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Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Concentrations of 17 elements, including mercury, and their relationship to fitness measures in arctic shorebirds and their eggs

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ARTICLE INFO

Article history:

Received 6 November 2009

Received in revised form 15 March 2010

Accepted 16 March 2010

Keywords:

Charadriiformes

Heavy metal

Red blood cells

Primary feathers

Nearctic

ABSTRACT

Exposure to contaminants is one hypothesis proposed to explain the global decline in shorebirds, and this is of particular concern in the arctic. However, little information exists on contaminant levels in arctic-breeding shorebirds, especially in Canada. We studied potential contaminants in three biparental shorebird species nesting in Nunavut, Canada: ruddy turnstones (*Arenaria interpres*), black-bellied plovers (*Pluvialis squatarola*) and semipalmated plovers (*Charadrius semipalmatus*). Blood, feathers and eggs were analyzed for As, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, and Zn. We assessed whether element concentrations a) differed among species and sexes, b) were correlated among pairs and their eggs, and c) were related to fitness endpoints, namely body condition, blood-parasite load, nest survival days, and hatching success.

Non-essential elements were found at lower concentrations than essential elements, with the exception of Hg. Maximum Hg levels in blood approached those associated with toxicological effects in other bird species, but other elements were well below known toxicological thresholds. Reproductive success was negatively correlated with paternal Hg and maternal Pb, although these effects were generally weak and varied among tissues. Element levels were positively correlated within pairs for blood-Hg (turnstones) and feather-Ni and Cr (semipalmated plovers); concentrations in eggs and maternal blood were never correlated. Concentrations of many elements differed among species, but there was no evidence that any species had higher overall exposure to non-essential metals. In conclusion, whereas we found little evidence that exposure to the majority of these elements is leading to declines of the species studied here, Hg levels were of potential concern and both Hg and Pb warrant further monitoring.

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1. Introduction

Many shorebird species are in decline around the world (IWSC, 2003), including two thirds of those found in Canada (Donaldson et al., 2000). Several global causes for these declines have been suggested, including habitat loss and fragmentation, reduced prey availability, and increased exposure to environmental pollutants (Morrison et al., 1994). Shorebirds may be at particular risk of contaminant exposure as their primary feeding areas include coastal and estuary sediments and agricultural fields, where many contaminants accumulate disproportionately (White et al., 1983; Evans et al., 1987). Environmental contaminants can cause avian mortality directly, but more commonly exact their toll through sub-lethal effects such as altered behaviour and reduced reproductive success, immune function, and body condition (Koller, 1980; Scheuhammer, 1987; Wolfe et al., 1998; de Luca-Abbott et al., 2001; Clotfelter et al., 2004).

Shorebirds that nest in the arctic are apparently especially vulnerable to decline, likely due to the risks associated with their lengthy migration (Zöckler et al., 2003), habitat disturbance due to climate change (Drent et al., 2006; Johnson et al., 2007), and/or contaminant exposure. The latter is of increasing concern for arctic wildlife, as pollution from industrialised areas is deposited disproportionately in the arctic via long-range air and water transport (MacDonald et al., 2000; AMAP, 2002). 'Legacy' organochlorines (e.g., DDTs) have decreased in arctic biota over the past decades (Braune et al., 2005). However some non-essential (i.e. toxic) elements such as mercury (Hg) have increased, particularly in Canada (Braune et al., 2005). Despite growing concern about shorebird declines and the effects of pollution on arctic wildlife (Braune et al., 2005; Fisk et al., 2005), there is a paucity of information about the contaminant loads of shorebirds nesting on Canada's arctic shores (Braune and Noble, 2009).

Whereas toxicology studies historically required lethal collection to evaluate contaminants in internal organs, sampling tissues that can be collected non-lethally has become increasingly common, with obvious advantages for threatened species. Blood samples provide the

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most direct, non-lethal assessment of dietary exposure, reflecting recent exposure primarily, and recently metabolised contaminants to a lesser extent (Moriarty, 1975; Pain, 1996; Evers et al., 2005). Egg contaminants reflect those in maternal blood and organs during egg formation. As arctic shorebirds provision eggs using primarily arctic-based resources (Klaassen et al., 2001; Morrison, 2006), egg elements should reflect those in the arctic environment. Feather contaminants accumulate via depuration of internal loads during feather growth, and/or exogenous absorption from the environment, to varying degrees depending on the contaminant. For example, feathers are an important depuration route for internal Hg and feather-Hg remains stable for the life of the feather (Burger et al., 1993), reliably indicating blood-Hg during growth (Goede and de Bruin, 1984; Thompson et al., 1991; Tavares et al., 2008). As arctic-nesting shorebirds grow most flight feathers on wintering grounds (Prater et al., 1977), feather levels of such endogenously accumulated elements provide otherwise hard to obtain information on contaminant exposure at the opposite end of the bird's range. Feather levels of elements that can be absorbed exogenously, such as cadmium (Cd), iron (Fe), lead (Pb), selenium (Se), manganese (Mn) and chromium (Cr) (Goede et al., 1989; Burger et al., 1993; Burger et al., 2008b), provide a measure of overall environmental exposure but are less reliable as indicators of internal levels (Goede and de Bruin, 1984; Goede and de Voogt, 1985; Scheuhammer, 1987; Pilastro et al., 1993; Pain et al., 2005).

We assessed the levels of 16 metals and Se in blood, feathers and eggs of three biparental shorebird species at a breeding site in Nunavut, Canada. Of these elements, As, beryllium (Be), Cd, Hg, nickel (Ni), Pb, antimony (Sb) and thallium (Tl) are toxic with no known biological use, whereas the others [cobalt (Co), copper (Cu), Cr, iron (Fe), Mn, molybdenum (Mo), Se, vanadium (V) and zinc (Zn)] are essential in small concentrations but toxic at higher levels. We compared element concentrations to measures of adult fitness, reproductive success, and established avian toxic effects thresholds to assess whether pollutants may pose a threat to East Bay shorebird populations. All three study species have experienced recent population declines in Canada (Donaldson et al., 2000), and local Inuit have noted overall declines in shorebird populations in the study area (Aiviq Hunters and Trappers Association, Coral Harbour, personal communication). To assess whether paired birds experienced similar contaminant exposure in the arctic and elsewhere, we tested for positive element correlations in blood and feathers. These data provide the first baseline element levels for non-lethally collected tissues of shorebirds nesting in Canada's arctic, and help fill a recognized knowledge gap regarding metals other than Hg and Cd in arctic wildlife (NCP, 2003).

