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Ecotoxicoparasitology: Understanding mercury concentrations in gut contents, intestinal helminths and host tissues of Alaskan gray wolves (*Canis lupus*)

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Abstract

Some gastrointestinal helminths acquire nutrients from the lumen contents in which they live; thus, they may be exposed to non-essential elements, such as mercury (Hg), during feeding. The objectives of this study were: 1) determine the total mercury concentrations ([THg]) in Gray wolves (*Canis lupus*) and their parasites, and 2) use stable isotopes to evaluate the trophic relationships within the host. [THg] and stable isotopes (C and N) were determined for helminths, host tissues, and lumen contents from 88 wolves. Sixty-three wolves contained grossly visible helminths (71.5%). The prevalence of taeniids and ascarids was 63.6% (56/88) and 20.5% (18/88), respectively. Nine of these 63 wolves contained both taeniids and ascarids (14.3%). All ascarids were determined to be *Toxascaris leonina*. *Taenia* species present included *T. krabbei* and *T. hydatigena*. Within the GI tract, [THg] in the lumen contents of the proximal small intestine were significantly lower than in the distal small intestine. There was a significant positive association between hepatic and taeniid [THg]. Bioaccumulation factors (BAF) ranged from <1 to 22.9 in taeniids, and 1.1 to 12.3 in *Toxascaris leonina*. Taeniid and ascarid BAF were significantly higher than 1, suggesting that both groups are capable of THg accumulation in their wolf host. $\delta^{13}\text{C}$ in taeniids was significantly lower than in host liver and skeletal muscle. [THg] in helminths and host tissues, in conjunction with stable isotope (C and N) values, provides insight into food-web dynamics of the host GI tract, and aids in elucidating ecotoxicoparasitologic relationships. Variation of [THg] throughout the GI tract, and between parasitic groups, underscores the need to

further evaluate the effect(s) of feeding niche, and the nutritional needs of parasites, as they relate to toxicant exposure and distribution within the host.

1. Introduction

Mercury (Hg) is a non-essential element that exists in numerous chemical forms, occurring both naturally in the environment and as the result of human activities. Major anthropogenic sources include the burning of fossil fuels (especially coal), municipal waste incineration (Virtanen et al., 2007), and other point sources such as amalgam waste from dental clinics (Shraim et al., 2011). Emissions from geological sources (e.g. cinnabar), as well as emissions from forest fires and volcanoes, also contribute Hg to the environment. Hg is not only ubiquitous, but also environmentally persistent (Balshaw et al., 2007), and can have detrimental effects on the health of organisms. Methylmercury (MeHg) is the highly toxic form (Kruzikova et al., 2008; Virtanen et al., 2007), which includes monomethylmercury (MeHg⁺) and dimethylmercury. The MeHg⁺ form has been shown to bioaccumulate and/or biomagnify in food webs, and often reaches high concentrations in specific tissues of long-lived fish eating animals (Clarkson & Magos, 2006; Balshaw et al., 2007; Bridges & Zalups, 2010; Virtanen et al., 2007).

Historically, studies on food webs have rarely included parasites, partly due to the complex nature of host-parasite interactions (Gómez-Díaz & González-Solís, 2010). The term *ecotoxicology* has been applied to the field of study focused on the effects of toxic chemicals on biological organisms, at the population, community, ecosystem level (Moriarty, 1988); *Ecotoxicoparasitology*, however, relates to the interface of three disciplines, and is concerned with complex systems including parasites, their hosts, and the toxicants to which they are exposed.

Several gastrointestinal helminths feed on the intestinal contents of the hosts in which they live, thereby, putting them in direct competition with their host for nutrients (Coop & Kyriazakis, 1999). These parasites are exposed to the same dietary and elimination pathways (e.g. bile) of the host; thus, uptake of toxicants by the parasites likely occurs as a direct result of their feeding activities within the host's gastrointestinal (GI) tract. One previous study showed that GI helminths are capable of bioaccumulation and/or biomagnification of non-essential elements at concentrations that are orders of magnitude higher than those in host tissues (Sures et al., 1999). However, the process by which parasites acquire, concentrate, and/or biotransform non-essential elements, such as mercury (Hg), and their potential impact on host-toxicant dynamics, is not well understood.

One tool that may be used to explore aspects of host-parasite relationships and mercury exposure is stable isotope analysis. In many systems, C and N stable isotopes have been used in feeding ecology studies to determine food web length and structure (Gómez-Díaz & González-Solís, 2010), often facilitating assessment of consumer feeding habits (Daugherty & Briggs, 2007). Because parasites utilize various feeding strategies, it may be challenging to interpret trophic relationships using isolated approaches (Gómez-Díaz & González-Solís, 2010). Previously stable isotope and THg analysis have been used to provide insight into sources of Hg exposure in gray wolves (McGrew et al., 2014). Nitrogen signatures have also

been shown to have a positive association with total mercury concentrations [THg] in arctic food webs (Atwell et al., 1998). The aim of this study was, therefore, to use stable isotope signatures (C and N) and [THg] together, to better understand the influence of the host-parasite relationship on [THg], and provide insight into the trophic relationships that exist within gray wolves from Alaska.

