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Implications of mercury and lead concentrations on breeding physiology and phenology in an Arctic bird☆

J.F. Provencher [a, *], M.R. Forbes [a], H.L. Hennin [b], O.P. Love [b], B.M. Braune [c], M.L. Mallory [d], H.G. Gilchrist [c]

[a] Department of Biology, Carleton University, Ottawa, ON, Canada
[b] Department of Biological Sciences and Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON, Canada
[c] WLSD, Environment and Climate Change Canada, Ottawa, ON, Canada
[d] Department of Biology, Acadia University, Wolfville, NS, Canada

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ABSTRACT

Although physiological traits and phenology are thought to be evolved traits, they often show marked variation within populations, which may be related to extrinsic factors. For example, trace elements such as mercury (Hg) and lead (Pb) alter biochemical processes within wildlife that may affect migration and breeding. While there is a growing understanding of how contaminants may influence wildlife physiology, studies addressing these interactions in free-living species are still limited. We examined how four non-essential trace elements (cadmium, Hg, Pb and selenium) interacted with physiological and breeding measures known to influence breeding in a free-living population of common eider ducks (*Somateria mollissima*). We collected blood from female eiders as they arrived at a breeding colony in northern Canada. Blood was subsequently assessed for baseline corticosterone (CORT), immunoglobulin Y (IgY), and the four trace elements. We used model selection to identify which elements varied most with CORT, IgY, arrival condition, and arrival timing. We then used path analysis to assess how the top two elements from the model selection process (Hg and Pb) varied with metrics known to influence reproduction. We found that arrival date, blood Hg, CORT, and IgY showed significant inter-annual variation. While blood Pb concentrations were low, blood Pb levels significantly increased with later arrival date of the birds, and varied negatively with eider body condition, suggesting that even at low blood concentrations, Pb may be related to lower investment in reproduction in eiders. In contrast, blood Hg concentrations were positively correlated with eider body condition, indicating that fatter birds also had higher Hg burdens. Overall, our results suggest that although blood Hg and Pb concentrations were below no-effect levels, these low level concentrations of known toxic metals show significant relationships with breeding onset and condition in female eider ducks, factors that could influence reproductive success in this species.

1. Introduction

There is increasing interest in determining how measures of immunity and stress relate to survival and reproduction (Hennin et al., 2012; Humborstad et al., 2016; Madeira et al., 2016). Since there is an expectation that environmental contaminants may influence the condition and reproductive success of an individual directly or indirectly by altering physiological traits (Blévin et al., 2014; Tartu et al., 2015), there is a need for integration of toxicology into this expanding field. Mercury (Hg) is of particular concern for marine birds in the Arctic, due to high levels of this trace element in the environment (Provencher et al., 2014b; Riget et al., 2011). Importantly, Hg has been shown to be an endocrine disruptor in both captive (Jayasena et al., 2011) and wild birds (Tartu et al., 2016a, 2013), even when exposure is low but chronic. In addition to Hg, marine birds are also exposed to other trace elements that are known to be toxic. For example, marine birds are exposed to lead (Pb) through shot from hunting activities, which can also have negative impacts (Merkel et al., 2006; Scheuhammer, 2009). Thus, it is important to consider trace metals in relation to

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* Corresponding author.
E-mail address: jennifpro@gmail.com (J.F. Provencher).

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each other to investigate possible interactions (Sarigiannis and Hansen, 2012). As a result, research exploring the links between physiological traits, contaminants, survival and reproduction are needed to better understand how individuals, and populations, may respond to changes in predation and disease, among other ecological interactions, in sensitive environments (Guindre-Parker et al., 2013; Harms et al., 2015; Hennin et al., 2016).

The impacts of Hg and Pb may be linked directly to behaviours associated with breeding. For example, female mallard ducks (*Anas platyrhynchos*) with higher Hg exposure laid fewer eggs and produced fewer ducklings compared with hens with lower Hg exposure (Heinz, 1979). Importantly, Hg and Pb poisoning may also cause changes in attributes that have negative subsequent effects on reproduction, such as reduced adult health and body condition. Increased levels of Pb in mallard ducks in the Mediterranean were correlated with increased humoral immune response and negatively correlated with cellular immune response (Vallverdú-Coll et al., 2015). In free-living Arctic black-legged kittiwakes (*Rissa tridactyla*) higher levels of Hg have been linked with decreased levels of prolactin, an important hormone in reproduction (Tartu et al., 2016b). Thus, even chronic moderate exposure to some trace elements can potentially impact physiological traits that have been linked with individual condition and fitness. While there are an increasing number of studies demonstrating the links between contaminants and physiological traits (Fallacara et al., 2011; Lewis et al., 2013a; Pollock and Machin, 2009; Tartu et al., 2016b), few studies have examined whether potential deleterious, synergistic effects of trace elements and physiological states can influence condition, reproduction and survival (although see Tartu et al., 2013).

