

Sources of Variation in Mercury Levels in Arctic-breeding Shorebirds

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in the Department of Biology
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By
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ABSTRACT

After long-range transport, atmospheric mercury is deposited in Arctic ecosystems via precipitation and can then accumulate in wetlands, where it is subject to methylation at spring thaw. Arctic-nesting shorebirds that forage in wet areas can thus be exposed to significant amounts of methylmercury – the most toxic form of mercury – as it is released from melting snow. Shorebirds that breed during this early period of snow melt and those that forage at higher trophic levels may have increased mercury levels. To my knowledge however, relationships between timing of breeding, trophic status, and mercury levels have not been evaluated in shorebirds. To investigate the extent to which mercury levels change through time and across trophic levels, I analysed blood and egg samples from two northern-breeding shorebird species – Semipalmated Sandpiper (*Calidris pusilla*; hereafter sandpiper) and Semipalmated Plover (*Charadrius semipalmatus*; hereafter plover). Both species are locally abundant and have similar arrival times to their breeding grounds, but differ in foraging preference, with plovers generally feeding at higher trophic levels than sandpipers. Blood and egg samples were collected from incubating shorebirds and analyzed for total mercury content (THg). In addition, blood samples were analysed for stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$).

Mean egg THg ranged from 0.07 – 0.52 $\mu\text{g/g}$ wet weight and did not reach published thresholds associated with reduced reproduction (0.70 $\mu\text{g/g}$ wet weight). Consistent with an effect of phenology, I observed a decline in mercury with later breeding. For every day that clutch initiation was delayed, THg declined by 0.006 ± 0.003 $\mu\text{g/g}$ wet weight in eggs. Among adult birds, sandpipers had higher THg levels than plovers ($p < 0.001$), and males had higher THg than females in both species ($p < 0.001$). Mean blood THg concentrations in male sandpipers approached thresholds associated with adverse effects; 37.5% of initial blood samples ($n = 3$) exceeded the described 1.0 $\mu\text{g/g}$ wet weight threshold. Sandpipers had higher $\delta^{15}\text{N}$ values than plovers, suggesting that sandpipers forage at a higher trophic level at Karrak Lake than originally described elsewhere. When species was used as a proxy for trophic status in egg mercury analyses, the same trend was apparent; sandpipers had higher mean egg THg (0.22 ± 0.09 $\mu\text{g/g}$ wet weight) than plovers (0.16 ± 0.05 $\mu\text{g/g}$ wet weight). Taken together, these results suggest that contrasting avian life history strategies, such as timing of breeding and foraging habits, can have relevance to THg exposure that warrant further consideration. Future research

should focus on adjusting methodology to reduce variation among samples (i.e. collecting first-laid eggs only) and determining whether observed mercury concentrations are impacting the survival of breeding Arctic shorebirds.

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LIST OF ABBRIVIATIONS

AIC_c	Akaike's Information Criterion (adjusted for small sample sizes).
AMDE	Atmospheric Mercury Depletion Event
ASDN	Arctic Shorebird Demographics Network
CI	Confidence Interval
CID	Clutch Initiation Date
DMA	Direct Mercury Analyzer
DW	Dry Weight
MDL	Method Detection Limit
MeHg	Methylmercury
MRL	Method Reporting Limit
RPD	Relative Percent Difference
SCID	Standardized Clutch Initiation Date
SD	Standard Deviation
SE	Standard Error
SEPL	Semipalmated Plover
SESA	Semipalmated Sandpiper
SRM	Standard Reference Materials
THg	Total Mercury
tMELT	Time Since Melt
WW	Wet Weight

INTRODUCTION

Mercury from anthropogenic sources is an emerging concern in Arctic ecosystems; this toxic metal disproportionately accumulates at high latitudes compared to temperate regions (Ariya et al. 2004; Poissant et al. 2008; Carignan and Sonke 2010). Although estimates of atmospheric mercury emissions have remained constant or decreased over the last two decades in the Western world, there is still significant mercury emitted from developing countries that rely heavily on fossil fuels (Pacyna et al. 2006). Elemental mercury in the atmosphere is capable of long-distance transport and has a relatively long residence time of 1-2 years in the environment (Schroeder et al. 1998). For these reasons, areas with mercury pollution are frequently remote from major pollution sources.

One explanation for disproportionate mercury levels in Arctic systems is the observance of Atmospheric Mercury Depletion Events (AMDEs). AMDEs occur in the spring at polar sunrise and trigger deposition of elemental mercury from the atmosphere into the arctic terrestrial system via precipitation and dry deposition (Schroeder et al. 1998). These depletion events significantly increase mercury deposition on the Arctic landscape: mercury concentrations in snow from Barrow, Alaska reported a 99% increase in total mercury concentrations after AMDEs (Lindberg et al. 2002; Poissant et al. 2008). Atmospheric depletion events continue until late spring, as the snow pack melts (Ariya et al. 2004; Lindberg et al. 2002; Schroeder et al. 1998; Steffen et al. 2008). Between 90 and 450 tonnes of mercury are deposited annually through atmospheric transport across the circumpolar Arctic (Ariya et al. 2004; Poissant et al. 2008).

Methylmercury is the most biologically active and toxic form of mercury, and in high concentrations it can reduce the reproductive success, cognitive function, and survival of animals (Ackerman et al. 2016; Shore et al. 2011). The mechanism by which mercury is methylated in Arctic systems is not well understood, but may involve sulfate-reducing bacteria, other methylating microbes, or abiotic processes (Compeau and Bartha 1985; Loseto, Siciliano, and Lean 2004; Loseto, Lean, and Siciliano 2004; Weber 1993). The Canadian Arctic includes areas with extensive wetlands and some research suggests that humic substrates – like those found in tundra wetlands – may readily methylate mercury in Arctic systems (Weber 1993). Levels of methylmercury in water overlaying inland sediments can increase 14-fold in as little as five days,

and in some Arctic systems, water from snowmelt is the most important source of methylmercury (Loseto et al. 2004a; Poissant et al. 2008).

Elevated mercury levels have been reported in many species of Arctic wildlife including seabirds, fish, and marine and terrestrial mammals (Gamberg et al. 2005; Stern 2009; Tran et al. 2016; Wagemann et al. 1998). Shorebirds also tend to have elevated mercury levels – in some cases, above concentrations that may reduce hatch success (Perkins et al. 2016), but most research about contaminants in Arctic-nesting shorebirds has occurred either in Alaska, or in the Hudson Bay basin (Hargreaves et al. 2010, 2011; McCloskey et al. 2013; Perkins et al. 2016; Saalfeld et al. 2016). Whether concentrations in birds from these locations are representative of mercury levels in shorebirds across the Arctic is unknown.

In addition to potential impacts of mercury exposure, shorebirds breeding in northern areas may also be particularly susceptible to potential phenological mismatch, due to disproportionate warming at high latitudes compared to more temperate areas (IPCC 2007). Timing of snowmelt is the most significant factor influencing nest initiation dates in Arctic-nesting shorebirds (Reneerkens et al. 2016; Smith et al. 2010), and some species can adjust timing of breeding in response to earlier spring onset (Liebezeit et al. 2014), but, to my knowledge, no research has yet evaluated how changes in timing of breeding by Arctic shorebirds influence their exposure to environmental contaminants. Differences in trophic status might also influence mercury levels in shorebirds. Specifically, because mercury is bioaccumulative, birds that forage at higher trophic levels should have higher mercury concentrations (Akearok et al. 2010; Bearhop et al. 2000; Evers et al. 2005; Provencher et al. 2014), and relationships between trophic status and contaminant concentrations have been described in other Arctic systems (Campbell et al. 2005). Finally, sex differences can significantly influence mercury concentrations in adult birds. In many species, female birds can use egg-laying as a method for excreting contaminants, and during the breeding period, females frequently have lower contaminant concentrations than males (Robinson et al. 2012). Information on sex differences in contaminant levels for shorebirds is limited, but does suggest there are differences between males and females for some species and that such differences can potentially affect fitness (Hargreaves et al. 2010), particularly for species that are biparental incubators (Rodewald 2015; Perkins et al. 2016). However, much uncertainty remains about the

relative influence of factors, like trophic status or sex, on mercury levels in Arctic-breeding shorebirds (McCloskey et al. 2013; Perkins et al. 2016).

1. 1. THESIS OBJECTIVES, HYPOTHESES AND PREDICTIONS

To evaluate potential sources of variation in observed mercury concentrations in Arctic-nesting shorebirds, I collected samples from two locally abundant shorebird species that nest in the Canadian Arctic; Semipalmated Sandpiper (*Calidris pusilla*, hereafter sandpiper), and Semipalmated Plover (*Charadrius semipalmatus*, hereafter plover). Both species arrive on their breeding grounds at the end of May and initiate clutches 5 – 10 days after arrival (Gratto-Trevor 1991; Rodewald 2015; Smith et al. 2010; *pers. obs.*, Figure 1.1). Sandpipers and plovers, however, differ in their foraging behaviour, with plovers more likely to prey on larger bodied, higher trophic level arthropods than sandpipers, which tend to forage on dipteran larvae (Gratto-Trevor 1991; Hargreaves et al. 2011; Holmes and Pitelka 1968; Rodewald 2015; Smith et al. 2010). These comparable and contrasting life history characteristics allowed me to evaluate competing hypotheses regarding nesting phenology and trophic status on mercury concentration (Table 1.1).

1. 1. 1. Nesting Phenology

Mercury pollution on the Arctic landscape is most pronounced during snowmelt in the spring, where it can accumulate in low-lying wet areas where shorebirds often forage. Birds nesting earliest in the breeding season should exhibit the highest levels of mercury in their eggs, because they forage in these areas for the longest period while the snow melts. For this reason, I anticipated that egg mercury levels would decline as nest initiation dates advanced in both sandpipers and plovers. For both species, I also predicted that adult blood mercury would decline over the breeding period, once the snowpack melts and no further atmospheric mercury is deposited until next winter.

1. 1. 2. Trophic Status

Mercury levels are highly variable across the Arctic ecosystem, and levels in Arctic wildlife depend on foraging behaviour (Akearok et al. 2010; Provencher et al. 2014). Species that occupy a higher trophic level should be exposed to higher mercury levels than species that forage at lower trophic levels (Bearhop et al. 2000; Evers et al. 2005). I expected plovers to

occupy a higher trophic level than sandpipers, because plovers tend to forage visually, and probably select for large bodied arthropods such as spiders while sandpipers tend to forage on smaller dipteran larvae (Hargreaves et al. 2011; Holmes and Pitelka 1968; Rodewald 2015). I therefore predicted that plovers would have higher mercury concentrations in their tissues than sandpipers. Differences in mercury concentration based on trophic status should further be reflected in geographic variation. Coastal locations, in particular, may be more strongly influenced by AMDEs, and marine environments are commonly ranked as areas with high mercury concentrations (Lindberg et al. 2002; Constant et al. 2007; Carignan and Sonke 2010; Ackerman et al. 2016). Because of the coastal influence at East Bay, I predicted that plovers sampled here would have distinct stable isotope profiles (specifically increased $\delta^{13}\text{C}$) and increased mercury concentrations, compared to plovers nesting at Karrak Lake.

1. 1. 3. Sex Differences

Mercury levels in adult birds should differ between the sexes and should be lower in females than in males after nest initiation, because females are capable of depurating excess mercury into their eggs (Robinson et al. 2012). Therefore, I predicted lower mercury levels for females than males in both species.

Table 1.1: Summary of predictions associated with each hypothesis which may influence variability of mercury concentrations in tissues of Arctic-breeding shorebird species. Hypotheses include trophic status hypothesis, nesting Phenology hypothesis, and sex differences hypothesis. SCID refers to standardized clutch initiation date in relation to snowmelt for egg sampling, while tMELT refers to time since snowmelt in blood sampling. Species codes are SESA, Semipalmated Sandpiper; SEPL, Semipalmated Plover.

Hypothesis Tested	Prediction	Tissue	Analysis	Species	Location
Nesting Phenology	Hg ↓ as SCID ↑	Eggs	Total Mercury Analysis	SESA	Coats Island Karrak Lake
Trophic Status	SEPL > SESA			SEPL	Karrak Lake
Nesting Phenology	Hg ↓ as tMELT ↑	Whole Blood	Total Mercury Analysis	SESA	Karrak Lake
Trophic Status	SEPL > SESA			SEPL	Karrak Lake East Bay
Sex Differences	Males > Females				
Trophic Status	SEPL > SESA	Whole Blood	Stable Isotope Analysis	SESA SEPL	Karrak Lake Karrak Lake East Bay
	$\delta^{13}\text{C}$ East Bay > $\delta^{13}\text{C}$ Karrak Lake				

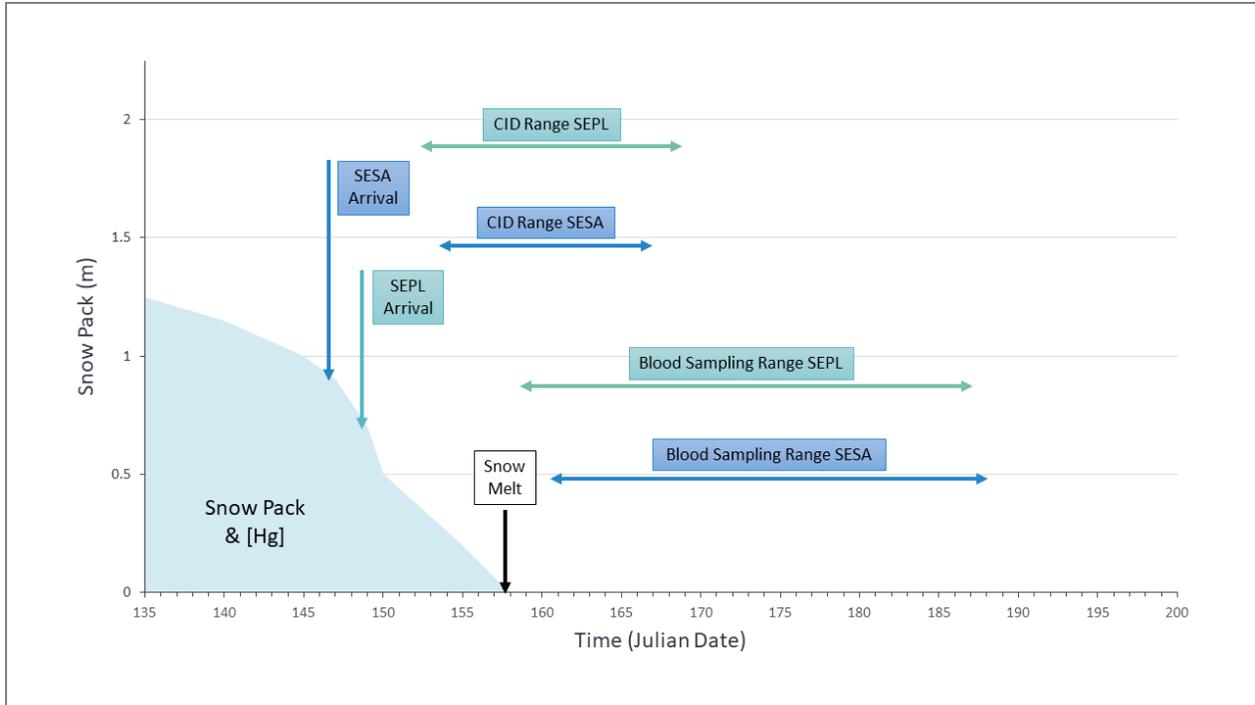


Figure 1.1: Visual representation of Timing of Breeding Hypothesis and levels of mercury in the snow pack in two shorebird species (SESA = Semipalmated Sandpiper, and SEPL = Semipalmated Plover), breeding at the Karrak Lake site in 2017. Arrival estimates were based on the first time a species was seen at the field site. It is expected that mercury concentrations in eggs will decline with increasing clutch initiation date as additional mercury is not being released after the snowpack melts.

METHODS

2. 1. STUDY SITES

2.1.1 Ahiak-Queen Maud Migratory Bird Sanctuary, Nunavut

Research took place within the Ahiak-Queen Maud Gulf Migratory Bird Sanctuary (AQMGMBS), in central Nunavut (Figure 2.1). The AQMGMBS is the largest protected area in Canada, and its 62,920 km² area protects some of the most extensive wetlands in the central Arctic (Environment and Climate Change Canada 2019a). Initially established to protect the only known nesting grounds of Ross's Geese (*Chen rossii*), this sanctuary is an important area for a plethora of other migratory birds, including several species of shorebirds, which has led to its designation as an Important Bird Area and a Wetland of International Importance under the Ramsar Convention (Bird Studies Canada; Ramsar Sites Information Service 2001). Operations within AQMGMBS took place out of the Karrak Lake research camp (hereafter, Karrak Lake). Samples from sandpipers were collected from this site in both 2016 and 2017, while samples from plovers were collected there in 2017 only.

2.1.2 Coats Island, Nunavut

Coats Island is listed as an Important Birding Area in Canada, for its seabird nesting colonies. This island is composed of mostly flat sedge tundra, wetlands, and raised beaches (Bird Studies Canada). Sandpiper egg samples were collected from this island in 2016, as this species is more prevalent here than in the East Bay Migratory Bird Sanctuary.