2. Materials and Methods

2.1 Sample Collection

Samples from gray wolves from across the state of Alaska were collected by the Alaska Department of Fish & Game, between 2006 and 2009, as part of ongoing studies. Previously, McGrew et al. (2014) measured C and N stable isotope signatures and [THg] from the liver, kidney, and skeletal muscle of this wolf population. In this work, the GI tracts, GI helminths, and host tissues were evaluated from a subset of these individuals, as well as other wolves from Alaska (n = 88). At necropsy, sex, and age class were determined, and tissues, including liver, skeletal muscle, and GI tracts (with lumen contents and parasites) were collected. Nine of 88 GI tracts were processed immediately, whereas the remaining GI tracts were transferred to the University of Alaska Fairbanks (UAF) and stored at -80 C until further processing could take place. Wolves were divided into two age classes, <12 months, and ≥ 12 months, based on previous studies (Gese et al., 1997; McGrew et al., 2014; Zarnke et al., 2004).

During processing, the large and small intestines were opened longitudinally using clean stainless steel instruments, and all grossly visible parasites were removed, rinsed with ultrapure water, and weighed. Due to freezing, only the ascarids were able to be enumerated. A limited number of representative ascarids were fixed in 10% formalin for morphologic identification; likewise, a single individual, terminal, taeniid proglottid was frozen for molecular identification (Obwaller et al., 2004). All remaining ascarids and taeniids were frozen for subsequent THg analysis, and C and N stable isotope (SI) analysis. Lumen contents and full-thickness sections of GI wall were collected from proximal small intestine (pSI), distal small intestine (dSI), and colon, and were weighed prior to freezing at -80 C. Determination of [THg] and stable isotopes was carried out for host tissues, lumen contents, and parasites.

2.2 Total mercury (THg) analysis

Host liver and skeletal muscle, are commonly sampled tissues in THg and SI studies due to their intrinsic properties and rate of cellular turnover. In this study, these tissues were thawed at room temperature, and sub-sampled (70-150 mg) using clean stainless steel forceps and scissors. Instruments were washed with ultrapure water and dried between each sample. Samples were analyzed with a direct mercury analyzer (DMA) using a Milestone DMA-80 instrument as described previously (Butala et al., 2006; McGrew et al., 2014; EPA 600-R-04-012). Groups of ascarids or taeniids from each host were freeze-dried, homogenized using a mortar and pestle, and analyzed for THg on a dry weight basis. Ascarids and taeniids were homogenized and analyzed separately in instances of mixed infection. The homogeneity ensured [THg] were representative of the parasite group(s)

collected from each host. [THg] were not determined for taeniids from 6/56 wolves due to low biomass. While host tissue [THg] were determined on a wet weight (ww) basis, both ww and dry weight (dw) values are presented for comparative purposes. Dry weight [THg] was calculated as $ww [THg] / (1 - \text{fraction moisture content})$. Percent moisture was also used to calculate [THg] in parasites on a wet weight basis.

2.3 Stable isotope analysis

SI values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were determined as previously described (McGrew et al., 2014). Briefly, all tissues to be analyzed were freeze-dried and homogenized prior to analysis, which was carried out with continuous-flow isotope-ratio mass spectrometry using an elemental analyzer (Carlo Erba NC1500 or Thermo Flash 2000) interfaced to a mass spectrometer (Micromass Optima or Thermo-Finnigan Delta Plus XP). Isotope values are reported in delta (δ) notation:

$$\delta X = (R_{\text{sample}} / R_{\text{standard}}) - 1$$

where X represents ^{13}C or ^{15}N in parts per thousand (‰) deviation relative to a standard (monitoring) gas and R_{sample} and R_{standard} represent the ratio of $^{13}\text{C}/^{12}\text{C}$, or $^{15}\text{N}/^{14}\text{N}$, for sample and standard, respectively. Analytical error was assessed by replicate measures of primary standards (<0.2‰ for all three isotopes across all analytical sequences) and quality control was assessed using several secondary standards, analyzed several times within individual analytical sequences (<0.3 ‰); accuracy was assessed using primary standards as unknowns, and was within 0.2‰ for both isotopes. Reproducibility of values was generally better than 0.2‰.

2.4 Helminth Identification

Prevalence was defined as the proportion of wolves infected with a given parasite, and intensity was defined as the number of individuals of a particular parasite species, in a given host (Bush et al., 1997). Ninety-five percent confidence intervals were determined using the Clopper-Pearson method (Newcombe, 1998).