Importantly, wildlife are often exposed to multiple trace elements that can have implications on physiology. In particular, selenium (Se) has been shown to interact with Hg within biota, and can provide protective effects against the toxicity of Hg, thus it is important to examine Hg concentrations within the context of Se (Wiener et al., 2003). Additionally, recent high levels of Cd have been detected in eiders in northern Canada (Mallory et al., 2014), as well as in Alaska (Lovvorn et al., 2013). Therefore it is important to consider trace elements in context of each other to investigate possible interactions and cumulative effects (Sarigiannis and Hansen, 2012).

Global models currently predict that Arctic ecosystems will undergo rapid changes in the coming decades (Blume-Werry et al., 2016; Wang et al., 2016), therefore the relationship between contaminants and reproduction in polar ecosystems is particularly important. Alongside changes in climatic conditions, including rain and snow melt, pollutant levels in the Arctic are changing (Stern et al., 2012). Additionally more acidic conditions in the marine environment due to ocean acidification may also alter how Hg is taken up by organisms (AMAP, 2013). Mercury is of particular concern for Arctic ecosystems because Hg biomagnification in food webs may be exacerbated in ecosystems with low productivity (Kidd et al., 2011; Provencher et al., 2014b). Furthermore, while Hg concentrations in many Arctic wildlife species in Canada are relatively low and appear to be leveling off, concentrations in many species have risen over the last several decades despite declines in Hg production in North America (Braune et al., 2015, 2016a; Riget et al., 2011). In light of the changing environment in Canada's north, determining how contaminants drive reproduction in wildlife is a key to predicting potential temporal changes in their populations.

The overall objective of this study was to investigate four biologically-important trace metals (Hg, Cd, Pb and Se) in relation to the health and condition of a free-living bird, the common eider duck (*Somateria mollissima borealis*; hereafter, eider ducks). Eider ducks are common and abundant throughout the pan-Arctic region (Goudie et al., 2000), forming part of a traditional subsistence hunt in many regions (Mallory et al., 2004). Eider ducks migrate to the Canadian Arctic each spring where females actively feed in the region before nesting and fasting during the incubation period (Hennin et al., 2014; Mosbech et al., 2006). We focus our study on examining trace elements in blood, a tissue which is indicative of exposure over the previous weeks (Hobson and Clark, 1992; the pre-breeding time period in the Arctic region). Thus, both blood metal concentrations and condition of the birds reflect the immediate time period before the breeding season.

Importantly, long-term monitoring of populations in Canada and other regions have yielded a wealth of information about eider, physiology, breeding metrics and contaminant burdens (Hennin et al., 2013; Love et al., 2010; Mallory and Braune, 2012). Specifically, Hennin et al. (2016) have shown that reproduction is linked with female corticosterone (CORT) levels during the arrival period, while immunoglobulin Y (Igy) is also related to reproduction in eiders (Counihan et al., 2015). Additionally, previous work has shown that in eider ducks earlier laying and higher arrival condition are associated with higher nest success (Descamps et al., 2011). Thus, eider ducks are an excellent species to investigate how trace elements interact with physiological parameters and their subsequent relations to breeding parameters.

To examine how Cd, Hg, Pb and Se vary with physiological traits that have been shown to influence reproductive onset and success (IgY, CORT, arrival date and arrival condition), a multi-step analysis was used. First, we examined which trace metal concentrations contributed significantly to the selected reproductive traits using general liner models and Akaike's information criterion (AIC) model selection. Second, a path analytic approach was used to investigate how the trace elements found to be the most associated with the metrics of interest (Hg and Pb) varied directly and indirectly with reproduction. In assessing candidate paths for our model, we relied on what has been demonstrated in previous studies. As Hg and Pb are transferred trophically (Franson and Pain, 2011; Shore et al., 2011) via the eiders benthic prey items, we predicted by analogy that both elements may show inter-annual variation as has been described in other avian species in the region (McCloskey et al., 2013). We also expected that arrival date and arrival condition of female eiders would show inter-annual variation between the two sampling years, since these metrics are influenced by a variety of environmental conditions that often differ annually (Descamps et al., 2011; Love et al., 2010). We expected inter-annual variation in IgY as found in other northern eider species (Counihan et al., 2015), and inter-annual variation in CORT as several species of marine birds have highly variable CORT levels from year to year (Ninnes et al., 2011; Satterthwaite et al., 2012). We predicted that Hg would show a negative relationship with IgY, as Hg has been associated with depressed immune responses (Lewis et al., 2013b). Conversely, we predicted that Pb would be positively correlated with IgY, as Pb poisoning can lead to increases in humoral immune responses (Vallverdú-Coll et al., 2015). We also expected that Hg and Pb could be negatively related to body condition, as higher concentrations of these metals are related to lower body condition in mallard and eiders (Vallverdú-Coll et al., 2015; Wayland et al., 2002). We expected that both Hg and Pb would be positively correlated to arrival date, with birds arriving later in the breeding season having higher concentrations of these metals as are known to have neurotoxin effects that alter behaviours deleteriously (Franson and Pain, 2011; Wiener et al., 2003). We predicted that both Hg and Pb would negatively correlate with CORT, as Hg and other contaminants can act as endocrine disruptors in birds (Jayasena et al., 2011; Moore et al., 2014), and could therefore negatively impact the secretion of

CORT (Love et al., 2003).

