Published in final edited form as:
Sci Total Environ. 2014 January 15; 0: . doi:10.1016/j.scitotenv.2013.08.045.

Mercury in gray wolves (Canis lupus) in Alaska: Increased exposure through consumption of marine prey

Ashley K. McGrew[a,1], **Lora R. Ballweber**[a], **Sara K. Moses**[b,2], **Craig A. Stricker**[c], **Kimberlee B. Beckmen**[d], **Mo D. Salman**[e], and **Todd M. O'Hara**[a,b]

[a]Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523-1619

[b]Wildlife Toxicology Laboratory, Department of Veterinary Medicine, University of Alaska Fairbanks, Fairbanks, 99775, AK

[c]U. S. Geological Survey, Fort Collins Science Center, Denver, CO 80225, USA

[d]Alaska Department of Fish & Game, Fairbanks, AK

[e]Animal Population Health Institute, Department of Clinical Sciences, Colorado State University, Fort Collins, CO

Abstract

Mercury (Hg) bioaccumulates in the tissues of organisms and biomagnifies within food-webs. Gray wolves (Canis lupus) in Alaska primarily acquire Hg through diet; therefore, comparing the extent of Hg exposure in wolves, in conjunction with stable isotopes, from interior and coastal regions of Alaska offers important insight into their feeding ecology. Liver, kidney, and skeletal muscle samples from 162 gray wolves were analyzed for total mercury (THg) concentrations and stable isotopic signatures ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$). Median hepatic THg concentrations were significantly higher in wolves with coastal access compared to wolves from interior Alaska. Stable isotope ratios, in conjunction with THg concentrations, provide strong evidence that coastal wolves are utilizing marine prey representing several trophic levels. The utilization of cross-ecosystem food resources by coastal wolves is clearly contributing to increased THg exposure, and may ultimately have negative health implications for these animals.

Keywords

bioaccumulation; biomagnification; mercury; Canis lupus; stable isotopes; feeding ecology

1. Introduction

A variety of stressors exist in the environment that may ultimately affect the health and functioning of organisms (Lafferty and Holt, 2003). Monomethylmercury ($MeHg^+$)

Corresponding author: Ashley McGrew, 970-297-5142, Ashley.mcgrew@colostate.edu.
[1]Present address: Veterinary Diagnostic Laboratory, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1644
[2]Present address: Biological Services Division, Great Lakes Indian Fish & Wildlife Commission, Odanah, WI 54861

bioaccumulates in the tissues of organisms, and elevated exposure to this form of mercury (Hg) can be particularly harmful to the fetus or neonate, especially in consumers that occupy high trophic positions (Chumchal et al., 2011). In some areas of the Arctic, Hg concentrations in marine food-webs have dramatically increased (Braune et al., 2005) to concentrations at which adverse biological effects might be expected (Dietz et al., 2013). While gray wolves (Canis lupus) have commonly been recognized as obligate carnivores that prey primarily on ungulates, recent studies have shown that the diets of some wolf populations are subsidized with marine organisms (Adams et al., 2010, Christensen et al., 2005; Watts et al., 2010). Therefore, gray wolves in Alaska serve as an optimal species for studying Hg exposure in apex-predator wildlife populations. While utilization of cross-ecosystem food sources (which has been demonstrated in a variety of higher-level consumers and predators) can be beneficial to species comprising recipient food-webs, the potential negative implications (low nutrients, infectious and toxic agents) of dietary components are not commonly explored (Christensen et al., 2005; Walters, Fritz, and Otter, 2008).

Stable isotope analysis is an integrated approach used to improve the overall understanding of food-webs (Hansen, Burmeister, and Sommer, 2009). Stable carbon (C)-, nitrogen (N)-, and sulfur (S)-isotopic compositions, in particular, have been widely recognized as established proxies of the trophic architecture. Enrichment of the heavier isotope of N (^{15}N) compared to the lighter form (^{14}N), occurs with each trophic level (Minagawa and Wada, 1984; Newsome et al., 2010), and marine organisms tend to have higher N isotope signatures relative to terrestrial biota (Benson and Parker, 1961; Miyake and Wada, 1967). Thus, N stable isotopes have been used extensively to quantify marine-based dietary sources for a variety of mammalian consumers, including wolves (Adams et al., 2010), grizzly bears (Ursus arctos horribills) (Christensen et al., 2011), polar bears (Ursus maritimus) (Dehn et al., 2006), and mink (Mustela vison) (Lake et al., 2007). Because isotopic enrichment in marine versus terrestrial biomes also exists for C and S, feeding ecology studies have additionally incorporated these isotopes, to determine the contribution of marine versus terrestrial prey sources in consumer diets (Inger et al., 2006). The purpose of this study was, therefore, to evaluate the feeding ecology of gray wolves in Alaska, as determined by stable isotopes signature, and total Hg (THg) tissue concentrations.

