Investigating the impact of helminths on mercury in Arctic foxes

by

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Abstract

Mercury (Hg) pollution in the Arctic poses a growing threat to the health of local wildlife, yet many factors behind Hg bioaccumulation remain poorly understood. Primarily ingested through diet, Hg bioaccumulates in animal tissues and can impair neurological and reproductive functions. Intestinal helminths have demonstrated the ability to take up Hg while residing in their hosts and have consequently been suggested to benefit their hosts through mitigating toxin exposure. In this study, I used Arctic foxes harvested over five years from Churchill, MB to analyze fox muscle total mercury concentrations ([THg]) (ng/g), intestinal helminth [THg], and helminth biomasses to determine if (i) helminth group (taxa and size) influences helminth Hg uptake, and (ii) helminths benefit their host by decreasing host Hg uptake. Results showed that [THg] did not significantly vary with fox sex, age, or harvest year. Cestodes demonstrated higher [THg] than nematodes, though size did not influence [THg] for either taxon. Both cestodes and nematodes demonstrated [THg] increases relative to host [THg], though only the cestodes had significantly higher [THg] than their hosts, and no significant effect was seen for either cestode or nematode biomass on fox [THg]. Overall, this study provides valuable insight into host-helminth-Hg dynamics. Further research is needed to elucidate the mechanisms and significance of helminth Hg uptake to help understand their potential applications in mitigating toxin exposure in wildlife.

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Introduction

Since industrialization began, mercury (Hg) levels in several Arctic animals have increased (Dietz et al. 2009). Though primarily emitted at mid-latitudes, long-range atmospheric transport carries, concentrates and deposits global atmospheric Hg in northern environments (Obrist et al. 2017). While natural processes such as volcanic eruptions and forest fires release some Hg, 30% of atmospheric Hg emissions are from anthropogenic processes, including the burning of fossil fuels and municipal waste (Clarkson and Magos 2006; Kumar and Wu 2019; Obrist et al. 2017). Approximately 243 tons of Hg are deposited from the atmosphere to Arctic environments each year (AMAP 2021), with over 92% of Hg in higher trophic level Arctic species resulting from anthropogenic Hg emissions alone (Dietz et al. 2009). However, these numbers can be expected to increase with rising global temperatures. For instance, while more frequent forest fires around the globe enhance atmospheric Hg emissions, substantial amounts of permafrost-locked Hg will proceed to thaw and be remobilized in Arctic ecosystems (Schuster et al. 2018). Since Hg is a toxic, non-essential element widely recognized for its negative effects on animal health, current and future increases in Hg in the Arctic raise concerns for the health of local wildlife.

Among the diverse forms of mercury, methylmercury (MeHg) has the highest toxic potential due to its unique ability to cross blood-brain and blood-placental barriers (Clarkson and Magos 2006; McCabe et al. 2005). These characteristics allow MeHg to devastate the central nervous system by causing structural degeneration, as well as impair and inhibit fetal brain development (Clarkson and Magos 2006). Furthermore, following consumption, 95% of MeHg remains in organisms due to its lipophilicity and affinity for proteins (AMAP 1998; Kirk et al. 2012). MeHg then proceeds to amass in individuals as they age (bioaccumulation) and in orders of magnitude up trophic levels (biomagnification), reaching the highest levels in apex predators (AMAP 1998; Kirk et al. 2012). Longer food chains, as often occur in aquatic ecosystems, have amplified biomagnification, burdening consumers of marine resources with greater Hg (Dietz et al. 2000; Pacyna et al. 2018). Past reports have found several Arctic marine predators to contain MeHg concentrations exceeding thresholds for biological effects (Dietz et al. 2013) and have observed neurochemical changes in wild fish-eating species from MeHg exposure (Basu et al. 2006, 2005).

In terrestrial ecosystems, lemmings (*Dicrostonyx* and *Lemmus*) are an important resource for many carnivores and serve as a primary prey for Arctic foxes (*Vulpes lagopus*). However, lemming populations undergo 3- to 4-year cycles (Krebs 2011), which are becoming damped in some locations (Ehrich et al. 2020). In response to periods of low lemming numbers, species such as Arctic foxes rely more heavily on scavenged marine resources, exposing them to high levels of MeHg (Dudenhoeffer et al. 2021). As climate-change-induced alterations in snowpack can lead to reduced winter survival of lemmings (Kausrud et al. 2008), further lemming cycle damping and declines may occur in coming years. These declines could force Arctic foxes to have increased reliance on marine resources, exposing them to similar concerning levels of MeHg as those seen in other Arctic marine predators (Dietz et al. 2013).

Aside from diet, bioaccumulation may be affected by organism size, sex, physiology, and physical maladies (e.g. Bocharova et al. 2013; Dobrzański et al. 2014; Gamberg et al. 2020; Hallanger et al. 2019). However, factors such as helminths have historically been overlooked in contaminant studies despite their recognized importance in food webs and their ability to influence amounts of essential and non-essential elements in a host (Lafferty et al. 2008; Brožová et al. 2015). Intestinal helminths are primarily acquired through food and occupy gastrointestinal tracts where they may take up nutrients in the host gut contents (Coop and Kyriazakis 1999). Although this helminth behaviour has traditionally been deemed as detrimental to vertebrate health, hosts may benefit from their interaction with parasites when pollutants are present. In intestines, many species of helminths consume/absorb intestinal contents, exposing them to metal pollutants that are consumed by the host (McGrew et al. 2018) and excreted to the intestine in bile as a detoxification attempt of mammals (Jankovská et al. 2008). This exposure may allow helminths to pre-emptively bioaccumulate Hg, providing a protective advantage to their hosts (Sures and Siddall 1999; Sures et al. 2003; Brožová et al. 2015). In fact, over 50 parasite species have been shown to take up toxins and have been suggested as sentinels for metal pollution in hosts (Sures 2017).

Large variations in morphology and resource acquisition between helminth taxa may lead to differences in metal pollutant uptake. Of the four major taxa of helminths, only nematodes and trematodes consume and digest resources using gastro-intestinal tracts complete with a mouth, intestine, and anus (Roberts and Janovy 2009). Acanthocephalans and cestodes instead absorb nutrients across their tegument (Roberts and Janovy 2009), potentially increasing their risk of contaminant uptake. Further, of observed parasites, cestodes and acanthocephalans have reported the highest levels of toxin concentrations compared to hosts (Sures 2017). Helminths also demonstrate large variations in size within and between taxa. Size variation may further influence the ability of helminths to take up Hg, especially for taxa that absorb nutrients across their body surface, due to larger helminths having decreased surface area compared to their mass (Read 2000).