Ascarids were identified based on morphological criteria (Anderson et al., 1974). Three of 15 wolves harbored only a single ascarid; thus, species identification could not be performed without adversely affecting [THg] and stable isotope determination. Taeniids were identified using molecular techniques. Individual proglottids were taken from the taeniids of 54/56 wolves. Genomic DNA was isolated from individual proglottids using a commercially available kit, according to manufacturer's instructions (Qiagen, DNA Blood and Tissue Kit). Primers targeting a fragment of the NADH dehydrogenase subunit I (ND1) gene, based on the protocol of Obwallner et al. (2004), were used to amplify an approximately 471 bp product. Amplification products were purified (Qiagen, QIAquick Gel Extraction Kit) prior to direct sequencing (Proteomics and Metabolomics Facility, Colorado State University, Fort Collins, CO). Nucleotide sequence comparisons were made using the BLAST software available from the NCBI website. Five sequences were unreadable, therefore 49/54 of the proglottids were identified to species.

2.5 Statistical Analyses

Chi-square tests were used to evaluate potential associations between pairs of dichotomous variables: age class, sex, the presence of taeniids, and the presence of ascarids. A Bonferroni-Holm adjustment was used on the p-values to control the Family-Wise error rate for multiple comparisons.

[THg] in GI tracts were compared separately for dw and ww using a repeated measures one-way ANOVA with unstructured covariance. A $\log_{10}$ transformation of [THg] was used to meet model assumptions of residual normality and homoscedasticity. Differences were estimated using least-squares means (Ostle, 1976) and 95% confidence intervals, with adjustments to p-values made using Tukey's Honest Significant Difference to control Family-Wise error.

Data from both taeniid species were combined and analyzed together based biologic similarities that exist between these two taeniid species within a given geographic region (e.g. dominant definitive and intermediate hosts, host specificity, distribution) (Samuel et al., 1971). Differences in [THg] between parasitic groups were analyzed for wolves harboring both taeniids and ascarids, as well as for hosts harboring only taeniids or ascarids. This was evaluated using the Welch's paired t-test, or the Wilcoxin rank sum test, respectively. Bioaccumulation factors (BAFs) were calculated by dividing the [THg] in either taeniids or ascarids by the [THg] in the lumen contents from where the parasites were derived. BAFs are defined as the ratio of a certain chemical or toxicant in an organism, to the concentration of that same chemical or toxicant in the food source or environment of that organism. To test whether $BAF > 1$, data were transformed prior to using a t-test of whether the mean BAF was greater than 1. A log-transformation of taeniid BAF data and an inverse transformation of ascarid BAF data (i.e. $1/\text{ascarid BAF}$) were used to best approximate a normal distribution. A onesided Wilcoxon signed rank test was used to test whether the values were symmetric about 1. Linear regression analysis comparing [THg] in taeniids and host liver was carried out on 47 wolves using $\log_{10}$ -transformed data. An ANOVA was conducted on the $\delta^{13}\text{C}$ levels in the host tissues and taeniids. Comparisons with ascarids were not possible due to low sample numbers and/or biomass. R and SAS statistical software were used for the statistical analyses described. Statistical values in which $p < 0.05$ were considered to be significant.

3. Results

3.1 Demographic data

Sex information was available for all 88 wolves, and estimated age class information was available for 85/88 individuals. Among wolves that were <12 months of age ($n = 26$), 13 were female and 13 were male; of wolves that were ≥ 12 months ($n = 59$), 30 were females and 29 were males. There was no significant association between the variables of age, sex, presence of ascarids and presence of taeniids; therefore, all data from all wolves were combined for subsequent analyses.

3.2 Prevalence, mean intensity, and biomass

Prevalence of GI macrohelminths was 63/88 (71.5%; 95% CI=61.0%, 80.7%). Taeniids were present in 56/88 (63.6%; 95% CI=52.7%, 73.6%) animals, and ascarids were present in 18/88 (20.5%; 95% CI=12.6%, 30.4%). Nine of 63 parasitized wolves contained both ascarids and taeniids (14.3%; 95% CI=6.7%, 25.4%). All ascarids were identified as *Toxascaris leonina*. Twenty of the 49 cestodes were identified as *Taenia krabbei* (40.8%; 95% CI=27.0%, 55.8%), and 29/49 were *Taenia hydatigena* (59.2%; 95% CI=44.2%, 73.0%). Mean intensity of ascarids was 27.1 (1 - 91), whereas mean intensity of taeniids could not be determined. Median ascarid biomass per animal was 1.2g (0.6g - 9.2g), and median taeniid biomass per animal was 10.5g (0.01g - 45.5g). There were no significant associations between ascarid mean intensity and [THg], or biomass of either parasite group and [THg].