2. Materials and Methods

2.1 Sample collection

Tissues subsamples from a total of 162 gray wolves were collected by the Alaska Department of Fish and Game (ADF&G) from 2006 through 2009 as part of ongoing projects. Samples of liver (n=145), kidney (n=143), skeletal muscle (n=60), and cardiac muscle (n=16) were placed into individual Whirlpac™ bags and frozen immediately at −20° C. Samples were transferred to the University of Alaska Fairbanks (UAF) and stored at −80° C until laboratory analyses. Tissues were not systematically collected from all organs of each animal due to logistical constraints. The collection site for each wolf was assigned based on Game Management Unit (GMU) as defined by ADF&G (http://www.adfg.alaska.gov/index.cfm?adfg=huntingmaps.gmuinfo). Sex and age class (van Belle et al., 2004) were determined for each animal. Animals were classified as <12 months or ≥12 months.

2.2 Stable isotope analyses

In preparation for stable isotope analysis (C, N, and S), liver and skeletal muscle samples were freeze-dried and ground to a fine powder using a mortar and pestle, followed by further homogenization using a ball mill (mini-bead beater, BioSpec). Approximately 1.5–2.0 mg of

tissue was loaded into 5 × 9 mm tin capsules for C and N isotope analyses; 5.5 mg of sample amended with 2.0 mg of Vanadium oxide V_2O_5 was loaded into tin capsules for S isotope analysis.

Stable isotope ratios were analyzed by 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), as described elsewhere (Butala, Scanlan, and Chandhuri, 2006). Isotope values are reported in delta (δ) notation:

$$\delta \mathrm{X} = (\mathrm{R}_{\mathrm{sample}} / \mathrm{R}_{\mathrm{standard}}) - 1$$

where X represents ^{13}C, ^{15}N, or ^{34}S in parts per thousand (‰) deviation relative to a standard (monitoring) gas and R_{sample} and $R_{standard}$ represent the ratio of $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$ for sample and standard, respectively. Isotopic data were normalized to V-PDB, Air, and V-CDT using the primary standards USGS 40 (−26.24‰ and −4.52‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively), USGS 41 (37.76‰ and 47.57‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively), NBS127 (21.1‰ for $\delta^{34}S$), and IAEA-SO6 (−34.05‰ for $\delta^{34}S$). 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 secondary standards analyzed within individual analytical sequences (<0.3 ‰). Accuracy was assessed using primary standards as unknowns, and was within 0.2‰ for all three isotopes. Sample reproducibility, determined via duplicate measurements, was better than 0.2‰ for all three isotopes.

2.3 Total mercury (THg) analysis

All samples were thawed to room temperature and sub-sampled (70–150 mg) in duplicate, using stainless steel forceps and scissors. Instruments were washed with ultrapure water and dried between each sample. THg concentrations are reported on a wet weight (ww) basis. Samples were analyzed using a Milestone DMA-80 instrument (Butala, Scanlan, and Chandhuri, 2006; EPA 600-R-04-012, 2004). The method detection limit for THg determination was 0.005 ng/g, ww. Quality assurance and quality control were based on method blanks, standard reference materials (SRMs), check standards, and sample duplicates. All samples were run in duplicate and re-analyzed if the percent difference between samples was >10%. The SRM utilized was DORM-3 (National Resource Council Canada; 0.382 +/− 0.060 ng THg/g). Percent recovery for check standards (5, 20, and 100 ng aqueous Hg) was >90%. Analysis of the standard reference material was within 10% of the certified value for THg.

2.4 Statistical analyses

Collection sites were grouped into two location categories based on whether or not a coastline was present in the assigned GMU. Thus, wolves in GMUs that contained coastline were defined as “coastal” and those in GMUS that had no coastline were defined as “interior” animals. Specific pack assignment was not possible based on sample collection design.

Data distributions were assessed for normality using graphical techniques including histograms and box-and-whisker plots, supplemented by quantile-quantile plots (Henderson, 2006). Log-transformation did not satisfy the assumptions of normality; therefore, non-parametric statistical analyses were employed and median values and ranges are reported. Chi-square analyses were first used to evaluate the dichotomous variables of age class, sex, and location. The Mann-Whitney U test was used to evaluate differences in liver, kidney,

and muscle THg concentrations based on location, sex, and age class. Isotope values were also compared based on location, sex, and age class. Alpha was set ≥ 0.05. Statistical analyses were performed using StatCrunch5.0 statistical software (Integrated Analytics LLC, Pearson Education, 2007–2009).