A handful of studies have investigated the effects of helminths on the toxic metal concentrations of hosts. Fish infected with cestodes and acanthocephalans have demonstrated lower levels of toxic metals than their uninfected counterparts (Hassanine and Al-Hasawi 2021; Sures and Siddal 1999). Similarly, mammals infected with cestodes, nematodes, and acanthocephalans have been observed to have lower toxic metal concentrations than uninfected individuals (Brožová et al. 2015; Jankovská et al. 2008; Jankovská et al. 2010; Sloup et al. 2018). Some authors have attributed the decreased Hg in parasitized individuals to parasitism-induced changes in the host's behaviour and physiology that alter feeding and metabolism (Bergey et al. 2002; Martin et al. 2011). Yet, the observed variation in bioaccumulation between helminth and host suggests helminths may actively reduce heavy metal availability in the intestine. Intestinal cestodes and acanthocephalans of various fish species have demonstrated the ability to bioaccumulate toxic metals to higher degrees than their host (Brázová et al. 2015; Sures et al. 1994; Sures and Taraschewski 1995). Similar results have been seen for intestinal cestodes and nematodes of red foxes (*Vulpes vulpes*) (Binkowski et al. 2016; Jankovská et al. 2010), cestodes of wood mice (*Apodemus sylvaticus*) (Torres et al. 2006), and nematodes of double-crested cormorants (*Phalacrocorax auritus*) (Robinson et al. 2010). However, studies observing host-helminth relationships in the context of Hg remain scarce, though cestodes, acanthocephalans, and nematodes have all been shown to take up Hg and affect Hg bioavailability in certain wild mammals (McGrew et al. 2015, 2018).

As climate change continues to alter the availability of Hg to Arctic wildlife, investigating potential bioaccumulation factors such as helminths is of increased importance. In Arctic foxes, intestinal cestode and nematode infections are common. Prevalent cestodes in Arctic foxes include *Echinococcus multilocularis*, *Taenia crassiceps,* and *Taenia polyacantha arctica* (Friesen et al. 2015). All of these cestodes absorb intestinal contents and are obtained by foxes through consumption of intermediate hosts (e.g. lemmings and other rodents) that have ingested food contaminated with cestode eggs from carnivore feces (Loos Frank 2000; Zajac and Conboy 2012). In contrast to *Taenia* spp., adult *Echinococcus* spp. are characteristically small, with *E. multilocularis,* for example, not exceeding 0.5cm (Roberts and Janovy 2009). Ascarid nematodes, particularly *Toxascaris leonina,* are frequently observed in Arctic fox intestines as well (Eaton and Secord 1979; Elmore et al. 2013). These nematodes also tend to feed on intestinal contents and may be contracted as larvae through consumption of paratenic rodent hosts or as eggs with the infective 3rd larval stage through exposure to areas and food contaminated by infected feces (Okulewicz et al. 2012). Since Arctic foxes spend the first 3-4 weeks of their lives confined to dens hosting litters of up to 25 pups (Audet et al. 2002), there may be a high risk for nematode transmission between individuals. In addition to cestode and nematode infections, Arctic foxes are exposed to Hg through the aforementioned supplemental scavenging of marine resources (Hallanger et al. 2019; Roth 2002). Combined, helminth abundance and Hg exposure make Arctic foxes an ideal candidate for host-helminth-Hg studies. By deepening our understanding of helminth impacts on host Hg concentrations, we will be able to better predict how Arctic marine-feeding animals will respond to future climate-change-induced changes in Hg contamination.

Objectives and Hypotheses

I examined whether intestinal helminths impact total mercury concentrations (hereafter [THg]) in Arctic fox hosts. Thus, I analyzed relationships between [THg] in helminths, [THg] in fox muscle tissue, and helminth group biomass. I hypothesized that (i) helminth morphology (taxa and size) influences helminth Hg uptake and (ii) helminths influence host Hg uptake. With my first hypothesis, I predicted that (i) helminths without digestive tracts would have higher

[THg] (ng/g) than those with digestive tracts, and (ii) for helminths that take up resources across their body surface only, [THg] (ng/g) would be lower for larger individuals due to less surface area compared to their body mass. With my second hypothesis, I predicted that (i) helminth [THg] would increase with host [THg], (ii) helminths would have higher [THg] than host tissue, and (iii) host [THg] would decrease with increasing helminth abundance.

Methods

Study design

I used carcasses of 132 Arctic foxes legally harvested during trapping seasons (November – March) in 2017, 2018, 2019, 2021, 2022, and 2024 from the Churchill registered trapline district on the west coast of Hudson Bay (58°N, 94°W) in Manitoba, Canada. All foxes were tested for mercury, while a subsample of 30 foxes was used to collect parasite data. The Churchill area is a transitional zone between the marine environment, tundra, and boreal forest, providing access to both terrestrial and marine resources. Arctic fox diets in this region consist of collared lemmings (*Dicrostonyx richardsonii*), supplemented by seasonal resources including lesser snow (*Chen caerulescens*) and Canada geese (*Branta canadensis*), many shorebird species, and ringed and harbour seals (*Pusa hispida* and *Phoca vitulina*) (Dudenhoeffer et al. 2021; Roth 2002, 2003). Dietary variation in Arctic foxes in this area affects both their helminth loads and [THg] (Friesen et al. 2015; Warret Rodrigues 2022).

Initial tissue harvesting

For each year, Arctic fox carcasses (with pelts removed) were collected from fur trappers by Churchill Northern Studies Centre staff and stored frozen on-site for up to four months before tissue sampling in June and July of the respective year. After thawing the carcass, we identified and recorded sex, since sex differences in diet and mercury concentrations have been seen in Arctic foxes (Dobrzański et al. 2014; Friesen et al. 2015). We collected muscle samples from the same location of the quadriceps for each fox. Samples were collected in microcentrifuge tubes, avoiding the top 2-4mm to exclude external contamination that may have occurred during skinning and transport (Warret Rodrigues et al. 2023). Muscle tissue [THg] is strongly related to the [THg] of other organs commonly used in mercury studies (e.g. brain, liver, kidney) (Warret Rodrigues et al. 2024). As it was the most consistently available tissue from foxes harvested in previous years, I used muscle tissue to indicate fox internal [THg].