We made four predictions: 1) blood levels would be positively correlated between paired birds, as pairs tend to feed close to their nest, but no correlation would exist for feathers, as pairs do not necessarily winter or migrate together; 2) levels would be positively correlated between females (blood) and their eggs, as shorebirds typically acquire the energy used for egg production in the arctic; 3) hatching success would be negatively correlated with toxic element levels in eggs and/or adults, as egg contaminants can influence embryo health directly and those in adults can decrease anti-predator behaviour (Clotfelter et al., 2004); and 4) adult toxic element levels would be correlated positively with blood-parasite loads and negatively with body condition.

2. Methods

2.1. Study site and species

Field work was conducted within the East Bay Migratory Bird Sanctuary on Southampton Island, Nunavut, (63°59'N, 81°40'W) during June and July 2008. We assessed contaminant loads of three shorebird species: ruddy turnstones (*Arenaria interpres*, hereafter

turnstones), black-bellied plovers (*Pluvialis squatarola*) and semipalmated plovers (*Charadrius semipalmatus*). Black-bellied plovers and turnstones were the focal species of this study, whereas blood and feathers were collected opportunistically from semipalmated plovers trapped for a separate study (DB Edwards, unpublished data). Tissue types vary slightly as a result (see below); samples sizes are presented in Table 1.

2.2. Adult condition and reproductive success

A long-term 3 × 4 km study plot was searched for nests of all shorebird species (ca. 450 person h), which were then monitored until they either failed or hatched (see Smith et al., 2007). We calculated two measures of reproductive success: a binary response for nests of known fate (hatched or failed; hereafter hatching success), and nest duration for all nests. Nest duration is the days of incubation completed, and was calculated as the Mayfield end date (Mayfield, 1975) minus the date of clutch completion, where the latter was either known or estimated by floating eggs (Liebezeit et al., 2007). Nests found later during incubation are more likely to be successful than those found at earlier stages (Mayfield, 1975); we accounted for this bias by including the estimated days of incubation completed when each nest was found as a covariate in models of reproductive success.

We trapped adult birds on the nest using Potter, bow-net or small Fundy pull traps (Gratto-Trevor, 2004). Birds were banded, weighed, and their exposed culmen measured. Since body condition can influence contaminant concentration (Wienburg and Shore, 2004; Jaspers et al., 2007), we quantified condition as mass/culmen (mass controlled for skeletal size; O'Hara et al., 2007). This was included as a covariate in analyses of reproductive success to control for parental body condition.

2.3. Blood

Blood samples (0.4 to 2 µL, always <1% of body weight) were collected from the brachial vein of trapped birds using a pre-heparinized syringe with a 25 gauge needle. Blood smears were made the same day, and once dry were fixed and stained with Diff-Quik. The entire area of each smear was later examined under a compound microscope (1000×) for blood parasites, particularly *Leucocytozoon* sp., *Hemoproteus* sp., and microfilarial species.

Entire blood samples from turnstones and black-bellied plovers were analyzed, whereas plasma from semipalmated plover blood was removed for a separate study, leaving only red blood cells (RBC) for contaminant analysis. To facilitate multi-species comparisons, we analyzed RBC and plasma separately for seven black-bellied plovers and six turnstones. This enabled us to compare RBC concentrations across all three species, and to calculate the proportion of each

Table 1

Sample sizes available for the study. Non-viable eggs were those found ejected from the nest or that failed to hatch in otherwise successful nests. For toxicological sampling, each egg was collected from a separate clutch. A complete family means that both adults of a pair and one of their eggs were sampled.

| Species | Reproductive success | | | Toxicological sampling | | | |
|----------------------|----------------------|---------------|-----------------|------------------------|----------------|-------|-------------------|
| | Nests found | Nests hatched | Non-viable eggs | Eggs | Females | Males | Complete families |
| Black-bellied plover | 15 | 4 | 1 | 13 | 7 | 5 | 5 |
| Ruddy turnstone | 29 | 20 | 4 | 27 | 21 | 15 | 10 |
| Semipalmated plover | 8 | 6 | 1 | 6 | 6 ^a | 6 | 6 |

^a One female semipalmated plover blood sample discarded; $n = 5$ for blood, $n = 6$ for feathers.

element in plasma vs. RBC, which we used to estimate whole-blood element concentrations for semipalmated plovers. To separate blood components, samples were centrifuged at 3100 rpm for 5 min, after which plasma was aliquoted into separate cryovials. Plasma was replaced with an equivalent volume of 0.9% saline solution added to the RBC. Samples were stored frozen until analyses. For split samples we calculated the concentration of each element in whole blood, $[W_{\text{split}}]$, as:

$$[W_{\text{split}}] = (\text{volP} * [P] + \text{volRBC} * [\text{RBC}]) / (\text{volP} + \text{volRBC}),$$

where volP is the volume of plasma, $[P]$ is the element concentration in plasma, volRBC is the volume of RBC, and $[\text{RBC}]$ is the element concentration in RBC, after accounting for saline added. Non-detect (ND) samples were set to half the detection limit and included in analyses if the element was detected in one blood component, otherwise W_{split} was set at half the detection limit for that element. Detection limits were 2 ng/mL for As, Cd, Tl, Pb, and <10 ng/mL for other elements. Whole-blood concentrations calculated from split samples did not differ from those obtained from intact whole blood for any element ($P > 0.1$ in all cases); whole and split samples were therefore pooled for analyses. One female semipalmated plover blood sample had to be discarded due to a recording error in the amount of saline added, leaving $n = 5$ samples.

