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

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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.

1. 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, molting, 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

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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 increase in *p,p′*-DDT or its metabolites, *p,p′*-DDD and *p,p′*-DDE, in various tissues following 20 min, 4 or 9 h 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 h 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.

2. Materials and methods

2.1. 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 ± 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 ($\pm$ 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 ± 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 behavior 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 h 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.

2.2. 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 ($\pm$5–10 m), velocity, date, and time at 4-minute intervals for two to three days (Caron-