To collect the intestine, we made incisions along the fox's ventral surface and removed the entire small and large intestines (pyloric sphincter to anus). Intestines were not collected in 2021. We also removed the lower jaw of each fox for later use of teeth in aging. To avoid cross-contamination between individuals, all samples were sealed and labeled immediately following removal and instruments were disinfected using 95% ethanol between foxes. All samples were transported to the University of Manitoba and stored at -20°C. Intestines were initially stored at -80ºC for 2-4 weeks to kill parasites before being moved to -20°C freezers.

Age determination

We used the relative size of the pulp cavity of a canine tooth from each fox to determine the age class (juvenile or adult). To extract the teeth, we cooked the jaws in hot water overnight to loosen the teeth, then carefully removed canine teeth using dental tools and x-rayed them using standard radiography techniques. Using ImageJ, we measured the ratio of the pulp cavity to tooth width at the widest point of the tooth (Schneider et al. 2012). When the ratio was <41%, foxes were considered adults (>1 year old), and when the ratio was >41%, foxes were considered juveniles (<1 year old) (Warret Rodrigues 2022).

Fox [THg] determination

Since nearly all Hg in muscle tissue is in the form of MeHg (Campbell et al. 2005), I measured the total mercury in tissues as it is less expensive and complex than measuring MeHg alone. To account for frozen samples losing moisture over time (Warret Rodrigues et al. 2023), I analyzed all tissues as dry weights. I freeze-dried muscle tissue samples for 48 hours at -50ºC, then homogenized them using a mortar and pestle.

I weighed out subsamples of approximately 0.01-0.03g and measured [THg] using a Hydra IIc (Teledyne Leeman Laboratories, Hudson, NH) mercury analyzer at the Centre for Earth Observation Science, University of Manitoba. The Hydra IIc uses thermal decomposition to directly analyze total mercury concentrations in solid samples by pyrolyzing all forms of mercury into elemental mercury, amalgamating it, and then detecting it using atomic absorption spectroscopy (Warret Rodrigues et al. 2023). This machine has a detection range from 0.001 - 1500ng Hg (Teledyne Leeman Labs 2015). I generated low and high linear calibration curves of a minimum of five determination points each, which were validated when $R^2>0.995$. My low-detection linear calibration curve was generated using blank sample boats (blank correction) and the certified reference material MESS-4 with 95% confidence (90 ± 40 ng/g). My high-detection linear calibration curve was generated using the certified reference material PACS-3 with 95% confidence (2980 ± 360 ng/g). While running muscle samples, I ran certified reference materials (including MESS-4, TORT-3 (292 ± 12 ng/g), DORM-4 (412 ± 36 ng/g), DOLT-5 (440±180 ng/g), NIST2709a (900 ± 200 ng/g), and PACS-3) 2-3 times every 14 samples to maintain quality control and quality assurance (Warret Rodrigues et al. 2023).

Sample selection

Of the foxes with intestines available, I used data on [THg] and age to select individuals for further analyses. Arctic fox harvest is highly juvenile-biased (Friesen et al. 2015), so I included all adult foxes, which would have had greater time for Hg accumulation. I also selected all foxes with exceptionally high [THg] (>3500 ng/g dry weight) to maximize the range of [THg] within my sample, as only four foxes fit this criterion. For each year, I grouped the remaining foxes into equal-width low, medium, and high [THg] bins based on the highest fox [THg] values that year. To employ a stratified random sampling approach, I selected up to three foxes from each bin based on availability to obtain a total sample group of 30 foxes. This procedure ensured that my sample group represented foxes with different ages, [THg], and harvest years.

Helminth identification and quantification

To extract helminths, I thawed the intestines of each selected fox and placed them in a glass dish containing deionized water. I split the intestines longitudinally, extracted large helminths using forceps, and then scraped and rinsed out all contents from the intestinal wall using deionized water. I added intestinal contents to a glass beaker and diluted them to 1000 mL with deionized water. To isolate helminths from the intestinal contents, I performed a sedimentation technique by decanting approximately 5 mL of the solution at a time and observing it under a stereoscope (Eckert 2003; Friesen et al. 2015). Upon detection, I removed helminths and placed them temporarily in deionized water.

Only cestode and nematode taxa were observed in foxes. I differentiated between cestodes and nematodes using differences in morphology (Roberts and Janovy 2009). Due to the degradation and damage of many crucial identification structures of the helminths from freezing and extraction, further morphological classification was not attempted. I split each taxon into small and large size categories: small nematodes (≤5cm), large nematodes (>5cm), small cestodes (≤0.5cm), and large cestodes (>0.5cm). For cestodes, this size threshold was chosen in an attempt to separate *Echinococcus* spp. (adults <0.5cm) from *Taenia* spp. (adults >0.5cm) (Roberts and Janovy 2009). The nematode size threshold was chosen based on observed nematode lengths to split individuals into two groups. I added the individuals of each group to vials containing 95% ethanol (Friesen et al. 2015) and stored the vials at -20˚C for up to eight weeks.

To determine the dry biomass of each helminth group, I placed helminths from each vial into pre-weighed aluminum foil dishes at room temperature (approximately 20˚C) for 48-72 hours to allow all water and ethanol to evaporate. I weighed dried samples using a microbalance and confirmed complete evaporation when consistent weights (± 0.01mg) were obtained for samples weighed eight hours apart.

Determination of helminth [THg]

I analyzed [THg] in helminths in the same manner as muscle samples using the Hydra IIc. For helminth groups >0.10g, I cut all helminths into pieces <1mm in length and width using surgical scissors and mixed them to homogenize the sample, then analyzed subsamples of 0.01-0.03g, depending on availability. Since slight [THg] variation may have existed between individuals in the same group, homogenization ensured subsamples taken from a helminth group represented the average [THg] of the group (McGrew et al. 2015). For helminth groups <0.01g, I analyzed all available helminth tissue to maximize the likelihood of samples containing sufficient Hg (ng) for detection by Hydra IIc (detection limit of 0.005 – 1000 ng Hg).