3. Results

Of the 162 wolves, 30 occupied regions with coastal access and 132 wolves were from interior locales. Sex information was available for 161/162 wolves and age for 159/162 wolves. Among interior wolves, 20 males and 25 females were < 12 months, whereas 40 males and 44 females were ≥12 months. Among coastal wolves, 4 males and 2 females were <12 months, whereas 11 males and 12 females were ≥ 12 months. Chi-Square analyses demonstrated there was no significant association between age class and location, location and sex, or age class and sex.

Among all wolves, THg concentrations varied widely among the four tissues examined, particularly for liver and kidney (Table 1). No significant differences in THg concentrations were observed based on sex (Table 2); however, wolves that were ≥ 12 months had significantly higher hepatic and renal THg concentrations, and coastal wolves had significantly higher THg concentrations in all tissues (Tables 3–4).

C and N isotope analysis was conducted on 132 liver samples and 59 skeletal muscle samples (Figs. 1A & 1B, 2A & 2B). S isotope analysis was carried out on 131 liver samples and 56 skeletal muscle samples. Median liver $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ signatures (and ranges) were −24.4 ‰ (−20.0 to −26.8), 7.2 ‰ (5.7 to 15.9), and 5.2 ‰ (−1.64 to 17.1), respectively. Median values for all stable isotopic compositions were found to be significantly higher in coastal animals for all tissues ($p<0.01$). Interior wolves had significantly lower S values and liver THg concentrations (Fig. 3A). A similar trend was observed for skeletal muscle (Fig. 3B).

4. Discussion

Stable isotope analysis has been shown to be an excellent technique for assessing questions relating to food webs and trophic structure, and in understanding the basic foraging habits of mammals (Crawford, McDonald, and Bearhop, 2008). Few studies have used mammalian stable isotope signatures and Hg tissue concentrations together to evaluate foraging ecology questions in apex-predator systems (Cardona-Marek et al., 2009; Horton et al., 2009; Young et al., 2010); and typically, only C and N isotope ratios are assessed. This work is unique in that THg concentrations and stable isotope ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) signatures were used in conjunction to better understand the feeding ecology of Alaskan gray wolves and how it relates to dietary-based toxicant exposure. Wolves have been described as opportunistic, generalist carnivores (Watts et al., 2010), with considerable dietary plasticity among and within populations. This feeding strategy has been proposed to play a role in the success of wolves in parts of Alaska (Szepanski, 1999). However, the importance of marine resources in coastal wolves, and the effect of prey choices on individuals and populations, remains unknown. A recent study by Bocharova et al. (2013) showed that THg concentrations in Arctic foxes were reflective of foraging strategies, rather than variation in overall THg concentrations in the environment, and that high THg concentrations acquired predominantly through diet may represent a prominent risk for top predators. The present study, which is the first to evaluate Hg in Alaskan gray wolves, also suggests that cross-ecosystem utilization of food resources may contribute to increased Hg exposure, particularly in coastal populations.

The stable isotope values (C, N, and S) in liver and skeletal muscle tissue from coastal wolves were significantly higher than values in interior wolves, suggesting a marine-based diet in coastal wolves. Carbon and nitrogen isotopes are two measures that have been used to demonstrate marine resource utilization in populations (Adams et al., 2010), as $\delta^{13}C$ and $\delta^{15}N$ increase with the marine content of diet (Hilderbrand et al., 1996). The additional measurement of $\delta^{34}S$ was implemented in this study, since it can further aid in differentiating between the dietary contributions of marine and terrestrial resources (Crawford, McDonald, and Bearhop, 2008; Hansen, Burmeister, and Sommer, 2009). Our isotope analyses strengthen the contention that marine resources are contributing to the diets of coastal wolves in Alaska.

Among all wolves, THg concentrations varied based on tissue type. The deposition of Hg in tissues may be dependent on a number of biological factors including diet, age, sex, body condition, and health. It has been suggested that the affinity for Hg in kidney and liver tissue may be due to the preferential bonds of the organic Hg compounds to SH-groups of the plasma proteins in these organs (Kacmár et al., 1992). The highest THg concentrations in this study were observed in liver and kidney, which has also been reported elsewhere (Agusa et al., 2011; Misztal-Szkudlińska et al., 2010; Sures, 2004). Isotopic differences among tissues within an individual are largely due to differential routing of macronutrients to the various organs and differences in tissue turnover rates (Martínez del Rio et al., 2009). Liver turns over faster than skeletal muscle, and therefore has a shorter dietary integration window. For this reason, it tends to be more reflective of recent feeding, and could explain some of the variance noted in both stable isotopes and THg.