We also included paths between IgY, arrival date, arrival condition and CORT to assess how Hg and Pb may influence CORT indirectly. We used IgY as a general indictor of health and immune status in the arriving eider ducks (Apanius and Nisbet, 2006; Counihan et al., 2015). Based on this framework, we predicted that IgY would be negatively correlated with arrival condition, with birds in better condition arriving with lower IgY levels as compared with birds with poorer condition (Bourgeon et al., 2010). We also expected IgY to be negatively correlated with arrival date, based on work by Counihan et al. (2015), demonstrating that reproductive timing and IgY can be negatively correlated in eider ducks. We measured baseline CORT levels in this study, using it as a metric of general physiological condition; higher CORT levels relate to greater energetic demand (Hennin et al., 2016, 2014). Based on this interpretation, we predicted that arrival condition would be positively correlated with CORT, with birds arriving in better condition having higher baseline CORT levels (Hennin et al., 2014). We also expected arrival date to positively correlate with CORT, with birds arriving earlier in the year having lower CORT levels, and those arriving later, and thus closer to breeding, having higher baseline CORT levels (Hennin et al., 2014). A summary of our predictions upon which our framework was based is provided in Fig. 1.

2. Materials and methods

2.1. Capture

Female eider ducks were caught at the breeding colony on Mittivik Island (East Bay Migratory Bird Sanctuary) in Northern Hudson Bay (64°01′04′N, 82°07′49′W) in 2013 ($n = 98$) and 2014 ($n = 92$). These female eiders spend the non-breeding season in waters off of Greenland and Newfoundland each year. They return to northern Canada between May and June (Mosbech et al., 2006), and lay their eggs between June and July (Love et al., 2010). Females were caught using monofilament flight nets as they arrive on the island, and before nest initiation. Only the initial capture data in each year were reported in this study. Each female was subsequently banded, marked using temporary nasal tags using UV degradable monofilament, and several samples were taken from the birds (see below). Several body metrics were also collected from each female at the banding station: individuals were weighed (g) using a pesola scale, and total head length (mm) was measured using calipers. All appropriate animal care permits were in place and approved by the Environment and Climate Change Canada Animal Care Committee.

2.2. Blood sampling

A small blood sample (maximum 1 ml) was taken immediately from the tarsal vein after capture at the net and within 3 min of the birds hitting the net using a 23G thin wall, 1-inch (c. 25-mm) needle attached to a heparinized 1-ml syringe. This sample was used to assess baseline CORT (Hennin et al., 2014; Romero and Reed, 2005; Wingfield et al., 1982) and immunoglobulin Y (IgY; Legagneux et al., 2014). All blood samples were kept at 4 °C and centrifuged at 10,000 rpm for 10 min within 6 h of collection. Upon separation, the plasma component of the blood was collected and stored at −20 °C for further analysis. A second blood sample was taken from the jugular vein while the bird was at the banding station (maximum 1 ml) to assess individual trace element concentrations. Whole blood samples were placed in acid-rinsed vials, kept at 4 °C, and frozen within 6 h of collection.

2.3. Trace element analysis

Analyses were conducted by RPC Laboratories (Fredericton, New Brunswick) for total Hg and Pb (Table 1; also shows detection limits). Each blood sample was prepared by microwave-assisted digestion in nitric acid (based on EPA Method 3051). The resulting solutions were then analyzed for Cd, Pb and Se by inductively coupled plasma mass spectrometry (ICP-MS; Thermo Elemental – X7 Quadrupole ICP-MS), with sample responses compared against standard calibration curves (based on EPA Method 200.8). Mercury was analyzed by cold vapour atomic absorption spectroscopy (AAS; based on EPA Method 245.6). Quality assurance/quality control (QA/QC) procedures included analysis of three reagent blanks, two certified biological reference tissues (DORM-4 and DOLT-4; National Research Council, Canada), two standard samples (Laked Horse blood; to assess between batch reproducibility), and two randomly selected duplicate samples per batch of 35 samples. All QA/QC measures were in compliance with the normal laboratory operating procedures at the time of analysis. All trace element concentrations are presented in wet weight µg/g, unless otherwise indicated for comparison purposes (Table 1).