Statistical analyses

I performed statistical analyses using R software v.4.2.2 (R Core Team 2022) loaded with the 'tidyverse' package for data wrangling, and all graphs were created using the 'ggplot2' package. I used a significance level of 0.05 for all statistical analyses. I normalized fox [THg] data using a log transformation. I performed a general linear model to test for an effect of age (adults: >1 year vs. juveniles: <1 year), sex (male, female), and year of harvest (2017, 2018, 2019, 2021, 2022, 2024) on log-transformed fox [THg]. I confirmed that my model met the assumptions of linearity, normality, independence, and homoscedasticity by generating and analyzing a scatterplot of model residuals against predicted log fox [THg] values, and a histogram of model residuals. Slight variations from normality and homoscedasticity were accepted due to the robustness of linear models. To determine if helminth [THg] varied with taxon or size, I generated a general linear mixed effects model using the R package 'lme4'. Since helminth [THg] results demonstrated inaccurate [THg] determination by the Hydra IIc at very small sample sizes (potentially due to insufficient Hg for detection in small samples), I excluded [THg] data from any helminth samples <0.0001g to maintain confidence in [THg] readings. Since Hydra IIc also tends to report samples with very low to no mercury as negative [THg], I corrected any remaining negative [THg] values to 0 ng/g. I then square-root-transformed helminth [THg] with sqrt(x+0.5) to achieve normality and homoscedasticity. I generated my linear mixed effects model with square-root transformed helminth [THg] as my response variable and helminth group (small cestode, large cestode, small nematode, large nematode) and fox [THg], including their interaction, as my predictor variables, controlling for a random effect of fox ID. I confirmed that my model met appropriate assumptions with a graphical analysis of the residuals. To determine which helminth groups differed in [THg], I performed pairwise comparisons on model results using the 'emmeans' package, with p-values adjusted for multiple comparisons using the Tukey adjustment. I determined the marginal and conditional R^2 of the model using the 'MuMIn' package.

To test if helminth [THg] increased with fox [THg], I used the previous linear mixed effects model for square-root transformed helminth [THg] but changed the independent variable of "helminth group" to "taxon" (cestode, nematode) to eliminate the variable of size. To determine if helminth and fox [THg] differed, I performed Wilcoxon signed-rank tests comparing fox [THg] to cestode [THg] and nematode [THg] separately. To analyze if helminth biomass impacted fox [THg], I ran a general linear model with log-transformed fox [THg] data as the response variable and biomass of cestodes (mg) and nematodes (mg) as predictor variables. I checked for outliers using a Cook's distance plot and removed data for two foxes with Cook's distance >0.5. I then confirmed the assumptions of the model by graphical analysis of residuals.

Results

Helminth biomass and [THg]

All foxes contained both cestodes and nematodes, though eight foxes only had three of four helminth groups and one fox contained just two of the helminth groups (small cestode and large nematode) (Table 1). The helminth biomass between foxes was highly variable (Table 1).

Small cestode biomass was <0.001g in five foxes, so these cestode groups were excluded from further helminth [THg] data analysis.

Table 1. Data for four helminth categories (large cestodes, small cestodes, large nematodes, and small nematodes) extracted from 30 Arctic foxes harvested from Churchill, MB in 2017, 2018, 2019, 2022 and 2024. All helminth biomasses (mg) were measured as dry weights. Maximum (max) and minimum (min) biomasses indicate total amounts of that category extracted from a single fox.

Helminth Type	Foxes with category present	Min biomass (mg)	Max biomass (mg)	Mean biomass (mg) ± SE	Median biomass (mg)
Large Cestode	27	0.11	2143.05	124.70 ± 71.33	11.42
Small Cestode	26	0.04	12.63	1.78 ± 0.48	0.67
Large Nematode	28	23.83	1145.61	174.71 ± 39.65	41.85
Small Nematode	29	0.18	227.30	50.05 ± 9.62	40.98

Following [THg] analysis, four small cestodes had negative [THg] values despite having sufficient biomass for Hg detection. These were corrected to 0 ng/g. Helminth [THg] differed significantly with group (GLM, $F_{3, 73.9} = 3.78$, $p<0.014$). Post-hoc pairwise comparisons of helminth groups indicated that [THg] was higher in large cestodes than large nematodes (emmeans Tukey-adjusted, $p<0.0001$) and small nematodes ($p<0.0001$), and was also higher in small cestodes than large nematodes ($p=0.016$) and small nematodes ($p=0.0034$), while no difference in [THg] occurred between large and small cestodes ($p=0.46$) or between large and small nematodes ($p=0.95$). These results indicated that cestodes had higher [THg] than nematodes, but within taxa, size did not have an effect (Figure 1). Since helminth size did not affect [THg], I combined small and large helminth biomasses for cestodes and nematodes in subsequent analyses.

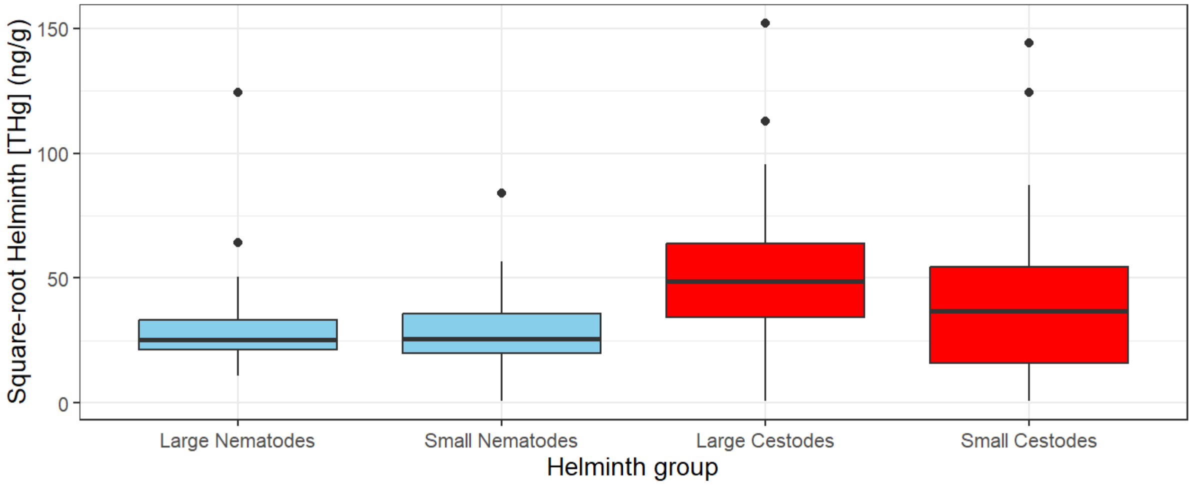

Figure 1. Total mercury concentrations ([THg]) (square-root transformed) for four helminth groups obtained from intestines of 30 Arctic foxes harvested from Churchill, MB in 2017, 2018, 2019, 2022, and 2024. Helminth groups were large cestodes (>0.5cm), small cestodes (<0.5cm), large nematodes (>5cm), and small nematodes (<5cm).