Coastal wolves in this study had significantly higher THg concentrations in all tissues examined, relative to interior wolves. In wildlife populations, Hg has been reported to cause reproductive impairment, alterations in growth and behavior, and even death (Facemire et al., 1995; Osowski et al., 1995; Wren, 1986). As obligate predators and opportunists, gray wolves have been previously described to eat a variety of small mammals, fish, and birds, when available (Darimont et al., 2004). Alaskan wolves with coastal access have been reported to utilize a wide variety of marine sources of prey, including harbor seals (Phoca vitulina) (Klein, 1995, Szepanski, 1999), various marine mammal carrion (Darimont et al., 2002; Mech, 1970), anadromous eulachon smelt (Thaleichthys pacificus), seabirds, marine invertebrates (Mech et al., 1998), and salmon (Adams et al., 2010; Darimont et al., 2002; Szepanski, 1999). Our results are in agreement with the work by Szepanski (1999), in which the diets of coastal wolves in Alaska were more varied, relative to interior animals, and trophic diversity was broader; likewise, these results are in agreement with findings for Arctic foxes, where coastal animals had higher variance in C isotopes, compared to those from inland regions (Angerbjorn, 1994).

In the present study, interior wolves tended to subsist on terrestrial resources, consequently resulting in low THg tissue concentrations. In contrast, coastal wolves tended to utilize marine resources resulting in higher THg concentrations. However, individual variation did exist. A few interior wolves with high THg had stable isotope signatures more similar to the coastal animals; conversely, there were 6 coastal wolves with relatively low S values (<5 ‰), more reflective of the interior wolves. While wolves belong to packs that tend to hunt cooperatively, not all pack members have access to the same resources. Unstable packs and lone individuals often have larger home ranges, are more mobile than established, stable packs (Mech and Botani, 2003), and may have a more varied or specialized diet (Urton and Hobson, 2005). These particular coastal animals may have occupied more inland regions of the coastal GMU without access, or limited access, to marine resources. Furthermore, landscape modifications and anthropogenic influences may alter prey distribution and

abundance, and behavioral interactions within and across species may affect accessibility to prey.

Our integrated approach provides insight into how toxicant exposure relates to foraging ecology in Alaskan gray wolves. However, evaluation of the physiological, environmental, and social factors affecting diet variation of wolves is needed in order to more clearly elucidate possible exposure pathways in individuals.

5. Conclusion

THg concentrations and C, N and S isotope values in various tissues provide four separate measures supporting the contention that, when accessible, Alaskan gray wolves exploit marine resources. If apex predators such as Alaskan gray wolves increase their utilization of marine subsidies, individuals and/or populations may be at risk for harmful effects associated with Hg exposure (e.g., in utero exposure of fetus) and other marine-based food-web toxicants. This work, and other reports, clearly demonstrates the need to better understand foraging behaviors of gray wolves in Alaska, and how THg exposure varies temporally and spatially based on foraging ecology. Stable isotope mixing model studies (Debridge et al., 2012; Fortin et al., 2007; Semmens et al., 2009; Szepanski, 1999; Urton and Hobson, 2005) have been used to quantify diet composition in wildlife populations, and may be one useful approach in addressing this need in the future.

Acknowledgments

The authors thank personnel at the Alaska Department of Fish and Game, all individuals who assisted in sample collection, and personnel in the Wildlife Toxicology Laboratory. This publication was made possible by grants from the National Center for Research Resources (P20RR016466) and the National Institute of General Medical Sciences (P20GM103395) from the National Institutes of Health. The senior author was partially supported by the Colorado State University – Program of Economically Important Infectious Animal Diseases through a special fund from the USDA:NIF. We also thank Cayce Gulbransen and Matthew Emmons for their involvement with the isotope analyses. The use of any trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. government.

References

Adams LG, Farley SD, Stricker CA, Demma DJ, Roffler GH, Miller DC, Rye RO. Are wolf-ungulate systems influenced by marine subsidies of Pacific salmon? Ecol Appl. 2010; 20(1):251–262. [PubMed: 20349845]

Angerbjorn A, Hersteinsson P, Liden K, Nelson E. Dietary variation in Arctic foxes (Alopex lagopus): an analysis of stable carbon isotopes. Oecologia. 1994; 99:226–232.