For each element we determined the mean ratio in shorebird plasma:RBC as $[P]/[\text{RBC}]$ using split samples from 19 shorebirds (ratios did not vary among species so a single ratio was calculated; full details and calculations given in online Appendix A). Using these ratios and the relative volume of plasma:RBC in each blood sample as measured from packed cell volumes (DB Edwards unpublished data), we estimated the whole-blood element levels in semipalmated plover RBC samples. Arsenic was only detected in five samples and was not consistently higher in one blood component than the other, so a reliable ratio could not be determined; whole blood data are therefore presented only for black-bellied plovers and turnstones.

2.4. Feathers

A primary feather was plucked from each wing of each bird: the 7th primary (counted from the proximal primary outwards) from turnstones and black-bellied plovers, and the 6th primary from semipalmated plovers, as the 7th was being used for a separate study. Paired 6th and 7th primaries from other species did not differ in concentrations of any element (ALH unpublished data). These primaries are generally grown on wintering grounds rather than during fall migration, and are far enough away from outer primaries that their removal should not affect flying ability (Atkinson et al., 2005, E. Nol personal communication). Detection limits were 25 ng/g (Mn, Mo, and Co), 50 ng/g (As, Cd, Tl, and Pb), 100 ng/g (Hg, Cu, Zn, and Fe), and 250 ng/g (Be, Fe, V, Cr, Sb, and Ni).

2.5. Eggs

To assess contaminant exposure to developing embryos, one egg was collected from each complete clutch found before hatching had started. To account for potential variation in contaminant loads with laying order, we attempted to collect the oldest egg from each clutch. If clutches were found with a single egg this egg was marked with pencil and collected once the clutch was complete, otherwise eggs were floated (Liebezeit et al., 2007) and the oldest collected. Egg contents were homogenised for element analysis. Egg-content detection limits were the same as for feathers.

2.6. Element analyses

Samples were analyzed at the Diagnostic Centre for Population and Animal Health, Michigan State University. To remove as many surface contaminants as possible, feathers were washed in a 5% sodium dodecylbenzenesulfonate (Liquinox™) solution, rinsed with acetone and oven dried for 14 h at 75 °C. To obtain a liquid digest for analyses, all solid samples were acid digested with nitric acid, added to samples at a ratio of 0.5 g sample to 5 mL acid. Samples were then digested at 100 °C for 14 h in a closed cell, polytetrafluoroethylene (Teflon™) lined digestion vessel. Digests were diluted with 18 MΩ water to a ratio of 0.5 g sample to 25 g final solution, yielding a 50 time analytical dilution. 200 μL of each digest and blood sample was diluted with 5 mL of a solution containing 0.5% EDTA and Triton X-100, 1% ammonia hydroxide, 2% propanol and 20 ppb of scandium, rhodium, indium and bismuth as internal standards. An Agilent 7500ce inductively coupled plasma-mass spectrometer (ICP-MS) was used for the analysis. The ICP-MS was tuned to yield a minimum of 8000 cps sensitivity for 1 ppb yttrium (mass 89), <1.0% oxide level as determined by the 156/140 mass ratio and <2.0% double charged ions as determined by the 70/140 mass ratio. Each element was calibrated using a 4-point linear curve of the analyte to internal standard response ratio. Concentrations are given as dry weight for feathers and eggs and wet weight for blood.

2.7. Statistical analyses

We tested whether element concentrations differed among species and between sexes in each tissue: blood (whole blood and RBC), feathers, and egg contents. Analyses involved randomized general linear models (glms) since response variables (element concentrations) did not meet parametric assumptions. Separate randomization tests were performed for each element, in which F -statistics from 1000 linear models conducted on randomized data were compared to F -statistics from the linear model for the original data (Manly, 1997: SAS 9.2). The P values we report for these analyses represent the proportion of randomized F -tests that exceeded those obtained for the original model (Manly, 1997).

To assess the relationship between elements and fitness, we tested whether adult body condition was related to blood and feather concentrations of each element, and whether nest duration and hatching success were related to element concentrations in paternal tissues, maternal tissues, and egg contents. The first three analyses involved randomized glms, whereas analyses of hatching success (hatch or fail) considered binomial distributions and a logit-link function (SAS proc genmod). Sex and species were included as factors in all models that considered >1 sex (body condition) or species (all models), respectively. Models of reproductive success included the age of the nest when found (all models) and adult body condition (models considering parental tissue levels) as covariates. Models initially considered all possible interactions between independent variables, but non-significant interactions were dropped from models using backward elimination ($\alpha = 0.05$), unless they were integral to the study design (i.e. species × sex in body condition models). Significant factors and interactions were explored further using multiple-comparison tests, using $\alpha = 0.05$ for *a priori* orthogonal contrasts (species differences) and the Dunn-Šidák procedure for all others (Kirk, 1995). Models comparing binary hatching success to female tissue concentrations would not converge for semipalmated plovers, as all five semipalmated plover nests for which we had female blood data hatched (the blood sample from the only semipalmated plover nest to fail was discarded due to problems with saline addition). These models therefore do not consider this species.

Tests for correlations (i.e. no causal relationship) were performed on ranked data using the Spearman non-parametric correlation (ρ). When considering all species together, we avoided spurious correlations resulting from species-level differences by standardizing data before ranking, subtracting the species' mean from all observations for that species.

3. Results

Of the 17 elements tested, five were below detection limits in all samples; Be, V, Sb, Cd and Tl. Positive correlations among essential elements were common in blood and eggs, and less so in feathers (Tables B1 and B2, online Appendix B). Non-essential elements (As, Hg, Ni and Pb) were occasionally positively correlated with essential elements, but rarely with each other, the only exception being Hg and Ni in blood (Table B1). Hg and Se were never significantly correlated.

3.1. Species and sex differences

Interspecific and sex differences in blood element concentrations were consistent whether models considered RBC or whole blood (including whole-blood equivalents calculated from semipalmated plover RBC samples) as the response variable (Table 2, Fig. 1A and B; for full summary statistics see Table C1, online Appendix C). In whole blood, turnstones had the highest Hg and As, whereas black-bellied plovers had the highest levels of Cu and Pb; semipalmated plovers had the lowest blood-Hg and Ni (Table 2). In RBC samples, As was detected in most semipalmated plovers but in neither of the other species. Males had significantly lower blood-Pb than females, but higher blood-Mn (Table 2).