Fox [THg] and the impact of helminth biomass

All fox muscle tissue analyzed had detectable levels of Hg, with concentrations ranging from 40.6-6987.3 ng/g (Table 2). The average ± SE [THg] over all years was 589.6 ± 79.4 ng/g and the median was 334.4 ng/g. Overall, age, sex, and harvest year explained 2.1% of the variation in fox [THg] ($R^2 = 0.021$), but the model was not significant (GLM, $F_{3,126} = 1.03$, $p = 0.38$). Despite most of the foxes with [THg] >3500 ng/g being harvested in 2017 (four out of five), there was no significant effect of year on fox [THg] (GLM, $F_{1,128} = 1.93$, $p=0.17$) (Figure 2). Fox [THg] also did not vary with sex (GLM, $F_{1,128}= 0.41$, $p_{sex}=0.53$) or age class (GLM, $F_{1,128}= 0.35$, $p_{age} = 0.56$) (Figure 3).

Table 2. Age, sex, and total mercury concentration ([THg]) for Arctic foxes harvested near Churchill, MB (n=132).

Year	Total foxes	Male	Female	Adult	Juvenile	Min. [THg]	Max. [THg]	Mean [THg] ± SE	Median [THg]
2017	28	28	0	6	22	40.6	6987.3	953.5 ± 301.2	374.8
2018	46	31	15	7	39	81.2	4150.2	513.0 ± 98.8	321.3
2019	4	3	1	4	0	284.3	458.6	348.8 ± 38.8	326.1
2021	9	8	1	0	9	242.7	3436.1	1005.6 ± 317.8	547.9
2022	43	15	28	2	41	44.1	1832.1	371.2 ± 52.7	243.7
2024	2	1	1	0	2	347.3	770.8	559.0 ± 211.8	559.0
Total	132	86	46	19	113	82.6	6987.3	589.6 ± 79.4	334.4

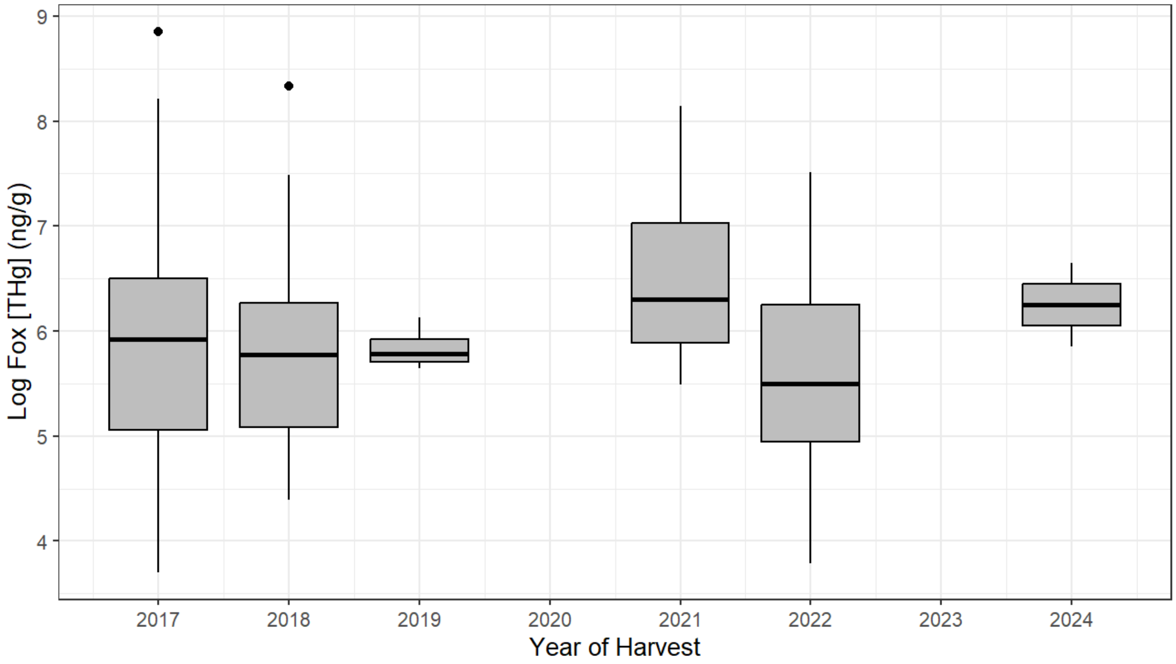

Figure 2. Variation in total mercury concentration ([THg]) (log transformed) in Arctic fox muscle from Churchill, MB in 2017 (n=28), 2018 (n=46), 2019 (n=4), 2021 (n=9), 2022 (n=43), and 2024 (n=2).