Agusa T, Yasugi SY, Iida A, Ikemoto T, Anan Y, Kuiken T, Osterhaus AD, Tanabe S, Iwata H. Accumulation features of trace elements in mass-stranded harbor seals (Phoca vitulina) in the North Sea coast in 2002: the body distribution and association with growth and nutrition status. Mar Pollut Bull. 2011; 62(5):963–975. [PubMed: 21429529]

van Belle, G.; Fisher, LD.; Heagerty, PJ.; Lumley, T. A Methodology for the Health Sciences. 2. Hoboken, NJ: John Wiley & Sons, Inc; 2004. Biostatistics.

Benson BB, Parker PDM. Nitrogen/argon and nitrogen isotope ratios in aerobic sea water. Deep-sea Res. 1961; 7:237–253.

Bocharova N, Treu G, Czirják GÁ, Krone O, Stefanski V, et al. Correlates between feeding ecology and mercury levels in historical and modern arctic foxes (Vulpes lagopus). PLoS ONE. 8(5):e60879.10.1371/journal.pone.0060879 [PubMed: 23671561]

Braune BM, Outridge PM, Fisk AT, Muir DCG, Helm PA, Hobbs K, Hoekstra PF, Kuzyk ZA, Kwan M, Letcher RJ, Lockhart WL, Norstrom RJ, Stern GA, Stirling I. Persistent organic pollutants and mercury in marine biota of the Canadian Arctic: An overview of spatial and temporal trends. Sci Tot Environ. 2005; 351–352:4–56.

Butala SJ, Scanlan LP, Chaudhuri SN. 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]

Cardona-Marek T, Knott K, Meyer BE, O'Hara TM. Mercury concentrations in Southern Beaufort Sea polar bears: Variation based on stable isotopes of carbon and nitrogen. Environ Toxicol Chem. 2009; 28(7):1416–1424. [PubMed: 19226182]

Chumchal MM, Rainwater TR, Osborn SC, Roberts AP, Abel MT, Cobb GP, Smith PN, Bailey FC. Mercury speciation and biomagnifications in the food web of Caddo Lake, Texas and Louisiana, USA, a subtropical freshwater ecosystem. Environ Toxicol Chem. 2011; 30(5):1153–1162. [PubMed: 21305578]

Christensen JR, MacDuffee M, MacDonald RW, Whiticar M, Ross PS. Persistent organic pollutants in British Columbia grizzly bears: consequence of divergent diets. Environ Sci Technol. 2005; 15(39):6952–60. [PubMed: 16201616]

Crawford K, McDonald RA, Bearhop S. Applications of stable isotope techniques to the ecology of mammals. Mammal Review. 2008; 38(1):87–107.

Darimont CT, Reimchen TE. Intra-hair stable isotope analysis implies seasonal shift to salmon in gray wolf diet. Can J Zool. 2002; 80:1638–1642.

Darimont CT, Price MHH, Winchester NN, Gordon-Walker J, Paquet PC. Predators in natural fragments: foraging ecology of wolves in British Columbia's central and north coast archipelago. Journal of Biogeography. 2004; 31(11):1867–1877.

Debridge JJ, Krausman PR, Darimont CT. Using Bayesian stable isotope mixing models to estimate wolf diet in a multi-prey ecosystem. The Journal of Wildlife Management. 2012; 76(6):1277–1289.

Dehn L, Follmann EH, Thomas DL, Scheffield GG, Rosa C, Duffy LK, O'Hara TM. Trophic relationships in an arctic food web and implications for trace metal transfer. Sci Tot Environ. 2006; 362:103–123.

Dietz, et al. What are toxicological effects of mercury in Arctic biota? Sci Tot Environ. 2013; 443:775–90.

Facemire C, Augspurger T, Bateman D, Brim M, Conzelmann P, Delchamps S, Douglas E, Inmon L, Looney K, Lopez F, Masson G, Morrison D, Morse N, Robison A. Impacts of mercury contamination in the southeastern United States. Water, Air, and Soil Pollution. 1995; 80:923–926.

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]

Fortin JK, Farley SD, Rode KD, Robbins CT. Dietary and spatial overlap between sympatric ursids relative to salmon use. BioOne. 2007; 18(1):19–29. http://dx.doi.org/10.2192/1537-6176(2007)18[19:DASOBS]2.0.CO;2.