2.1.3 East Bay (Qaqsauqtuuq) Migratory Bird Sanctuary, Nunavut

East Bay (Qaqsauqtuuq) Migratory Bird Sanctuary (hereafter East Bay) is situated in the northern portion of Hudson Bay, Nunavut. This sanctuary is comprised largely of sedge meadow lowlands and shallow lakes and was established in 1959 to protect breeding colonies of light geese (Environment and Climate Change Canada 2019b). Since its formation as a Migratory Bird Sanctuary, several species of shorebirds have been recorded breeding in the area (Bird Studies Canada). Blood samples from plovers were collected from here in 2017.

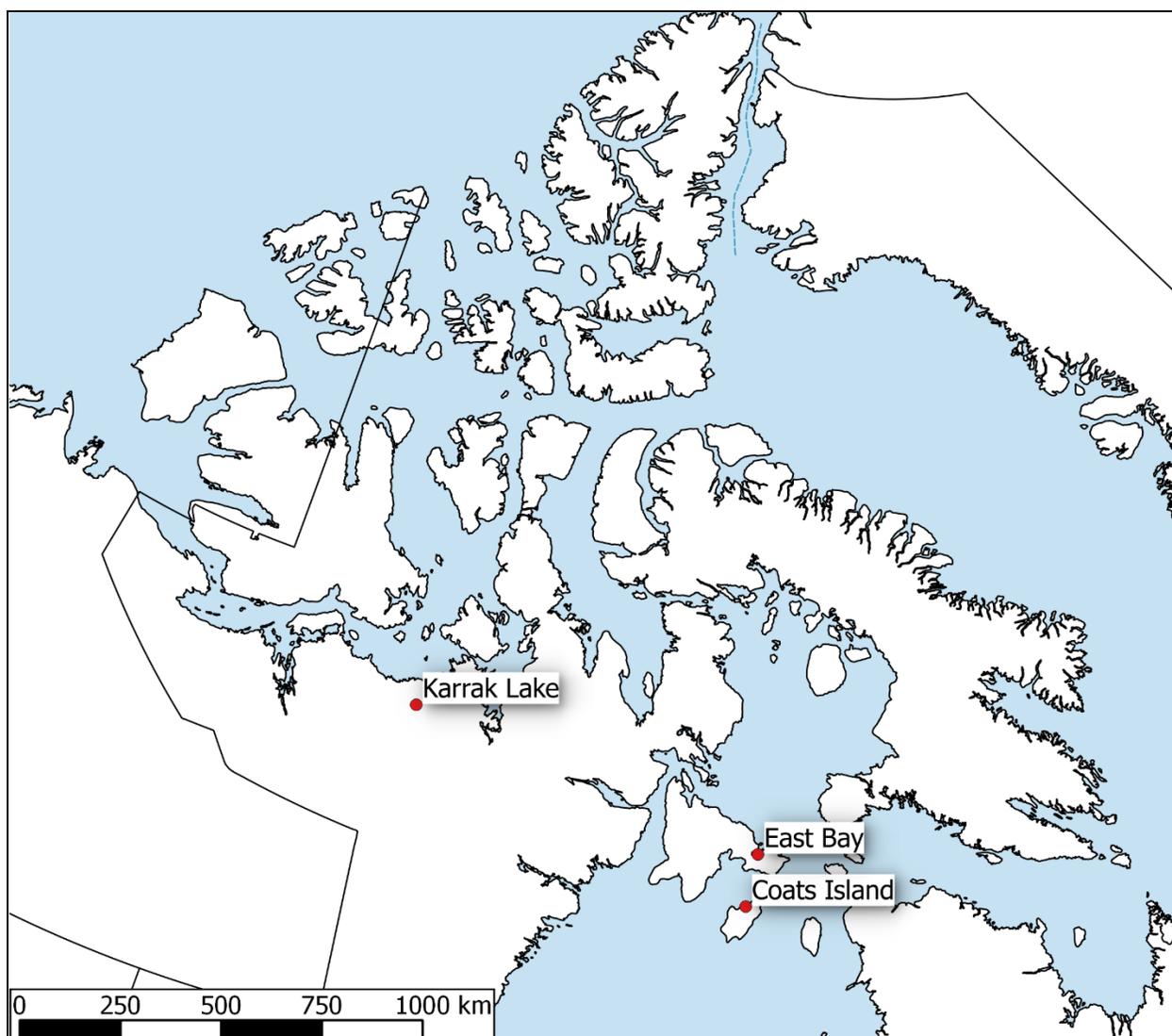


Figure 2.1: General locations of shorebird tissue collection sites across the Canadian Arctic. Karrak Lake refers to the research camp located in the Ahik-Queen Maud Gulf Migratory Bird Sanctuary. The East Bay site is located within the East Bay Migratory Bird Sanctuary. Coats Island is accessed by crews from the East Bay site but is not within the MBS boundaries. Map was created utilizing QGIS software (QGIS Development Team 2018).

2. 2. FIELD METHODS

All samples were collected in accordance with permits issued by Environment and Climate Change Canada, and the Nunavut Department of the Environment (0).

2.2.1 Egg Collections

Eggs of two shorebird species were collected over two years, at two sites in the Canadian Arctic: Karrak Lake, NU and Coats Island, NU. A total of 43 eggs were collected over two field

seasons from the Karrak Lake site, (SESA = 30, SEPL = 13). Nest-searching followed protocols set in place by the Arctic Shorebird Demographic Network (Brown et al. 2014). Briefly: I searched for birds flushing from nests or exhibiting typical territorial behaviour. Ten metre ropes were dragged systematically over the study area to help flush birds. Eggs were only collected if the clutch was complete (4 eggs within the nest cup) and selected randomly. If a nest was found as a partial clutch, clutch initiation date (CID) was determined by estimating that one egg was laid per day since initiation to determine the initiation date. Otherwise, at least 2 eggs were floated in the field to determine float angle, and therefore incubation start date, CID was then determined by subtracting three from this date (Liebezeit et al. 2007; Brown et al. 2014). Collected eggs were recorded by species, nest ID, date collected, and float angle (Liebezeit et al. 2007). All eggs were shipped to the National Wildlife Research Centre (NWRC) in Ottawa for contaminant analysis.

Egg collections were conducted on Coats Island, Nunavut in 2016 under the supervision of Dr. Paul Smith, and 10 sandpiper eggs were collected. Small deviations in sampling techniques at the Coats Island site required attention in order to keep sampling efforts consistent between sites: two nests had multiple eggs removed (one nest was abandoned, so a total of 3 eggs were collected/salvaged, a second nest had two eggs removed due to human error). Of these eggs, one egg was randomly selected as sample representative of the nest using a random number selector in R (R Core Team 2018). In total, three eggs were removed from analysis in order to keep sampling efforts consistent. A fourth egg from this site was removed from analysis because of discrepancies on sample submission forms; as this egg could not be traced back to an original nest. In total, six eggs were analysed from this site.

Eggs were placed in Whirl-pak bags after collection and stored in foam-lined coolers while at camp. All eggs were shipped to the National Wildlife Research Centre (NWRC) in Ottawa for contaminant analysis.

2.2.2 Trapping of Adult Birds

A maximum of 40 birds per species were permitted to be captured at the Karrak Lake site via spring-loaded bownet traps (Lamarre Bownets, Quebec Canada) placed over the nest. Eight plovers at East Bay were captured using the same method. Bownet traps are 61cm wide and were centred over the nest bowl to avoid damaging eggs; traps were also tested prior to being set to

ensure they deployed smoothly. An effort was made to capture both parents, and the trap was reset as one bird was processed if the other was thought to be nearby. This tactic worked better with plovers than with sandpipers. Once a bird was trapped, it was immediately removed from the trap to avoid injury. Birds were then measured and banded, and blood samples were collected. Adults spent no more than 30 minutes in captivity and were released near the nest.

Nesting adults were trapped twice during the incubation period to collect contaminant samples at two intervals within the breeding season. Nests were first trapped at the “early” stage: when first found and as close to clutch initiation as possible; and then again at the “late” stage prior to hatch, the date of which was predicted through egg floatation (Liebezeit et al. 2007). If trapping both parents at once was unsuccessful, the nest was revisited again once an incubation switch was likely (3-4 hours later). During the initial trapping period, unbanded adults could be easily re-sighted near the nest, which was advantageous as this decreased direct disturbance to the nest caused by repeated trapping. During the later recaptures, all attempts were made to trap both adults at one time to avoid additional disturbance. In situations where this was not possible, the adult that was trapped first had non-toxic, food colouring applied on their chest feathers for easier identification in the field. The tinted dye was temporary (lasting no more than 36 hours) and did not affect their behaviour (*pers. obs.*). Adult sandpipers and plovers were fitted with a 1B aluminum band on the upper left leg, and a butt-end plastic colour band on the upper right leg. Band numbers were used to identify individuals during the recapture period.

2.2.3 Blood Collections

Blood was collected twice from adult shorebirds near the start and near the end of the incubation period (early June to early July). Blood was collected from the brachial vein, under the wing of adult birds, with no more than 250 μL being collected from a bird at any one time – this adheres with the 1% blood collection to body volume rule for these birds (Owen 2011). A minimum of 50 μL was useful for mercury analysis (NWRC, *per. comm*). A 27G needle was used to prick the vein, and heparinized microcapillary tubes were used to collect the blood. Blood was transferred to an acid-washed cryovial and stored in a conventional freezer for the duration of the field season (approximately 6 weeks).

2.2.4 Environmental Data

Timing of snowmelt was determined through established protocols at the Karrak Lake field site. Briefly: these surveys comprise of twelve, 100m long transects, which were visited every-other-day in the spring, snow depth was recorded every 5 m along the transect and transects were revisited until all intervals recorded 0 cm of snow (Kellett and Alisauskas 2019). In the scope of this study, the date at which all transects recorded 0 cm of snow along all intervals was referred to as date of snowmelt. At the Coats Island site in 2016, snowmelt surveys also took place, but monitoring efforts were modified to accommodate the constraints associated with a smaller crew size. Snow cover was estimated weekly at each shorebird plot. On July 7, 2016 all plots visited by crews had 0% snow cover (Scott Flemming, *per. comm*), and this date was used as date of snowmelt. (Table 2.1). Clutch initiation dates estimated from egg floatation were standardized against the date of snowmelt to account for variation in melt and initiation dates based on year and site differences according to:

$$SCID = X - \mu$$

Where X = Clutch Initiation Date of a given nest, and μ = snowmelt date.

Adult blood mercury concentrations were standardized as time since snowmelt ($tMELT$) utilizing the same formula:

$$tMELT = X - \mu$$

Where X = Julian date of blood draw, and μ = snowmelt date.

Table 2.1: Summary table of average clutch initiation dates (CIDs) for both shorebird species breeding at two locations in the Canadian Arctic. Snowmelt date was determined when all snow depths reached 0 cm in a predetermined area and is reported as Julian date (calendar dates reported in brackets). CIDs were determined either from nest monitoring (when nests were found as partial clutches) or by egg floatation (when nests were found as full clutches) and refers to when the first egg was laid.

Species	Location	Year	Snowmelt Date	N	\bar{x} CID \pm SD	Range	\bar{x} SCID	SCID Range
Semipalmated Sandpiper	Coats Island, Nunavut	2016	183 (July 7)	6	173.5 ± 1.38	172 – 176	-9.50 ± 1.38	-11 – -7
	Karrak Lake, Nunavut	2016	167 (June 15)	10	163.3 ± 3.16	159 – 168	-3.70 ± 3.16	-8 – 1
	Karrak Lake, Nunavut	2017	158 (June 7)	20	159.7 ± 3.76	154 – 167	1.70 ± 3.76	-4 – 9
Semipalmated Plover	Karrak Lake, Nunavut	2017	158 (June 7)	13	158 ± 4.65	153 – 169	0 ± 4.65	-5 – -11

2. 3. LABORATORY METHODS

2. 3. 1. Mercury Analysis

Whole egg and blood samples were transported in refrigerated coolers to the National Wildlife Research Centre (Ottawa, Ontario), for total mercury (THg) analysis. More than 95% of mercury found in bird tissues is in its methylated form, so analyzing for THg is a viable proxy for more expensive MeHg analysis (Bond and Robertson 2015; Evers et al. 2003; Scheuhammer, Perrault, and Bond 2001). Whole eggs were kept at 4°C once received, and whole blood was stored at -20°C. Eggs were cracked, and the contents homogenized and stored at -40°C. Mercury analysis took place at the Metals Lab at the NWRC, where egg and blood samples were stored at -30°C until they were freeze-dried following standard protocols [SOP-MET-PROC-05F]. Moisture content was determined during the drying process. After being freeze-dried, all samples were stored in a desiccator until analysis.

All samples were analysed for total mercury via a Direct Mercury Analyzer (DMA-80, Milestone SRL, Italy). Samples were thermally and chemically decomposed in quartz catalytic tube, under a continuous flow of ultra-pure oxygen. A gold amalgamator selectively traps mercury from the decomposition products. After the system was flushed with oxygen to remove by-products, the amalgamator was rapidly heated to release mercury vapour, which was then transported via an oxygen flow through absorbance cells in path of a single wavelength atomic absorption spectrometer. Absorption was measured at 253.7nm as a function of mercury amount, instrument software directly calculated mercury concentration based initial sample weight. All resulting mercury concentrations were reported as dry weights with associated moisture content. Mercury concentrations were then back calculated to wet weight format according to:

$$Wet\ Weight = \frac{DW \times (100 - PM)}{100}$$

Where, DW = dry weight of sample and PM = percent moisture of sample. Reporting mercury concentrations in both wet and dry weights allow for the broadest comparisons of mercury levels in birds.

2. 3. 2. Quality Assurance

Accuracy of the DMA-80 was evaluated at the start of each day by running at least four analyses on standard reference materials (SRMs). SRMs were analyzed throughout the day at regular intervals, and at the end of day to ensure that accuracy of the instrument was maintained. Precision was determined by randomly selecting aliquots and performing replicate analyses and quantified by Relative Percent Difference (RPD).

Method Detection Limits (MDL) and Method Reporting Limits (MRL) were determined after each calibration of the instrument by utilizing an SRM with a low mercury concentration. Ten consecutive analyses were performed with SRM Oyster Tissue 1566b for each calibration (in 2016 for egg samples, and in 2017 for egg and blood samples), the MDL was 0.015 – 0.018 ng and 0.032 ng for eggs and blood respectively. MRL was reported at 0.075 – 0.090 ng and 0.159 ng for eggs and blood respectively.

To account for the potential of background contamination, five empty nickel boats were analysed at the start of each day. Sample analyses only proceeded if blank results reported <0.07 ng mercury.

2. 3. 3. Stable Isotope Analysis

To assess trophic status, blood samples from all shorebirds (n = 64) were analysed for stable isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Whole blood samples were dried according to protocols outlined above for mercury analysis. Dried samples were then ground to a homogenous powder and weighed (approximately 1 mg) into tin cups for analysis at the National Hydrology Research Centre Isotope Laboratory. Standard continuous flow–isotope ratio mass spectrometry techniques were employed, with measurements made using a Costech elemental analyzer coupled to a Delta mass spectrometer. By convention, isotope values are reported per mil (‰) in delta notation as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ according to:

$$\delta X = \left(\frac{R_{\text{Sample}}}{R_{\text{std}}} - 1 \right) \text{‰}$$

where X = ^{13}C or ^{15}N , R= $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$, and standards (std) were Vienna PeeDee Belemnite (VPDB) for ^{13}C and atmospheric N_2 (AIR) for ^{15}N .

Casein Protein (B2155, Elemental Microanalysis Ltd.) was used as a standard reference material. Standards (in the same mass range as the test samples) were processed before and between every ten tissue samples within each analytical run. Replicate standard measurements within a run were used to estimate analytical precision: mean $\delta^{13}\text{C}$ (± 1 standard deviation, SD) within runs = $-22.4\text{‰} \pm 0.0\text{‰}$, and mean $\delta^{15}\text{N}$ (± 1 SD) within runs = $6.8\text{‰} \pm 0.1\text{‰}$ for egg albumen. Measurement error estimates (± 1 SD) were $\pm 0.1\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.1\text{‰}$ for $\delta^{15}\text{N}$, based on measurements of replicates and laboratory standards across multiple runs.

2. 4. STATISTICAL ANALYSES

All statistical analyses were conducted using R statistical software (R Core Team 2018). Before analyses, data was examined for normality. The mean, standard deviation, and range of mercury levels found in whole blood and egg samples were reported for all sample sites and years.

2. 4. 1. Egg Mercury

I created a set of candidate general linear models from data collected during the 2016 and 2017 seasons to evaluate potential predictor variables (species, site, standardized clutch initiation date (SCID), and year) associated with total mercury concentrations in eggs. Akaike's Information Criterion, corrected for small sample size (AIC_c), was used to rank models using the R package MuMIn (Barton 2018). Models which did not outcompete the null or included meaningless interactions or non-informative parameters were dropped (Arnold 2010; 0), and remaining models were reevaluated to estimate AIC_c values and model weights.

2. 4. 2. Blood Mercury

Analysis of mercury levels in blood of adult shorebirds involved two steps. First, I investigated the factors that influenced THg concentration in initial blood samples of shorebirds. To do this, I created a set of candidate general linear models that included sex, species, and site as predictor variables. The variable of site was removed to reduce model complexity because it was only available for plover, and blood mercury levels did not differ between sites for this species (Welch's t-test, $p = 0.41$, 0). AIC_c values were used to rank models as described in section 2.4.1.