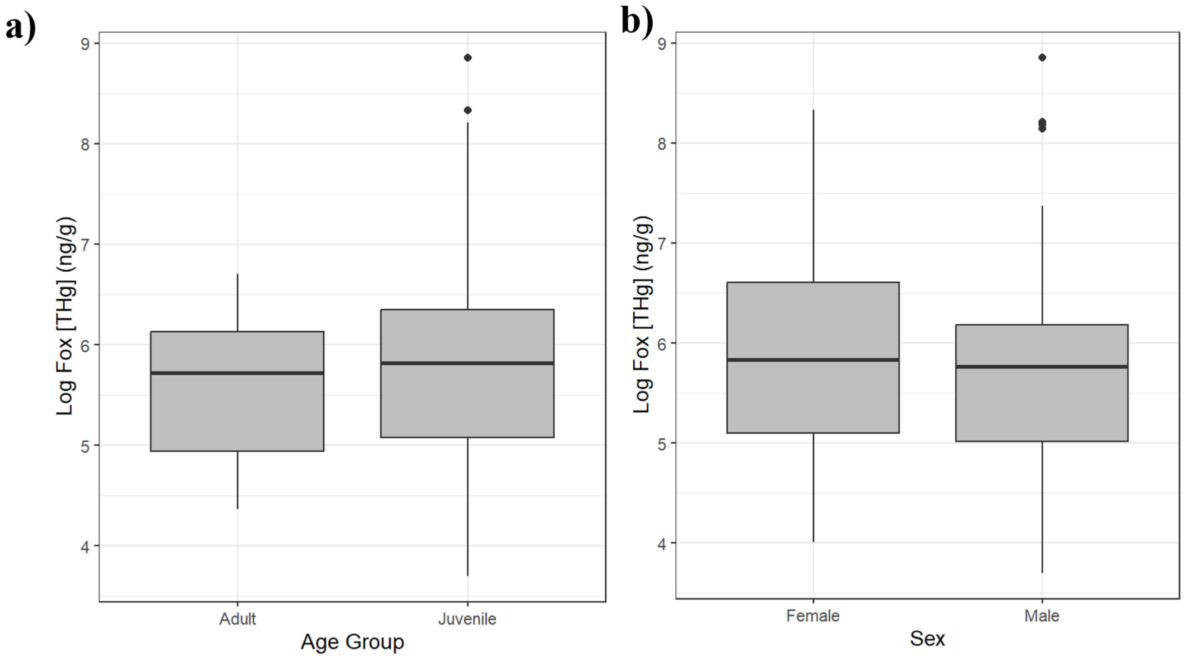

Figure 3. Variation in total mercury concentration ([THg]) (log transformed) in a) adult (n=19) and juvenile (n=113) and b) male (n=86) and female (n=46) Arctic foxes harvested from Churchill, MB in 2017, 2018, 2019, 2021, 2022 and 2024.

Fox [THg] significantly affected helminth [THg] (GLM, $F_{1, 78.2}$= 170.7, p<0.0001), with an interaction between helminth taxon and fox [THg] (GLM, $F_{1, 77.2}$ = 26.1, p<0.0001) (Figure 4). This model explained 76% of the variation in the helminth [THg] (Conditional R^2 = 0.76), with 73% of the variation explained by the helminth group, fox [THg] and the interaction between them (Marginal R^2 = 0.73). Comparisons of helminth and fox tissue [THg] found that cestode tissue had significantly higher [THg] than fox muscle tissue (Wilcoxon signed rank test, V = 66, p<0.0001) (Figure 4). In contrast, nematode and fox tissue did not differ in [THg] (Wilcoxon signed rank test, V = 903, p = 0.55) (Figure 4). The model for the analyses of helminth biomass and fox [THg] was not significant ($F_{2,25}$ = 0.74, p=0.49, R^2 = 0.056) and fox [THg] was not affected by cestode biomass (GLM, $t_{cestode}$=0.43, $p_{cestode}$=0.67) or nematode biomass (GLM, $t_{nematode}$ = -1.11, $p_{nematode}$=0.28) (Figure 5).

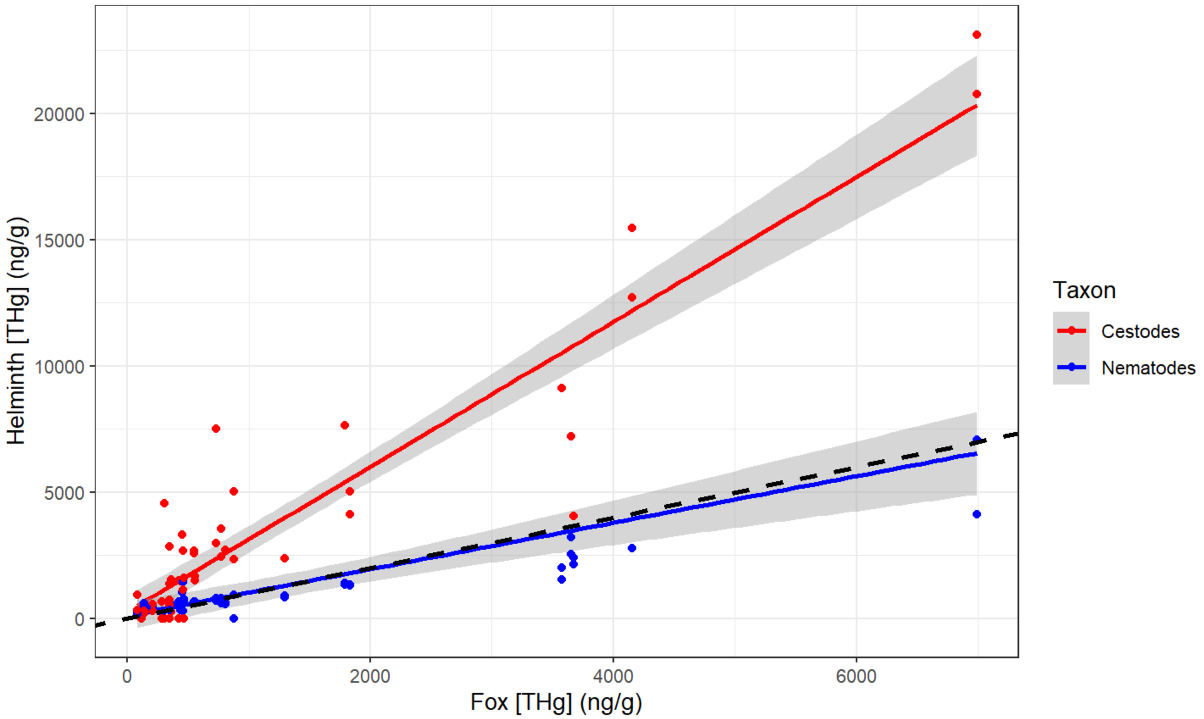

Figure 4. The overall relationship between helminth total mercury concentration ([THg]) (ng/g) and fox [THg] (ng/g) for cestodes (red) and nematodes (blue) collected from Arctic fox intestines (n=28) near Churchill, MB. 95% confidence intervals are shown in grey. The relationship that would indicate no difference between fox and helminth [THg] (one to one ratio) is indicated as a black dashed line.