In feather samples, species differed in almost every element detected, but males and females did not, nor did sex interact significantly with species (Table 2). Consequently, we present feather data combined across sexes (Fig. 1C). Black-bellied plovers had the highest levels of As and Pb, whereas semipalmated plovers had the highest Co, Cr, Ni and Zn but the lowest Hg (Table 2). Turnstones were often intermediate to the other two species, but as with blood samples, tended to have the highest levels of Hg (Fig. 1C; full summary statistics available in Table C2, online Appendix C).

Unlike blood and feathers, few elements differed among species in eggs (Table 2) and Hg was the only toxic element detected. Of egg elements that differed among species, Fe, Mo and Zn were highest in black-bellied plover eggs and lowest in semipalmated plover eggs, whereas Hg showed the opposite pattern (Fig. 1D; full summary statistics see Table C3, online Appendix).

3.2. Patterns among tissues

We found that across all species and sexes, feather concentrations were not significant predictors of whole-blood concentrations for any element ($P > 0.5$). Further, species with the highest blood levels of a given element did not always have the highest feather-levels as well. For example, semipalmated plovers had the lowest blood-Fe but the highest feather-Fe (Table 2). Results did not change when considering RBC concentrations.

Mercury sequestration in eggs varied significantly among species whether measured as whole-blood:egg ($F_2 = 4.11, P < 0.05$) or RBC:egg ($F_2 = 3.99, P < 0.05$). The mean whole-blood:egg Hg ratio was 3:1 for black-bellied plovers and turnstones and 1:1 for semipalmated plovers ($t > 2.9, P < 0.05$; results did not change when considering RBC: egg).

3.3. Family relationships

Element concentrations were not generally correlated among pairs in either blood or feathers. When considering all three species together using standardized data, no elements were significantly correlated between paired birds in either tissue. Considering non-standardized data analyzed separately for each species, no elements were significantly positively correlated among pairs in blood, although Hg was weakly correlated among turnstones pairs ($\rho_{10} = 0.564, P < 0.1$). Feather concentrations were correlated among semipalmated plover pairs for Cr ($\rho_6 = 0.841, P < 0.5$) and Ni ($\rho_6 = 0.886, P < 0.05, n = 6$ pairs).

Considering all species together, elements in eggs were never correlated with levels in whole blood or feathers of either male birds

Table 2

Species and sex differences in element concentrations from red blood cells (RBC), whole blood, and feathers, and species differences for elements detected in egg contents. Different letters denote significant differences ($P < 0.05$), those in brackets denote marginally significant differences (factor effect $P < 0.1$). Sex differences are only given for metals that varied between sexes for at least one tissue.

| Metal | Tissue type | Species | | | Sex | | F statistic | | |
|-------|--------------------------|---------|------|------|-----|-----|-------------|--------|---------------|
| | | BBPL | RUTU | SEPL | F | M | Species | Sex | Species × sex |
| As | RBC | ND | ND | a | | | 4.17* | 0.766 | 0.914 |
| | Whole blood ^a | b | a | . | | | 4.52* | 1.98 | 0.872 |
| | Feather | a | b | c | | | 17.64*** | 0.1705 | 1.58 |
| Co | RBC | a | b | a | | | 8.14* | 3.34 | 0.728 |
| | Whole blood | ab | b | a | | | 10.6*** | 2.04 | 0.983 |
| | Feather | b | b | a | | | 6.64** | 1.31 | 0.799 |
| Cu | Egg | a | a | a | | | 1.38 | . | . |
| | RBC | (a) | (b) | (b) | | | 4.06† | 1.90 | 0.085 |
| | Whole blood | a | b | ab | | | 3.38* | 0.919 | 0.853 |
| Fe | Feather | a | b | a | | | 25.2*** | 0.611 | 0.839 |
| | Egg | a | a | a | | | 1.34 | . | . |
| | RBC | a | a | a | | | 1.18 | 0.525 | 0.651 |
| Hg | Whole blood | a | ab | b | | | 4.57* | 0.301 | 3.64† |
| | Feather | b | b | a | | | 12.6*** | 2.40 | 0.637 |
| | Egg | a | ab | b | | | 5.68** | . | . |
| Mn | RBC | ab | a | b | | | 6.31* | 1.58 | 1.20 |
| | Whole blood | b | a | c | | | 33.3*** | 2.34 | 1.15 |
| | Feather | a | a | b | | | 12.4*** | 1.60 | 0.270 |
| Mo | Egg | b | a | a | | | 13.9*** | . | . |
| | RBC | a | a | a | | | 1.43 | 2.13 | 1.55 |
| | Whole blood | a | a | a | a | b | 0.328 | 8.94** | 0.983 |
| Ni | Feather | b | b | a | (a) | (b) | 11.5** | 3.55† | 0.159 |
| | Egg | a | a | a | | | 2.41 | . | . |
| | RBC | ab | b | a | | | 5.83* | 1.30 | 0.583 |
| Pb | Whole blood | a | b | a | | | 15.2*** | 1.33 | 0.328 |
| | Feather | a | a | a | | | 0.459 | 0.606 | 1.33 |
| | Egg | a | b | b | | | 28.6*** | . | . |
| Se | RBC | a | ab | b | | | 4.25* | 0.977 | 0.611 |
| | Whole blood | a | a | b | | | 7.66** | 1.23 | 1.04 |
| | Feather | c | b | a | | | 16.1*** | 0.074 | 0.038 |
| Zn | RBC | a | a | a | a | b | 2.70 | 7.54† | 0.506 |
| | Whole blood | a | b | ab | a | b | 5.95** | 7.82† | 0.126 |
| | Feather | a | ab | b | | | 5.98** | 0.050 | 0.980 |
| Zn | RBC | a | a | a | | | 2.98 | 0.102 | 2.41 |
| | Whole blood | b | a | a | | | 6.12* | 0.211 | 1.76 |
| | Feather | b | a | a | | | 19.1*** | 2.08 | 0.600 |
| Zn | Egg | a | a | a | | | 1.63 | . | . |
| | RBC | a | a | a | | | 1.14 | 1.84 | 0.534 |
| | Whole blood | a | a | a | | | 0.710 | 0.241 | 2.46 |
| Zn | Feather | c | b | a | | | 14.7*** | 0.024 | 1.50 |
| | Egg | a | a | b | | | 4.27* | . | . |

† $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ND = not detectable.