2.4. Physiological assays

Baseline plasma CORT was analyzed using an enzyme-linked immunoassay (EIA; Assay Designs, Ann Arbor, MI, USA), previously validated in common eiders (Hennin et al., 2014). Samples were run in triplicate at a 1:20 dilution with 1.5% of kit-provided steroid displacement buffer (Hennin et al., 2014). Plates (Biotek Synergy H1) were run with a kit-provided standard curve by

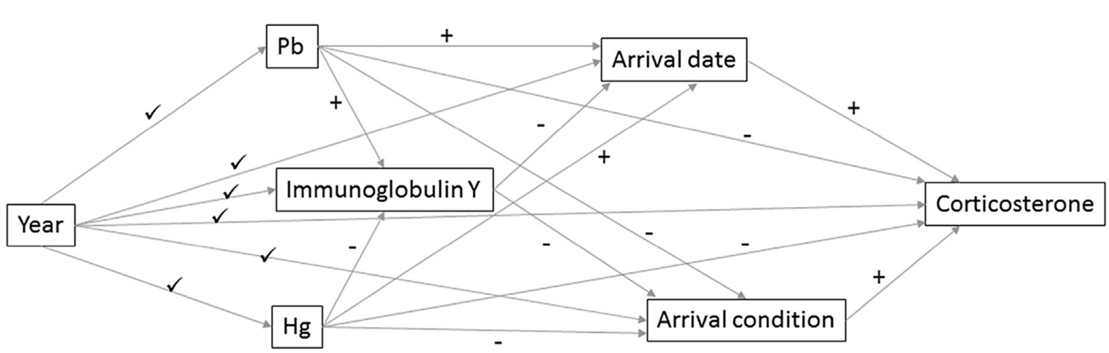

Fig. 1. Hypothesized global path model between two trace metals (mercury and lead) and known breeding precursors in breeding female eider ducks (year, arrival date, arrival condition, immunoglobulin Y and corticosterone) over two years of sampling (2013 and 2014).+ signs indicate a predicted positive relationship between the variables and - indicates a predicted negative relationship. ✓ indicates were an inter-annual differences was expected.

Table 1
Trace element concentrations in whole blood from female common eider ducks (*Somateria mollissima*) upon their arrival at a breeding colony in northern Hudson Bay ($n = 193$). Arithmetic mean with standard deviation (SD), geometric mean with 95% confidence intervals (CI), minimum detected values and maximum detected values are given along with the detection limits for each element. All concentrations given in μg/g wet weights (ww).

Elements	Detection limit	Mean (SD)	Geometric mean (95% CI)	Minimum, maximum levels
Cadmium (Cd)				
Overall	0.0005	0.003 (0.001)	0.0023 (0.0002)	0.0005, 0.0108
2013		0.002 (0.001)	0.0022 (0.0001)	0.0004, 0.0059
2014		0.003 (0.001)	0.0025 (0.0003)	0.0004, 0.0108
Lead (Pb)				
Overall	0.005	0.009 (0.006)	0.008 (0.001)	0.005, 0.043
2013		0.009 (0.006)	0.008 (0.001)	0.0045, 0.043
2014		0.009 (0.006)	0.008 (0.001)	0.0045, 0.039
Mercury (Hg)				
Overall	0.01	0.21 (0.06)	0.20 (0.008)	0.08, 0.43
2013		0.19 (0.05)	0.18 (0.010)	0.08, 0.34
2014		0.22 (0.06)	0.21 (0.012)	0.09, 0.43
Selenium (Se)				
Overall	0.05	4.13 (1.11)	3.96 (0.18)	1.01, 8.11
2013		4.11 (1.13)	3.97 (0.22)	1.77, 8.11
2014		4.15 (1.36)	3.94 (0.27)	1.01, 7.26

serially diluting a 200,000 pg ml^{-1} CORT standard and a control of laying hen plasma (Sigma-Aldrich Canada, Oakville, ON, Canada). Assay plates were read on a spectrophotometer plate reader at 405 nM and the mean inter- and intra-assay coefficients of variation across all plates were 8.17 and 7.99%, respectively. All CORT samples were run in the year of sampling.

The level of plasma IgY was used as an indicator of overall humoral adaptive immune function (Bourgeon et al., 2009, 2006). A sensitive, in-house, ELISA (enzyme-linked immunosorbent assay) method previously validated in common eiders (Bourgeon et al., 2006) was used to determine the amount of total IgY in eider plasma (diluted to 1/32,000 in carbonate–bicarbonate buffer; Bourgeon et al., 2006). The mean intra-assay coefficient of variation across all plates was 3.08%.

2.5. Statistical approaches

Levels of the four trace elements (Cd, Hg, Pb and Se) were examined in female eider blood. The arithmetic means with standard deviation (SD) and the geometric means with 95% confidence intervals along with the range of values are presented (Table 1). When values of less than the detection limit occurred, values were set at 10% below the detection limit to complete the multivariate analysis and geometric mean calculations. Levene's test was used to confirm homogeneity of variances and the Shapiro-Wilk test was used to confirm data normality. Data were log (Pb, CORT, Hg molar mass, Se molar mass) or square–root (Hg) transformed to approach the assumptions for parametric multivariate analysis where necessary. We used body mass divided by the total head length as an index of body condition as these two variables have been shown to reflect overall body condition (Jamieson et al., 2006). Since samples were collected over two breeding seasons, we also included sampling year as a predictor variable. We also assessed the relationship between the molar mass of Hg and Se using a GLM as these trace elements are known to co-vary in some species.