To analyse whether blood mercury in adult shorebirds changed through the breeding period at the Karrak Lake site, I created a candidate set of generalized linear models that included species, sex, and time since snow melt as predictor variables. To account for repeated measures, individual was included in each model as a random effect. Since this analysis evaluated potential changes in blood mercury concentrations over time, birds with incomplete sampling were removed from this analysis (i.e. where nests failed, and adults could not be sampled a second time). All candidate models then went through the same model selection process as described above.

2. 4. 3. Stable Isotopes and Trophic Status

Stable isotopic composition in whole blood samples of nesting sandpiper and plover were analysed to evaluate the influence of trophic status on THg levels. Isotope analysis was only available for blood samples, and the data that were used for the above blood mercury analyses were again used here. One sample from a sandpiper was removed from this iteration, because of anomalous carbon and nitrogen ratios flagged by the lab. This sample was too small to be reanalysed. In total, 64 blood samples were used for stable isotope analysis.

There were small variations in what data was collected at which site, so the larger data set relating to blood mercury and stable isotopic levels was subset into 3 smaller data sets of; initial blood samples of both species at Karrak Lake only (sex, and species differences), initial blood samples of plovers at both locations (site differences), and blood samples over time at Karrak Lake (temporal trends). A multivariate ANOVA was conducted on each subset to determine whether isotopic profiles differed based on species, sex, or site differences, and whether they changed through time. Univariate analyses utilizing Tukey's HSD (Honest Significant Difference) test were run on significant variables determined from the MANOVA to understand which level of each factor was causing significant change in isotopic profiles.

Finally, stable isotope profiles were added as explanatory variables to the candidate models sets outlined above to determine whether differences in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ profiles influenced the trends seen in blood mercury concentrations between species, sexes and sites, as well as through time. Candidate models were then rerun with these variables, where they were ranked by AIC_c values and model weights were estimated.

RESULTS

I analysed egg samples for total mercury concentrations from six sandpiper nests collected from Coats Island, Nunavut in 2016, and from 43 shorebird nests collected from Karrak Lake, Nunavut between 2016 and 2017 (Table 3.1) – mean values, standard deviation, and range of mercury concentrations are reported. I also analysed whole blood samples from adult birds for total mercury, and samples were collected from eight plovers from East Bay Nunavut, and from 36 shorebirds from Karrak Lake Nunavut in 2017 (Table 3.5).

Annual comparisons of mercury concentrations were only available for eggs of sandpiper nesting at Karrak Lake, but mean mercury concentrations did not differ between years in this study (0).

Site differences in mercury concentrations were variable. Sandpiper eggs collected at Coats Island had higher mean mercury concentrations (0.27 ± 0.08 $\mu\text{g/g}$ wet weight) than those collected at Karrak Lake (0.20 ± 0.09 $\mu\text{g/g}$ ww), but mercury concentrations in blood collected from plovers at Karrak Lake and East Bay did not differ (0.30 ± 0.11 $\mu\text{g/g}$ ww and 0.27 ± 0.09 $\mu\text{g/g}$ ww, respectively; 0).

As predicted, I detected an effect of species on egg mercury concentrations: sandpipers had higher (0.22 ± 0.09 $\mu\text{g/g}$ ww, sites pooled), and more variable egg mercury concentrations, ranging between 0.07 – 0.52 $\mu\text{g/g}$ ww; samples on both ends of this range were collected at Karrak Lake. Plovers had lower (0.16 ± 0.05 $\mu\text{g/g}$ ww), and less variable egg mercury concentrations ranging between 0.08 – 0.26 $\mu\text{g/g}$ ww (Table 3.1). Mercury concentrations in shorebird blood were variable and dependent on sex as well as species. Male sandpipers had the highest mean blood mercury concentrations (0.92 ± 0.36 $\mu\text{g/g}$ ww), followed by females (0.50 ± 0.21 $\mu\text{g/g}$ ww). Plovers generally had lower blood mercury concentrations, but followed a similar trend with males having more elevated concentrations than females (0.34 ± 0.11 and 0.24 ± 0.08 $\mu\text{g/g}$ ww, respectively; Table 3.5).

3. 1. NESTING PHENOLOGY

3. 1. 1. Influence of Clutch Initiation Date

All egg samples collected during this study were used for this analysis; in total 36 eggs were collected from sandpipers at two sites, while 13 eggs were collected from plovers at Karrak Lake. SCIDs were consistent between species at the Karrak Lake site in 2017, but differed between sites and years, with Coats Island having later initiation dates than Karrak Lake in 2016 (-9.50 ± 1.38 and -3.70 ± 3.16 , respectively), and Karrak Lake having earlier spring onset and SCIDs in 2017 than in 2016 (Julian dates 1.70 ± 3.76 and -9.50 ± 1.38 , respectively, Table 2.1). Mean (\bar{x}), number of samples (n), standard deviation (SD) and range of mercury concentrations of data used in this analysis are summarized in Table 3.1.

Variability in the candidate models was high, but the influence of Standardized CID was present in the top three models (Table 3.2). SCID and species differences account for 60% of the cumulative weight of evidence (Arnold 2010). While the effect was small, mercury concentrations in eggs decreased as SCID increased ($\beta = -0.006 \pm 0.003$ SE, Figure 3.1), with overall levels being higher in sandpiper eggs than plovers ($\beta_{\text{SESA}} = 0.052 \pm 0.030$ SE).

Table 3.1: Eggs of two shorebird species were collected at two sites in the Canadian Arctic over two years and analyzed for total mercury concentration. Number of samples (n), mean (\bar{x}), standard deviation (SD) and range of mercury concentrations found in egg samples are summarised below, and reported in $\mu\text{g/g}$ wet weight.

Species	Site	Year	Tissue	n	\bar{x}	SD	Range
Semipalmated	Coats Island	2016	Egg	6	0.27	0.08	0.13 – 0.36
Sandpiper	NU						
	Karrak Lake	2016	Egg	10	0.20	0.08	0.07 – 0.31
	NU	2017		20	0.20	0.10	0.10 – 0.52
Semipalmated	Karrak Lake	2017	Egg	13	0.16	0.05	0.08 – 0.26
Plover	NU						

Table 3.2: Model selection process for general linear models estimating effects of standardized clutch initiation dates (SCID), and species differences on egg mercury concentrations (EggHgWW), at two sites in the Canadian Arctic. Variability in the models were high, but the influence of SCID was present in the top 3 models. Standardized SCID and species differences account for 60% of the cumulative variation seen in the model (Arnold, 2010). Mercury concentrations in eggs decreased as SCID increased ($\beta = -0.006 \pm 0.003$ SE) and were higher in Semipalmated Sandpiper eggs than Semipalmated Plovers ($\beta_{\text{SESA}} = 0.052 \pm 0.030$ SE).

Model	Df	Log-likelihood	Dev	AIC _c	Δ_c	Weight
Egg_{HgWW} ~ SCID + Species	4	55.18	0.30	-101.46	0.00	0.28
Egg_{HgWW} ~ SCID	3	53.73	0.32	-100.92	0.54	0.21
Egg_{HgWW} ~ SCID * Species	5	55.47	0.30	-99.54	1.91	0.11
Egg_{HgWW} ~ Species + Site + SCID	5	55.27	0.30	-99.14	2.31	0.09
Egg_{HgWW} ~ Site + SCID	4	53.97	0.32	-99.03	2.43	0.08
Egg_{HgWW} ~ Site + Species	4	53.67	0.32	-98.42	3.03	0.06
Egg_{HgWW} ~ Site	3	52.42	0.34	-98.30	3.16	0.06
Egg_{HgWW} ~ Site * SCID	5	54.55	0.31	-97.71	3.74	0.04
Egg_{HgWW} ~ Species	3	52.11	0.34	-97.68	3.77	0.04
Egg_{HgWW} ~ 1	2	50.20	0.37	-96.14	5.31	0.02

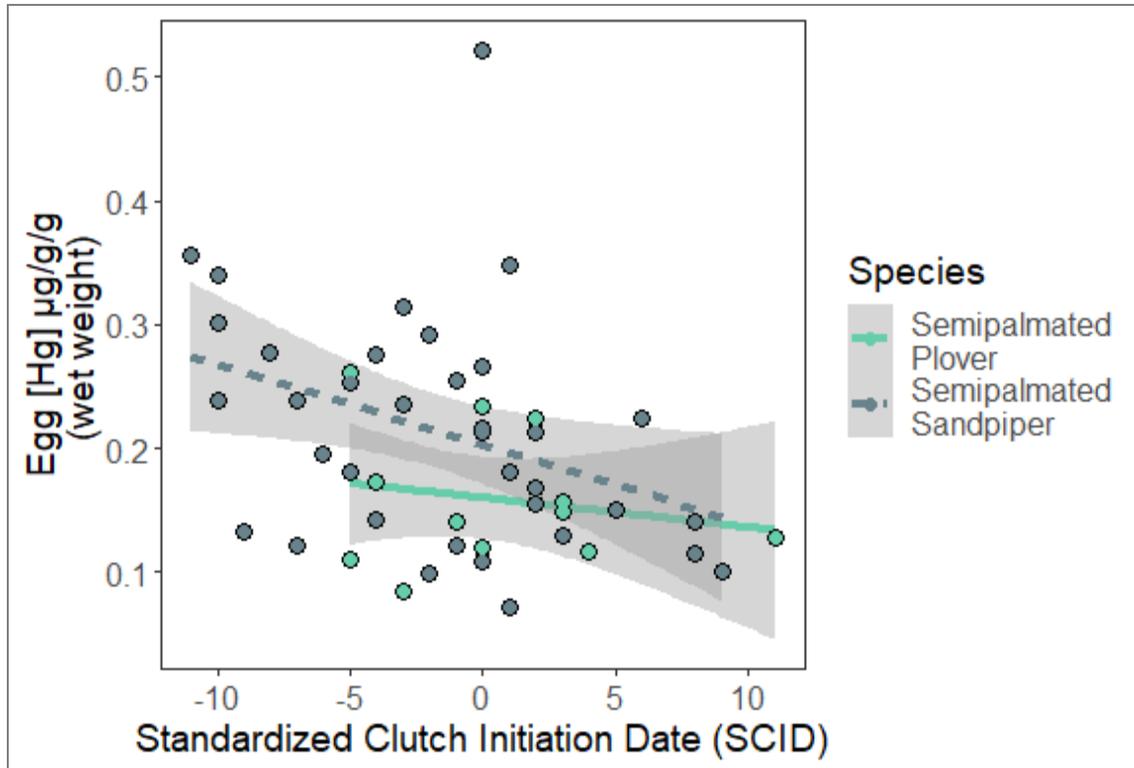


Figure 3.1: The top model result (60% Akaike Weight) of a model selection process indicating standardized clutch initiation date (SCID) and species differences contribute to differences in egg total mercury concentration [Hg]. CID was standardized against snowmelt, and SCID = 0 indicates a clutch was initiated at snowmelt completion. Eggs were analyzed from two sites in the Canadian Arctic, but site did not influence trends seen in these data. For every daily increase in SCID egg [Hg] declines by 0.006 (± 0.003 95% CI) $\mu\text{g/g}$ wet weight.

3. 3. 2. Mercury Levels in Adult Shorebirds Through Time

In order to analyse the change in mercury concentrations in Arctic-breeding shorebirds across the breeding season, data were filtered, and single blood samples were excluded (i.e. birds that were only sampled initially). Average interval between blood collections was 14.6 days for both species. Mean (\bar{x}), number of samples (n), standard deviation (SD) and range of mercury concentrations of data used in this analysis are summarized in Table 3.3.

Variability in the candidate model set was high. Species was present as an explanatory variable in the top two models, and species differences accounted for 75% of the cumulative model weight (Arnold 2010). However, there was considerable variation in mercury concentrations within birds that was not accounted for by the variables that I considered. There was a small increase in average mercury concentrations between first and second sampling

periods, but this trend was small and not significant. There was no effect of time since melt on mercury concentrations in adult shorebird blood (Table 3.4, 0). Plovers had lower blood mercury concentrations on average than sandpipers ($P = < 0.001$), and male sandpipers had higher mercury concentrations than females ($P = 0.02$, Figure 3.2).

Table 3.3: Whole blood samples collected over the breeding period for two shorebird species nesting at Karrak Lake. Only paired blood samples are included (i.e. birds which only had one sample drawn were excluded). Initial blood samples were collected soon after clutches were initiated, secondary samples were collected prior to estimated hatch date, average interval between sampling dates was 14.6 days. Number of samples (n), mean (\bar{x}), standard deviation (SD) and range of mercury concentrations in blood samples are reported in $\mu\text{g/g}$ wet weight.

Species	Sex	Blood Sample	n	\bar{x}	SD	Range
Semipalmated	Male	Initial	8	0.92	0.35	0.40 – 1.41
		Secondary	8	1.03	0.69	0.35 – 2.22
Sandpiper	Female	Initial	7	0.53	0.21	0.22 – 0.78
		Secondary	7	0.67	0.32	0.32 – 1.12
Semipalmated	Male	Initial	3	0.45	0.08	0.36 – 0.52
		Secondary	3	0.41	0.04	0.36 – 0.45
Plover	Female	Initial	3	0.23	0.07	0.16 – 0.28
		Secondary	3	0.36	0.14	0.25 – 0.52

Table 3.4: Model selection process for generalized mixed models estimating effects of time since melt (tMELT), species, and sex differences on adult blood mercury concentrations (BldHg_{WW}) over time, at Karrak Lake. Data was blocked by individual (ID) to account for individual differences. Species differences accounted for 75% of cumulative model weight (Arnold, 2010). However, highest ranked models did not exceed $\Delta < 2$ and indicates weak support. There was no discernible effect of tMELT on mercury concentrations in the blood of adult shorebirds, as none of the models containing this variable surpassed the null model (0).

Model	Df	Log-Likelihood	Deviance	AIC_c	Δ_c	Weight
Bld_{Hg_{WW}} ~ Species, (1 ID)	4	-14.76	24.78	38.59	0.00	0.44
Bld_{Hg_{WW}} ~ Sex + Species, (1 ID)	5	-13.82	20.66	39.30	0.71	0.31
Bld_{Hg_{WW}} ~ 1, (1 ID)	3	-16.58	30.22	39.78	1.19	0.24

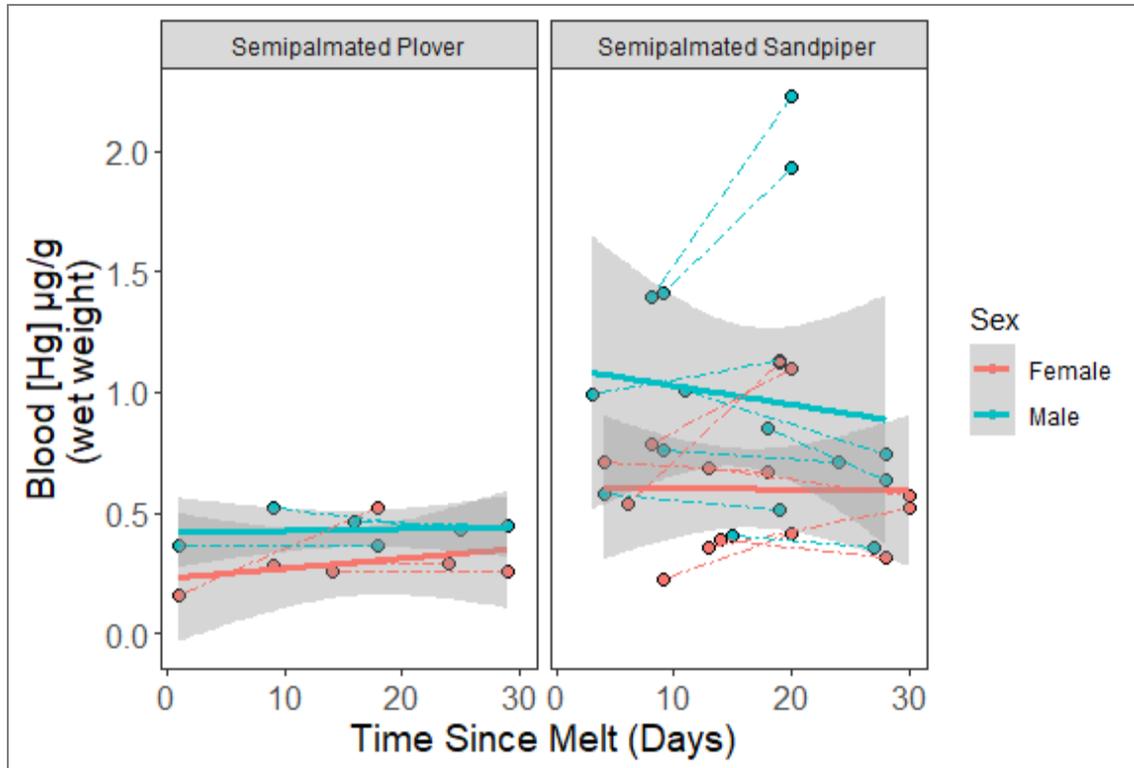


Figure 3.2: Time since melt has no discernible effect on blood mercury concentrations of two shorebird species sampled at Karrak Lake, NU in 2017. Sandpipers generally had higher mercury concentrations than plovers ($P = 0.02$), with male sandpipers being higher than females ($P = 0.02$) but an overall change in mercury concentrations over time was not reported. Individual data points are denoted by filled circles, dashed lines indicate blocking associated with first and second blood sampling from an individual. Overall trend line denoted by solid line; shading indicates 95% CI).