^a Arsenic concentrations in whole blood could not be reliably calculated from those in RBC, so this model considers black-bellied plovers and ruddy turnstones only.

($P > 0.05$ in all cases, $n = 26$ sire-egg pairs), or female birds ($P > 0.2$ in all cases, $n = 34$ dam-egg pairs). These patterns were consistent when species were analyzed separately using non-standardized data.

3.4. Contaminants and condition

Body condition (mass/culmen) was not significantly related to concentrations of any non-essential element. Whole-blood Se was significantly related to body condition for black-bellied plovers, but only for females (sex × [blood]: $F_1 = 4.44, P < 0.05$; Table 3). Body

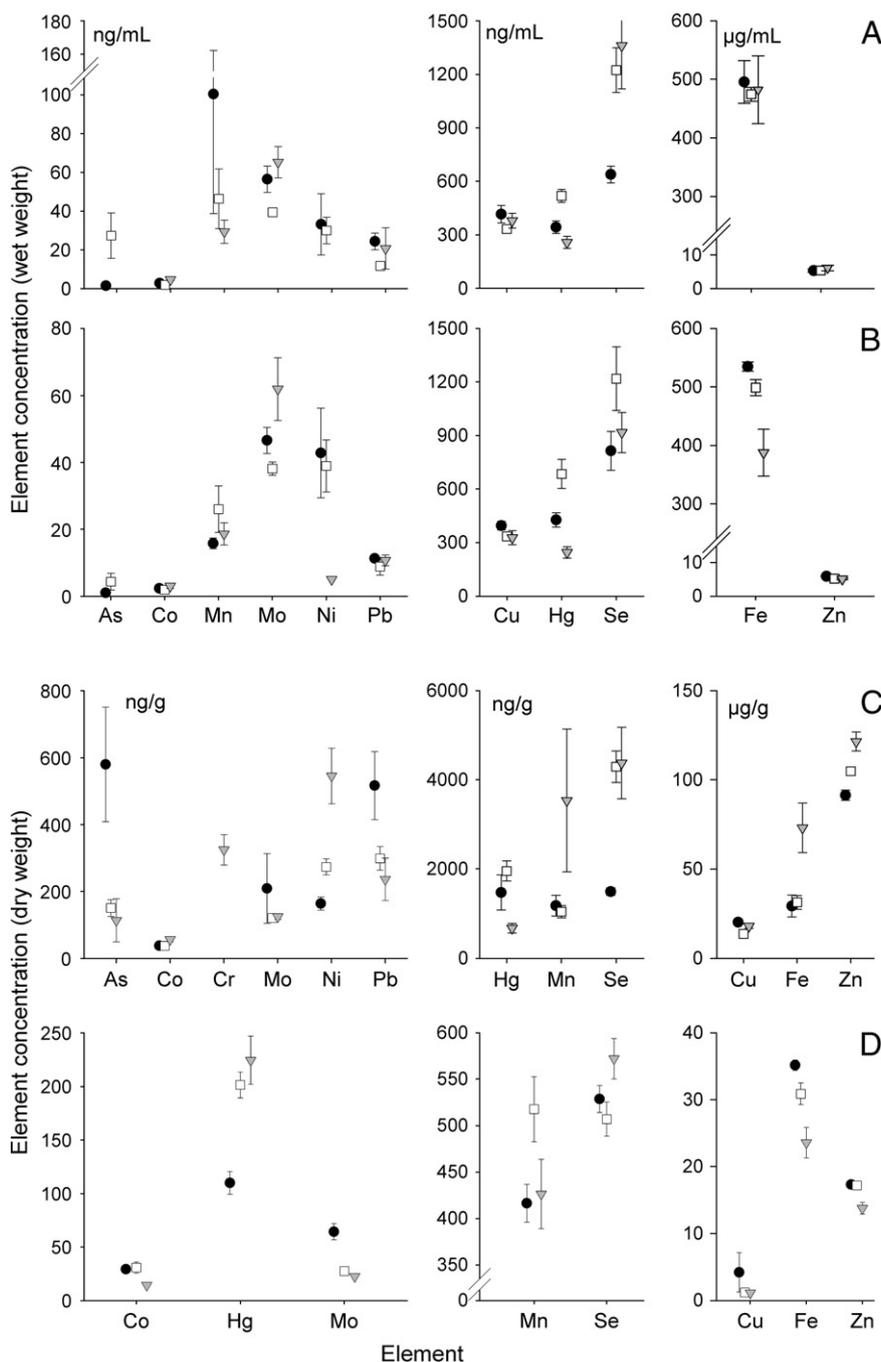


Fig. 1. Mean (\pm SE) element concentrations in whole blood (semipalmated plover levels calculated from RBC values) of females (A) and males (B), primary feathers (sexes combined; C) and eggs (D) of black-bellied plovers (●), ruddy turnstones (□), and semipalmated plovers (▼). In blood, As was ND in all male black-bellied plovers and Ni was ND in all male semipalmated plovers, whereas Cr was ND for all black-bellied plover and turnstone feathers. Arsenic, Cr, Pb and Ni were ND in all eggs.

condition did not vary between males and females for any species ($F_1 = 0.67, P > 0.5$). No blood parasites were found.

3.5. Egg contaminants and reproductive success

When hatching success was modelled as a binary variable (hatch or fail), it was not significantly predicted by egg concentrations of any element. Only a small number of eggs from each species were unviable (i.e. failed to hatch in otherwise successful nests; Table 1), and no nests were abandoned. Thus, predation appeared to be the main cause of hatching failure.

After accounting for species differences and the age of the nest when found, the number of days for which each nest was active was related to

egg concentrations of Fe, Mn and Mo. Egg-Fe varied negatively with nest duration, but this was only significant for black-bellied plovers (species \times [egg]: $F_1 = 4.99, P < 0.01$; Table 3). Nest duration was negatively associated with egg-Mn, but positively associated with egg-Mo (Table 3).