First we evaluated how the four trace elements (Cd, Hg, Pb and Se) as predictor variables to our four predictor variables (arrival condition, arrival time, IgY and CORT) using general linear models (GLM). For individual predictor variable, the global model included all the interactions possible between the predictor variables (the trace element concentrations). We then compared the global model series to a series of reduced models, including the null model which contained only an intercept value. We used maximum-likelihood methods, evidence ratios and ΔAICc (Akaike's information criterion corrected for small sample sizes) to compare the relative weight of support between the models and select the top model. The trace elements that were found repeatedly in the top models for each predictor variable were considered within the following step to evaluate the interactions. Importantly, we repeated this procedure including Hg and Se as separate independent variables, and as a single combined variable of Hg:Se molar mass ratio to examine in the relationship between Hg and Se varied when considered together. Only models with ΔAIC < 2.0 are presented in addition to the null and global models. Model parameter estimates were calculated across all models, and used to assess the average sign (positive or negative relationship between the predictor and response variables) and the relative magnitude.

Second, we used a path analytic approach (Shipley, 2009, 2000) to further explore the relationships between the trace elements identified as significant in the AIC model selection process (Hg and Pb), and the physiological variables (CORT, IgY), in relation to arrival date and body condition. A path analytic approach allows for the user to draw stronger inferences from correlational data than linear models (LM) and generalized linear models (GLM) do on their own (Shipley, 2009, 2000). This method was also chosen because it allows us to conceptualize and evaluate both direct and indirect affects that Hg and Pb may have on other variables known to be associated with breeding in eider ducks. Path analysis is also robust to non-normal data through the use of different model parameters based on individual data sets (Shipley, 2009), as transformations did not completely normalize all the data. Data were analyzed using R3.1.1 statistical software (R Development Core Team, 2013). All statistical tests were evaluated at $\alpha = 0.05$ and means are presented ± SD.

The global path model was constructed based on previous knowledge of how trace elements, arrival date, condition metrics and physiological parameters are known to interact (Fig. 1). The fit of the model was evaluated through directed separation (d-sep) tests (Shipley, 2009). Global model fit was evaluated using Fisher's C statistic: $C = -2\sum \ln(p)$, which follows a chi-squared distribution with $2 \times k$ df, where p is the null probability of each d-sep test ($n = k$; Shipley, 2009). The global model is rejected if the C value is below a significant p-value ($p < 0.05$), which is interpreted as the hypothesized causal structure of the model differing significantly from the correlational structure in the data. Each path was fit as appropriate to the data. To calculate relative path coefficients for each path (i.e., standardized partial regression coefficients), each variable (V) was standardized using z-score scaling (V − mean of V/ standard deviation of V), except for paths that related to year. All year paths were scored using d statistics which allow for better

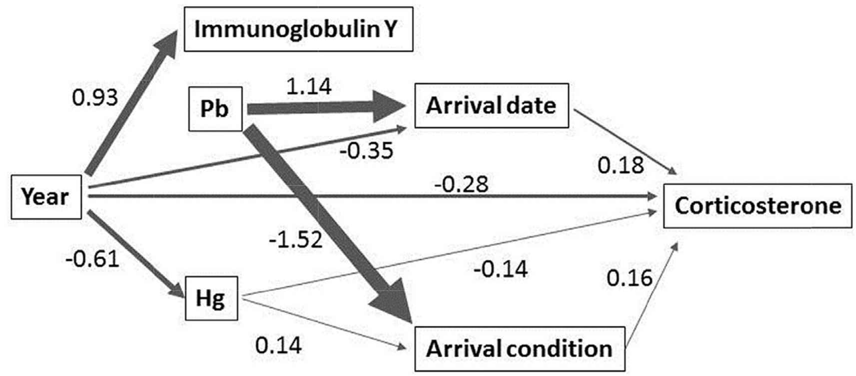

Fig. 2. Simplified path model showing the significant relationships between two trace metals (mercury and lead) and known breeding precursors in eider ducks (year, arrival date, arrival condition, immunoglobulin Y and corticosterone) over two years of sampling (2013 and 2014). Only significant paths are shown ($p < 0.05$). Standardized path coefficients are given for each path, with arrow size scaled to illustrate relative effect sizes. The full hypothesized model can be found in the Supplemental material.

estimates when a predictor variable is categorical (Nakagawa and Cuthill, 2007). Total effect sizes were calculated by summing the direct effect and the products of the indirect effect sizes (Shipley, 2000). All data from this project are archived and generally available through the Polar Data Catalogue (CCIN reference number: 11810).

3. Results

In 2013 all birds ($n = 98$) were caught between June 15th and July 3rd, and in 2014 ($n = 92$) birds were caught between June 11th and July 1st. We detected blood Cd concentrations above the detection limit for 181 female eiders with mean and median values well below reported values known to be associated with deleterious effects in birds (Table 1; Wayland and Scheuhammer, 2011). Blood Se concentrations were reported for all 190 female eiders, and were higher than those reported as adequate in birds (Table 1; Puls, 1994). Additionally, both Cd and Se concentrations in eider blood were much lower in this study than reported previously at this breeding colony (Wayland et al., 2001).