3.3 [THg] in host tissues

Median (range) hepatic [THg] was 37.8 (5.7-2226.8) ppb ww. Median (range) [THg] in the lumen contents of the pSI, dSI, and colon were 5.0 (<1.0 - 64.0), 7.8 (1.2 - 57.9), and 22.0 (2.8 - 463.0) ppb ww, respectively. In proximal small intestine (pSI) lumen contents, [THg] were <1ppb in 2/88 samples. Median (range) [THg] in the wall of the pSI, distal small intestine (dSI), and colon were 4.1 (<1 - 177.8), 3.5 (<1 - 79.6), and 3.5 (<1 - 49.4) ppb, respectively. [THg] was <1 ppb in 9/81 pSI wall samples, 13/83 dSI wall samples, and 18/73 colon wall samples. [THg] in lumen contents differed significantly based on location along the GI tract for both ww and dw. Least-squares means for [THg] (log₁₀) and 95% confidence intervals for the means show [THg] in pSI lumen contents were significantly lower than in dSI lumen contents (Table 1).

3.4 [THg] in parasites and host liver

Mean [THg] in taeniids and in *Toxascaris leonina* were determined (Table 2). No significant differences in [THg] were noted between these two parasite groups. Hepatic [THg] in hosts infected with taeniids (n = 57) ranged from 5.70 to 2,226.83 ppb (ww). [THg] in taeniids from these hosts ranged from 0.31 to 107.34 ppb (ww). Regression analysis showed a highly significant positive association between taeniid and host hepatic [THg] ($r^2 = 0.38$, slope = 0.696, $p < 0.0001$) (Figure 1). The relationship between [THg] in ascarids and host hepatic tissue could not be analyzed due to sample size constraints.

3.5 [THg] in parasites relative to host lumen contents

BAF ranged from <1 to 22.9 in taeniids, and 1.1 to 12.3 in *Toxascaris leonina* (Table 3), and there was no significant difference between BAFs of taeniids and ascarids. Mean cestode BAF was found to be significantly higher than 1, as was mean nematode BAF. For both taeniids and ascarids, significantly more values were higher than one than were lower than one.

3.6 Stable isotope analysis

Stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were measured in host tissues as well as in the taeniids and ascarids (Table 4). $\delta^{13}\text{C}$ values in taeniids were significantly lower than host

livers and skeletal muscle. The low number of ascarids on which isotope signatures were measured precluded statistical comparison between the two parasite groups, as well as between the ascarids and their host.

4. Discussion

While previous studies have explored isolated aspects of host-parasite relationships and C and N stable isotopes in mammals (Sinisalo et al., 2006), and stable isotopes have been extensively used in mammalian ecology (Crawford et al., 2008), studies integrating the use of stable isotopes and THg in order to examine the interface of the toxicant-parasite interactions and host feeding ecology in wolves have not been carried out. Furthermore, as few studies have explored [THg] and SI values in parasites, determining which species of parasites were present in these wolves was important, as it provides ecological and biological context for the [THg] and SI values obtained.

In this study, wolves were infected with *Toxascaris leonina*, *Taenia krabbei*, and *Taenia hydatigena*, all of which have been previously reported in this host species (Irovi et al., 2015; Guerra et al., 2012; Lavikainen et al., 2011; Rausch, 1959; Segovia et al., 2001). Both prevalence and mean intensity of ascarids was higher in these wolves compared to wolves elsewhere (Abdybekova & Torgerson, 2012; Gori et al., 2015; Guberti et al., 1993; Popiolek et al., 2007; Stronen et al., 2011). The prevalence of these GI helminths was expected, given the dietary trends of these wolf populations, the transmission cycles of these parasites, and the presence of appropriate intermediate and/or paratenic hosts in this geographic region.

Within the host, [THg] in host lumen contents and GI wall were consistently low. However, trends were still evident, with concentrations increasing, moving aborally through the intestinal tract. Taeniids and ascarids commonly reside in the proximal small intestine of their definitive host, and appear to be inhabiting an ecological niche that is relatively lower in THg concentrations than elsewhere in the GI tract, as indicated in this study. Whether the [THg] in that particular area of the GI tract is lower due to the presence of the parasites is not known. Studies focused on understanding the mechanisms by which these parasites take up and/or excrete non-essential elements such as Hg, as well as whether inter- or intra-specific competition exists between parasite groups, are clearly needed. In these wolves, [THg] in the GI tract do not appear to be altering the typical site preferences of these parasites.