3.6. Parental contaminants and reproductive success

Several elements in maternal tissues were related to reproductive success, although these relationships were generally weak (Table 3). Maternal blood-Pb was negatively associated with both binary hatching success (black-bellied plovers and turnstones) and nest duration (all species), as was blood-Cu (Table 3; Fig. 2). Hatching success was

Table 3

Significant relationships between fitness measures and element concentrations in biparental shorebirds [black-bellied plovers (BBPL), ruddy turnstones (RUTU), semipalmated plovers (SEPL)]. Separate analyses were conducted for females, males and eggs; analyses of toxic elements in adults considered both feather and blood concentrations, whereas analyses of essential elements considered blood only. Test statistics are *F*-statistics for models considering all species, and *t* statistics for species-specific contrasts.

| Fitness endpoint | | | | Test statistic | Partial regression |
|---|-------------|-----------|-----|----------------------|--------------------|
| Element | Tissue | Species | Sex | (<i>df</i> =1) | coefficient ± SE |
| <i>Body condition</i> | | | | | |
| Se | Whole blood | BBPL | F | 2.29 [†] | 0.003 ± 0.0013 |
| <i>Hatching success (hatch or fail)^a</i> | | | | | |
| Cu | Whole blood | All | F | 7.93 ^{**} | -0.0392 ± 0.0199 |
| | | | M | 5.05 [*] | 0.0436 ± 0.0243 |
| Hg | Feather | All | M | 4.85 [*] | -0.0013 ± 0.0007 |
| | Whole blood | BBPL&RUTU | F | 3.78 [†] | -0.0088 ± 0.0052 |
| Mo | Whole blood | All | M | 9.04 ^{**} | 0.263 ± 0.135 |
| Ni | Whole blood | BBPL&RUTU | F | 5.60 [*] | 0.0886 ± 0.0501 |
| | Feather | BBPL | F | 35.68 ^{***} | 0.196 ± 0.0328 |
| Pb | Whole blood | BBPL&RUTU | F | 8.13 ^{**} | -0.265 ± 0.0044 |
| Se | Whole blood | All | M | 4.22 [*] | 0.0056 ± 0.0039 |
| Zn | Feather | All | M | 3.93 [*] | 0.0002 ± 0.0001 |
| <i>Nest duration</i> | | | | | |
| Cu | Whole blood | All | F | 3.77 [*] | -0.021 ± 0.011 |
| Fe | Egg | BBPL | . | 3.16 ^{**} | -0.0017 ± 0.0005 |
| Hg | Whole blood | All | M | 0.654 [†] | 0.0031 ± 0.0038 |
| Mn | Egg | All | . | 1.41 [†] | -0.0075 ± 0.006 |
| Mo | Egg | All | . | 9.63 ^{***} | 0.128 ± 0.05 |
| Pb | Whole blood | All | F | 4.75 ^{**} | -0.175 ± 0.081 |
| Se | Whole blood | All | M | 2.27 [*] | 0.0026 ± 0.0017 |

[†]*P*<0.08, **P*<0.05, ***P*<0.01, ****P*<0.001.

^a Models comparing binary hatching success to female tissue concentrations included only BBPL and RUTU, as all nests for which female SEPL blood concentrations were available hatched due to the exclusion of one SEPL blood sample.

positively related to maternal feather-Ni for black-bellied plovers (species × [feather] interaction: *F*₁ = 3.77, *P* = 0.05; Table 3).

Paternal element levels were not strongly related to reproductive success. Once species differences, nest age and sire body condition were accounted for, hatching success was positively related to paternal blood levels of Cu, Se and Mo, and nest duration was positively related to paternal blood levels of Se and Hg (Table 3). In contrast, hatching success was negatively related to paternal feather-Hg (Table 3; Fig. 2).

4. Discussion

4.1. Elements and fitness

We found no negative relationships between adult body condition and toxic element concentrations. The lack of blood parasites found in this study may in fact be common among shorebirds (Borg, 1992; Earle and Underhill, 1993; Figuerola et al., 1996), suggesting that blood-parasite load is not a good measure of condition for these species.

The only negative correlations we detected between toxic elements and fitness measures occurred between reproductive success and Hg and Pb in parental tissues. These relationships were generally weak, inconsistent among sexes, and in the case of paternal Hg, inconsistent between blood and feathers (Table 3). Although these inconsistencies may be due to our small sample size, for now it remains unclear whether these correlations are biologically significant. A more sensitive endpoint, such as immune system response (Koller, 1980), might illuminate more subtle relationships between contaminants and shorebird fitness.

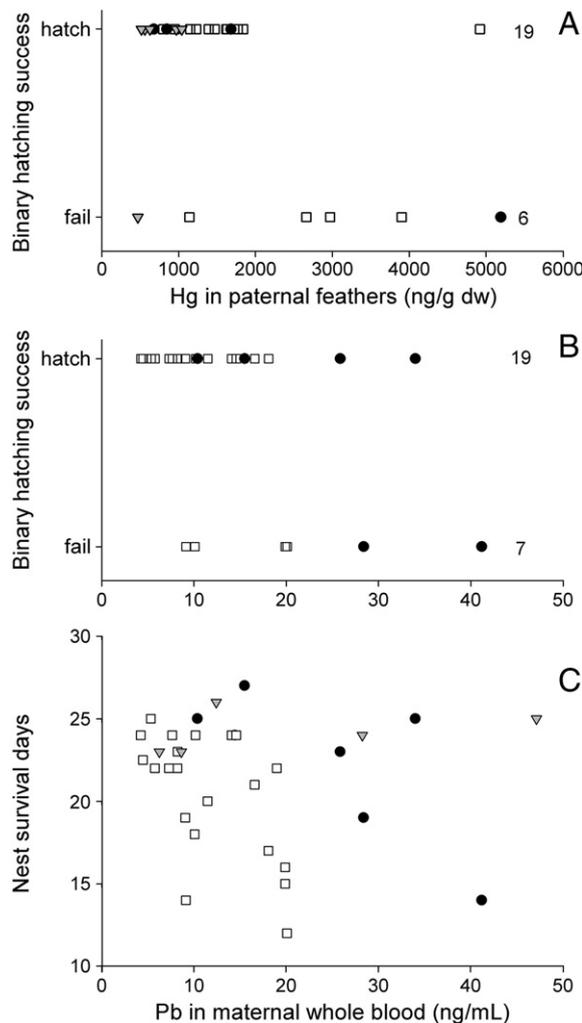


Fig. 2. Significant relationships ($\alpha=0.05$) between reproductive success and parental levels of mercury (A) and lead (B and C) in three shorebird species; black-bellied plovers (●), ruddy turnstones (□), and semipalmated plovers (▽). Nest survival analysis includes all nests (C), whereas binary hatching success includes only nests whose fate was known; numbers indicate the number of nests in each fate (A and B). No maternal blood samples were available from failed semipalmated plover nests, thus the model presented in B includes only black-bellied plovers and turnstones.

