188 \]
Results

Figure S2: Concentrations of major PBDE congeners determined in liver and plasma of ring-billed gulls (*Larus delawarensis*) during the incubation phase near Montreal (QC, Canada).
**Figure S3**: Concentrations of major PCB congeners determined in liver of ring-billed gulls (*Larus delawarensis*) during the incubation phase near Montreal (QC, Canada).
Figure S4: Concentrations of $\Sigma_{37}$PBDE vs. $\Sigma_{38}$PCBs in liver of ring-billed gulls (*Larus delawarensis*) nesting near Montreal (QC, Canada). Points above the dashed line represent birds for which $\Sigma$PBDE exceed $\Sigma$PCB 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 Σ37PBDEs 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 Σ37PBDEs ($r = 0.61$, $p = 0.001$; 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 Σ37PBDE concentrations in plasma of incubating ring-billed gulls (Larus delawarensis) near Montreal (QC, Canada).