[THg] did not differ significantly between taeniids and ascarids, which was somewhat surprising, given that these parasite groups assimilate nutrients in different ways and utilize different feeding strategies (Hall, 1985; Halton, 1997). Taeniids do not possess a GI tract, and acquire host-derived nutrients directly across their tegument (Pappas, 1983). Whether certain cestode (taeniid) species are able to discriminate between different types of food sources remains unknown (Persson, 2007). Our results also suggest that some parasite groups may be more effective at THg bioaccumulation, relative to others, as evidenced by the greater variation of [THg] and BAFs seen in taeniids. The fact that [THg] in taeniids were associated with [THg] in the host liver suggests that [THg] in some groups of parasites may be reflective of trends seen at the level of the host. In the future, it would be worth

exploring antemortem approaches to measuring [THg] in taeniid proglottids that have been passed into the environment.

BAFs in both parasite groups were significantly higher than 1 which signifies that THg is higher in the parasite than in the lumen contents in which it lives (i.e. source of nutrients). The possibility that parasites have the potential to affect host contaminant uptake and retention has been previously explored (Provencher, 2013; Robinson et al., 2010).

C and N stable isotope analysis is a valuable tool in understanding food-webs; however, their application in parasitology has only been explored on a limited basis. It is critical to recognize how parasitism differs from predation as a trophic strategy, and how parasites differ from each other when interpreting host-parasite-toxicant interactions in a complex system. We found that ^{15}N was depleted in taeniids and ascarids relative to all wolf tissues and GI lumen contents. ^{15}N depletion relative to host tissues has been previously reported for both nematodes and cestodes (Boag, 1998; Pinnegar et al., 2001; Xu et al., 2007) as well as in a number of studies on fish-host-parasite systems (Deudero et al., 2002; Iken et al., 2001; Pinnegar et al., 2001; Xu et al., 2007). To our knowledge, ^{15}N depletion in parasites, relative to the host, has not been described in wolves, although it has been reported in non-mammalian species (Robinson et al., 2010).

It has been noted previously that isotopic fractionation in parasites is not well understood (Boag et al., 1998; Neilson & Brown, 1999) and parasites have been shown to have both higher and lower isotope ratios (C & N) relative to their host (Boag 1998; Neilson & Brown, 1999; Deudero et al., 2002). It has also been suggested that, for parasites, which assimilate only specific host-derived biosubstrates, their isotopic composition will be based only on those particular nutrient components (Behrmann-Godel & Yohannes, 2013). In typical predator-prey relationships, ^{13}C and ^{15}N are enriched $\sim 1\text{‰}$ and 0-6‰, respectively (Deniro & Epstein, 1978; Minagawa & Wada, 1984); however, the magnitude and direction of fractionation in parasites is thought to be affected by numerous factors, including feeding strategy, nutrient requirements, rate of feeding, rate of excretion, assimilation efficiency, feeding selectivity, and life stage (Behrmann-Godel & Yohannes, 2013; Deudero et al., 2002; Olive et al., 2003; Lafferty et al., 2008).

5. Conclusions

The results of this study suggest that parasites may potentially be playing a beneficial role, serving as a biological 'sink' for non-essential elements to which the host is exposed, which supports the findings of Sures (2008). It is plausible that parasites are also evolving mechanisms to survive and perpetuate their life cycle in the face of increasing exposure to toxicants and changing environmental stressors. For these reasons, further studies are needed to explore the dynamics of parasite populations within the host, and particularly, the effects of ecological niche on toxicant distribution and bioavailability within the host.