4.2. Toxicological levels and comparisons with other species and sites

To date, non-lethally collected tissues have not been used extensively in toxicological assessments of shorebirds, nor are avian toxicological thresholds available for most metals. Published shorebird blood levels are available only for Cd, Hg, Pb and Se. With the exception of Hg, blood levels detected in this study were much lower than those documented elsewhere. Although blood-Pb was negatively associated with reproductive success at East Bay, mean blood-Pb concentrations were only 20–50% of those found in oystercatchers wintering in urban areas in New Zealand (Thompson and Dowding, 1999). Maximum blood-Pb (47 ng/mL; Appendix C) was well below the 250–400 ng/mL avian level associated with exposure to elevated environmental Pb (Wobeser, 1997). Blood-Cd (always <2 ng/mL) was below the median oystercatcher level (Thompson and Dowding, 1999). Mean blood-Se was <30% of that from European shorebirds at inland sites and <10% of that from the Wadden Sea, an area polluted with Se (Goede, 1993; Goede and Wolterbeek, 1994).

In contrast, mean blood-Hg levels in this study were comparable to those from polluted sites. Blood-Hg levels were ten times higher than those of shorebirds living in areas with more direct pollution input

(Thompson and Dowding, 1999; Tavares et al., 2008), and turnstone blood-Hg (max. 1734 ng/mL) was similar to that of oystercatchers from highly polluted areas of the Wadden Sea (mean 1400–2500 ng/mL; Goede and Wolterbeek, 1994). As Hg in blood largely reflects recent dietary exposure (Fowler, 1990; DesGranges et al., 1998; Evers et al., 2005), this suggests that Hg may be of concern for arctic shorebirds, as seen in other avian species from the Canadian arctic (Braune et al., 2005; Braune, 2007). Although all blood-Hg levels detected in this study were well below the adverse effect threshold for avian piscivores (i.e. common loon, *Gavia immer*) of 3000 ng/mL (Evers et al., 2008) species vary considerably in their sensitivity to toxic elements and threshold levels have yet to be established for shorebirds.

More data on element levels exist for egg contaminants, especially Hg, which is one of few non-essential metals transmitted to eggs and the only one detected in this study. Egg-Hg levels were lower than those from shorebird populations in putatively polluted locations (Mattig et al., 2000; Harding et al., 2005; Roodbergen et al., 2008) and seabird eggs from other islands in the Canadian arctic (Braune, 2007; Burger et al., 2008a), but higher than those in seabird eggs from a mainland arctic site (mean 70 ng/g dw; Henny et al., 1995). Embryo health and survival decline at egg-Hg concentrations ranging from 50 to 1300 ng/g fresh weight (or 200–5200 ng/g dw, assuming eggs are ca. 75% moisture) across a broad range of bird taxa (Fimreite, 1974; Heinz, 1979; Evers et al., 2003; Heinz et al., 2009). Mean Hg levels in turnstone and semipalmated plover eggs (ca. 200 ng/g; Fig. 1B) approached toxic effect thresholds of more sensitive species, although we found that egg-Hg was not correlated with reproductive success. Egg-Se levels (max. 740 ng/g dw; online Appendix Table C3) were below the 1000 ng/g dw adverse effect threshold suggested for mallards (Heinz et al., 1989), and below those found in eggs of arctic-nesting seabirds (Braune, 2007) and ducks (Henny et al., 1995; Burger et al., 2008a). Egg-Mo (max. 134 ng/g dw; Table C3) was also well below levels known to cause mortality in chicken embryos (19000 ng/g ww) (Puls, 1994).

Feather elements are more difficult to compare among populations, because they reflect multiple pathways of contamination [almost entirely endogenous (e.g. Hg, As and Zn), exogenous (e.g. Cd) or a combination (e.g. Pb, Se and Fe)], and therefore multiple potential exposure locations (Burger et al., 1993; Jaspers et al., 2004). Shorebirds in Se-polluted areas rapidly accumulate feather-Se exogenously (Goede and de Bruin, 1984), developing mean concentrations as high as 5800 ng/g (Goede, 1988; Goede et al., 1989). However, Se in organs rapidly decreased after birds left polluted areas, and no adverse effects on subsequent reproduction were found (Goede et al., 1989). As these levels were much higher than those found in this study (max. 1190 ng/g; Fig. 1A), Se levels were likely well below adverse effect thresholds. Similarly, the highest mean feather-Pb detected in this study (520 ng/g for black-bellied plovers; Fig. 1A) was $\leq 50\%$ of that in shorebird primaries from polluted areas of the Wadden Sea (Goede and de Voogt, 1985) or body feathers from Delaware Bay (Burger et al., 1993) and Alaska (Burger et al., 2008b). The maximum feather-Pb level (1350 ng/g; Table C2 in online Appendix) was well below the 4000 ng/g level associated with adverse effects in gulls (Burger and Gochfeld, 1993; Burger, 1998). Feather-As levels were similar to those in shorebird primaries collected or grown on relatively pristine sites and below those formed on the Wadden Sea (Goede, 1985; Goede et al., 1989). The highest mean feather-Hg found at East Bay (1900 ng/g, turnstones) was below levels from shorebird feathers grown in polluted areas in Europe, which had means as high as 7400 ng/g (Goede et al., 1989), but higher than those grown in Siberia (mean 500 ng/g; Goede, 1988). Although toxic effect thresholds vary by species, feather-Hg found in this study (max. 6150 ng/g; Table C2) was well below levels associated with toxic effects in loons (40 000 ng/g fresh weight; Evers et al., 2008) and generally below those associated with reproductive impairment

birds in general (>5000 ng/g dw; Eisler, 2000). Feather Zn and Ni (max 170 $\mu\text{g/g}$ and 1190 ng/g, respectively) were also well below toxic levels for birds (400 $\mu\text{g/g}$ and 30 000 ng/g, respectively) (Puls, 1994).