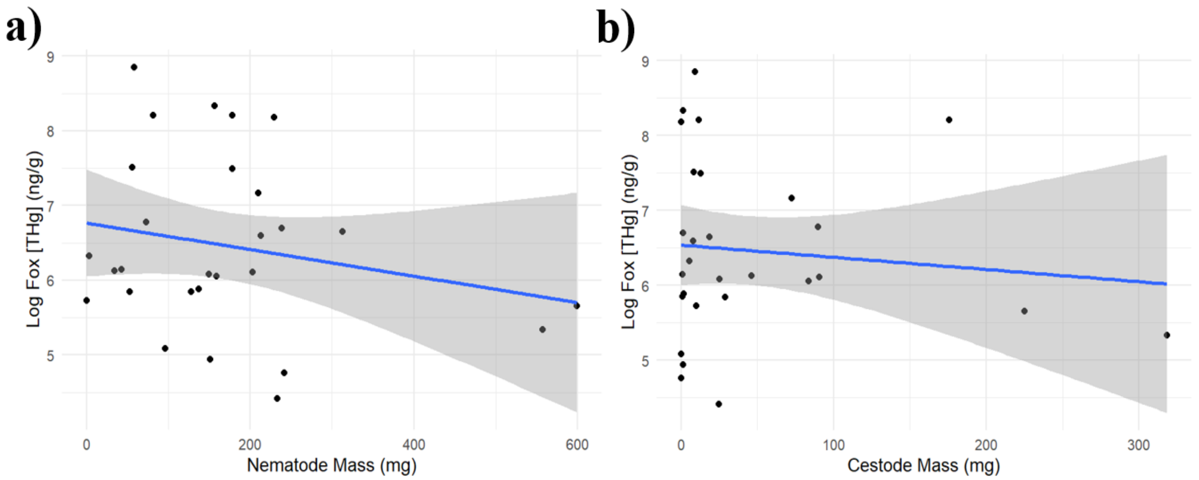

Figure 5. Relationships between total mercury concentration ([THg]) (log transformed) in Arctic fox muscle and biomass of a) nematodes and b) cestodes (mg dry weight) extracted from fox intestines (n=28).

Discussion

This study demonstrated that Arctic fox helminths can accumulate Hg in their tissues to varying extents. Though it is possible that minimal Hg was obtained by helminths as larvae before entering foxes, the positive relationship observed between helminth [THg] and fox host [THg] is consistent with most or all helminth Hg being host-sourced (Figure 4). Despite this result, helminth biomass did not affect host [THg] (Figure 5). Further, helminths in this study demonstrated taxonomic differences in [THg]. Cestodes demonstrated higher [THg] than both nematodes and Arctic fox muscle tissue (Figure 1, 4), as well as displayed a relationship with a steeper slope to fox muscle [THg] than nematodes (Figure 4).

All Arctic foxes used in this study were confirmed to have detectable levels of Hg, as anticipated, with muscle [THg] ranging from 40.6 - 6987.3 ng/g with a standard error of 79.4 ng/g. As previous studies have found a positive relationship between the amount of marine resources consumed and mercury concentrations in Arctic fox tissues (Hallanger et al. 2019), the variation in [THg] of foxes in this study likely resulted from different extents of marine resource use. Marine resource consumption by foxes has further been observed to vary based on yearly variations in lemming abundance (Roth, 2002, 2003; Tarroux et al. 2012; Dudenhoeffer et al. 2021), but the year of harvest did not affect [THg] of Arctic foxes in this study. In Churchill, lemming populations peaked in 2017 (Warret Rodrigues 2022) and would be expected to decline throughout 2018 and 2019 due to their 3- to 4-year population cycles (Krebs 2011). Based on these lemming trends, consumption of marine resources (and Hg) would be expected to be lower in 2017 than in the following two years, which should be reflected in fox [THg]. However, these patterns were not seen in fox [THg]. Damped lemming cycles in Churchill have been suggested (Ehrich et al. 2020; Warret Rodrigues 2023) and may have contributed to the lack of patterns in yearly fox [THg] by increasing Arctic fox reliance on marine resources even in peak lemming years, consequently decreasing yearly [THg] fluctuations.

Arctic fox [THg] in this study was not impacted by sex or age. Though lack of sex differences was consistent with several other studies (Bocharova et al. 2013; Hallanger et al. 2019; Treu et al. 2018), Warret Rodrigues (2022) observed female Arctic foxes in the Churchill area to have higher [THg] than males. Though it is possible that sex differences in [THg] resulted from differences in genetic and metabolic activity (Dobrzański et al. 2014), variation in diet may have played a role, as female Arctic foxes in this region have been suggested to consume fewer rodents than males (Friesen et al. 2015). Females may, therefore, have higher Hg exposure through heavier marine resource use. The lack of difference in [THg] between adult and juvenile foxes was unexpected due to the bioaccumulative nature of Hg and previous observations of age variations in Churchill Arctic foxes (Warret Rodrigues 2022). However, a lack of relationship between age and mercury concentrations in Arctic foxes is not unheard of (Hallanger et al. 2019; Treu et al. 2018). As previously described, the diets of Arctic foxes are heavily influenced by lemming cycles, and it is possible that inconsistent observations of age differences in [THg] in past studies were, at least in part, due to changing lemming densities and behavioural differences between adults and juveniles (Warret Rodrigues 2022). Warret Rodrigues (2022) suggested that in years of lemming declines, adult foxes may have increased Hg exposure through marine resource consumption, while in years of lemming increases, adult Hg exposure may decline while pups take up Hg from their mothers through milk and placental transfer (Knott et al. 2012; Wagemann et al.1988; Warret Rodrigues 2022). This maternal transfer may blur differences in [THg] between adult females and juveniles. Maternal Hg transfer to offspring may also serve as an excretory strategy for females, which could further explain the lack of difference I observed between males and females despite the suggested increase in marine resource use by females (Friesen et al. 2015). It is also possible that sex and age differences were not detected due to unbalanced ratios in my study, with male and juvenile foxes being most abundant, and insufficient age ranges (most adults did not exceed one year old). More research is needed to fully understand the interacting factors influencing [THg] across ages and sexes in Arctic foxes.

Consistent with the predictions for my first hypothesis, cestodes had higher [THg] than nematodes within foxes. Similar patterns have been seen for other metals, including lead (Pb), manganese (Mn), copper (Cu), zinc (Zn), and nickel (Ni) in a previous study on red fox helminths (Jankovská et al. 2010). It is possible that these patterns resulted from different feeding mechanisms between the groups. Intestinal cestodes take up nutrients from gut contents through diffusion and active transport across their tegument, though it is unknown if certain cestodes can discriminate the type of food they take in (Persson et al. 2007). In contrast, the external cuticle layer of nematodes may prevent Hg uptake through their body wall, and nematodes may be able to use their mouths to select which resources they consume (Roberts and Janovy 2009). It should be cautioned that though the nematode species in my study were plausibly ascarids that feed on intestinal contents (Eaton and Secord 1979; Elmore et al. 2013; Friesen et al. 2015), species were not identified. As a result, the presence of blood- and tissue-feeding nematodes such as hookworms was possible (despite these species having low prevalence and abundance compared to *T. leonina* (Elmore et al. 2013)) and may have influenced nematode [THg] due to different routes and levels of Hg exposure from alternate diets.