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References

- Abdybekova AM, Torgerson PR. Frequency distributions of helminths of wolves in Kazakhstan. *Veterinary Parasitology*. 2012; 184(2-4):348–351. [PubMed: 21962968]
- Anderson RC, Chabaud AG, Willmott S. *CIH Keys to the Nematode Parasites of Vertebrates*. Commonwealth Agricultural Beureaux. 1974
- Atwell L, Hobson KA, Welch HE. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences*. 1998; 55(5):1114–1121.
- Balshaw S, Edwards J, Daugherty B, Ross K. Mercury in seafood: mechanisms of accumulation and consequences for consumer health. *Rev Environ Health*. 2007; 22(2):91–113. [PubMed: 17894202]
- Behrmann-Godel J, Yohannes E. Multiple isotope analyses of the pike tapeworm *Triaenophorus nodulosus* reveal peculiarities in consumer-diet discrimination patterns. *Journal of Helminthology*. 2013 CJO2014. 10.1017/S0022149X13000849
- Boag B, Nielson, Robinson D, Scrimgeour CM, Handley LL. Wild rabbit host and some parasites show trophic level relationships for ^{13}C and ^{15}N : a first report. *Isot in Environ Health Stud*. 1998; 34:81–85.
- Bridges CC, Zalups RK. Transport of inorganic mercury and methylmercury in target tissues and organs. *J Toxicol and Environ Health*. 2010; 13:385–410.
- Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*. 1997; 83(4):575–583. [PubMed: 9267395]
- Butala SJ, Scanlan LP, Chaudhuri S. A detailed study of thermal decomposition, amalgamation/atomic absorption spectrophotometry methodology for the quantitative analysis of mercury in fish and hair. *J Food Prot*. 2006; 69(11):2720–2728. [PubMed: 17133817]
- irovi D, Pavlovi I, Penezi A. Intestinal helminth parasites of the grey wolf (*Canis lupus* L.) in Serbia. *Acta Vet Hung*. 2015; 63(2):189–98. [PubMed: 26051257]
- Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. *Critical Reviews in Toxicology*. 2006; 36:609–662. [PubMed: 16973445]
- Coop RL, Kyriazakis I. Nutrition-parasite interaction. *Veterinary Parasitology*. 1999; 84:187–204. [PubMed: 10456415]
- Crawford K, McDonald RA, Bearhop S. Applications of stable isotope techniques to the ecology of mammals. *Mammal Review*. 2008; 38(1):87–107.
- Daugherty MP, Briggs CJ. Multiple sources of isotopic variation in a terrestrial arthropod community: challenges for disentangling food webs. *Environmental Entomology*. 2007; 36(4):776–791. [PubMed: 17716468]
- DeNiro MJ, Epstein S. Mechanism of stable isotope fractionation associated with lipid synthesis. *Science*. 1977; 197:261–263. [PubMed: 327543]
- Deudero S, Pinnegar JK, Polunin NVC. Insights into fish host-parasite trophic relationships revealed by stable isotope analysis. *Diseases of Aquatic Organisms*. 2002; 52:77–86. [PubMed: 12517008]
- Field measurement technology for mercury in soil and sediment: Milestone Inc's Direct Mercury Analyzer (DMA-80). Office of Research and Development; Washington, DC: May 2004. EPA 600-R-04-012
- Gese EM, Schultz RD, Johnson MR, Williams ES, Crabtree RL, Ruff RL. Serological survey for diseases in free-ranging coyotes (*Canis latrans*) in Yellowstone National Park, Wyoming. *Journal of Wildlife Diseases*. 1997; 33(1):47–56. [PubMed: 9027690]
- Gómez-Díaz E, González-Solís J. Trophic structure in a seabird host-parasite food web: insights from stable isotope analyses. *PLoS One*. 2010; 5(5):e10454. [PubMed: 20454612]