3. 2. TROPHIC STATUS

3. 2. 1. Whole Blood Analysis

Whole blood samples were collected from two breeding shorebird species at two sites in the Canadian Arctic in 2017. Both sandpipers and plovers were sampled at the Karrak Lake site, while only plovers were sampled at the East Bay site. In total, 65 blood samples comprising of both initial and secondary samples from both sexes were collected in 2017 and analyzed for total mercury concentration. Mean, standard deviation, and range of THg concentrations found in initial blood samples are reported in Table 3.5. Mean, standard deviation and range of stable isotopic values for each species at each site are reported in Table 3.6.

Table 3.5: Initial whole blood samples collected from two shorebird species at two sites in the Canadian Arctic analyzed for total mercury concentration in 2017. Number of samples (n), mean (\bar{x}), standard deviation (SD) and range of mercury concentrations found in initial blood samples are summarised below, and reported in $\mu\text{g/g}$ wet weight.

Site	Species	Tissue	Sex	n	\bar{x}	SD	Range
Karrak	Semipalmated	Whole	Male	8	0.92	0.36	0.40 – 1.41
Lake, NU	Sandpiper	Blood	Female	8	0.50	0.21	0.22 – 0.78
	Semipalmated	Whole	Male	10	0.36	0.09	0.24 – 0.52
	Plover	Blood	Female	10	0.24	0.10	0.08 – 0.40
East Bay, NU	Semipalmated	Whole	Male	3	0.31	0.13	0.19 – 0.45
	Plover	Blood	Female	5	0.24	0.06	0.17 – 0.33

Table 3.6: Stable isotopic profiles of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the initial blood samples of two shorebird species (Semipalmated Sandpiper = SESA, Semipalmated Plover = SEPL) from two locations in the Canadian Arctic. Number of samples (n), mean values (\bar{x}) \pm standard deviation (SD), and range are summarised below. Sexes and blood samples are pooled. Stable isotopes are reported as per mil ‰.

Species	Site	n	\bar{x} $\delta^{13}\text{C} \pm \text{SD}$	Range	\bar{x} $\delta^{15}\text{N} \pm \text{SD}$	Range
SESA	Karrak Lake	15	-22.88 ± 1.41	-25.54 – -20.28	9.21 ± 0.87	7.92 – 11.53
SEPL	Karrak Lake	20	-23.49 ± 0.79	-25.37 – -22.17	8.56 ± 0.78	7.65 – 10.99
	East Bay	8	-19.19 ± 0.70	-20.02 – -18.12	9.17 ± 0.35	8.70 – 9.88

3. 2. 1. 1. Species Differences

$\delta^{15}\text{N}$ differed between shorebird species at Karrak Lake in 2017. However, contrary to what I expected, sandpipers averaged higher $\delta^{15}\text{N}$ in their blood relative to that of plover ($\delta^{15}\text{N} = 9.21$ and $\delta^{15}\text{N} = 8.56$, respectively, Table 3.6, $P = 0.03$). Likewise, sandpipers had higher mean THg concentrations in blood ($0.20 \pm 0.09 \mu\text{g ww}$) than plover ($0.16 \pm 0.05 \mu\text{g ww}$). Stable isotopic composition in adult blood was unrelated to mercury concentrations. The addition of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ as predictor variables did not improve model rankings (see 3.3 Sex Differences), such that models containing $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were not supported during the model selection process (0).

3. 2. 1. 2. Site Differences

Site comparisons were only available for plovers in 2017. As expected, isotope signatures in plovers differed by sampling location. Plovers nesting at the East Bay site had significantly higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ than plovers nesting at Karrak Lake ($\delta^{15}\text{N}$ $P = 0.04$; $\delta^{13}\text{C}$ $P = < 0.001$, Figure 3.3). Although there were significant differences in isotope profiles based on site, I did not detect an effect of stable isotopic composition of adult blood on mercury concentrations in these birds. Plover blood mercury concentrations were comparable between sites (mean THg 0.27 ± 0.09 and 0.30 ± 0.11 μg wet weight, East Bay and Karrak Lake respectively; $P = 0.41$; 0).

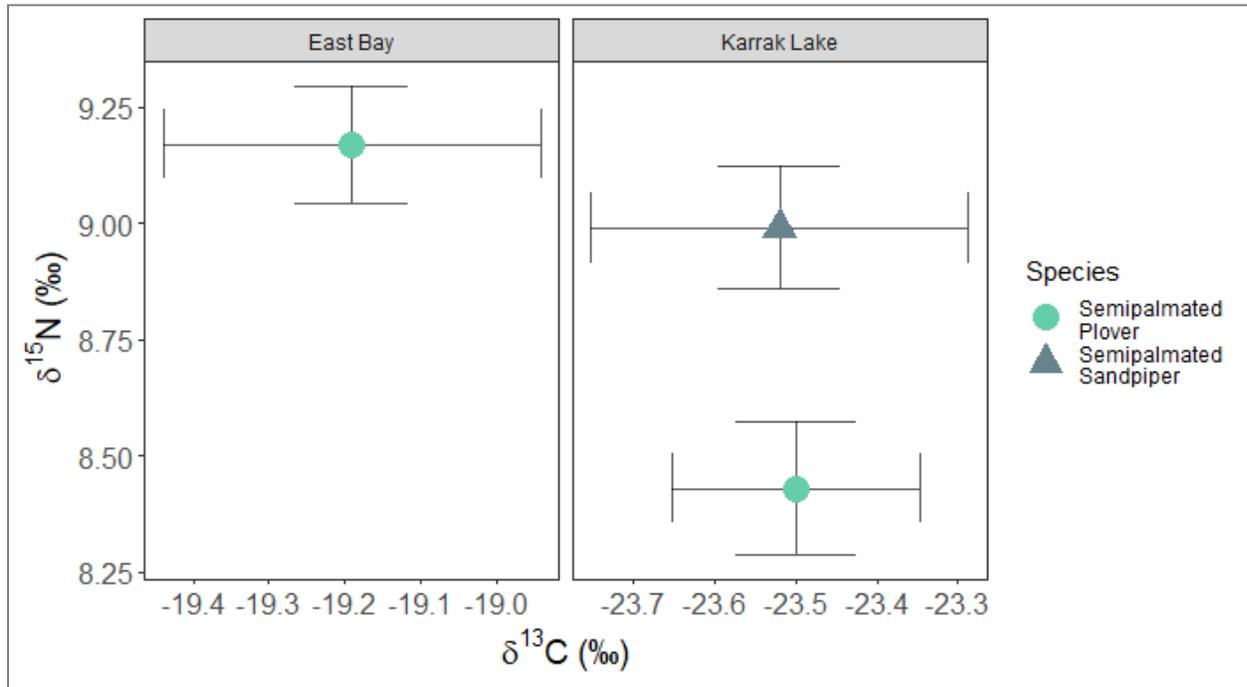


Figure 3.3: Biplot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in blood (means \pm SE reported) of two shorebird species sampled from two locations in the Canadian Arctic (East Bay NU, $n = 8$ (Semipalmated Plover only); Karrak Lake NU, $n = 20$ Semipalmated Plover, $n = 15$ Semipalmated Sandpiper). Initial blood samples only, sexes are pooled. Plovers nesting at East Bay had significantly higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than plovers at Karrak Lake ($\delta^{15}\text{N}$ $P = 0.04$; $\delta^{13}\text{C}$ $P = < 0.001$), whereas sandpipers had higher $\delta^{15}\text{N}$ than plovers nesting at the same site ($P = 0.03$).

3. 2. 2. Egg Analysis

3. 2. 2. 1. *Species Differences*

Species differences were used as a proxy for Trophic Status in egg analyses, as stable isotope values for eggs were not available. Only sandpipers were sampled at the same site (Karrak Lake) across years. However, THg concentrations in eggs between 2016 and 2017 did not differ ($P = 0.87, 0$), so data were pooled in subsequent analyses.

Eggs were collected from 30 sandpiper and 13 plover nests at Karrak Lake during this study. Sandpipers had more variable egg mercury concentrations (0.07 – 0.52 $\mu\text{g/g}$ wet weight) than plovers (0.08 – 0.26 $\mu\text{g/g}$ wet weight). Average egg mercury concentrations were higher ($P = 0.064$, Figure 3.4) in sandpiper ($0.20 \pm 0.09 \mu\text{g/g}$ wet weight) than in plover ($0.16 \pm 0.05 \mu\text{g/g}$ wet weight). Variability in the candidate model set was high, with only 39% of the weight of evidence in support of an effect of species (SEPL < SESA, $\beta_{\text{SESA}} = 0.043 \pm 0.028$, Table 3.2), such that a substantial amount of variation in egg mercury concentrations was not related to species differences.

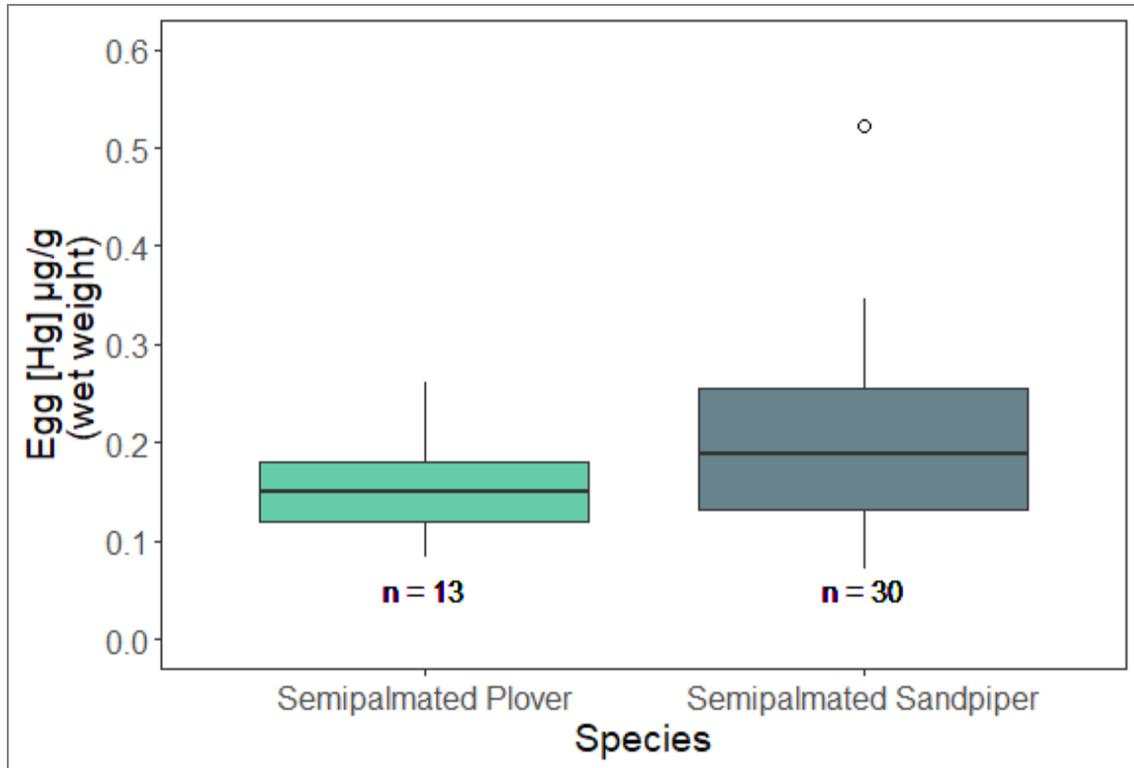


Figure 3.4: Total mercury concentrations in eggs of two shorebird species breeding at the Karrak Lake NU site. Mean values denoted by midline in boxplot, first and third quartiles denoted by boxes. Line represent standard deviation; outliers are denoted by empty circles. Mercury concentrations are reported in $\mu\text{g/g}$ wet weight. Sandpipers averaged higher egg mercury concentrations than plovers at the same site ($\beta_{\text{SESA}} = 0.043 \pm 0.028 \text{ SE}$, $P = 0.06$).

3. 2 .2. 2. Site Differences

Sandpiper eggs were sampled from Coats Island in 2016, and from Karrak Lake in 2016 and 2017, to compare mercury concentrations across the region. Since THg concentrations in eggs collected at Karrak Lake did not differ between years, they were pooled for analyses. In total six eggs were collected from Coats Island, and 30 eggs were collected from Karrak Lake. Eggs collected from birds nesting at the Karrak Lake site had higher variability in their egg THg ($0.07 - 0.52 \mu\text{g/g}$ wet weight) than those collected at the Coats Island site ($0.13 - 0.36 \mu\text{g/g}$ wet weight), which may be due to differences in sample size. Eggs from birds nesting at the Coats Island site had higher average THg levels than those nesting at the Karrak Lake site ($0.27 \pm 0.08 \mu\text{g/g}$ ww and $0.20 \pm 0.09 \mu\text{g/g}$ ww, respectively). Differences in egg THg concentrations between sites were small ($P = 0.12$, Karrak Lake < Coats Island, $\beta_{\text{Karrak}} = -0.065 \pm 0.041 \text{ SE}$,

Figure 3.5), and models containing site were consistently ranked low in the model selection process (Table 3.2).

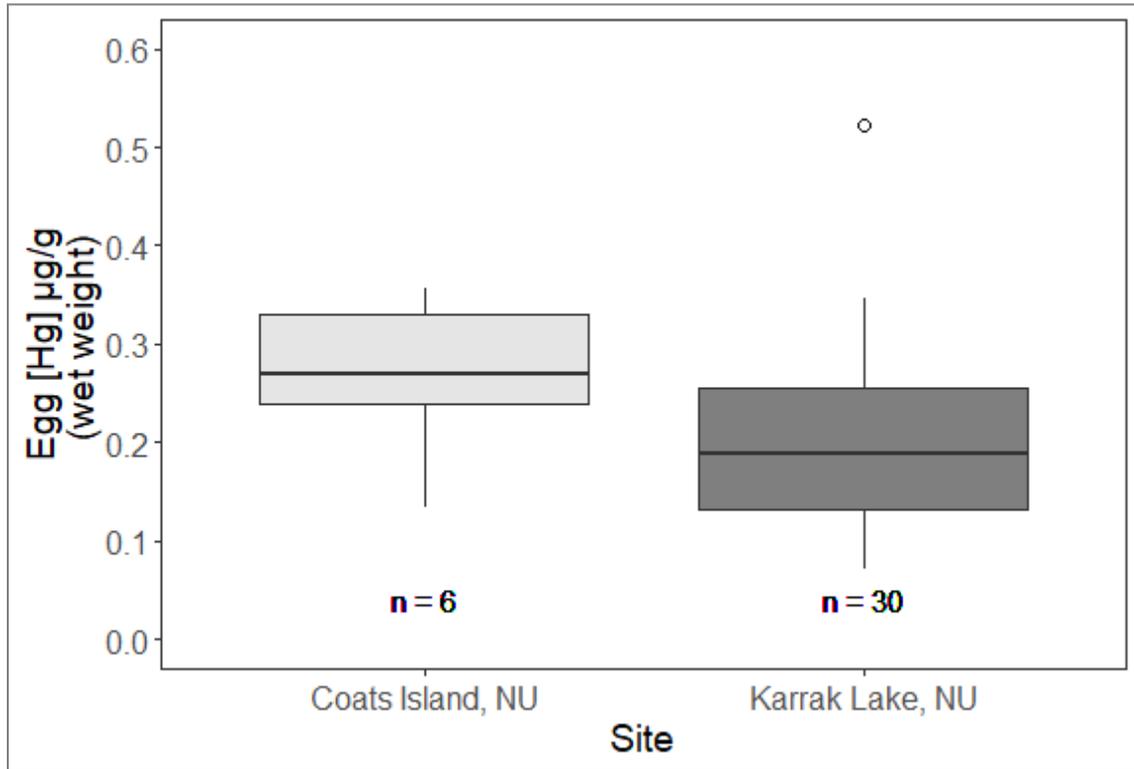


Figure 3.5: Total mercury concentrations in eggs of Semipalmated Sandpipers breeding at two sites in the Canadian Arctic, years pooled. Mean values denoted by midline in boxplot, first and third quartiles denoted by boxes. Line represent standard deviation; outliers are denoted by empty circles. Mercury concentrations are reported in $\mu\text{g/g}$ wet weight. Sandpipers at the Coats Island site averaged higher egg mercury concentrations than those at the Karrak Lake site ($\beta_{\text{Karrak}} = -0.065 \pm 0.041 \text{ SE}$, $P = 0.12$).

3. 3. SEX DIFFERENCES

Plover blood mercury concentrations did not differ between sites ($P = 0.41$, 0), and so plover data across sites were pooled for analysis. Sandpipers had higher initial blood mercury concentrations than plovers ($0.71 \pm 0.36 \mu\text{g/g}$ ww and $0.29 \pm 0.10 \mu\text{g/g}$ ww, respectively, $P = <0.001$) and were more variable (Table 3.5). Male birds had higher initial mercury concentrations than females in both species ($P = 0.01$), with this difference being more pronounced in sandpipers (SEPL < SESA, Female < Male; $\beta_{\text{SESA Male}} = 0.32 \pm 0.12 \text{ SE}$, Figure 3.6). Initial maternal blood mercury concentrations were correlated with egg mercury concentrations ($r = 0.58$, $P = 0.01$, 0). The most supported general linear model indicated that the

interaction of species and sex estimated differences in blood mercury concentrations (92% of model weight, Table 3.7).