Hansen T, Burmeister A, Sommer U. Simultaneous d15N, d13C and d34S measurements of low-biomass samples using a technically advanced high sensitivity elemental analyzer connected to an isotope ratio mass spectrometer. Rapid Commun Mass Spectrom. 2009; 23:3387–3393. [PubMed: 19813280]

Henderson AR. Testing experimental data for univariate normality. Clin Chim Acta. 2006; 366(1–2):112–129. [PubMed: 16388793]

Hilderbrand GV, Farley SD, Robbins CT, Hanley TA, Titus K, Servheen C. Use of stable isotopes to determine diets of living and extinct bears. Can J Zool. 1996; 74:2080–2088.

Horton TW, Blum JD, Xie Z, Hren M, Chamberlain CP. Stable isotope food-web analysis and mercury biomagnification in polar bears (Ursus maritimus). Polar Research. 2009; 28(3):443–454.

Inger R, Ruxton GD, Newton J, Colhoun K, Robinson JA, Jackson AL, Bearhop S. Temporal and intrapopulation variation in prey choice of wintering geese determined by stable isotope analysis. Journal of Animal Ecology. 2006; 75:1190–1200. [PubMed: 16922855]

Kacmár P, Legáth J, Neuschl J. Levels of mercury in the organs and tissues of sheep after administration of very low doses. Vet Med (Praha). 1992; 37(4):231–5. [PubMed: 1641953]

Klein, DR. The introduction, increase, and demise of wolves on Coronation Island, Alaska. In: Carbyn, LN.; Fritts, SH.; Seip, DR., editors. Ecology and Conservation of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta; Edmonton, Alberta, Canada: p. 275-280.

Lafferty KD, Holt RD. How should environmental stress affect the population dynamics of disease? Ecology Letters. 2003; 6:654–664.

Lake JL, Ryba SA, Serbst J, Brown CF 4th, Gibson L. Mercury and stable isotopes of carbon and nitrogen in mink. Environ Toxicol Chem. 2007; 26(12):2611–2619. [PubMed: 18020681]

Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ. Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev. 2009; 84:91–111. [PubMed: 19046398]

Mech, LD. The wolf: the ecology and behavior of an endangered species. Doubleday/Natural History; Garden City, NY: 1970.

Mech, LD.; Adams, LG.; Meier, TJ.; Burch, JW.; Dale, BW. The wolves of Denali. University of Minnesota Press; Minneapolis, Minnesota, USA: 1998.

Mech, LD.; Boitani, L. Wolf social ecology. In: Mech, LD.; Boitani, L., editors. Wolves: behavior, ecology, and conservation. The University of Chicago Press; Chicago, USA: 2003. p. 448

Minagawa M, Wada E. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between d^{15}N and animal age. Geochim Cosmochini Acta. 1984; 48:1135–1140.

Misztal-Szkudlińska M, Szefer P, Konieczka P, Namieśnik J. Biomagnification of mercury in trophic relation of Great Cormorant (Phalacrocorax carbo) and fish in the Vistula Lagoon, Poland. Environ Monit Assess. 2010; 176(1–4):439–449. [PubMed: 20623377]

Miyake Y, Wada E. The abundance ratio of $^{15}N/^{14}N$ in marine environments. Rec Oceanogr Works. 1967; 9:37–53.

Newsome SD, Bentall GB, Tinker MT, Oftedal OT, Ralls K, Estes JA, Fogel ML. Variation in delta13C and delta15N diet-vibrissae trophic discrimination factors in a wild population of California sea otters. Ecol Appl 2010. 2010; 20(6):1744–1752.

Osowski SL, Brewer LW, Baker OE, Cobb GP. The Decline of Mink in Georgia, North Carolina, and South Carolina: The Role of Contaminants. Arch Environ Contam Toxicol. 1995; 29:418–423. [PubMed: 7487161]

Semmens BX, Ward EJ, Moore JW, Darimont CT. Quantifying Inter- and Intra-Population Niche Variability Using Hierarchical Bayesian Stable Isotope Mixing Models. PLoS ONE. 2009; 4(7):e6187.10.1371/journal.pone.0006187 [PubMed: 19587790]

Sures B. Environmental parasitology: relevancy of parasites in monitoring environmental pollution. Trends Parasitol. 2004; 20(4):170–177. [PubMed: 15099556]

Szepanski MM, Ben-David M, Van Ballenberghe V. Assessmentof anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. Oecologia. 1999; 120(3): 327–335.