A total of 154 female eiders (or 81%) had blood Pb concentrations above the detection level, with concentrations on average below the normal range reported for waterfowl (0.02–0.50 ppm ww; Puls, 1994, Table 1). All female eiders had blood Hg concentrations within normal ranges reported for waterfowl and seabirds including previous studies at East Bay Island (Wayland et al., 2001; Appendix A), and below values reported to be associated with impaired reproduction (Evers et al., 2004; Puls, 1994; Shore et al., 2011).

We found no significant correlation between the molar mass of Hg and Se (GLM $F_{1,189} = 0.04$, $p = 0.84$). Although correlations between Hg and Se have been found more consistently in mammals, our results agree with those of Ohlendorf and Heinz (2011) and other studies from the Canadian Arctic (Provencher et al., 2014a) that no clear pattern between these two elements exists in marine birds.

While we found that blood Pb showed no significant interannual variation (GL M - $F_{1,188} = 0.16$, $p = 0.68$; Table 1; Fig. 2), blood Hg concentrations showed a significant difference between the two sampling years (GLM - $F_{1,188} = 17.8$, $p < 0.0001$; Table 1; Fig. 2).

3.1. AIC model selection

When the model sets were compared across the four predictor variables, Pb was found to often be included in the top model, and have the largest absolute averaged model estimate (Appendix B-I; online supplemental material). We also found that Hg was in the top three variables when parameter estimates were averaged across models, indicating that variability in the response variables was also influenced by Hg values. Importantly, when we included the Hg:Se ratio as a predictor variable, we did not find that the Hg:Se ratio significantly explained any of the response variables (Appendix B-I; online supplemental material). We examined this body of evidence, and chose to include Hg and Pb in our next analytical steps to further explore the relationships with the chosen reproductive variables.

3.2. Path model fit

Data from 190 female eider ducks were used to test and parameterize our path model (Fig. 1). Our final hypothesized model was consistent with the correlational structure of the data (Fisher's C statistic $= 5.01$, df $= 6$, $p = 0.54$), suggesting the global model constructed significantly fit the data. We found that Hg and Pb varied significantly with several variables in the hypothesized model, and while some relationships followed our predictions, not all relationships did (Fig. 2).

3.3. Variables related with IgY

IgY was measured in the blood of all females examined, and levels were significantly higher in females in 2013 compared with 2014 (GLM - $F_{3,186} = 14.56$, $p < 0.0001$; Table 2; Fig. 2). Contrary to our predictions, we found that IgY did not vary significantly with blood concentrations of either Hg or Pb (Hg - GLM - $F_{3,186} = 14.56$, $p < 0.30$; Pb - GLM - $F_{3,186} = 14.56$, $p = 0.24$).

3.4. Variables related to arrival condition

Mean (SD) female mass was 2171 ± 185 g, with no difference in arrival condition between the two sampling years (GLM - $F_{4,185} = 8.97$, $p = 0.79$; Table 2; Fig. 2). Contrary to our predictions, we found no relationship between IgY and arrival condition (GLM - $F_{4,185} = 8.97$, $p = 0.15$; Fig. 2). Both Hg and Pb varied significantly with arrival condition, but in opposite directions. Female eiders with higher blood Pb concentrations had lower arrival condition (GLM - $F_{4,185} = 8.97$, $p < 0.0001$; Fig. 2). Female eiders with higher blood Hg concentrations had higher arrival condition (GLM - $F_{4,185} = 8.97$, $p = 0.04$; Fig. 2).

Table 2
Biometric values for breeding female common eider ducks (*Somateria mollissima*) caught during the pre-breeding season (June to July) in 2013 and 2014 at East Bay Island, in northern Hudson Bay.

	Total n	Corticosterone (ng/ml)	Immunoglobulin Y (absorbance units)	Condition Index (body mass/head length)
2013	98	9.93 ± 14.09	0.73 ± 0.17	7.71 ± 0.70
2014	92	11.98 ± 12.70	0.59 ± 0.15	7.78 ± 0.64
Overall	190	10.94 ± 13.43	0.66 ± 0.18	7.75 ± 0.69

3.5. Relationships with arrival date

Median arrival date of female eiders was earlier in 2013 than 2014 (GLM - $F_{4,185} = 8.97$, $p = 0.04$), although the effect was small (Fig. 2). Concentrations of Hg (GLM - $F_{4,185} = 8.97$, $p = 0.88$) or IgY (GLM - $F_{4,185} = 8.97$, $p = 0.82$) were not related to female arrival date. However, there was a positive correlation between blood Pb levels and arrival date of female eiders (GLM - $F_{4,185} = 8.97$, $p > 0.0001$), with eiders arriving later in the season having higher blood Pb concentrations (Fig. 2).