At the level assessed, the size of helminths in this study did not affect cestode or nematode [THg]. This finding was consistent with my prediction for nematodes but inconsistent with my prediction for cestodes. Limits of the mercury analysis may have impacted these results. In five of the 26 foxes containing small cestodes, the total mass of small cestodes was insufficient for accurate [THg] determination, reducing the number of groups available for comparisons between small and large cestodes. The exclusion of these small cestode groups would have particularly impacted my results if these samples contained high [THg]. Crowding effects may have also prevented observations of size variations in cestode [THg]. It is known that the size of cestodes is inversely proportional to the number of cestodes in an infection (Read 2000). Therefore, in foxes with large numbers of cestodes, each cestode would be smaller, and Hg may be diluted between all individuals, whereas foxes with lower intensity infections would contain fewer and larger cestodes, exposing each cestode to higher amounts of Hg. Further, in large infections of small cestodes, increased contact with neighbours could reduce the amount of exposed body surface of individuals, reducing their Hg uptake (Read 2000). Though I could not find other studies observing the effects of helminth size on toxic metal uptake, a previous study attributed low cadmium (Cd), Pb, and Hg concentrations of wild rabbit (*Oryctolagus cuniculus*) cestodes to their large size, which could suggest that larger helminths accumulate metals to lower concentrations (Eira et al. 2005). Further studies should investigate if helminth size affects metal uptake to validate the results seen here.

As predicted with my second hypothesis, helminth [THg] increased with host [THg]. This relationship helped to support the assumption that helminths obtained Hg while occupying fox host tissues. Though both cestode [THg] and nematode [THg] increased with fox [THg], the relationship was stronger for the former. Similar results were seen in a past study where taeniid cestodes demonstrated stronger relationships in [THg] to host liver tissue than ascarid nematodes (McGrew et al. 2015). These observed differences in relationship strengths between helminth taxa may reflect the previously discussed resource acquisition and processing differences, with cestodes retaining rather than digesting and excreting toxins. The presence of positive relationships between host and helminth [THg] could have important implications for non-invasive methods of host [THg] monitoring. Eggs of some intestinal nematodes and cestodes, along with posterior segments of cestodes (a.k.a. proglottids), are excreted from the host via feces (Chenin 2000). As a result, further research should analyze if these fecal parasite tissues can be used to reflect and monitor [THg] in host species.

Partially consistent with my predictions, cestodes had higher [THg] when compared to fox muscle [THg], yet nematodes did not. The lack of difference between nematode and fox muscle [THg] may again be explained through variations in helminth resource acquisition. Though not as complex, nematodes have gastrointestinal tracts like vertebrates, which may allow these two groups to maintain more similar [THg] compared to species lacking digestive systems (e.g. cestodes). Mammalian studies comparing host and helminth [THg] are scarce. In red foxes, other toxic metals (Cr, Pb) are taken up to levels higher than the host kidney and liver tissue by both cestodes (*Mesocestoides* spp.) and nematodes (*Toxascaris leonina*) (Jankovská et al. 2008). Though these trends could have been similar for Hg, this metal was not observed by Jankovská et al. (2008), and assumptions should not be made due to variations in metal properties and how they interact with animal tissues. In contrast with my results, a study on wild rabbits did not find a difference between wild rabbit and cestode [THg] (Eira et al. 2005). However, observations by Eira et al. (2005) may have resulted from insufficient Hg uptake in herbivorous rabbits for detectable bioaccumulation in cestodes. Aside from mammals, other past research has observed higher [THg] in nematodes than muscle tissue of double-crested cormorants (Robinson et al. 2010), no difference in [THg] between cestodes and muscle tissue of feral pigeons (*Columba* *livia*), and highly variable results for cestode and nematode [THg] compared to [THg] in fish hosts (e.g. Mazhar et al. 2014; Mendes et al. 2013; Retief et al. 2006). More research is needed to fill the gaps in knowledge surrounding helminth-host [THg] comparisons, particularly for foxes and other carnivores.

Contrary to my predictions, host [THg] did not decrease with increasing helminth biomass in this study. Studies linking helminth infection intensities to metal concentrations are inconsistent and scarce. In one study, red foxes infected with cestodes had lower levels of toxic metals Cd and Pb than uninfected counterparts, though nematodes were not investigated (Brožová et al. 2015). In another, Cd and Pb did not vary between red foxes infected and uninfected with cestodes and nematodes (Binkowski et al. 2016). Though these studies did not investigate Hg, the latter was more consistent with my results since it found no difference in other toxic metals (Cd and Pb) for foxes with and without cestodes and nematodes (Binkowski et al. 2016). Similar Hg levels have further been seen in Arctic terns (*Sterna paradisaea*) with and without intestinal parasites (Provencher et al. 2014). In my study, the small sample size of 30 foxes may have influenced my results. Not only did a small sample size provide limited observations, but it also required my models to have minimal independent variables to avoid overfitting. In particular, I limited my model for helminth biomass impact on fox [THg] to only have predictor variables of nematode biomass and cestode biomass. Had I obtained a larger sample size, I would have used the Akaike information criterion in model selection to see if adding additional variables such as fox age, sex, year of harvest, and/or interaction terms improved this model. Though it was not included here, diet may also be an important factor for linking helminth and host [THg] since it is the primary route of both Hg and helminth uptake. Future research should aim to include diet indicators such as stable isotopes (e.g. $\delta^{15}N$) in their analyses (Tarroux et al. 2012). Lastly, it is possible that results for helminth biomass effects on fox [THg] were impacted by severe degradation of cestodes in certain foxes that may have led to losses in helminth biomass, though this impact would be minimal.

Arctic foxes are one of several species threatened by Arctic mercury pollution due to their scavenging of Hg-rich marine prey. As climate change continues to alter Hg pollution and species dynamics in Arctic environments, elucidating the factors influencing bioaccumulation in wildlife is of increased importance. This study demonstrates that intestinal helminths can take up Hg to varying extents, suggesting helminths' capacity to alter Hg bioavailability to hosts. Though not observed here, future research should continue to investigate the mechanisms by which helminths absorb and potentially sequester Hg and if these mechanisms play a role in mitigating host Hg exposure. As Hg availability in the Arctic continues to change, a better understanding of host-helminth-Hg interactions is crucial for assessing health risks to Arctic species and informing conservation strategies.

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