Urton EJM, Hobson KA. Intrapopulation variation in gray wolf isotope (d15N and d13C) profiles: implications for the ecology of individuals. Oecologia. 2005; 145:317–326. [PubMed: 15883848]

Walters DM, Fritz KM, Otter RR. The dark side of subsidies: adult stream insects export organic contaminants to riparian predators. Ecol Appl. 2008; 18(8):1835–41. [PubMed: 19263881]

Watts DE, Butler LG, Dale BW, Cox RD. The Ilnik wolf *Canis lupus* pack: use of marine mammals and off-shore sea ice. Wildl Biol. 2010; 16:144–149.

Wren CD. A review of metal accumulation and toxicity in wild mammals: I. Mercury. Environmental Research. 1986; 40(1):210–244. [PubMed: 3519207]

Young BG, Loseto LL, Ferguson SH. Diet differences among age classes of Arctic seals: evidence from stable isotope and mercury biomarkers. Polar Biol. 2010; 33:153–162.

Highlights

- Stable isotopes (C, N, & S) indicate the use of marine resources in Alaskan wolves
- Coastal wolves had significantly higher THg concentrations versus interior wolves
- Cross-ecosystem feeding behavior partly explains high THg concentrations in wolves

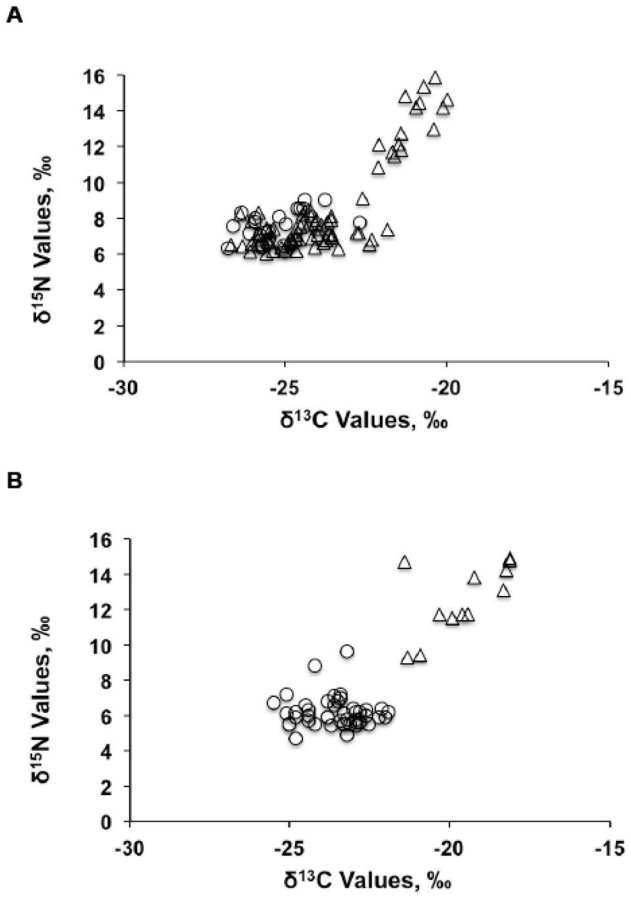

Figure 1.
Stable isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) in A) liver, and B) skeletal muscle, based on the designation of coastal versus interior gray wolves; △ = coastal wolves, ○ = interior wolves.

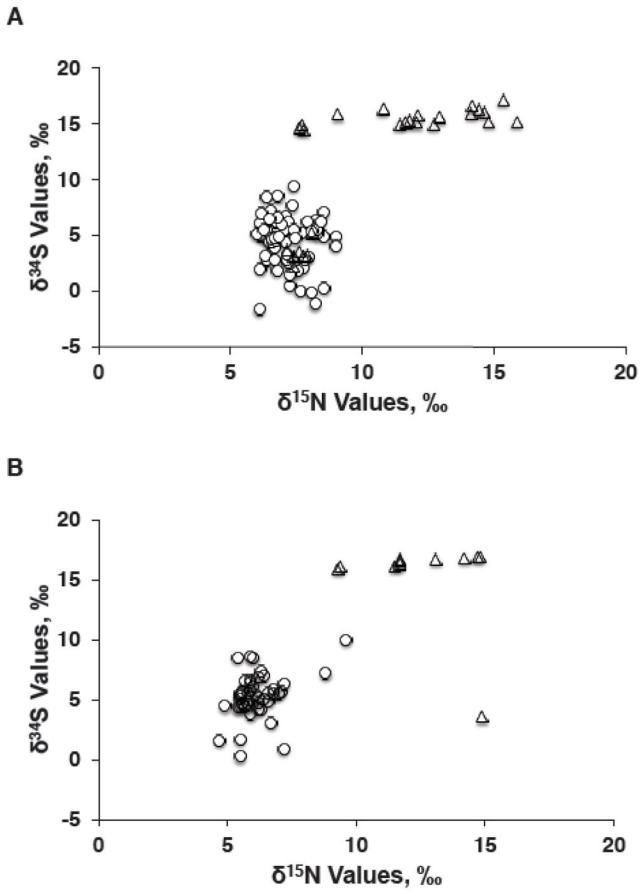

Figure 2.
Stable isotopic compositions ($\delta^{15}N$ and $\delta^{34}S$) in A) liver, and B) skeletal muscle, based on the designation of coastal versus interior gray wolves; △ = coastal wolves, ○ = interior wolves.