- Gori F, Armua-Fernandez MT, Milanesi P, Serafini M, Magi M, Deplazes P, Macchioni F. The occurrence of taeniids of wolves in Liguria (northern Italy). *Int J Parasitol Parasites Wildl*. 2015; 4(2):252–255. [PubMed: 26042204]
- Guberti V, Stancampiano L, Francisci F. Intestinal helminth parasite community in wolves (*Canis lupus*) in Italy. *Parassitologia*. 1993; 35(1):59–65. [PubMed: 8065823]
- Guerra D, Armua-Fernandez MT, Silva M, Bravo I, Santos N, Deplazes P, Carvalho LM. Taeniid species of the Iberian wolf (*Canis lupus signatus*) in Portugal with special focus on *Echinococcus* spp. *Int J Parasitol Parasites Wildl*. 2012; 2:50–53. [PubMed: 24533315]
- Hall A. Nutritional aspects of parasitic infection. *Progress in Food and Nutrition Science*. 1985; 9(3-4): 227–256. [PubMed: 3914654]
- Halton DW. Nutritional adaptations to parasitism within the Platyhelminthes. *International Journal for Parasitology*. 1997; 27(6):693–704. [PubMed: 9229252]
- Iken K, Brey T, Wand U, Voigt J, Junghans P. Food web structure of the benthic community at Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. *Prog Oceanogr*. 2001; 50:383–405.
- Kruzikova K, Modra H, Kensova R, Skocovska B, Wlasow T, Svoboda T, Svobodova Z. Mercury in human hair as an indicator of the fish consumption. *Neuro Endocrinol Lett*. 2008; 29(5):675–679. [PubMed: 18987591]
- Lafferty KD, Allesina S, Arim M, Briggs CJ, De Leo G, Dobson AP, Dunne JA, Johnson PT, Kuris AM, Marcogliese DJ, Martinez ND, Memmott J, Marquet PA, McLaughlin JP, Mordecai EA, Pascual M, Poulin R, Thielges DW. Parasites in food webs: the ultimate missing links. *Ecol Lett*. 2008; 11(6):533–546. [PubMed: 18462196]
- Lavikainen A, Laaksonen S, Beckmen K, Oksanen A, Isomursu M, Men S. Molecular identification of *Taenia* spp. in wolves (*Canis lupus*), brown bears (*Ursus arctos*) and cervids from North Europe and Alaska. *Parasitology International*. 2011; 60(3):289–295. [PubMed: 21571090]
- Minegawa M, Wada E. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim Cosmochim Acta*. 1984; 48:1135–1140.
- McGrew AK, Ballweber LR, Moses SK, Stricker CA, Beckmen KB, Salman MD, O'Hara TM. Mercury in gray wolves (*Canis lupus*) in Alaska: Increased exposure through consumption of marine prey. *Science of the Total Environment*. 2014; 468-469:609–613. [PubMed: 24056451]
- Moriarty F. *Ecotoxicology. Human and Experimental Toxicology*. 1988; 7(5):437–441.
- Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Statistics in Medicine*. 1998; 17:857–872. [PubMed: 9595616]
- Nielson R, Brown DJF. Feeding on different host plants alters the natural abundance of ^{13}C and ^{15}N in Longidoridae (Nematoda). *J Nematol*. 1999; 3. 1:20–26.
- Obwaller A, Schneider R, Walochnik J, Gollackner B, Deutz A, Janitschke K, Aspöck H, Auer H. *Echinococcus granulosus* strain differentiation based on sequence heterogeneity in mitochondrial genes of cytochrome c oxidase-1 and NADH dehydrogenase-1. *Parasitology*. 2004; 128:569–575. [PubMed: 15180325]
- Olive PJW, Pinnegar JK, Polunin NVC, Richards G, Welch R. Isotope trophic step fractionation: a dynamic equilibrium model. *J Anim Ecol*. 2003; 72:608–617.
- Ostle, B. *Statistics in Research: Basic Concepts and Techniques for Research Workers*. Second. Iowa University Press; 1976.
- Pappas, PW. The host parasite interface. In: Arme, C.; Pappas, PW., editors. *Biology of the Eucestoda*. Vol. 2. London: Academic Press; 1983. p. 297–341.
- Persson ME, Larsson P, Stenroth P. Fractionation of ^{15}N and ^{13}C for Atlantic salmon and its intestinal cestode *Eubothrium crassum*. *Journal of Fish Biology*. 2007; 71:441–452.
- Pinnegar JK, Campbell N, Polunin NVC. Unusual stable isotope fractionation patterns observed for fish host-parasite trophic relationships. *Journal of Fish Biology*. 2001; 59:494–503.
- Popiolek M, Szczesna J, Nowaka S, Myslajeka RW. Helminth infections in fecal samples of wolves *Canis lupus* L. from western Beskid Mountains in southern Poland. *J Helminthol*. 2007; 81(4): 339–344. [PubMed: 18251025]
- Provencher JF. Parasites and pollution: why both matter to marine bird conservation in the North. *Arctic Institute of North America*. 2013; 66(4):516–520.

- R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing; Vienna, Austria: 2014. URL
- Rausch R, Williamson FS. Studies on the helminth fauna of Alaska. XXXIV. The parasites of wolves, *Canis lupus* L. *J Parasitol*. 1959; 45(4, Part 1):395–403. [PubMed: 13673343]
- Robinson SA, Forbes MR, Hebert CE. Mercury in parasitic nematodes and trematodes and their double-crested cormorant hosts: bioaccumulation in the face of sequestration by nematodes. *Science of the Total Environment*. 2010; 408(22):5165–5618.
- Samuel, WM.; Pybus, MJ.; Kocan, AA. Parasitic Diseases of Wild Mammals. 2nd. Iowa State Press; Ames, IA: 2001.
- Segovia JM, Torres J, Miquel J, Llaneza L, Feliu C. Helminths in the wolf, *Canis lupus*, from north-western Spain. 2001; 75(2):183–192.
- Shraim A, Alsuhaime A, Al-Thakafy JT. Dental clinics: a point pollution source, not only of mercury but also of other amalgam constituents. *Chemosphere*. 2011; 84(8):1133–1139. [PubMed: 21543103]
- Sinisalo T, Tellervo V, Helle E, Jones RI. Combining stable isotope and intestinal parasite information to evaluate dietary differences between individual ringed seals (*Phoca hispida botnica*). *Can J Zool*. 2006; 84:823–831.
- Stronen AV, Sallows T, Forbes GJ, Wagner B, Paquet PC. Diseases and parasites in wolves of the Riding Mountain National Park region, Manitoba. *Canada J Wildl Dis*. 47(1):222–227. [PubMed: 21270013]
- Sures B, Siddall R, Taraschewski H. Parasites as accumulation indicators of heavy metal pollution. *Parasitology Today*. 1999; 15(1):16–21. [PubMed: 10234173]
- Sures B. Environmental parasitology. Interactions between parasites and pollutants in the aquatic environment. *Parasite*. 2008; 15:434–438. [PubMed: 18814718]
- Virtanen JK, Rissanen TH, Voutilainen S, Tuomainen TP. Mercury as a risk factor for cardiovascular diseases. *J Nutr Biochem*. 2007; 18(2):75–85. [PubMed: 16781863]
- Xu J, Zhang M, Xie P. Trophic relationship between the parasitic isopod *Ichthyoxenus japonensis* and the fish *Carassius auratus auratus* as revealed by stable isotopes. *Journal of Freshwater Ecology*. 2011; 22(2):333–337.
- Zarnke RL, Verltorf JM, DeLong RA. Serological survey for selected disease agents in wolves (*Canis lupus*) from Alaska and the Yukon Territory, 1984–2000. *Journal of Wildlife Diseases*. 2004; 40(4):632–638. [PubMed: 15650081]