3.6. Variables related with baseline CORT

Baseline CORT was detected in all 190 female eiders sampled (Table 2), and females sampled in 2014 had higher CORT than those caught in 2013 (GLM - $F_{6,183} = 3.37$, $p = 0.03$; Table 2; Fig. 2), although the effect size was small (Fig. 2). While no significant relationship between Pb and CORT was detected (GLM - $F_{6,183} = 3.37$, $p = 0.64$), there was a trend for females with higher Hg to have lower CORT (GLM - $F_{6,183} = 3.37$, $p = 0.05$; Table 2; Fig. 2). Additionally, female eiders with higher CORT arrived at the colony later (GLM - $F_{6,183} = 3.37$, $p = 0.02$) and in better body condition (GLM - $F_{6,183} = 3.37$, $p = 0.04$, Fig. 2).

4. Discussion

The purpose of this study was to examine how Cd, Hg, Pb and Se concentrations are directly or indirectly related to physiology, condition and reproduction in a free-living Arctic bird. Overall our findings suggest that blood concentrations of all four elements in female eider ducks in northern Hudson Bay are below reported levels associated with toxic effects (Puls, 1994), and similar or lower to what has been reported for this species at this location (Mallory et al., 2014; Wayland et al., 2001), as well as in other regions (Franson et al., 2004, 2000; Meattey et al., 2014). While we found that generally blood concentrations of Pb and Hg were low, we did find that these blood concentrations significantly varied with several parameters known to influence breeding success in the species.

4.1. Trace elements and corticosterone

We detected a significant negative relationship between Hg and baseline levels of CORT. Our findings support the growing body of literature that suggests that Hg may disrupt endocrine capabilities in birds (Herring et al., 2012; Pollock and Machin, 2008; Tartu et al., 2016b, 2013). While the size of the direct relationship between Hg and CORT was small (−0.14), the total indirect effect of year and Hg on CORT was more than five times greater (−0.75). This suggests that inter-annual variation in eider Hg blood concentrations had significant effects on CORT, and likely contributes to variation in endocrine system outputs in eider ducks. This is important as baseline CORT, among other physiological parameters dependent on endocrine systems, strongly influences reproductive success in eiders (Hennin et al., 2016, 2014) and other species (Lattin et al., 2016; Love et al., 2005).

Our findings should be interpreted within the larger rubric of endocrine biology when applying them to even relatively simple ecological systems. We chose to construct our hypothesized global path model with CORT downstream of arrival date and condition, but there are other interpretations of this complex and interrelated system. We recognize that CORT likely influences condition both before individuals arrive, and afterwards (e.g. Lovvorn et al., 2012). While altering the position of CORT in the model in relation to arrival date and condition alters the effect size slightly, the overall significance of the global model is not affected because the number and identity of the individual paths are not altered.

Interestingly, while our results show that higher Hg concentrations are related to lower levels of CORT (direct effect size −0.14; Fig. 2), we also found that Hg is positively, but indirectly associated with CORT via arrival condition, with an indirect effect size that is twice as large as the direct relationship (0.30; Fig. 2). This suggests that although Hg and CORT may be directly negatively correlated, the drive to increase in condition via increased foraging (and thus uptake of Hg), after arrival and in preparation for reproduction may have a larger positive effect on CORT secretion than the associated Hg that comes with it. Importantly, inter-annual variation in food availability (access to benthic invertebrates in ice-free regions) may also confound the effects of Hg on CORT, with potentially limited food resources (thus lower Hg levels) associated with increased CORT. This demonstrates how relationships between condition and trophically transferred factors can be complex and should be considered simultaneously in terms of their relations with endocrine levels that affect resource acquisition (Bulté et al., 2012; Marcogliese and Pietrock, 2011).

We note that our sample included female eiders that were at different physiological stages within the pre-breeding period. Recent work has demonstrated that female eiders that are captured and sampled during the rapid follicle growth period physiologically differ from females that are still in the pre-recruitment phase of breeding (Hennin et al., 2014). Thus, it is likely that any influence both Hg and Pb have on physiological parameters could affect these two groups differently, and thus breeding outcomes. While we recognize breeding stage as important, we were unable to test for these differences in a rigorous way due to limited sample size. Future work should take breeding stage into consideration to explore more fully how contaminants may differentially affect wildlife throughout their annual cycle.

We found no significant relationship between Pb blood concentrations and CORT levels. While the effects of Pb on CORT is less studied than Hg, some studies have shown no effect of Pb on CORT (Eeva et al., 2014), while other research has found correlations between Pb and maximum CORT levels in free-living birds (Baos et al., 2006). This suggests that Pb may affect CORT and other endocrine systems, but that it may be related to species or phase of the annual cycle.