Strong positive correlations among toxic elements might suggest that some shorebirds spent time in consistently more polluted (e.g. urban or industrial) areas. However, the only significant positive correlation among toxic elements was between Hg and Ni in blood. Thus, higher concentrations of toxic elements do not seem to reflect consistent differences in overall exposure to contaminants.

4.3. Sex and species differences

Sex and species differences in contaminant loads were common, and could have resulted from differences in factors that affect contaminant accumulation, such as age and/or trophic level (Evers et al. 2005). Based on maximum longevity of captive and recaptured wild birds, semipalmated plovers seem to be shorter-lived than the other, larger species (Rydzewski, 1978; Clapp et al., 1982; ISIS, 2008). All three species forage for invertebrates visually from surface sediments, and their diets (e.g. Iribarne and Martinez, 1999; Smith and Nol, 2000) and local feeding areas overlap considerably. Although adult semipalmated plovers had lower concentrations of certain bioaccumulating metals, such as Hg (Lindberg and Odsjö, 1983; Burger, 2002; Evers et al., 2005), as would be expected with a lower mean age or trophic level, other bioaccumulating metals, such as Pb (Burger, 1995; Swaileh and Sansur, 2006; Gangoso et al., 2009), and Zn (Borga et al., 2006; Taggart et al., 2006) did not show this pattern (Table 2). Because our sample size was small, individual variation in age and trophic level, which we did not quantify, may be masking species-level variation. Alternatively, interactions between species-level differences in accumulation and/or excretion may be causing the complex patterns seen here.

Excretion ability certainly varies between sexes, as females can excrete some elements into eggs, and may also vary among species. Sequestration into eggs is generally thought to explain higher Hg loads in male birds (Lewis et al., 1993; Goede and Wolterbeek, 1994; Hui, 1998), although female-blood Hg concentrations generally reach an equilibrium with dietary MeHg uptake from the breeding territory soon after laying (Evers et al., 2005). Sequestration into eggs seems to vary among species, as semipalmated plovers had the lowest blood and feather-Hg but highest egg-Hg, whereas the opposite was true of Mo (Table 2). However, females did not differ in contaminant concentrations more than males (no significant sex \times species interactions), and contaminants not sequestered into eggs, including most toxic elements, commonly differed among species in blood and feathers (Table 2). We conclude that differential transfer of elements to eggs cannot explain the majority of species differences we detected.

Finally, species and/or sexes may differ geographically in contaminant exposure through variation in stopover or wintering sites. Although these species breed at relatively narrow latitudinal ranges, they over-winter across a wide latitudinal range, throughout the Caribbean, along most of coastal Latin America, and parts of coastal North America. A female black-bellied plover banded during this study was resighted in Manitoba in September 2008, indicating that not all shorebirds nesting in the eastern arctic migrate south via coastal routes. Few banded shorebirds are recaptured in other parts of their range, so it is unknown whether individuals that nest in the same area also use similar migration routes and wintering grounds. Distinguishing the relative influence of arctic and wintering-ground exposure will require more information on the migration patterns of shorebird populations, evaluation of site-specific contaminants in soil or invertebrates, and would be aided by a reliable formula for calculating blood levels during feather growth from stable feather elements, such as Hg (e.g. Bearhop et al., 2000).

4.4. Tissue correlations

We detected few correlations in element levels among pairs, which likely reflects contrasting accumulation, excretion, or exposure (i.e. different feeding locations or prey), although it may also be related to small sample size. Differing location is the most likely explanation for the lack of pair correlations in feather elements, given that shorebird pairs are not known to winter or migrate together. Further, consistent sex differences in consumption, absorption or excretion should also have produced significant differences in mean feather levels between sexes, which were not observed for any element (Table 2). As blood-Hg was weakly correlated among turnstone pairs, lack of other significant blood correlations may simply reflect low sample sizes.

4.5. Relationship to shorebird declines

The lack of toxicological threshold information for the majority of metals highlights the importance of measuring fitness in addition to contaminant loads. We did not detect strong negative relationships between non-essential element concentrations and any measure of fitness considered for these arctic-breeding shorebirds. Although this may partially result from our relatively small sample sizes, the maximum toxic-element concentrations we detected were generally well below those associated with known pollution sources or toxicological thresholds. The only exceptions were Hg in blood and eggs; as feather-Hg was well below adverse effects thresholds, this suggests that Hg contamination is a greater problem on arctic-breeding grounds than on southern wintering grounds. We acknowledge that the birds sampled here represent the most successful individuals of each species, having completed migration, found a partner and produced eggs. Thus, although we did not find any negative effects of non-essential elements, they may affect shorebird populations in ways not detectable through studies of breeding adults such as ours (e.g. juvenile survival, migration success). While our findings suggest that arctic-shorebird declines in our study area are likely not related to concentrations of most toxic-elements studied, the negative relationship between reproductive success and paternal feather-Hg concentrations in concert with blood-Hg concentrations at levels associated with adverse effects indicate that further investigations are warranted.

Acknowledgments

The authors would like to thank Darryl Edwards for considerable help organizing fieldwork, Paul Woodard, Katrina Cook, Stephane Bourassa and Cedric Juillet for field assistance, Paul Smith for help initiating the project, and DE and Deb Perkins for sharing unpublished PCV data. Thanks also to Lawrence Harder and Nina Internicola for statistical advice and to Birgit Braune and two anonymous reviewers for helpful comments. Funding and logistical support were provided by the Calgary Zoo Centre for Conservation Research Fund, Environment Canada, the Polar Continental Shelf Project, Canadian North Airlines and the Nunavut Wildlife Trust.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.scitotenv.2010.03.027](https://doi.org/10.1016/j.scitotenv.2010.03.027).

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