Highlights

- [THg] and stable isotopes together provide insight on host-parasite-Hg interactions
- A significant positive association exists between host liver and taeniid [THg]
- [THg] varies within the GI tract and may be influenced by the presence of parasites
- [THg] is higher in ascarids & taeniids than in the lumen contents in the GI tract

Figure 1. Total mercury concentrations ([THg]) in taeniids ($\log_{10}$) against [THg] in the host liver ($\log_{10}$). There was an association between [THg], shown by the blue regression line ($r^2 = 0.38$, $p < 0.0001$). Age (shape) and sex (color) are included to illustrate the lack of influence of these variables.

Table 1

Least-squares means and estimated differences of log₁₀ total mercury concentrations ([THg]) throughout the gastrointestinal tract. Means are given for (THg) as determined by wet weight and by dry weight.

[THg] by Section	[THg] Mean $\pm$ 95% CI (log ₁₀)	
	Wet	Dry
Proximal SI lumen	0.69 $\pm$ 0.091	1.14 $\pm$ 0.12
Distal SI Lumen	0.99 $\pm$ 0.09	1.40 $\pm$ 0.15
Colon Contents	1.35 $\pm$ 0.10	1.63 $\pm$ 0.27
Differences in [THg]	[THg] Wet	[THg] Dry
	Mean $\pm$ 95% CI (log ₁₀)	Mean $\pm$ 95% CI (log ₁₀)
pSI lumen – dSI lumen	-0.30 $\pm$ 0.10 ^a	0.27 $\pm$ 0.15 ^a
pSI lumen – colon contents	-0.66 $\pm$ 0.11 ^a	0.49 $\pm$ 0.35 ^a
dSI lumen – colon contents	-0.36 $\pm$ 0.09 ^a	0.22 $\pm$ 0.25 ^a

^a Estimated differences are significant (p = 0.05); [THg] in GI tracts were compared separately for dw and ww using a repeated measures one-way ANOVA

Table 2

Mean, median, and range of [THg] in parasites from Alaskan wolves (n), expressed in ppb, wet weight (ww); standard deviation of the mean is indicated in parentheses.

Parasite	n	Mean	Median	Range
Taeniids	52	18.7 (± 24.4)	7.4	0.31-107.3
Ascarids	12	8.9 (± 3.7)	4.4	0.4-14.87

Table 3

Mean and median bioaccumulation factors (BAFs) of parasites, in wolves carrying either taeniids or ascarids.

Parasite	n	Mean	Median	Range
Taeniids	35	3.1	1.8	0.3 - 22.9
Ascarids	10	2.6	1.5	1.1 - 12.3

Nitrogen ($\delta^{15}\text{N}$) Carbon ($\delta^{13}\text{C}$) stable isotope values for ascarids, taeniids, and gray wolf tissues and GI lumen contents. Sample number is shown, as well as mean and median values (pSI = proximal small intestine (SI), dSI = distal SI). Values are expressed on a per mil basis.

Table 4

	$\delta^{15}\text{N}$				$\delta^{13}\text{C}$			
	n	Mean	Median	Range	n	Mean	Median	Range
Liver	72	7.0	6.9	5.7 to 8.6	73	-24.6	-24.6	-26.7 to -21.8
Muscle	42	6.1	5.9	4.9 to 8.8	43	-23.5	-23.4	-25.5 to -21.9
Taeniids	37	3.9	4.3	3.0 to 11.2	37	-25.5	-25.1	-28.0 to -22.8
Ascarids	3	5.0	4.7	4.4 to 6.0	3	-23.6	-23.6	-23.2 to -23.9
pSI wall	10	6	6.2	4.4 to 7.1	10	-23.8	-23.9	-25.2 to -22.3
dSI wall	10	7.1	7.1	5.9 to 8.2	10	-24.7	-24.0	-28.4 to -22.1
Colon wall	10	6.8	7.0	5.9 to 7.3	10	-24.7	-23.8	-29.2 to -22.1
pSI-lumen	4	6.4	6.4	5.9 to 6.8	4	-25.5	-25.5	-25.6 to -25.4
dSI-lumen	5	6.0	6.1	5.5 to 6.4	5	-24.8	-25.0	-25.4 to -23.5