Table 3.7: General linear models for estimating effects of species and sex differences on initial blood mercury concentrations ($\mu\text{g/g}$ wet weight) collected in 2017. The interaction between species and sex differences accounted for 92% of model weight. Semipalmated Plovers have lower blood mercury levels than Semipalmated Sandpipers (SEPL < SESA, $\beta_{\text{SESA}} = 0.26 \pm 0.08$ SE), and male sandpipers have higher levels than females (Female < Male, $\beta_{\text{Male}} = 0.11 \pm 0.07$ SE).

Model	Df	Log-Likelihood	Deviance	AIC _C	Δ_C	Weight
Bld_{HgWW} ~ Species * Sex	5	13.35	1.40	-15.13	0.00	0.92
Bld_{HgWW} ~ Species + Sex	4	9.69	1.66	-10.36	4.77	0.08
Bld_{HgWW} ~ Species	3	3.34	2.21	-0.09	15.04	0.00
Bld_{HgWW} ~ Sex	3	-5.98	3.38	18.59	33.69	0.00
Bld_{HgWW} ~ 1	2	-9.71	4.01	23.71	38.84	0.00

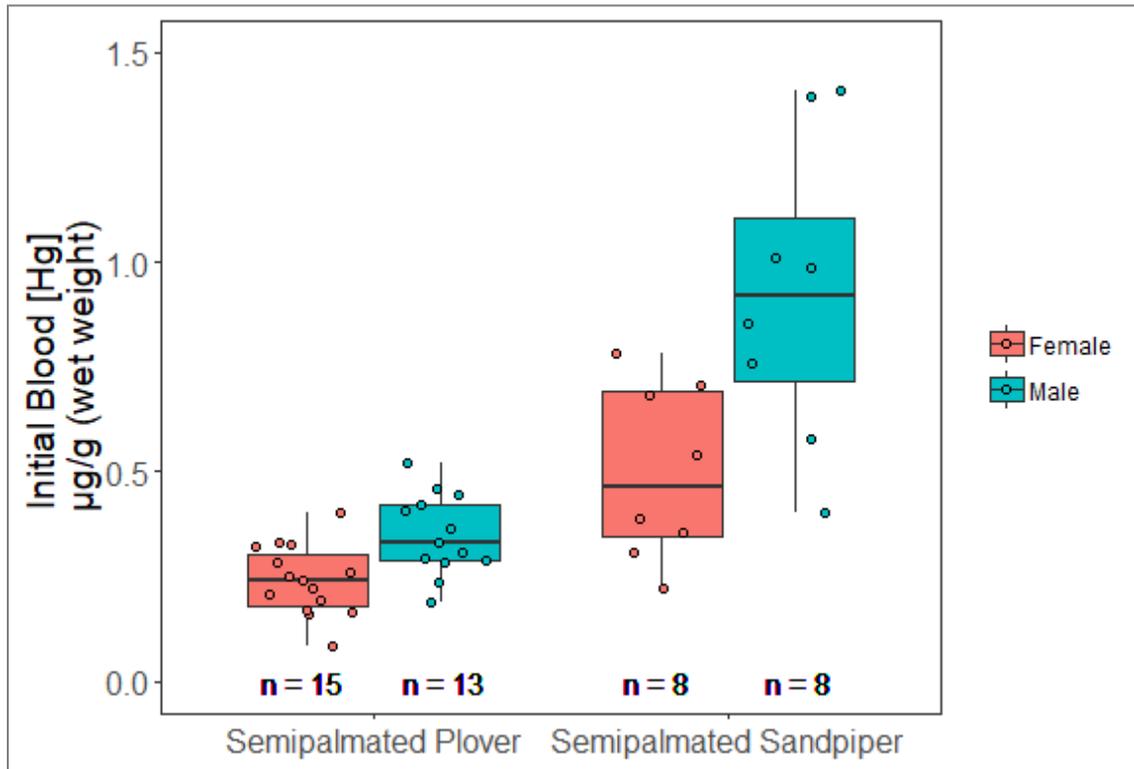


Figure 3.6: Variables that influence initial blood mercury levels in breeding Arctic shorebirds at two sites in Nunavut. Blood samples from Semipalmated Plovers at Karrak Lake and East Bay

MBS were pooled, as they did not differ significantly between sites ($P = 0.41$). The interactive effect of sex and species differences explain 92% of the variation. Results suggest that sex and species differences contribute to blood mercury levels found in sandpipers and plovers breeding in the Canadian Arctic. In both species, males had higher blood mercury levels than females ($P = 0.01$), while both sexes of Semipalmated Sandpipers had higher blood mercury levels and more variation in mercury levels than Semipalmated Plovers ($P = <0.001$).

DISCUSSION

4. 1. NESTING PHENOLOGY

4. 1. 1. Influences of Clutch Initiation Date

The influence of SCID on THg was small, but measurable and on average, egg mercury concentration declined as SCID increased, with this trend being more pronounced in sandpipers, which had more variable mercury levels than plovers. These results suggest that birds initiating nests earlier in the season are exposed to higher levels of mercury when foraging and producing eggs, relative to birds that initiate later in the breeding season.

Shorebirds are generally regarded as income breeders (Klaassen et al. 2001) with nutrients (and therefore contaminants) used for egg development obtained on the breeding grounds. However, there has been some discussion as to whether these birds fall on a continuum of capital and income strategies: Yohannes et al. (2010) reported that earlier breeding female Pectoral Sandpipers (*Calidris melanotos*) utilize some reserves obtained from staging grounds when producing the first egg, while later eggs are produced from nutrients acquired locally. This would then present the question as to whether potential contaminants reported in eggs are brought to the breeding grounds from wintering, or stopover locations. Sandpipers wintering in Suriname and stopping at locations in New Jersey were sampled by Burger and colleagues (2018). Wintering birds had mean mercury concentrations of $0.02 \pm 0.001 \mu\text{g/g ww}$, while mean concentrations of blood mercury collected from birds at stopover sites in New Jersey exhibited even lower levels of $0.01 \pm 0.003 \mu\text{g/g ww}$ (Burger et al. 2018). Currently, exact wintering locations of birds nesting at Karrak Lake are unknown, but if this trend is consistent across wintering grounds and stopover sites, it seems unlikely that significant levels of mercury from their wintering grounds would be present in birds by the time they arrive on their breeding grounds. Low exposure to mercury on the wintering grounds was corroborated by Hargreaves et al. (2010), who noted that mercury concentrations found in feathers grown on the wintering grounds were significantly lower than egg mercury concentrations.

Additional unaccounted variation reported in mercury levels could be related to collection methods; one egg was randomly selected for collection, only once a clutch was completed, and most nests were found once they were nearing or after completion. Shorebirds

only intermittently incubate their nests during the laying period (Norton 1972), and the first laid egg was not determined during this study. There was also no way to determine whether later initiated nests were the result of later breeding birds, or the result of previously unmarked pairs that had nested earlier, experienced a nest failure, and then re-nested. This is especially true for later initiated nests that showed lower mercury levels. It's unclear at what rate shorebirds experience partial nest failures and then choose to re-nest, but re-nesting could reduce mercury concentrations in successive eggs (Becker 1992; Braune et al. 2018; Kennamer et al. 2005). Conversely, age of nesting birds may influence mercury concentrations, as older, experienced birds tend to nest earlier than younger birds (Bauer et al. 2018). Age may also impact overall mercury burdens in nesting birds, with adults having higher mercury burdens than immatures (Burger et al. 2018).

Future work should further adjust methodologies in order to reduce the variability in reported mercury concentrations. Other research has recommended specifically collecting the first laid egg of a clutch in order to report contaminant levels (Braune et al. 2018), and I recommend that this method be extended to shorebirds as well. Intensively searching areas where nests were found in previous years may be a way of finding partial clutches (<2 eggs), as sandpipers exhibit nest site philopatry, especially if nests are successful (Gratto et al. 1985). Currently, the wintering grounds of the shorebirds nesting at Karrak Lake – as well as the contaminants they may be exposed to while there – are unknown, but the analyses of feather isotopes or the use of geolocators might provide insights regarding connectivity. Identifying and characterizing the contaminant loads at wintering sites, as well as migratory stopover sites, and potentially staging areas would be useful in determining the extent of mercury exposure throughout the yearly cycle of these birds, since there are differing points of view in terms of where nutrients and contaminants in eggs are being acquired from (Klaassen et al. 2001; Yohannes et al. 2010)

4. 1. 2. Mercury Levels in Adult Shorebirds Through Time

Sex and species consistently influenced blood mercury concentrations in this study, but concentrations did not change with time since melt, instead staying at a consistent level throughout the breeding season, possibly suggesting ongoing local exposure to mercury during this time frame. Alternatively, some birds may have commenced molt around the time of

secondary capture, and since mercury can be depurated into growing feathers (Monteiro and Furness 2001; Perkins et al. 2016), possible trends could have been obscured. In general, individual heterogeneity in sandpiper mercury concentrations remained high throughout the sampling period, but factors influencing differences among individuals remain unknown. Future research should study additional factors driving the variability in mercury levels over time, such as differences in foraging location, prey preferences, and timing of molt in relation to mercury sampling.

To my knowledge, this is the first study to explore how changes in breeding phenology influence contaminant levels in wild shorebirds and my results show that shifts in timing of breeding can have toxicological consequences. Although the specific half-life of THg in shorebird blood is unknown, previous studies suggest that THg in avian blood represents relatively recent exposure, ranging from days to weeks (Evers 2018), and potentially months (Monteiro and Furness 2001). It is therefore difficult to identify an exact period (i.e. location) of exposure for the birds I sampled. However, shorebirds begin arriving at Karrak Lake between May 27 and 29 (*pers. obs.*) and my mean blood sampling date was June 23, an interval that approaches the upper estimates for the half-life of THg in avian blood, suggesting that mercury I measured in shorebird blood was not acquired outside of the breeding area. Consistent with this idea, Burger et al. (2018) report low mercury concentrations in shorebirds sampled on wintering grounds, as well as at stopover sites, and suggest that shorebirds are unlikely to acquire mercury on the wintering grounds or migration areas.

My results likely represent a conservative estimate of blood mercury concentrations through time, as most blood samples selected for analyses were from birds for which initial and secondary blood samples were available (i.e., adults from nests that were either successful or persisted until just before hatch). Blood samples from individuals who experienced an early nest failure are thus not well represented, with my results biased to individuals that were robust enough to successfully migrate to breeding grounds, defend a territory, find a mate, and incubate a nest.

4. 2. TROPHIC STATUS

4. 2. 1. Whole Blood Samples

4. 2. 1. 1 *Species and Site Differences*

At Karrak Lake, nitrogen isotopes differed significantly between shorebird species, but I detected no effect of sex for either species. Surprisingly, sandpipers averaged higher $\delta^{15}\text{N}$ in their blood than plovers, suggesting that sandpipers may feed at a higher trophic level than originally expected, but overall differences in $\delta^{15}\text{N}$ between species were small and did not surpass the 3 – 4‰ enrichment factor typically associated with a change in trophic position (Atwell et al. 1998; Post 2002). Factors unrelated to dietary differences – staging areas, spatial variation in nitrogen baselines, or isotopic carry-over – may also play a role in the differences in $\delta^{15}\text{N}$ between species described here (Post 2002).

For plovers, site differences influenced isotopic signatures, with birds nesting at East Bay having significantly higher values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ than those at Karrak Lake, suggesting that plovers at East Bay may be foraging differently than those at Karrak Lake. Differences in $\delta^{13}\text{C}$ are typically used to distinguish prey from terrestrial or marine sources (Evans Ogden et al. 2004; Hobson 1999), and plovers at East Bay had significantly higher $\delta^{13}\text{C}$, indicating a marine influence on assimilated diet. Stable isotopes were not available for eggs collected from sandpipers at Coats Island, but they would also likely show a coastal influence, distinct from the inland birds sampled at Karrak Lake.

Since mercury bioaccumulates (Driscoll et al. 2007; Eagles-Smith et al. 2016), species that forage at higher trophic levels should consume more mercury from contaminated ecosystems than species that forage at lower levels (Atwell et al. 1998; Hargreaves et al. 2011).

Methylmercury readily accumulates in aquatic systems, and birds acquiring nutrients from marine sources could have elevated levels of mercury (Fitzgerald et al. 2007; Provencher et al. 2014). Yet neither $\delta^{15}\text{N}$ nor $\delta^{13}\text{C}$ values were related to blood mercury in either species of shorebird at either site. This is potentially due to the differences in half-lives of mercury and stable isotopes. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have a half-life of approximately 11 days in whole blood of captive Dunlin (*Calidris alpina pacifica*) after diets were switched from terrestrial to marine

carbon and nitrogen signatures (Evans Ogden et al. 2004). The half-life of mercury in birds is variable, but likely reflects exposure within days to weeks (Evers 2018). Since blood was sampled after snowmelt and often exceeded the 11 day half-life (Evans Ogden et al. 2004), stable isotopes sampled in blood may not reflect what was being consumed during the period of exposure, as has been described for Great Skuas (Bearhop et al. 2000).

This decoupling of isotopes and mercury may indicate a seasonal shift in foraging preference after the egg laying period, but data linking stable isotope values and foraging preferences in breeding shorebirds is somewhat limited. Because birds sampled here were captured while incubating nests, it is likely that stable isotope profiles and mercury concentrations in the blood of this birds both reflect locally consumed foods, but specific isotopic profiles of prey items would be required to verify this. Hargreaves et al. (2011) reported that plovers frequently foraged in coastal ecozones, on higher trophic level prey than in other ecozones, which is consistent with the enriched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in blood of plovers from this site that we report. However, despite differences in the trophic status of plovers between sites, mercury concentrations did not change. The variability reported in mercury concentrations and stable isotopic profiles between species and sites suggests that specific foraging locations, and prey preference may play a substantial role in mercury accumulation in shorebirds.

While stable isotopic profiles differed between species, and across sites, stable isotopic signatures did not help to explain variation in mercury concentrations in the blood of shorebirds studied. The stable isotopic analysis conducted here is preliminary: we currently do not have stable isotope profiles for invertebrate groups occurring at these sites. Additionally, we do not know to what extent mercury concentrations differ between invertebrate classes, between invertebrates in differing habitats, or what prey species shorebirds prefer to forage on. Such information would greatly improve our understanding of the variation in reported mercury levels. Future studies should aim to characterize the diets of breeding shorebirds, as well as to describe how mercury concentrations differ between prey species and foraging habitats. Determining where shorebirds spend their time during the breeding period and characterizing these habitats would also be beneficial in determining sources of mercury e.

4. 2. 2. Egg Samples

4. 2. 2. 1 *Species Differences*

Mercury was detected in all eggs sampled from the central Canadian low Arctic (Karrak Lake, NU). Contrary to my initial prediction, mercury concentrations in eggs were marginally higher from sandpiper eggs than from plovers ($P = 0.06$). Comparisons of egg mercury between years was available only for sandpipers and did not differ. These findings suggest that species differences and potentially differences in foraging behaviour during the pre-laying period play a role in mercury concentrations found in eggs, and I suggest that future work to determine the proportion of given prey items in shorebird diets and to link dietary variability to mercury concentrations will be valuable.

Species differences in mercury concentrations, however, may only be partially influenced by differences in trophic status (as indexed by $\delta^{15}\text{N}$). The species difference in $\delta^{15}\text{N}$ that I observed was small and did not fall within the 3 - 4‰ generally expected with an increase in trophic position (Post 2002), and coarse-scale geographic variability (i.e. site level) in egg mercury levels was weak. Whereas sandpiper eggs from Karrak Lake only had marginally lower mercury levels than those from Coats Island (Karrak Lake < Coats Island, $\beta_{\text{Karrak}} = -0.065 \pm 0.041$ SE), mercury levels in eggs from plovers at Karrak Lake were generally consistent with those reported for other sites (McCloskey et al. 2013; Perkins et al. 2016). Similarly, I did not detect annual differences in egg mercury concentrations for sandpipers, and although I did not compare eggs from plovers over multiple years, values from the literature (0.185 $\mu\text{g/g}$ wet weight; McCloskey et al. 2013), suggest that mercury concentrations in plover eggs contents were comparable between years, but more data would be needed to discern patterns of annual variation.

Difference in fine-scale foraging location, rather than geographic differences or differences in the specific prey types consumed, may have a stronger influence on observed mercury concentrations: sandpipers tend to forage in wetland areas, whereas plovers can be found in upland areas and near larger water bodies (Rodewald 2015; Perkins et al. 2016). Research studying this phenomenon in eggs is limited, but a study done by Perkins et al. (2016) in Alaska reported that mercury concentrations in shorebird blood differed with foraging

location, and species which preferred lower, wetter areas had higher mercury concentrations than upland foraging counterparts. This pattern was supported anecdotally here, as sandpipers at Karrak Lake were often seen foraging in small vegetated ponds, whereas plovers were more frequently encountered on dry eskers, or at the edge of larger lakes (*pers. obs.*). Since organic carbon and humic matter may play important roles in mercury methylation, specific, fine-scale foraging location should be considered when determining sources of mercury exposure in these systems (Weber 1993; Loseto et al. 2004b; Kainz and Lucotte 2006). Finally, as expected, egg mercury concentrations in this study ($r = 0.58$, $P = 0.01$; 0) were positively correlated with maternal blood mercury concentrations, suggesting females can readily depurate mercury into their eggs.