4.2. Trace elements and immune function

Although trace elements, such as Hg, are often associated with immunosuppressive effects in wildlife even at sublethal levels (Fallacara et al., 2011; Kenow et al., 2007; Lewis et al., 2013a), we found no evidence of a relationship between IgY levels and either Hg or Pb concentrations. Similar to our findings, a study examining immunity metrics in great skuas (*Stercorarius skua*) nesting in the Shetland Islands, Iceland and Bjørnøya also found no significant relationship between IgY and organochlorines or PBDEs (Bourgeon et al., 2012). While several captive studies have shown negative effects of Hg on immune metrics (Lewis et al., 2013b), they often use a suite of immune metrics (i.e. skin swelling assays, response to sheep red blood cells assays etc.) to determine systemic responses within individuals. To date, studies of free-living species using a wide selection of immune metrics are few. We found that IgY did not vary with arrival date or condition even though IgY showed significant inter-annual variation (Table 2). This suggests that general immune status may not be a large driver of timing of and condition at arrival on the breeding grounds in female eiders. Further, inter-annual IgY variation has been showed in other studies (Counihan et al., 2015; Hegemann et al., 2012; Staszewski et al., 2007), but there is little consensus on what causes this variation. While immunity likely plays some role in reproduction, IgY on its own may not be specific enough to detect any subtle differences in relation to contaminants and breeding metrics. To investigate further how Hg may influence northern birds' immunity, a broad suite of immune metrics including immune responses to novel immunological challenges may be required.

4.3. Trace elements, timing and condition

While Pb levels in females eiders did not vary with IgY or CORT, later arriving birds and birds in poorer condition did have higher blood Pb concentrations. Typically, eiders arriving on the breeding colonies earlier often perform better reproductively (Descamps et al., 2011), and thus our findings suggest that eider ducks with higher Pb levels would likely have had lower reproductive success. Taken together, these two findings suggest that while blood Pb levels may be very low in eider ducks in the Canadian Arctic in June, there are likely sublethal effects of Pb on eider reproduction. While Pb is thought to be both trophically transferred and originating from point sources of Pb in the environment (Finkelstein et al., 2003), high Pb concentrations in waterfowl have also been linked with embedded Pb shot (Johansen et al., 2001; Merkel et al., 2006; Sanderson et al., 1998; Scheuhammer, et al., 1998). Although Pb shot was banned for shooting waterfowl in North America since 1999, Pb shot is still used in Greenland where many eider ducks from Arctic Canada spend the non-breeding season (Mosbech et al., 2006). Migratory birds may also consume spent shot in the environment that is still used for hunting non-migratory bird species, mistaking it for grit, which is commonly ingested (Schummer et al., 2011). Additionally, waterfowl have been reported to ingest Pb fishing weights, another source of Pb in the aquatic environments (Schummer et al., 2011). Regardless of the source, our results suggest that even at blood Pb levels below toxic concentrations, the consistent patterns of higher Pb levels found in later-arriving females with lower body condition is suggestive of sublethal effects.

Interestingly, the effect of Pb on arrival condition was the largest effect detected in this study (−1.52; Fig. 2), indicating that this relationship warrants further consideration to understand the underlying causes and mechanisms. Since Pb exposure is known to cause increased oxidative stress and impaired constitutive immunity (Vallverdú-Coll et al., 2016, 2015), reduced condition in birds exposed to Pb may occur through several mechanistic pathways affecting foraging rates or energetics. Similar to the results presented here, Newth et al. (2016) found that body condition in whooper swans (*Cygnus cygnus*) to be negatively association with Pb concentrations at much lower levels than previously established clinical thresholds. Taken together these results suggest that even low levels of Pb exposure have sub-lethal effects on wild birds.

While we found that low Cd concentrations in blood from breeding female eider ducks, Mallory et al. (2014) reported high levels of Cd in common eider livers from the same colony only a few years earlier. Additionally, while we found that contemporary concentrations of blood Cd were lower when compared with historic samples, Mallory et al. (2014) found an increasing trend in liver Cd concentrations over time. These contrasting findings suggest that eider ducks may be increasingly being exposed to Cd on their wintering grounds leading to an increase in liver concentrations which are reflective of contaminant exposure over the previous months, but not on their breeding areas as seen in low levels of Cd in blood which is reflective of the exposure over days (Hobson and Clark, 1992). This highlights how migratory species can be differentially exposed throughout their annual cycles; an important component to consider when assessing how tissue concentrations are related to biological effects.

As mentioned, Hg concentrations detected in many Arctic wildlife species in Canada continued to increase over the last few decades (Riget et al., 2011), with only a recent leveling off of Hg levels in some species (Braune et al., 2015; 2016b). While many species may have low exposure to both Hg and Pb, this study suggests that sublethal levels of trace metals from the environment may be associated with reduced reproductive outputs. Additionally, those levels of contaminants, if maintained, might have sublethal or greater effects on wildlife during other times of the year, particularly during times of energetic stress such as winter months when lower temperatures are coincident with reduced foraging rates (Lemly, 1993). Also important to consider is that, in wildlife, relative contaminant concentrations may increase or become mobilized (for lipophilic contaminants), as body mass decreases during energetically demanding times, notably breeding and migration (Bustnes et al., 2010). Thus, future studies examining how sublethal effects of contaminants on wildlife need to focus on times of energetic stress rather than just times of resource abundance in trying to elucidate potential impacts of contaminants on hosts.

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Appendix. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.08.052.

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