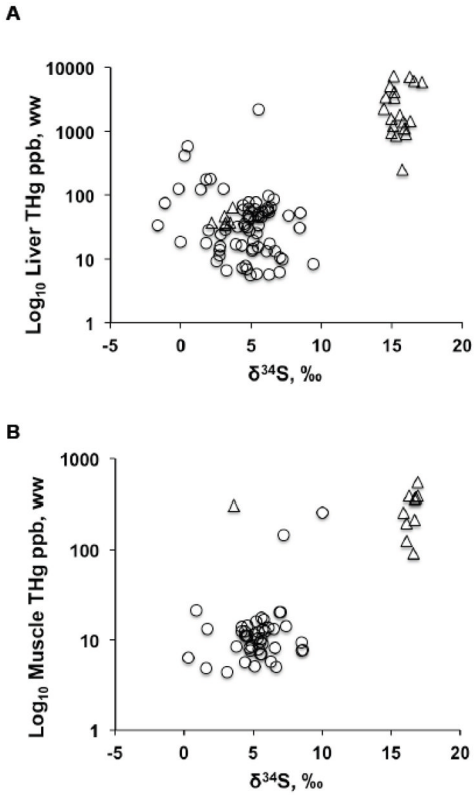

Figure 3.
THg and $\delta^{34}S$ in wolf liver, based on the designation of coastal versus interior gray wolves; △ = coastal wolves, ○ = interior wolves.

Table 1

Median and range of THg concentrations for all gray wolf (*Canis lupus*) tissues expressed in ug/kg wet weight (ww).

Organ/Tissue	n	Median	Range
Liver	145	34.7	5.5–7260.7
Kidney	143	95.2	8.3–11,173.3
Skeletal Muscle	60	12.5	4.4–546.0
Cardiac Muscle	16	206.7	3.9–640.0

Table 2

Median and range of THg concentrations in male and female gray wolves (*Canis lupus*), expressed in ug/kg wet weight (ww).

Tissue	n	Median	Range	p-value
Liver				0.39
Male	68	36.9	5.7–7260.7	
Female	76	30.9	5.5–7206.0	
Kidney				0.40
Male	68	106.4	13.5–11,173.3	
Female	74	92.9	8.3–4638.2	
Skeletal Muscle				0.39
Male	27	13.0	4.4–388.6	
Female	33	12.2	4.8–545.9	
Cardiac Muscle				0.84
Male	7	226.8	4.2–306.5	
Female	9	186.6	3.9–639.5	

Table 3

Median and range of THg concentrations in gray wolves (*Canis lupus*), expressed in ug/kg wet weight (ww) based on age class (<12 months or ≥ 12 months).

Tissue	n	Median	Range	p-value
Liver				<0.001
< 12 months	45	15.3	5.5–6086.0	
≥ 12 months	97	48.4	13.3–7260.7	
Kidney				<0.001
< 12 months	46	33.9	8.3–3373.0	
≥ 12 months	96	154.3	37.6–11,173.0	
Skeletal Muscle				0.07
< 12 months	9	8.1	4.4–545.9	
≥ 12 months	49	13.0	5.0–465.6	
Cardiac Muscle				0.125
< 12 months	1	3.9	3.9	
≥ 12 months	15	226.8	116.2–335.8	

Table 4

Median and range of THg concentrations in coastal and interior gray wolves (*Canis lupus*), expressed in ug/kg wet weight (ww).

Tissue	n	Median	Range	p-value
Liver				<0.001
Interior	117	28.4	5.5–2226.9	
Coastal	28	1159.0	33.8–7260.7	
Kidney				<0.001
Interior	116	80.5	8.3–4567.3	
Coastal	27	1939.0	78.3–11,173.0	
Skeletal Muscle				<0.001
Interior	47	10.9	4.4–258.4	
Coastal	13	317.2	90.3–545.9	
Cardiac Muscle				<0.001
Interior	3	4.2	3.9–179.0	
Coastal	13	232.8	70.5–639.5	