As stated previously, the mercury concentrations reported here represent a conservative estimate of mercury pollution in shorebird eggs. Concentrations reported in eggs were highly variable, in part due to the sampling methods employed. Laying order can influence contaminant levels in the eggs of other bird species, with the first laid egg often containing higher contaminant levels than consecutively laid eggs (Akearok et al. 2010). This trend has been described in Charadriiformes, including oystercatchers (Becker 1992), and could feasibly occur in the species that I studied as well.

4. 2. 2. 2 Site Differences

As previously described, evidence for coarse-scale variation in egg mercury levels was weak and the influence of trophic status on observed patterns is unclear, as are the effects of longitude. Research pertaining to AMDEs suggests that depletion of mercury onto the Arctic landscape is highest in the western Arctic and decreases towards the east (Lu et al. 2001; Ariya et al. 2004; Steffen et al. 2008), but available data for shorebirds show the opposite trend (i.e. increasing from west to east). Saalfeld et al. (2016) reported that sandpiper eggs collected in Alaska had mean mercury concentrations of 0.08 ± 0.03 $\mu\text{g/g}$ wet weight (assuming 75% moisture content), which was much lower than the levels reported at the Karrak Lake site (0.20 ± 0.09 $\mu\text{g/g}$ wet weight), approximately 2100 kilometres to the east. Furthermore, sandpipers nesting at Karrak Lake also had lower mercury concentrations in their eggs than those nesting at Coats Island (0.27 ± 0.08 $\mu\text{g/g}$ wet weight) which is an additional ~950 kilometres east of

Karrak Lake. Sandpiper eggs were not collected at East Bay during this study which is in close vicinity to Coats Island, but White-rumped Sandpiper (*Calidris fuscicollis*) eggs were collected at this site in 2011, and mean egg mercury concentrations in this species was reported at 0.236 µg/g wet weight (McCloskey et al. 2013). There is limited knowledge pertaining to shorebird eggs mercury concentrations at sites in the eastern Arctic in areas extending from the Baffin and Nunavik regions of Canada and into Greenland, and contaminant levels shorebird eggs from there are unavailable.

My sample size was small regarding spatial trends of mercury in shorebirds, but this west to east increase in mercury concentrations may be of conservation interest for sandpipers as they are estimated to be in decline, with eastern breeding populations showing the strongest downward trends based on surveys conducted on southbound migration stopover sites in the Bay of Fundy (Hicklin and Chardine 2012). These populations may have multiple stressors throughout their annual cycle, but with limited contaminant monitoring on eastern breeding grounds, the potential influence of mercury contamination on reproductive success or juvenile survival warrants further attention.

Research collecting Arctic-breeding shorebird eggs as a means for baseline monitoring of environmental contaminants across the Arctic is limited (Saalfeld et al. 2016). But shorebirds nest across the Arctic (Canadian Wildlife Service 2006) and are visible when nesting, making them a good candidate species for expanding initiatives both in terms of research, and community led programs – especially in the eastern Arctic, where data is deficient. Expanding this monitoring project to include support from the Arctic Shorebird Demographics Network (ASDN) would assist in filling some of the knowledge gaps associated with how mercury concentrations change across the Arctic landscape. The ASDN has research camps scattered around the western Arctic which monitor breeding shorebird populations, including those of sandpiper (Brown et al. 2014). Adding egg collections to established protocols would help to gain insight on how mercury pollution changes across the Arctic.

Additionally, there is some indication that influence of Arctic Mercury Depletion Events (AMDEs) are more pronounced in areas adjacent to the coast (Loseto et al. 2004a; Constant et al. 2007; Poissant et al. 2008; Dastoor et al. 2015). Some research has been done in describing mercury levels in lichens at increasing distances from Hudson Bay; with lichens nearest the

coastline having the highest concentrations of mercury (Carignan and Sonke 2010). A study of this magnitude has not been conducted with Arctic-breeding birds but could add an additional level of spatial complexity when trying to characterize mercury accumulation across the Arctic.

4. 3. SEX DIFFERENCES

Mercury was detected in all initial blood samples. Mercury concentrations were influenced by species differences, and this could be due to differences in trophic status, as described above, with mean initial blood mercury concentrations in sandpipers being twice that of plovers. Further, sex differences influenced the ranges of mercury concentrations reported, with males of both species having higher mercury concentrations in their initial blood samples than respective females. Comparisons in site differences in blood mercury concentrations were only available for plovers. Interestingly, initial blood mercury concentrations did not differ between sites in this species even as isotopic profiles changed ($p = 0.41, 0$). In plovers, the consistency in mercury concentrations between sites could allude to additional species-specific differences not explored in this study, such as precise differences in foraging ecology or differences in detoxification processes, like metallothionein regulation. Distinct adaptive responses to elevated trace element concentrations have been described in Red Knots (*Calidris canutus*) and Black-tailed Godwits (*Limosa limosa*) (Lucia et al. 2012). These species are within the same family (Scolopacidae) and differ in their detoxification processes (Lucia et al. 2012), therefore it is feasible that more divergent species (i.e. Semipalmated Sandpiper, Scolopacidae; Semipalmated Plover, Charadriidae) may also differ in their detoxification processes.

These results indicate both sex and species differences should be considered when selecting study species for environmental contaminant monitoring programs. Sandpipers have higher mercury concentrations overall, which may be influenced by their foraging behaviour (Perkins et al. 2016). Males of both species had higher mercury concentrations than females, as females are able to depurate mercury burdens into their eggs (Robinson et al. 2012). This sex difference may also be the result of differing arrival times: males of both species arrive on the breeding grounds before females, and would spend a longer time foraging in the local environment (Rodewald 2015).

Describing interspecific differences as well as sex differences in mercury concentrations of shorebirds presents a new facet of shorebird research. Blood mercury concentrations in sandpipers have previously been reported for collections in Alaska (Perkins et al. 2016), where mean blood mercury concentrations were $0.95 \pm 0.62 \mu\text{g/g}$ fresh weight. These levels were comparable to initial blood mercury concentrations observed in male sandpipers breeding at Karrak Lake ($0.92 \pm 0.36 \mu\text{g/g ww}$). Interestingly, sex was not determined to be an important variable influencing mercury concentrations in sandpipers (Perkins et al. 2016). But based on my results, sex differences can explain significant differences in mercury concentrations, and failing to determine the sex of a bird when conducting mercury analyses could lead to the over- or underestimate of mercury concentrations and the associated risk to a population (Robinson et al. 2012).

The initial blood samples reported here represent a conservative estimate on blood mercury concentrations. Due to budget constraints, and to fulfill the goals of this study by reporting mercury concentrations through time, most blood samples analysed for mercury were taken from birds where initial and secondary blood samples were available (i.e. adults from nests that were either successful or persisted until just prior to hatch). This approach may have excluded samples from adults that had high mercury concentrations and had nest failure prior to secondary sampling. Birds sampled in this study represent the most robust individuals, as they were able to migrate back to their breeding grounds, defend a territory, find a mate, and incubate a nest, and as such represent a conservative estimate in terms of mercury concentrations.

Several previous studies have reported mercury concentrations in blood of one, or both species studied here. Hargreaves et al. (2010, 2011), collected and analysed whole blood samples from plovers at East Bay for total mercury concentration. Hargreaves et al. (2011) reported mean plover blood mercury concentrations lower than the levels reported here for males but comparable to that of females, but these differences are confounded by differing analytical methods; Hargreaves et al. (2010, 2011) used inductively coupled plasma-mass spectrometry (ICP-MS), which underestimates true mercury concentrations, relative to the direct mercury analyzer that I used (Jian et al. 2000). To further assess temporal trends in blood mercury levels for shorebirds, more data are needed. Long-term increases in mercury concentrations have been described in Ivory Gulls (*Pagophila eburnea*) which live year-round in the Arctic; mercury

concentrations in their breast feathers rose from 0.09 µg/g dry weight in 1887 to 4.11 µg/g dry weight in 2007 (Bond et al. 2015). Conversely, weak year-to-year correlations of mercury concentrations were found in the blood of nesting King Eiders (*Somateria spectabilis*) found at Karrak Lake (Wayland et al. 2007), but this research is limited to three years of data collection.

Future monitoring of shorebirds should focus on characterizing foraging location preference at a species level, as well as determining prey preference, and mercury concentrations associated with different prey types. I recommend continuing to collect blood samples for mercury analysis, as well as stable isotope profiling. Additionally, blood samples should be collected from both sexes where possible, as sex differences have been shown to be significant, especially in sandpipers. Not reporting sex differences could potentially lead to over- or underestimation of mercury concentrations, especially in species that have high variability in reported concentrations. Initial blood mercury samples collected from birds which experienced nest failure should also be analysed in the future to determine whether parental mercury concentrations affect nest success. Finally, analysis of selenium concentrations should be carried out simultaneously with analysis of mercury. Selenium and mercury react antagonistically of one another, and presence of selenium may have a protective effect and aid in mercury demethylation; molar ratios between selenium and mercury may be important in understanding true mercury toxicity in wild birds (Scheuhammer et al. 2015).

4. 4. MERCURY THRESHOLDS IN ARCTIC WILDLIFE

4. 4. 1. Egg Mercury Thresholds

Egg mercury levels for both shorebird species were below described thresholds for reproductive impairment in avian species. The shorebird guild has limited literature associated with lethal and sub-lethal thresholds associated with tissue mercury concentrations. As such, more generalized threshold levels reported for most avian species have been used as a placeholder. A meta-analysis conducted by Shore et al. (2011), suggests hazardous concentration (HC5) threshold level of 0.60 µg/g wet weight for egg mercury concentrations, the authors predict that mercury concentrations exceeding this threshold may be indicative of adverse reproductive affects in 95% of species. None of the shorebird eggs collected in this study had THg concentrations that reached this threshold. However, it is pertinent to note that the levels in

eggs reported here suggest a conservative estimate in terms of mercury levels. Order of laying can play a significant role in the levels of contaminants found in eggs, with the first laid egg often having the highest levels of contamination. It was not possible to collect the first laid egg during this study, as shorebirds incubate the nest cup only sporadically while laying (Norton 1972). Most nests were found at a complete clutch, so egg selection was completely random. Variation in egg mercury concentrations was high throughout this study, and lay order remaining an unknown could be a reasonable explanation to this.

Descriptions of egg mercury concentrations in Arctic-breeding shorebird species are limited in the literature. The levels reported here are comparable to other instances where egg mercury concentrations were reported in other shorebirds in the Canadian Arctic (McCloskey et al. 2013; Table 10). But levels reported here were several times higher than concentrations reported by Saalfeld et al. (2016) in Alaska, and by Hargreaves et al. (2010) at East Bay NU. Although the discrepancies between the levels reported by Hargreaves et al. (2010), and those reported in this study may be due to differences in mercury analysis methods described previously.

Egg collections for mercury analysis remains a viable monitoring method for environmental contaminants. Egg mercury concentrations reflect those found in the laying female (0), and likely reflect local sources of mercury exposure (Klaassen et al. 2001; Morrison and Hobson 2004). Egg collections can be especially useful in areas where mercury concentrations are not well known; as a preliminary step in determining contaminant hotspots, or in community-led monitoring programs; since egg collections do not require specialist training as blood collections do, and birds and their nests are relatively easy to find and collect.

Table 4.1: Total mercury concentrations in eggs reported in Arctic wildlife ($\mu\text{g/g}$ wet weight).

Species	Location	Sample Period	\bar{x} (\pm SD) THg	<i>n</i>	Reference
Common Eider	Tern Island, NU	2008	0.21 ± 0.01^a	16	Akearok et al. 2010 ^b
	East Bay, NU		0.13 ± 0.01^a	9	
Long-tailed Duck	Tern Island, NU	2008	0.21 ± 0.06^a	4	Akearok et al. 2010 ^b
Black-bellied Plover	East Bay, NU	2011	0.134 ^c	5	McCloskey et al. 2013
	Various sites, AK	2002,2004	0.13 ± 0.03	8	Saalfeld et al. 2016
Semipalmated Plover	East Bay, NU	2008	0.05 ^d	6	Hargreaves et al. 2010
		2011	0.189 ^c	5	McCloskey et al. 2013
	Karrak Lake, NU	2017	0.16 ± 0.05	13	This study
Ruddy Turnstone	East Bay, NU	2011	0.159 ^c	5	McCloskey et al. 2013
Semipalmated Sandpiper	Various sites, AK	2004	0.08 ± 0.03	7	Saalfeld et al. 2016
	Coats Island, NU	2016	0.27 ± 0.08	6	This study
	Karrak Lake, NU	2016, 2017	0.20 ± 0.09	30	
White-rumped Sandpiper	East Bay, NU	2011	0.236 ^c	5	McCloskey et al. 2013
Arctic Tern	Tern Island, NU	2008	0.53 ± 0.03^a	17	Akearok et al. 2010 ^b
Ivory Gull	Seymour Island, NU	2004	1.59 ± 1.29^a	6	Braune et al. 2006
Thick-billed Murre	Coats Island, NU	2003	$0.15^{a, d}$	13	Braune et al. 2018

^a calculated from dry weight assuming 75% moisture content.

^b first laid egg concentrations reported.

^c source literature only reported mean values.

^d source literature reported the median value.

4. 4. 2. Blood Mercury Thresholds

Shorebirds have limited literature associated with lethal and sub-lethal thresholds of mercury concentrations. As such, more generalized threshold levels reported for most avian species has been used as a placeholder. A meta-analysis conducted by Ackerman et al. (2016), suggested a blood mercury threshold concentration of 1.0 µg/g wet weight, beyond which the elevated mercury levels may begin to affect overall health, physiology and reproductive functions.

Blood mercury concentrations reported here were variable and differed with species and sex. Females of both species had lower mean total mercury concentrations than males, and this is likely due to their ability to rid their systems of mercury by depurating it into their eggs (Robinson et al. 2012). The depuration as a form of reducing mercury was supported in my results, as female birds with higher initial mercury concentrations also laid eggs with higher mercury concentrations (0). This sex-based difference may also be attributed to males arriving earlier to the breeding grounds and foraging longer in the local environment (Rodewald 2015). This difference in arrival time may have increased male mercury concentrations, prior to sampling, which was not possible to capture in the sampling that took place.

Mean blood mercury concentrations in plovers were well below the threshold level described (Ackerman et al. 2016). Female plovers averaged 0.24 ± 0.06 µg/g wet weight, while male plovers averaged slightly higher with blood concentrations of 0.31 ± 0.13 µg/g wet weight, neither approach the mercury threshold of 1.0 µg/g wet weight, which suggests they are likely at low risk for mercury toxicity. Sandpipers were much more variable in their blood mercury concentrations, and males had significantly higher initial mean concentrations than females. Mean initial female blood mercury concentration was 0.50 ± 0.21 µg/g wet weight, while mean male concentrations were almost twice as high and approaching the threshold for potential detrimental effects; at 0.92 ± 0.36 µg/g wet weight. In addition, three male sandpipers had initial blood concentrations that exceeded this 1.0 µg/g wet weight threshold, which puts them at risk for potential mercury toxicity. Indeed, sandpipers – especially males – have mean blood mercury concentrations comparable to, or exceeding those of other larger, marine bird species which also breed in the Arctic (Table 11).

Results were variable and samples sizes were small when analysing blood mercury over time, which hindered the ability to identify a discernible trend in the data, but during secondary sampling male sandpipers which had high initial mercury concentrations saw an increase. As well, two female sandpipers had secondary blood samples surpassing the 1.0 µg/g wet weight threshold. High mercury concentrations in male sandpipers have the potential to negatively affect nest success, as this species is a biparental incubator (Jehl 2007). Since mercury is a neurotoxin (Evers 2018), and shorebirds rely heavily on distraction displays to protect their nests from predators (Hicklin and Gratto-Trevor 2010), elevated levels may reduce an incubating birds' ability to defend their eggs.

It is currently unclear at what concentration mercury becomes toxic to shorebirds, as previous work quantifying thresholds in American Avocets (*Recurvirostra americana*) were inconclusive (Ackerman et al. 2008), it is possible that shorebirds are less sensitive to mercury (McCloskey et al. 2013; Shore et al. 2011). But Hargreaves et al. (2010) recorded a small reduction in hatching success in shorebirds with increasing mercury concentrations. This coupled with the fact that these authors reported much lower blood mercury concentrations in shorebirds than what was reported in this study indicate that blood mercury concentrations in breeding shorebirds that approach the threshold for toxic effects warrant further study into reproductive success at sites with elevated mercury concentrations.

Table 4.2: Total mercury concentrations in whole blood reported in Arctic avian wildlife ($\mu\text{g/g}$ wet weight).

Species	Location	Sample Period	\bar{x} (\pm SD) THg	<i>n</i>	Reference
Common Eider	Nunavut	Not stated	0.31 ± 0.08^a	9	Mallory et al. 2018
	East Bay, NU	2013, 2014	0.21 ± 0.06	193	Provencher et al. 2016
Harlequin Duck	Kodiak Island, AK	2005, 2006, 2008	0.04 ± 0.02	33	Savoy et al. 2017
	Unalaska Island, AK		0.31 ± 0.19	82	
King Eider	Nunavut Karrak Lake, NU	Not stated	0.51 ± 0.19^a	6	Mallory et al. 2018
		2001	0.13 ± 0.01	63	Wayland et al. 2008
		2002	0.18 ± 0.01	69	
		2003	0.17 ± 0.01	74	
American Golden Plover	Barrow, AK	2009	0.18 ± 0.20	12	Perkins et al. 2016
Black-bellied Plover	East Bay, NU	2008	$0.38 (0.19-0.57)^b$	12	Hargreaves et al. 2011
Semipalmated Plover	East Bay, NU	2008	$0.24 (0.06 - 0.37)^b$	11	Hargreaves et al. 2011
		2017	0.31 ± 0.13^c	8	
	Karrak Lake, NU	2017	0.24 ± 0.06^d	20	
			0.36 ± 0.09^c		
Dunlin	Barrow, AK	2008	0.20 ± 0.09	19	Perkins et al. 2016
		2009	0.21 ± 0.09	23	
Purple Sandpiper	NW Greenland	2010 – 2012	0.53 ± 0.31	4	Burnham et al. 2018
Semipalmated Sandpiper	Barrow, AK	2008	0.95 ± 0.62	16	Perkins et al. 2016
	Karrak Lake, NU	2017	0.92 ± 0.36^c	16	This study.
			0.50 ± 0.21^d		
White-rumped Sandpiper	East Bay, NU	2008	$0.35 (0.13 - 0.68)^b$	15	Hargreaves et al. 2011
Black-legged Kittiwake	Nunavut	Not stated	0.76 ± 0.25^a	9	Mallory et al. 2018
Northern Fulmar			0.91 ± 0.52^a	7	
Thick-billed Murre			0.72 ± 0.125^a	6	

^a calculated from dry weight assuming 75% moisture content.

^b only mean concentration and range reported.

^c Male blood samples only.

^d Female blood samples only.

4. 5. CONCLUSIONS

The results of these research aim to help fill knowledge gaps associated with mercury contamination in breeding shorebirds in the Canadian Arctic by identifying species, sex, trophic and temporal differences that affect mercury concentrations in both blood and eggs.

4. 5. 1. Egg Mercury Concentrations

Clutch initiation date had a small but measurable effect on egg mercury concentrations in the shorebird species I studied, with later clutches having lower mercury concentrations. To my knowledge, this is the first study that has explored the relationship between nesting phenology, and egg mercury concentration. While egg mercury declines with increasing SCID, it is unclear if this is due to a reduction in mercury concentrations in prey items used for egg production, or whether nests initiated later are the result of renesting attempts, ideas that should be tested in future research.

Species differences (as a proxy for trophic status) affected mercury concentrations in egg samples. Sandpipers had higher mercury concentrations than plovers, which was contrary to my initial prediction. This difference is likely due to species foraging preferences, as sandpipers forage at higher trophic levels than plovers, and may prefer to forage in wet areas where mercury can accumulate (Ackerman et al. 2016; Perkins et al. 2016). This significant species difference indicates that care should be taken when selecting target species for contaminant monitoring as mercury concentrations can vary significantly at one site. Better yet, broadening research to include multiple species which utilize different habitats would help to fill some of the knowledge gaps associated with wildlife exposure to mercury in terrestrial Arctic systems. Currently, mercury concentrations in the eggs of shorebird species studied here are below the threshold for adverse reproductive effects estimated for most bird species (Shore et al. 2011). However, shorebird nests are challenging to find while they are actively laying, as the parents do not incubate during this time (Norton 1972). As such, eggs were randomly selected from a complete clutch. Contaminant concentrations decline with laying order in most bird species (Akearok et al. 2010) the concentrations reported here represent a conservative estimate. Additionally, there is some discussion as to whether shorebirds are purely income breeders, or whether they utilize a

mixture of capital and income strategies (Klaassen et al. 2001; Yohannes et al. 2010). The extent to which this occurs in shorebird species studied here remains unknown.

Differences in sites were marginal, with eggs collected from Coats Island having marginally higher average mercury concentrations than those collected at Karrak Lake. Year influence was not an important variable in predicting egg mercury concentration. Spatial and temporal trends in mercury concentrations of wildlife – while not significant in this study – are variable, and elevated mercury concentrations have been reported in shorebird species across the Arctic (Hargreaves et al. 2010, 2011; McCloskey et al. 2013; Perkins et al. 2016; Saalfeld et al. 2016). This study was small both in terms of sites studied and in duration and expanding monitoring to encompass a longer time scale, as well as including other field sites would help to clarify patterns of mercury exposure for shorebirds across the Arctic.

Egg mercury concentrations were variable across the species and sites studied, as well as through time, with small declines in mercury concentrations seen as clutch initiation dates increased. Future research should aim to reduce some of this variability by attempting to collect the first laid egg in a clutch, as well as analysing egg contents for stable isotopic profiles in addition to mercury, in order to determine whether they were produced from local resources. Eggs are a relatively easy biological sample to collect and based on the results here egg mercury concentrations are correlated with maternal mercury concentrations making them useful for mercury monitoring across the Arctic.

4. 5. 2. Blood Mercury Concentrations

Blood mercury concentrations differed with species, and sandpipers had higher mercury concentrations overall. Mercury concentrations were highly variable across the incubation period and with time since melt, which reduced the ability to ascertain a trend. But the lack of a decline in blood mercury over time suggests that these birds are being exposed to elevated mercury concentrations in the local environment. While stable isotopes indicated that sandpipers had higher $\delta^{15}\text{N}$ profiles than plovers this was not supported in a model selection process and could be due to a decoupling between the half-lives of mercury and stable isotopes (Bearhop et al. 2000; Evans Ogden et al. 2004; Evers 2018). We do not know how these birds are being exposed

to mercury, and prey or foraging preference may play a substantial role in an individual's contaminant profile.

Furthermore, sex differences were highly significant in predicting mercury concentrations: females of both species had lower levels than males of the same species, likely because they are capable of depurating mercury into their eggs (Robinson et al. 2012), and indeed maternal blood and egg mercury concentrations were positively correlated. Differences in arrival time may also be a contributing factor as males arrive on the breeding grounds earlier (Rodewald 2015). These results highlight the importance of reporting sex differences when monitoring contaminant levels in breeding birds, as failure to do so could over- or underestimate contaminant risk in a population.

Finally, site differences did not influence mercury concentrations, and mercury in the blood of plovers nesting at Karrak Lake were comparable to those nesting at East Bay. However, this was a small study and trends in mercury across the Arctic are not well understood. Contaminant monitoring programs would benefit from sampling avian species across the Arctic.

Future work should continue to monitor mercury concentrations in nesting shorebirds. Several male sandpipers were above the threshold for adverse reproduction effects, and Hargreaves et al. (2011) reported small reductions in nest success in shorebirds with elevated mercury concentrations during their study. This is concerning, because elevated mercury concentrations described by Hargreaves et al. (2011) were a fraction of those reported here, suggesting that shorebirds, and particularly sandpipers at this site may be at risk. Currently, potential sublethal effects of mercury in shorebirds has not been described, but future research should focus on reporting nest success of these birds in relation to mercury loads reported in both blood and eggs.

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APPENDIX A: PERMITS REQUIRED FOR SAMPLE COLLECTION

- Environment and Climate Change Canada
 - o NUN-SCI-15-02
 - o NUN-SCI-17-01
 - o NUN-MBS-17-04
- Nunavut Department of the Environment
 - o 2016-024
 - o 2017-041

APPENDIX B: CANDIDATE MODEL SETS INCLUDING UNINFORMATIVE PARAMETERS AND MODELS BELOW THE NULL

Table B.1: General linear model selection process of species and year effects on mercury concentrations in egg contents (Hg_{Egg}) of two shorebird species breeding at Karrak Lake. This table reports all candidate models run during analyses, including those that did not outperform the null. Species differences account for 39% of model weight in this analysis ($SEPL < SESA$, $\beta_{SESA} = 0.043 \pm 0.028$), however there is a substantial amount of variation in egg mercury concentrations not explained by the model. Year differences did not influence egg mercury concentrations at this site.

Model	Df	Log-Likelihood	Dev	AIC _C	Δ_c	Weight
$Hg_{Egg} \sim \text{Species}$	3	46.69	0.29	-86.77	0.00	0.39
$Hg_{Egg} \sim 1$	2	45.47	0.30	-86.64	0.13	0.37
$Hg_{Egg} \sim \text{Year}$	3	45.55	0.30	-84.49	2.29	0.12
$Hg_{Egg} \sim \text{Species} + \text{Year}$	4	46.71	0.29	-84.36	0.41	0.12

Table B.2: General linear model selection process of year and site effects on mercury concentrations in egg contents of Semipalmated Sandpipers breeding at two sites in the Canadian Arctic. This table reports all candidate models run during analyses, including those that did not outperform the null. Site differences account for 39% of model weight in this analysis (Karrak Lake < Coats Island, $\beta_{Karrak} = -0.065 \pm 0.041$ SE), however there is a substantial amount of variation in egg mercury concentrations not explained by this model. Year differences did not influence egg mercury concentrations.

Model	Df	Log-Likelihood	Dev	AIC _C	Δ_c	Weight
$Hg_{Egg} \sim \text{Site}$	3	35.89	0.29	-65.03	0.00	0.39
$Hg_{Egg} \sim 1$	2	34.61	0.31	-64.86	0.16	0.36
$Hg_{Egg} \sim \text{Year}$	3	34.83	0.30	-62.90	2.12	0.14
$Hg_{Egg} \sim \text{Site} + \text{Year}$	4	35.90	0.29	-62.51	2.51	0.11

Table B.3: Model selection process for general linear models estimating effects of standardized clutch initiation dates (SCID), and species differences on egg mercury concentrations ($Egg_{Hg_{WW}}$), at two sites in the Canadian Arctic. This table reports all candidate models run during analyses, including those with uninformative parameters, and those that did not outperform the null model. Variability in the models were high, but the influence of SCID was present in the top 3 models. Standardized SCID and species differences account for 48% of the cumulative variation seen in the model (Arnold, 2010). Mercury concentrations in eggs decreased as SCID increased ($\beta = -0.006 \pm 0.003$ SE) and were higher in Semipalmated Sandpiper eggs than Semipalmated Plovers ($\beta_{SESA} = 0.052 \pm 0.030$ SE).

Model	Df	Log-likelihood	Deviance	AIC _c	Δ_c	Weight
Egg_{Hg_{WW}} ~ SCID + Species	4	55.18	0.30	-101.46	0.00	0.22
Egg_{Hg_{WW}} ~ SCID	3	53.73	0.32	-100.92	0.54	0.17
Egg_{Hg_{WW}} ~ SCID * Species	5	55.47	0.30	-99.54	1.91	0.09
Egg_{Hg_{WW}} ~ Species + Site + SCID	5	55.27	0.30	-99.14	2.31	0.07
Egg_{Hg_{WW}} ~ Site + SCID	4	53.97	0.32	-99.03	2.43	0.07
Egg_{Hg_{WW}} ~ Year + SCID	4	53.76	0.32	-98.61	2.84	0.05
Egg_{Hg_{WW}} ~ Site + Species	4	53.67	0.32	-98.42	3.03	0.05
Egg_{Hg_{WW}} ~ Site	3	52.42	0.34	-98.30	3.16	0.05
Egg_{Hg_{WW}} ~ SCID + Species + Site + Year	6	56.03	0.29	-98.06	3.40	0.04
Egg_{Hg_{WW}} ~ Site * SCID	5	54.55	0.31	-97.71	3.74	0.03
Egg_{Hg_{WW}} ~ Species	3	52.11	0.34	-97.68	3.77	0.03
Egg_{Hg_{WW}} ~ Year * SCID	5	54.51	0.31	-97.63	3.83	0.03
Egg_{Hg_{WW}} ~ SCID + Site + Year	5	54.07	0.32	-96.74	4.71	0.02
Egg_{Hg_{WW}} ~ 1	2	50.20	0.37	-96.14	5.31	0.02
Egg_{Hg_{WW}} ~ Site + Year	4	52.50	0.34	-96.09	5.37	0.02
Egg_{Hg_{WW}} ~ Species + Site + Year	5	53.68	0.32	-95.97	5.49	0.01
Egg_{Hg_{WW}} ~ Year + Species	3	51.25	0.35	-95.96	5.50	0.01
Egg_{Hg_{WW}} ~ Year	4	52.37	0.34	-95.83	5.63	0.01

Table B.4: Model selection process for generalized mixed models estimating effects of time since melt (tMELT), species, and sex differences on adult blood mercury concentrations ($Bld_{Hg_{WW}}$) over time at Karrak Lake. Data was blocked by individual (ID) to account for individual differences. This table reports all candidate models run during analyses, including those that did not outperform the null. Species differences accounted for 65% of cumulative model weight (Arnold, 2010). However, highest ranked models did not exceed $\Delta < 2$ and indicates weak support. There was no discernible effect of time since melt on mercury concentrations in the blood of adult shorebirds.

Model	Df	Log-Likelihood	Deviance	AIC _c	Δ_c	Weight
Bld_{Hg_{WW}} ~ Species, (1 ID)	4	-14.76	24.78	38.59	0.00	0.38
Bld_{Hg_{WW}} ~ Sex + Species, (1 ID)	5	-13.82	20.66	39.30	0.71	0.27
Bld_{Hg_{WW}} ~ 1, (1 ID)	3	-16.58	30.22	39.78	1.19	0.21
Bld_{Hg_{WW}} ~ Sex, (1 ID)	4	-15.78	26.83	40.65	2.06	0.14
Bld_{Hg_{WW}} ~ tMELT + Species, (1 ID)	5	-18.71	23.59	49.08	10.49	0.00
Bld_{Hg_{WW}} ~ tMELT, (1 ID)	4	-20.44	28.84	49.96	11.37	0.00
Bld_{Hg_{WW}} ~ tMELT + Sex + Species, (1 ID)	6	-17.82	19.60	50.05	11.46	0.00
Bld_{Hg_{WW}} ~ tMELT + Sex, (1 ID)	5	-19.68	25.53	51.03	12.44	0.00

APPENDIX C: INTER YEAR VARIATION OF MERCURY CONCENTRATIONS IN SHOREBIRD EGGS AT A SITE IN THE CENTRAL CANADIAN LOW ARCTIC.

Table C.5: Data set associated with analysis of inter-year variation of mercury at the Karrak Lake NU site. Number of samples (n), mean (x), standard deviation (SD) and range of mercury concentrations in egg samples of Semipalmated Sandpipers are reported in $\mu\text{g/g}$ wet weight.

Species	Site	Year	Tissue	n	x	SD	Range
Semipalmated Sandpiper	Karrak Lake, NU	2016	Eggs	10	0.20	0.08	0.07 – 0.31
Semipalmated Sandpiper	Karrak Lake, NU	2017	Eggs	20	0.20	0.10	0.10 – 0.52

Table C.6: Model selection process for general linear models estimating effect of year on egg mercury concentrations ($\mu\text{g/g}$ wet weight) in Semipalmated Sandpipers at the Karrak Lake field site. Year did not improve model estimates beyond the null.

Model	Df	Log-likelihood	Dev	AIC _c	Δ_c	Weight
$\text{Hg}_{\text{Egg}} \sim 1$	2	29.06	-58.1	-53.7	0.0	0.77
$\text{Hg}_{\text{Egg}} \sim \text{Year}$	3	29.07	-58.1	-51.2	2.5	0.23

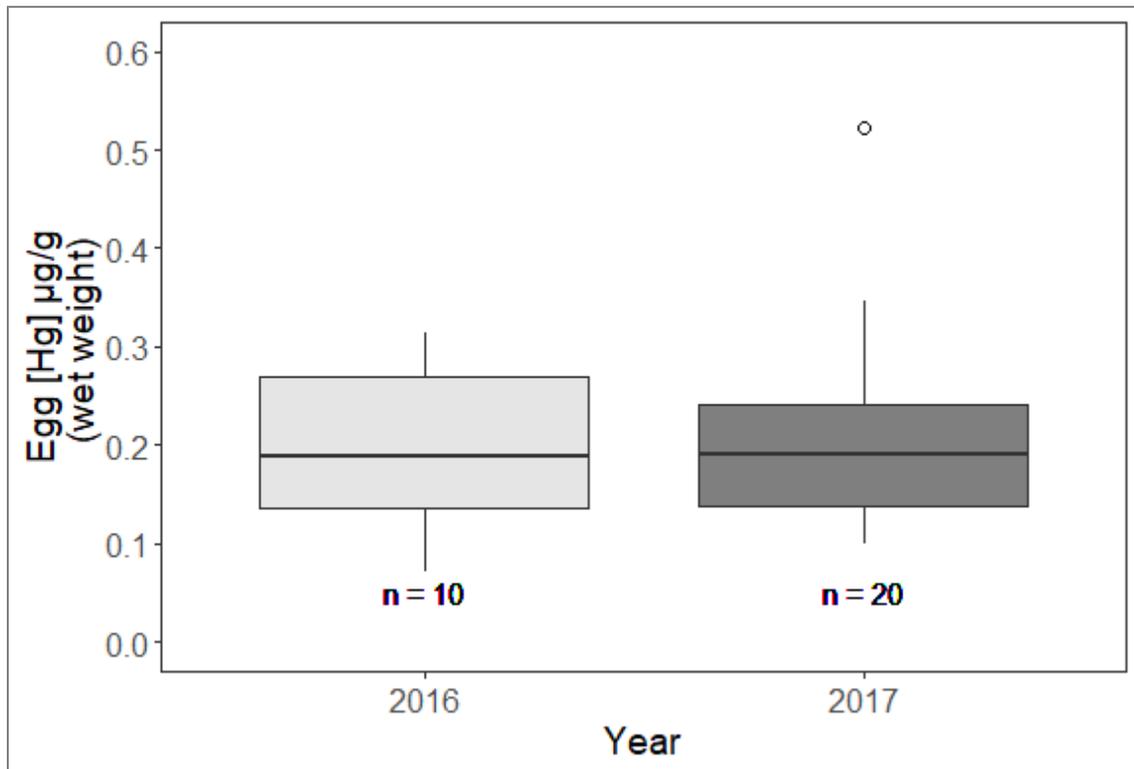


Figure C.1: Egg mercury concentration distribution across two years in Semipalmated Sandpipers at the Karrak Lake field site in the central Canadian Arctic. Mean values denoted by midline in boxplot, first and third quartiles denoted by boxes. Line represent standard deviation; outliers are denoted by empty circles. Mercury levels did not differ between years at this site ($p = 0.87$).

APPENDIX D: CORRELATION BETWEEN EGG MERCURY CONCENTRATIONS AND INITIAL MATERNAL BLOOD MERCURY CONCENTRATIONS

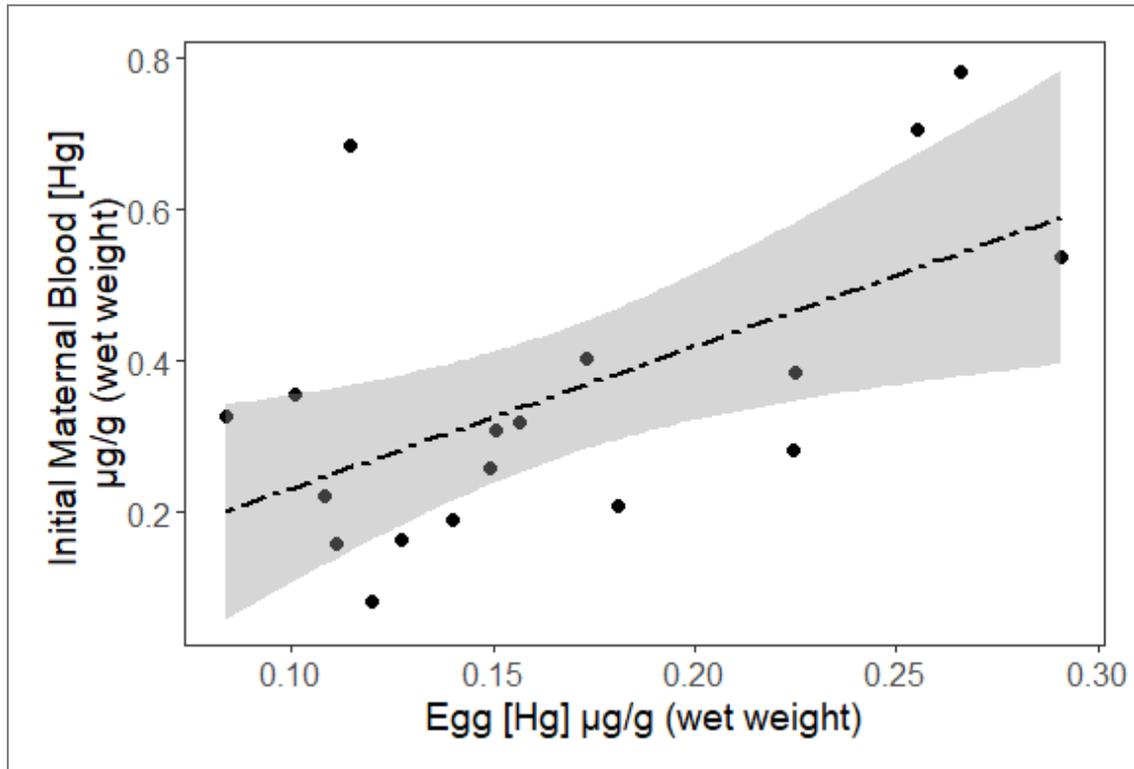


Figure D.2: Correlation (denoted by dashed line, shading indicates 95% CI) between initial maternal blood mercury concentration and egg mercury concentration ($\mu\text{g/g}$ wet weight) in both species of shorebird breeding at Karrak Lake, NU in 2017. Initial female blood mercury concentrations, and egg mercury concentrations are positively correlated ($r = 0.58$, $p = 0.01$). A model selection process indicated that species did not influence the relationship between maternal and egg mercury levels beyond that of the null model, and so for this analysis species were pooled ($n = 18$).

APPENDIX E: BETWEEN SITES VARIATION OF MERCURY CONCENTRATIONS IN INITIAL BLOOD SAMPLES IN SEMIPALMATED PLOVERS

Table E.7: Data set associated with this analysis. Initial blood mercury concentrations of Semipalmated Plovers were analyzed from two field sites in 2017. Number of samples (n), mean (\bar{x}), standard deviation (SD) and range of mercury concentrations in blood samples are reported in $\mu\text{g/g}$ wet weight.

Species	Site	Year	Tissue	n	\bar{x}	SD	Range
Semipalmated Plover	East Bay	2017	Whole	8	0.27	0.09	0.17 – 0.45
	MBS, NU		Blood				
	Karrak Lake, NU	2017	Whole Blood	20	0.30	0.11	0.08 – 0.52

Table E.8: General linear model of site effect on mercury levels ($\mu\text{g/g}$ wet weight) in initial blood samples (Hg_{Blood}) collected from Semipalmated Plovers at two sites in the Canadian Arctic. Site does not predict blood mercury levels in this species.

Model	Df	Log-Likelihood	Dev	AIC_c	Δ_c	Weight
$\text{Hg}_{\text{Blood}} \sim \text{Sex}$	3	28.78	0.21	-50.56	0.00	0.70
$\text{Hg}_{\text{Blood}} \sim \text{Sex} + \text{Site}$	4	28.94	0.21	-48.15	2.41	0.21
$\text{Hg}_{\text{Blood}} \sim \text{Sex} * \text{Site}$	5	29.25	0.20	-45.77	4.78	0.06
$\text{Hg}_{\text{Blood}} \sim 1$	2	24.09	0.29	-43.69	6.86	0.02
$\text{Hg}_{\text{Blood}} \sim \text{Site}$	3	24.41	0.29	-41.82	8.73	0.01

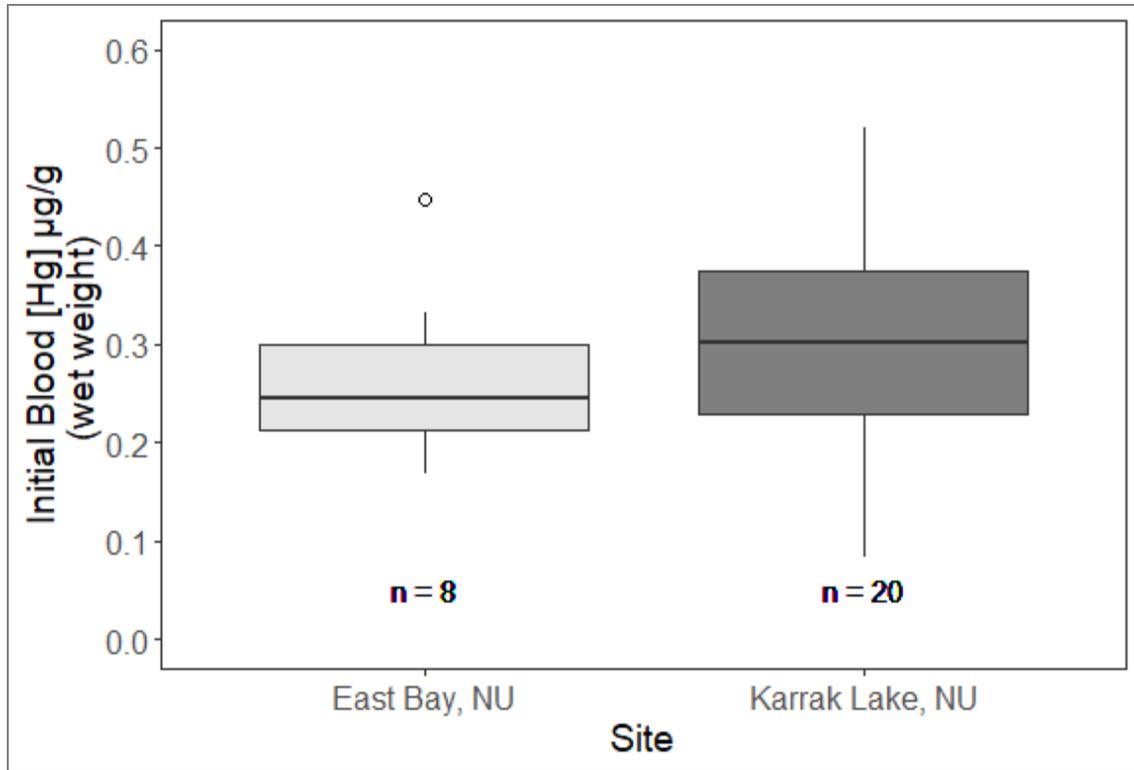


Figure E.3: Mercury concentrations found in initial blood samples of Semipalmated Plovers collected from two locations in the Canadian Arctic in 2017. Mean values denoted by midline in boxplot, first and third quartiles denoted by boxes. Line represent standard deviation; outliers are denoted by empty circles. Blood was collected from nesting Semipalmated Plover pairs at Karrak Lake NU and East Bay NU, sexes have been pooled. Total mercury levels in plover blood are not significantly different between sites ($p = 0.41$).

APPENDIX F: MODEL SELECTION PROCESS INCLUDING STABLE ISOTOPES AS
VARIABLES INFLUENCING MERCURY CONCENTRATIONS IN SHOREBIRD BLOOD

Table F.9: Candidate model set of general linear models estimating initial blood mercury concentrations (Hg_{Blood}) in two shorebird species at two sites in the Canadian Arctic. The interactive effect of species and sex differences account for 72% of model weight (SEPL<SESA, $\beta_{\text{SESA}} = 0.26 \pm 0.08$ SE). Isotopic profiles of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ influencing mercury concentration was not well supported in this analysis.

Model	Df	Log-Likelihood	Deviance	AIC _c	Δ_c	Weight
$Hg_{\text{Bld}} \sim \text{Species} * \text{Sex}$	5	12.63	1.40	-13.63	0.00	0.72
$Hg_{\text{Bld}} \sim \text{Species} + \text{Sex}$	4	9.36	1.63	-9.68	3.96	0.10
$Hg_{\text{Bld}} \sim \text{Species} * \text{Sex} + \text{Site} + \delta^{15}\text{N}$	7	12.69	1.40	-8.18	5.46	0.05
$Hg_{\text{Bld}} \sim \text{Species} * \text{Sex} + \text{Site} + \delta^{13}\text{C}$	7	12.67	1.40	-8.13	5.50	0.05
$Hg_{\text{Bld}} \sim \text{Species} + \text{Sex} + \delta^{13}\text{C}$	5	9.40	1.63	-7.19	6.45	0.03
$Hg_{\text{Bld}} \sim \text{Species} + \text{Sex} + \delta^{15}\text{N}$	5	9.37	1.63	-7.12	6.51	0.03
$Hg_{\text{Bld}} \sim \text{Species} * \text{Sex} + \text{Site} + \delta^{13}\text{C} + \delta^{15}\text{N}$	8	12.69	1.40	-5.14	8.49	0.01
$Hg_{\text{Bld}} \sim \text{Species} + \text{Sex} + \text{Site} + \delta^{13}\text{C}$	6	9.45	1.62	-4.56	9.07	0.01
$Hg_{\text{Bld}} \sim \text{Species} + \text{Sex} + \text{Site} + \delta^{15}\text{N}$	6	9.37	1.63	-4.41	9.22	0.01
$Hg_{\text{Bld}} \sim \text{Species} + \text{Sex} + \text{Site} + \delta^{13}\text{C} + \delta^{15}\text{N}$	7	9.45	1.62	-1.70	11.93	0.00
$Hg_{\text{Bld}} \sim \text{Species}$	3	3.58	2.13	-0.55	13.08	0.00
$Hg_{\text{Bld}} \sim \text{Species} + \delta^{15}\text{N}$	4	4.53	2.04	-0.01	13.62	0.00
$Hg_{\text{Bld}} \sim \text{Species} + \delta^{13}\text{C}$	4	4.31	2.06	0.44	14.07	0.00
$Hg_{\text{Bld}} \sim \text{Sex} + \text{Site} + \delta^{15}\text{N}$	5	-2.29	2.80	16.21	29.84	0.00
$Hg_{\text{Bld}} \sim \text{Sex} + \text{Site}$	4	-3.69	2.99	16.43	30.06	0.00
$Hg_{\text{Bld}} \sim \text{Sex}$	3	-5.13	3.20	16.88	30.51	0.00
$Hg_{\text{Bld}} \sim \text{Sex} + \delta^{15}\text{N}$	4	-4.21	3.06	17.48	31.11	0.00
$Hg_{\text{Bld}} \sim \text{Sex} + \text{Site} + \delta^{13}\text{C}$	5	-3.24	2.93	18.11	31.74	0.00
$Hg_{\text{Bld}} \sim \text{Sex} + \delta^{13}\text{C}$	4	-4.68	3.13	18.42	32.05	0.00
$Hg_{\text{Bld}} \sim \text{Site}$	3	-6.69	3.44	20.00	33.63	0.00
$Hg_{\text{Bld}} \sim \delta^{13}\text{C}$	3	-7.04	3.49	20.70	34.33	0.00
$Hg_{\text{Bld}} \sim 1$	2	-8.29	3.70	20.88	34.52	0.00
$Hg_{\text{Bld}} \sim \delta^{13}\text{C} + \delta^{15}\text{N}$	4	-6.75	3.45	22.55	36.18	0.00
$Hg_{\text{Bld}} \sim \delta^{15}\text{N}$	3	-8.27	3.70	23.15	36.78	0.00

Table F.10: Candidate model set of generalized linear models estimating blood mercury concentrations through time in two shorebird species nesting at Karrak Lake. Data was blocked by bird (ID) to account for individual differences. The effect of species differences accounts for 33% of model weight (SEPL<SESA, $\beta_{SESA} = 0.42 \pm 0.19$ SE). However, Δ_c of the top three models did not exceed 2 and indicates weak support. Isotopic profiles of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ influencing mercury concentration were not well supported in this analysis.

Model	Df	Log-Likelihood	Deviance	AIC _c	Δ_c	Weight
Hg _{Bld} ~ Species, (1 ID)	4	-14.70	24.78	38.53	0.00	0.33
Hg _{Bld} ~ 1, (1 ID)	3	-16.24	29.59	39.14	0.61	0.25
Hg _{Bld} ~ Sex + Species, (1 ID)	5	-13.99	21.26	39.74	1.21	0.18
Hg _{Bld} ~ Sex, (1 ID)	4	-15.72	26.87	40.59	2.06	0.12
Hg _{Bld} ~ $\delta^{15}\text{N}$, (1 ID)	4	-16.86	27.22	42.85	4.32	0.04
Hg _{Bld} ~ $\delta^{13}\text{C}$, (1 ID)	4	-17.12	26.67	43.39	4.86	0.03
Hg _{Bld} ~ Sex + Species + $\delta^{15}\text{N}$, (1 ID)	6	-14.44	18.61	43.42	4.89	0.03
Hg _{Bld} ~ Sex + Species + $\delta^{13}\text{C}$, (1 ID)	6	-15.10	18.65	44.75	6.22	0.01
Hg _{Bld} ~ tMELT + Species, (1 ID)	5	-18.67	23.74	49.11	10.57	0.00
Hg _{Bld} ~ tMELT, (1 ID)	4	-20.13	28.37	49.40	10.87	0.00
Hg _{Bld} ~ tMELT + Sex, (1 ID)	5	-19.66	25.73	51.07	12.54	0.00
Hg _{Bld} ~ tMELT + $\delta^{15}\text{N}$, (1 ID)	5	-21.12	27.16	54.00	15.47	0.00
Hg _{Bld} ~ tMELT + $\delta^{13}\text{C}$, (1 ID)	5	-21.42	26.62	54.61	16.08	0.00
Hg _{Bld} ~ tMELT + Sex + Species + $\delta^{15}\text{N}$, (1 ID)	7	-18.73	18.57	54.96	16.42	0.00
Hg _{Bld} ~ tMELT + Sex + Species + $\delta^{13}\text{C}$ (1 ID)	7	-19.43	18.60	56.37	17.84	0.00
Hg _{Bld} ~ tMELT + $\delta^{13}\text{C}$ + $\delta^{15}\text{N}$	6	-22.74	26.27	60.02	21.48	0.00
Hg _{Bld} ~ tMELT + Sex + Species + $\delta^{13}\text{C}$ + $\delta^{15}\text{N}$, (1 ID)	8	-20.49	17.73	61.62	23.08	0.00